Dietary grape pomace − effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens

Taiwo J. Erinle, Samson Oladokun, Janice MacIsaac, Bruce Rathgeber, and Deborah Adewole[1]

Department of Animal Science and Aquaculture, Dalhousie University, Truro, NS B2N 5E3, Canada

ABSTRACT The search for alternatives to antibiotics in poultry production is still on-going and has been directed towards investigation of the efficacy of different potential alternatives. However, it is important that the sought alternatives are cost-efficient and have no negative impact on meat quality, for ease of adoption and profit maximization. This study aimed at exploiting an agro-industrial waste, grape pomace (**GP**) as an alternative to in-feed antibiotics and assessing the effects on growth, intestinal morphology, ceca microbiota, ceca short-chain fatty acid (**SCFA**) concentration, blood biochemical parameters, and breast muscle myopathies of broiler chickens. A total of 576 one-day-old Cobb-500 broiler chicks were randomly allotted to 3 dietary treatments − Negative control (**NC**, a corn-wheat soybean-based diet), NC + 0.05% bacitracin methylene disalicylate (**BMD**), and NC + 2.5% GP. Each treatment was assigned to 8 replicate pens with 25 birds per pen. Body weight (**BW**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined weekly. On d 36, 2 chickens/pen were euthanized for measuring blood biochemical parameters, ceca SCFA, and ceca microbiota. White striping (**WS**) and wooden breast (**WB**) incidence were assessed in 4 chickens/pen on d 42. The GP diet increased ($P < 0.05$) average FI throughout the feeding phases compared to the other treatments, but overall FCR was similar. Birds in the GP treatment had higher ($P < 0.05$) villus height (**VH**) and increased VH:crypt depth ratio in the duodenum and jejunum compared to other treatments. The level of ceca SCFA and the incidence of WS and WB was the same for all treatments. Plasma Ca and P were significantly higher ($P < 0.05$) in birds fed GP and BMD, compared to the NC. Birds in the GP treatment had significantly reduced ($P < 0.05$) plasma aspartate transaminase than other treatments. Birds receiving GP had a higher ($P < 0.05$) relative abundance of the phylum *Bacteroidetes* and reduced ($P < 0.05$) *Firmicutes* compared to other treatments. The relative abundance of *Bacteroides* and *Lactobacillus* genera were higher ($P < 0.05$) among birds fed GP compared to other treatments. Inclusion of 2.5% GP in broiler chicken diets improved gut morphology and modified the cecal bacterial community and blood biochemical profiles with no adverse effect on growth performance and meat quality.

Key words: grape pomace, broiler chickens, growth performance, gut morphology, ceca microbiota

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INTRODUCTION

The advent of antibiotics and their adoption in livestock production has unequivocally contributed to improvements in growth performance and gastrointestinal functionality of many livestock species, including poultry. However, the constant use of antibiotics in livestock as disease prophylaxis rather than a curative measure has contributed to the evolution of pathogenic microbes that are resistant to antibiotics, including those used in human medicine (Mehdi et al., 2018). Public outcry regarding antibiotic-resistant infections have ushered strict restrictions placed on the use of antibiotics as growth promoters in livestock production in the Europe (European Parliament and the Council of the European Union, 2003), and other countries have taken the cue (Chicken Farmers of Canada, 2020). The embargo placed on the prophylactic use of antibiotics has contributed to the proliferation of pathogenic microbes and could negatively impact the economy of the poultry industry. Therefore, there is a need to identify not only a potent but also a relatively cost-efficient alternative to antibiotics that could afford performance optimization of the birds.

Grape (*Vitis vinifera*) pomace (**GP**) is a downstream product that can be obtained from the production of grape juice and wine (Muhlack et al., 2018). It is

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[1]Corresponding author: Deborah.adewole@dal.ca

comprised of residual seeds, skin, and stems of grapes. The global wine industry used roughly 60 million tons of grapes in the production of wine, while the Canadian fruit processing and winery industry in Ontario alone produced approximately 89,000 tonnes of grapes in 2017 (García−Lomillo and González−SanJosé, 2017; Gowman et al., 2019). About 20 to 25% of the weight of grape produced is attributable to the weight of GP after wine pressing (Muhlack et al., 2018; Gowman et al., 2019) and poses a challenge on how to safely dispose it. It is noteworthy that these grape by-products contain appreciable amounts of phenolic compounds, dietary insoluble fiber and protein (Dwyer et al., 2014; Hogervorst et al., 2017; Heuzé and Trans, 2020). Phenolic compounds have been harnessed in some poultry nutritional studies as potential alternatives to antibiotics because of their antioxidant and antimicrobial capacities. Over the years, grape by-products have been underexploited, with large portions used for unproductive purposes like disposal in landfills, thus generating environmental concerns.

Optimum exploitation of grape bio-waste as a nutraceutical for poultry birds could enhance the performance and general well-being of chickens and improve profit margin for both chicken farmers and wineries. Although previous studies have investigated the effect of GP on growth performance of broiler chickens, studies showing the possibility of GP to improve growth of broiler chickens are very scanty (Sáyago-Ayerdi et al., 2009; Viveros et al., 2011; Chamorro et al., 2015; Aditya et al., 2018; Ebrahimzadeh et al., 2018; Kumanda et al., 2019). This might be partly due to the high inclusion levels of GP (usually within the range of 5−10%) as mostly reported. Except for the report of Kumanda et al. (2019), dietary supplementation of GP within 5 to 10% has been reported to show no significant improvement on growth performance of broiler chickens. It is therefore imperative to investigate the effect of a lower inclusion level of GP, particularly in comparison to antibiotics. At the intestinal level, the use of grape by-products showed modulatory effects on gut morphology in the duodenal mucosa of pigs (Gressner et al., 2013; Wang et al., 2020) and the relative abundance of *Enterobacteriaceae, E. coli, Lactobacillus, Enterococcus, Clostridia, Campylobacter, Salmonella*, and *Helicobacter pylori* (Viveros et al., 2011; Chamorro et al., 2019; Nardoia et al., 2020).

Besides the digestive tract, antioxidants provide potential benefits in other systems of the body including circulatory and muscular systems. Fibrosis and oxidative damage resulting from tricarboxylic acid cycle, excess nitric oxide, and accumulation of long-chain fatty acids have been implicated in the incidence of breast muscle myopathies in poultry birds (Mogire, 2020). The incidence of myopathies in breast muscle, including white striping (**WS**) and wooden breast (**WB**), has been associated with heavier body weight of birds (Kuttappan et al., 2012), thus making broiler chickens highly susceptible. In the studies by Makris et al. (2007), Chamorro et al. (2015), and Brenes et al. (2016), GP supplementation was reported to reduce oxidative stress of blood and muscle tissues of monogastric animals. These studies suggest that dietary GP could be effective in preventing WS and WB in broiler chickens. To the best of our knowledge, no study has investigated the effect of dietary GP on the incidence of WS and WB and ceca SCFA concentration as an indicator of gut health in poultry. In addition, data on the effect of GP on other measures of chicken health such as gut microbiota, morphology, and blood biochemistry are limited.

Given the above, it was hypothesized that lower inclusion of dietary GP at 2.5% would improve growth performance, reduce breast muscle myopathies, and modulate gut health in the equal capacity of antibiotics. Therefore, the current study was aimed at investigating the impact of 2.5% dietary GP as an alternative to in-feed antibiotics, by evaluating its effect on cecal short-chain fatty acid concentration and breast muscle myopathies, in addition to growth performance, blood biochemistry, and intestinal morphology of broiler chickens.

MATERIALS AND METHODS

The experimental protocols (Animal Care Certification Number 2020-027) were subjected to approval by Dalhousie University Animal Care and Use Committee, and birds were handled in accordance with the guidelines established by the Canadian Council on Animal Care (2009).

Diets and Experimental Design

A total of 576 one-day-old mixed-sex Cobb-500 broiler chicks were obtained from Atlantic Poultry Incorporated, Port Williams, Nova Scotia, and were raised on floor pens. Room temperature was monitored daily and was gradually reduced from 30°C to 22.6°C from d 0 to 42. The lighting program was set to produce 18 h of light and 6 h of darkness throughout the experimental period, and illumination was gradually reduced from 20 1x on d 0 to 5 1x on d 39.

Diets and Experimental Design

The GP used in this study was obtained from Gaspereau Vineyards, Nova Scotia. The product was freeze-dried using a Supermodulyo freeze-dryer (Model:220 Thermo Savant; Holbrook, NY) and grinded using a coffee grinder. The birds were randomly allotted to 3 treatments groups containing 8 replicates, with 25 birds per replicate and fed the following diets: 1) A corn-wheat-soybean meal diet (**NC**); 2) NC + 0.05% in-feed bacitracin methylene disalicylate (**BMD**); and 3) NC + 2.5% GP (**GP**). The experimental diets were formulated to meet the nutrient requirements of broiler chickens as recommended by NRC (1994), and birds were fed on a phase-feeding program as follows: starter (1−14 d of age), grower (14−24 d of age), and finisher (24−42 d of age). The ingredient and nutrient compositions of the

Table 1. Gross and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated).[1]

Ingredients	Starter phase (1−14 d)			Grower phase (14−28 d)			Finisher phase (28−42 d)		
	NC	BMD	GP	NC	BMD	GP	NC	BMD	GP
Corn	42.77	42.67	40.03	45.92	45.82	41.80	50.71	50.62	46.71
Soybean meal	39.95	39.96	38.64	36.22	36.24	36.31	31.17	31.19	31.24
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Animal fat/vegetable oil blend	2.72	2.76	4.26	3.75	3.78	5.28	4.34	4.37	5.77
Grape pomace[2]	–	–	2.50	–	–	2.50	–	–	2.50
BMD 110 G[3]	–	0.05	–	–	0.05	–	–	0.05	–
Limestone	1.81	1.81	1.77	1.65	1.65	1.62	1.50	1.50	1.47
Dicalcium phosphate	1.22	1.22	1.24	1.05	1.05	1.07	0.90	0.90	0.91
DL methionine premix[4]	0.61	0.61	0.63	0.53	0.53	0.55	0.49	0.49	0.51
Starter vitamin/mineral premix[5]	0.50	0.50	0.50	–	–	–	–	–	–
Grower/Finisher vitamin/mineral premix[6]	–	–	–	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.37	0.37	0.38	0.38	0.38	0.38
Lysine HCl	0.03	0.03	0.04	–	–	–	0.01	0.01	0.01
Calculated analysis									
Crude protein	23	23	23	21.5	21.5	21.5	19.5	19.5	19.5
Metabolizable energy (kcal kg^{-1})	3,000	3,000	3,000	3,100	3,100	3,100	3,200	3,200	3,200
Calcium	0.96	0.96	0.96	0.87	0.87	0.87	0.78	0.78	0.78
Available phosphorus	0.48	0.48	0.48	0.44	0.44	0.44	0.39	0.39	0.39
Digestible lysine	1.28	1.28	1.28	1.15	1.15	1.15	1.02	1.02	1.02
Digestible methionine + Cystine	0.95	0.95	0.95	0.87	0.87	0.87	0.8	0.8	0.8
Sodium	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18
analyzed analysis									
Crude protein	20.24	18.90	22.04	19.46	19.52	21.72	18.66	19.51	19.31
Calcium	0.92	0.80	0.94	1.06	0.87	0.89	0.89	0.86	0.80
Total phosphorus	0.60	0.54	0.61	0.53	0.55	0.52	0.53	0.51	0.50
Sodium	0.15	0.17	0.19	0.14	0.18	0.15	0.16	0.18	0.16
Crude fat	4.50	5.03	6.53	7.22	6.98	7.42	5.23	4.74	6.90

[1]Abbreviations: BMD, antibiotic diet; GP, diet containing 2.5% grape pomace; NC, negative control diet.
[2]Grape pomace: 93.29% dry matter; 10.43% crude protein; 10.05% crude fat; 48.44% acid detergent fibre; 46.27% neutral detergent fibre; 0.47% calcium; 1.56% potassium; 0.08% magnesium; 0.24% phosphorus; 12.40 ppm copper; 11.73 ppm zinc.
[3]Bacitracin methylene disalicylate (providing 55 mg/kg mixed feed); Alpharma, Inc., Fort Lee, NJ.
[4]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.
[5]Starter vitamin-mineral premix contained the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B12; 29.7 mg niacin; 1.0 mg folic acid, 801 mg choline;0. 3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc; 25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone.
[6]Grower and Finisher vitamin-mineral premix contained the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B12; 29.7 mg niacin; 1.0 mg folic acid, 801 mg choline;0. 3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc; 25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone.

diets for the 3 phases are shown in Table 1. The chemical composition of GP was presented in Supplementary Table 1. The chemical composition of the diets was determined following AOAC (1994) procedure. Total polyphenols in the GP and control diets and polyphenols profile of GP (Figure 1) were determined using ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) at the Institute of Nutrition and Functional Foods, Quebec, Canada.

Growth Performance

Average body weight (**ABW**) and average feed intake (**AFI**) were determined weekly on a pen basis, and mortality was recorded daily to correct for AFI and feed conversion ratio (**FCR**). Birds that died were sent to the Veterinary Pathology Laboratory, Dalhousie University for postmortem.

Blood Biochemistry Analysis

On d 36, 2 birds were randomly selected from each pen, individually weighed, and euthanized by electrical stunning and exsanguination. Blood samples were collected from each bird into 5 mL heparinized tubes and were centrifuged at 5,000 rpm for 10 m and shipped on ice to Atlantic Veterinary College, University of Prince Edward Island Pathology Laboratory, where samples were analyzed using Cobas 6000 analyzer series. Serum immunoglobulin G and M were analyzed using enzyme-link immunosorbent assay (**ELISA**) kits from Bethyl Laboratories, Inc. (catalog number E33-104-200218 and E33-102-180410, respectively) following manufacturer instructions.

Short-Chain Fatty Acid Concentrations and Total Eubacteria Count

Digesta from the pair of ceca were mixed and divided into 2 subsamples; one portion was placed in BioFreeze sampling kits (Alimetric Diagnostics, Espoo, Finland) for the determination of short-chain fatty acid (SCFA) profile and quantity. In addition to the cecal SCFA concentration, the analysis of the most prevalent bacterial species was performed by Alimetrics Diagnostics Ltd.

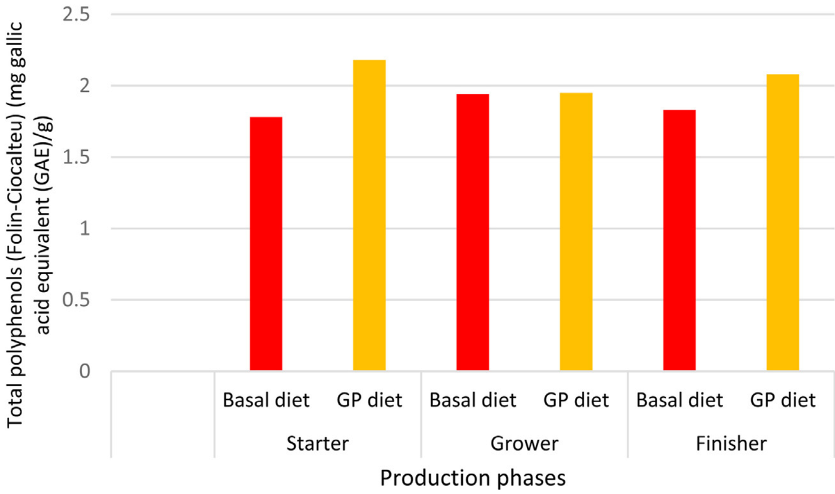

Figure 1. Total polyphenols content (mg gallic acid equivalent GAE/g) in treatments offered to broiler chicken according to production phases. NC, BMD, and GP diets per production phase. NC = negative control diet (corn-wheat-soybean meal diet), NC + 0.05% in-feed bacitracin methylene disalicylate diet = BMD, and GP = diet containing 2.5% grape pomace.

Gut Morphology

One centimeter of the duodenal, jejunal, and ileal midpoints was removed from each euthanized bird, and preserved in formalin for 3 d. The intestinal segments were then immersed in paraffin and cut at the thickness of 2 μm. Each of the cut excised segments was mounted on a glass slide (n = 16 per treatment) and stained with Alcian blue and periodic acid-Schiff (**PAS**) reagents. The morphological slides were examined using a microscope coupled with a digital camera. Ten well-oriented and distinct villi on each slide were identified and measured for villus height (**VH**), villus width (**VW**), and crypt depth (**CD**). Villus height was measured from the tip of the villus to the villus crypt junction, that is, top of the lamina propria of each villus. Crypt depth was measured from the villus crypt junction to the tip of the muscularis mucosa (Shang et al., 2020). The villus height:crypt depth (**VH:CD)** was subsequently calculated.

Gut Microbiota

The second portion of the mixed cecal digesta was stored in plastic RNAse and DNAse-free tubes, placed in liquid nitrogen, and afterward kept at -80°C for analysis of gut microbiota. Specimens were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate (Qiagen, Carlsbad, CA). DNA was extracted following MoBio's instructions on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5'-GTGCCAGCMGCCGCGGTAA-3', and 806R 5'-GGACTACHVGGGTWTCTAAT-3'), as per the protocol of Kozich et al. (2013). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). Sequences were denoised, taxonomically classified using Greengenes (v. 13_8) as the reference database, and clustered into 97%-similarity operational taxonomic units (**OTU**) with the mothur software package (v. 1.39.5) (Schloss, 2009), following the recommended procedure (https://www.mothur.org/wiki /MiSeq_ SOP; accessed Nov 2017). Bioinformatics analyses were conducted in the R statistical environment (R Development Core Team. 2013).

Breast Muscle Myopathy

Breast muscle samples were collected on 4 birds (2 males and 2 females) per pen (32 birds per treatment) on d 42 and were evaluated visually and scored by one observer for precision. The breast muscle samples were also sliced into fillets. The visual myopathies were scored based on the incidence of white striping (**WS**) and wooden breast (**WB**) scores following a method modified from Kuttappan et al. (2012).

Statistical Analysis

One-way ANOVA was carried out using Minitab LLC (2019) software with treatments (NC, BMD, and GP). Following ANOVA, differences between significant means were tested using Tukey's honest significant difference (**HSD**) test in the same statistical package. Parametric dataset was analyzed by one-way ANOVA, while nonparametric data were analyzed by Kruskal Wallis' median test in the same statistical package. Analyzed data were presented as means, standard error of the mean (SEM), and probability values. Values were considered statistically different at $P < 0.05$.

RESULTS

Total Polyphenol Content (TPC)

The results of the TPC (Folin-Ciocalteu) (mg gallic acid equivalent GAE/g) in the NC and GP diets are presented in Figure 1. The TPC of diets supplemented with 2.5% GP at the starter, grower, and finisher diets were

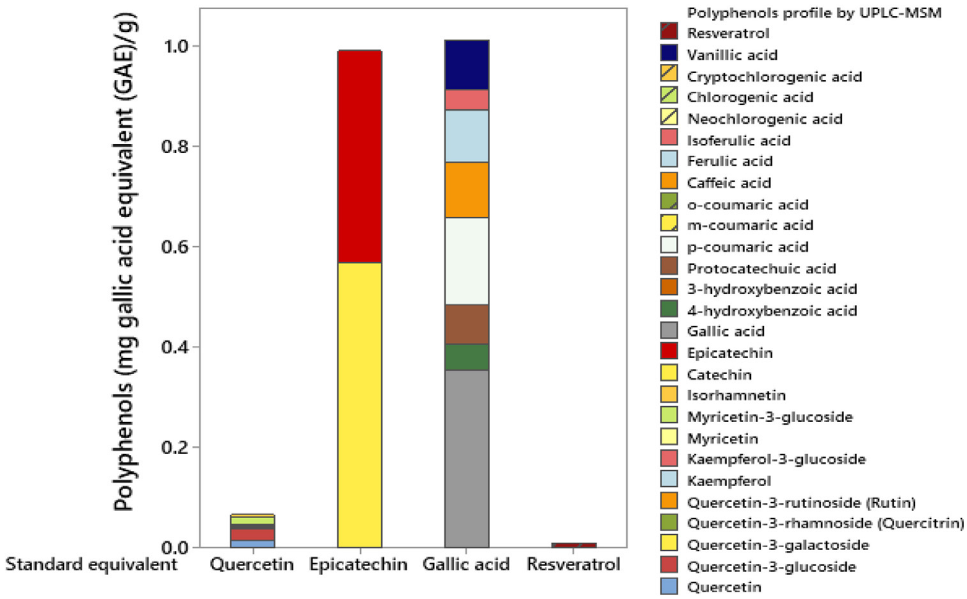

Figure 2. Polyphenols profile of whole grape pomace by UPLC-MSMS (mg standard equivalent/g).

2.18, 1.95 and 2.08 mg GAE/g, respectively. In the NC treatment, TPC in the starter, grower, and finisher diets were 1.78, 1.94, and 1.83 mg GAE/g, respectively. The profile of polyphenols (mg gallic acid equivalent GAE/g) in whole GP was present in Figure 2. Epicatechin, catechin, and gallic acid were observed to most abundant polyphenols in whole GP.

Growth Performance

The growth performance of broiler chickens fed GP as an in-feed antibiotic replacement is presented in Table 2. At the end of the starter phase, AFI, and AWG were significantly higher ($P < 0.05$) among birds fed GP and antibiotic diets compared to the control-fed birds. The FCR of birds was not significantly affected by the dietary treatments. In the grower phase, all the growth parameters were significantly influenced ($P < 0.05$) by the dietary treatments. The average feed intake of birds fed GP and antibiotic diets was statistically similar and higher ($P < 0.05$) than the birds receiving the control diet. The AWG of birds fed GP and control were statistically similar but significantly lower ($P < 0.05$) compared to the antibiotic fed birds. In the finisher phase, GP and antibiotic-fed birds had higher ($P < 0.05$) AFI compared to the control birds; however, average AWG and FCR were similar across all treatments. On an overall performance basis, FCR was not significantly influenced by the dietary treatments; however, GP and antibiotic treatments had higher ($P < 0.05$) AFI compared to the control. Compared to the GP and control diet, average AWG was significantly higher ($P < 0.05$) in birds fed the antibiotic diet.

Gut Morphology

The effects of the dietary GP on the morphology of intestinal segments of broiler chickens is presented in

Table 2. Effect of dietary supplementation of grape pomace as a substitute for synthetic antibiotics on growth performance of broiler chickens examined at phase levels.

Phases	Parameters	Treatments[1]			SEM	P-value
		NC	BMD	GP		
Starter (d 1–14)	Average feed intake (g/bird)	942[b]	1,017[a]	1,008[a]	10.50	0.002
	Average weight gain (g/bird)	296[b]	342[a]	333[a]	5.72	<0.050
	FCR	1.58	1.48	1.50	0.02	0.116
Grower (d 14–28)	Average feed intake (g/bird)	2,811[b]	3,086[a]	2,930[ab]	38.60	0.004
	Average weight gain (g/bird)	907[b]	1,028[a]	894[b]	15.30	<0.050
	FCR	1.57[ab]	1.50[b]	1.64[a]	0.02	0.020
Finisher (d 28–42)	Average feed intake (g/bird)	5,143[b]	5,411[a]	5,196[ab]	40.80	0.012
	Average weight gain (g/bird)	1,422	1,455	1,418	12.40	0.413
	FCR	1.80	1.85	1.82	0.02	0.422
Overall	Average feed intake (g/bird)	4,354[b]	4,743[a]	4,571[a]	45.70	<0.050
	Average weight gain (g/bird)	2,629[b]	2,828[a]	2,648[b]	25.80	<0.050
	FCR	1.72	1.65	1.69	0.02	0.358

[1]Abbreviations: BMD, (bacitracin methylene disalicylate) antibiotic diet; GP, diet containing 2.5% grape pomace; NC, Negative control diet.
[a,b]In a row, means assigned different lowercase superscript letters are significantly different, $P < 0.05$ (Tukey's procedure).

Table 3. Effect of dietary grape pomace on intestinal morphology of broiler chickens.

Parameters (mm)	Treatment effect[1]			SEM	*P*-value
	NC	BMD	GP		
Duodenum					
Villus height	2.11[b]	2.10[b]	2.42[a]	0.02	0.000
Villus width	0.21	0.22	0.22	0.00	0.358
Crypt depth	0.18	0.18	0.18	0.00	0.742
VH:CD	11.02[b]	11.20[b]	13.13[a]	0.17	0.000
Jejunum					
Villus height	1.36[a]	1.15[b]	1.36[a]	0.02	0.000
Villus width	0.19[a]	0.17[b]	0.19[a]	0.61	0.002
Crypt depth	0.16[a]	0.13[b]	0.13[b]	0.00	0.001
VH:CD	9.71[b]	9.58[b]	10.83[a]	0.16	0.001
Ileum					
Villus height	0.89[a]	0.84[ab]	0.83[b]	0.01	0.025
Villus width	0.17[ab]	0.16[b]	0.17[a]	0.00	0.022
Crypt depth	0.16[a]	0.14[b]	0.15[ab]	0.00	0.039
VH:CD	5.76	5.79	5.58	0.10	0.544

[1]Abbreviations: BMD (bacitracin methylene disalicylate) antibiotic diet; GP, diet containing 2.5% grape pomace; NC, negative control diet; VH:CD = villus height:crypt depth ratio.
[a,b]In a row, means assigned different lowercase superscript letters are significantly different, $P < 0.05$ (Tukey's procedure).

Table 3. In the duodenal section, GP significantly increased ($P < 0.05$) VH and VH:CD of the birds compared to other treatments, while VW and CD were similar across the treatments. In the jejunal segment, VH and VW were higher ($P < 0.05$) in the GP and control treatments compared to the antibiotic treatment. Although CD was lower ($P < 0.05$) in birds fed GP and antibiotic treatment compared to the control, VH:CD was the highest ($P < 0.05$) in bird fed GP compared to other treatments. In the ileal portion, VH was significantly higher ($P < 0.05$) among birds fed the control diet compared to the GP and antibiotic treatments. Crypt depth was similar between the NC and GP groups; however, it was significantly higher ($P < 0.05$) in the NC group when compared to the antibiotic treatment. Also, in this gut section, VH:CD was similar across the treatments.

Plasma Biochemistry and Serum Immunoglobulins

The effect of dietary GP supplementation on blood biochemical indices of broiler chickens is shown in Table 4. Dietary GP supplementation had significant ($P < 0.05$) effects on Ca, P, ALP, and AST. Ca and *P* were significantly higher ($P < 0.05$) in birds fed GP and antibiotic diets compared to the NC diet. Cholesterol was not significantly affected by the dietary treatments. Both the control and GP-fed birds had lower ($P < 0.05$) ALP compared to the antibiotic treatment. Birds in the GP treatment had significantly reduced ($P < 0.05$) AST than other treatments. Although ALT was not significantly affected by the diets, it was lowest among the GP birds compared to the birds in the antibiotic and control treatments. Serum IgG and IgM were not affected by dietary treatments.

Table 4. Effect of dietary grape pomace on blood biochemistry and immunoglobulin profiles of broiler chickens.

Parameters	Treatment effect[1]			SEM	*P*-value
	NC	BMD	GP		
Sodium (mmol/L)	150	150	151	0.90	0.797
Potassium (mmol/L)	4.78	4.95	4.54	0.19	0.443
Na:K ratio	31.32	29.40	33.44	0.98	0.178
Chloride (mmol/L)	111	110	111	0.75	0.929
Calcium (mmol/L)	2.66[ab]	2.48[b]	2.76[a]	0.04	0.007
Phosphorus (mmol/L)	1.78[b]	2.08[a]	1.79[b]	0.05	0.022
Magnesium (mmol/L)	0.80	0.83	0.78	0.01	0.146
Urea (mmol/L)	0.33	0.29	0.29	0.01	0.179
Glucose (mmol/L)	13.94	13.70	14.60	0.17	0.092
Cholesterol (mmol/L)	2.70	2.79	2.55	0.06	0.236
Amylase (U/L)	454	522	678	63.6	0.174
ALP (U/L)	3,063[a]	1,866[b]	3,222[a]	222	0.001
ALT (U/L)	4.64	4.30	4.16	0.32	0.690
AST (U/L)	252[ab]	305[a]	230[b]	31.5	0.01
CK (U/L)	16,304	21,057	10,890	4,733	0.095
GGT (U/L)	13.42	11.56	14.60	0.71	0.198
Lipase (U/L)	25.55	25.05	23.72	1.29	0.825
T. Protein (g/L)	27.88	27.00	29.19	0.47	0.122
Albumin (g/L)	10.61	10.78	11.09	0.16	0.368
Globulin (g/L)	17.16	16.08	17.80	0.36	0.116
A:G	0.67	0.69	0.61	0.02	0.075
Iron (umol/L)	16.69	16.53	16.85	0.44	0.934
Uric Acid (umol/L)	332.71	284.64	313.82	11.0	0.213
Bile Acids (umol/L)	14.35	13.60	12.94	0.56	0.576
Creatinine (umol/L)	0.00	0.00	0.00	0.07	0.319
T. Bilirubin (umol/L)	0.00	0.00	0.00	0.03	0.600
Serum IgG (mg/mL)	6.17	5.49	5.16	0.47	0.699
Serum IgM (mg/mL)	0.40	0.30	0.28	0.02	0.064

[1]Abbreviations: A:G, Albumin Globulin ratio; ALP, Alkaline Phosphatas; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMD, (bacitracin methylene disalicylate) antibiotic diet; CK, Creatine kinase; GGT, Gamma-glutamyl transferase; GP, diet containing 2.5% grape pomace; Na:K, Sodium:Potassium ratio; NC, Negative control diet; T. Protein, Total Protein; T. bilirubin, Total bilirubin.
[a,b]In a row, means assigned different lowercase superscript letters are significantly different, $P < 0.05$ (Tukey's procedure).

Cecal Microbiota

A total of 6,169 OTU were detected, with an average of 43,773 quality-filtered reads generated per sample and clustered into 97% similarity. Information on the sequencing quality profile is presented in Supplementary Figure 1. There was an effect of dietary treatment on the total number of quality filtered read counts, as illustrated in Supplementary Figure 2. Aggregation of OTU into each taxonomic rank and the relative abundance of the most abundant phyla, genera (classified and unclassified) are presented in Figures 3-6. Supplementation of 2.5% GP significantly reduced ($P < 0.05$) the abundance of phylum Firmicutes, Proteobacteria, and Bacteria_unclassified but increased ($P < 0.05$) the abundance of phylum Bacteroidota (also known as Bacteroidetes). Compared to the antibiotic treatment, relative abundance of genera was significantly higher ($P < 0.05$) in the GP and NC treatments. Genera *Bacteroides*, and *Lactobacillus* were significantly ($P < 0.05$) increased among birds fed GP compared to other treatments. However, genera *Oscillospirales, Escherichia, Lachnospiraceae, CAG-352, Blautia, UCG-005, NK4A214_group* and

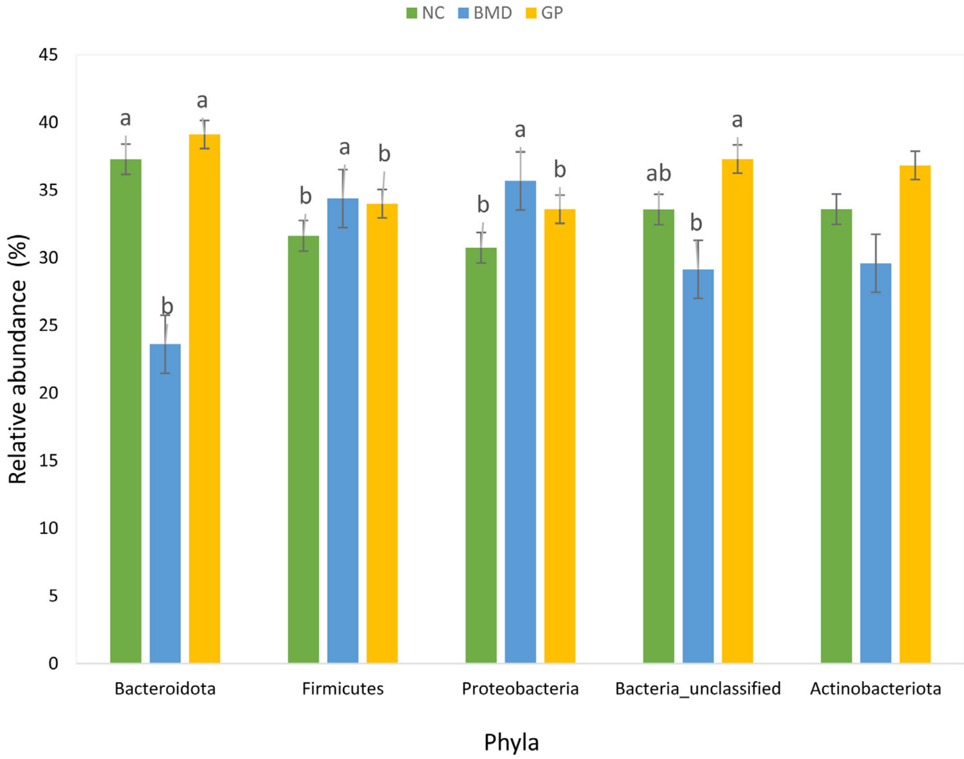

Figure 3. Percentage relative abundance of the most abundance bacteria Phyla in the ceca of broiler chickens fed grape pomace as substitute to in-feed antibiotics. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

Anaerovoracaea were significantly ($P < 0.05$) higher among antibiotic-treated birds compared to other treatments. For the Shannon diversity and richness, the antibiotic treatment had the highest ($P < 0.05$) alpha diversity (Figure 7). Permutational analysis of variance shows a significant ($P < 0.05$) difference in beta diversity, with the birds fed antibiotic diet being higher than other treatment groups, as shown in Figure 8.

Ceca Short-Chain Fatty Acid Concentration

The effect of dietary GP supplementation on total eubacteria counts and short-chain fatty acids concentration in the ceca is presented in Table 5. Compared to antibiotic and control diets, supplementation of 2.5% GP did not have significant ($P > 0.05$) effect on the total eubacteria count, SCFA, AA, PA, BA, VA, LA, BCFA, and VFA.

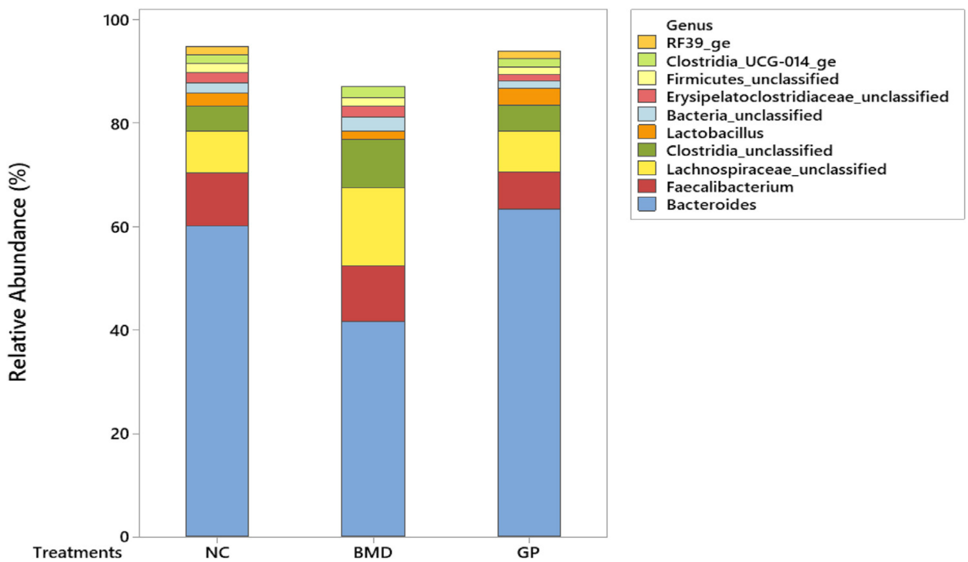

Figure 4. Percentage relative abundance of the most abundance bacteria genera in the ceca samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

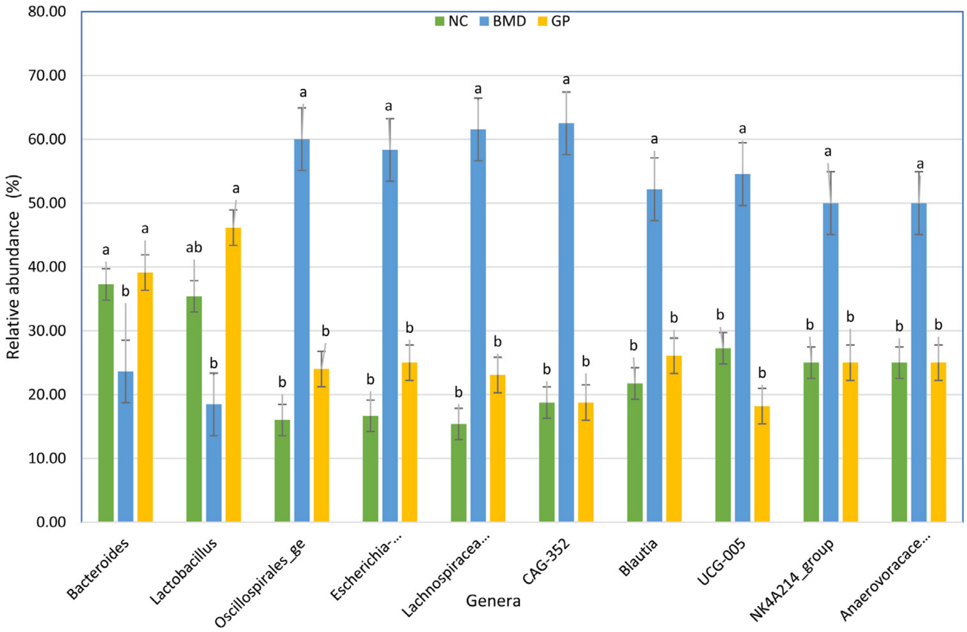

Figure 5. Percentage relative abundance of the most abundance classified genera of bacteria in the ceca samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

Breast Muscle Myopathy

White striping and WB scores of broiler chickens fed dietary GP are presented in Table 6. The result shows no dietary treatment or sex effect on WS and WB score. However, male chickens had higher breast muscle and slaughter weights compared to female chickens; while breast weight expressed as a percentage of body weight was higher in female chickens compared to the males. Wooden breast score was generally low across all treatments with only few birds WB incidence.

DISCUSSION

Grape pomace contains bioactive substances which have been recognized to possess antioxidative and antimicrobial properties. In this regard, GP has been sought

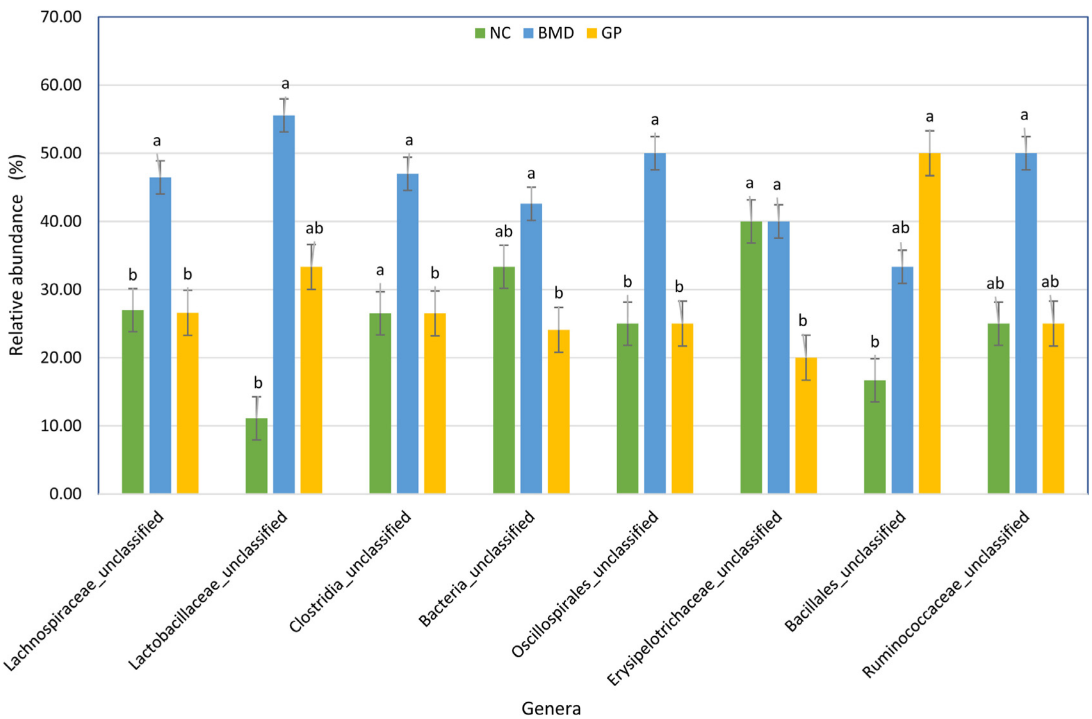

Figure 6. Percentage relative abundance of the most abundance unclassified genera of bacteria in the cecal samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

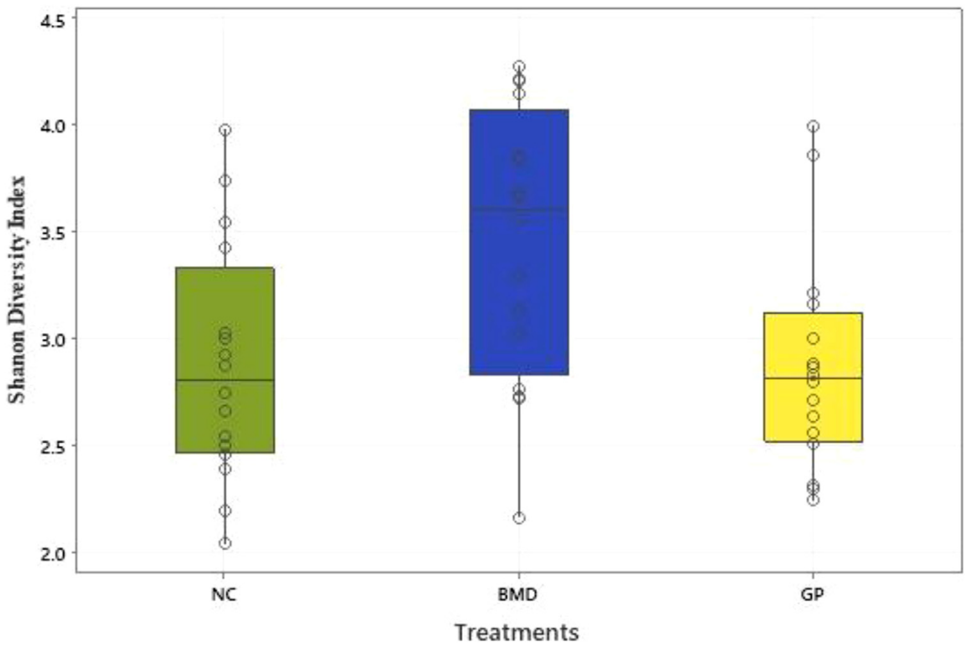

Figure 7. Box-and-whisker plot showing significant differences in the Shannon diversity index (Alpha diversity) ($P > 0.05$; F Value- 0.723). Ceca content was collected from 36-day-old broiler chickens offered 3 different dietary treatments. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

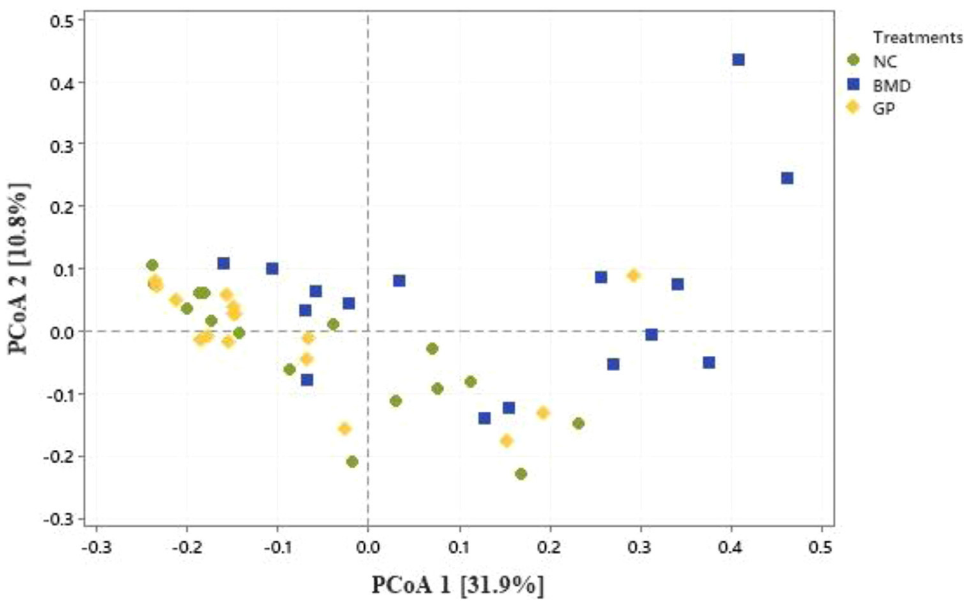

Figure 8. Multivariance analysis determined significant differences ($P < 0.05$) in beta-diversity among treatments. Treatment groups: NC = negative control diet, BMD = (bacitracin methylene disalicylate) antibiotic diet, and GP = diet containing 2.5% grape pomace.

Table 5. Effect of dietary supplementation of grape pomace on total eubacteria count and short-chain fatty acids concentration in the ceca of broiler chickens.

Parameters	Treatment effect[1]			SEM	*P*-value
	NC	BMD	GP		
Total eubacteria (16S rDNA copies/g)	2.58E+12	2.26E+12	2.33E+12	4.89E+11	0.819
Short chain fatty acids (mM)	72.51	78.82	77.94	3.49	0.736
Acetic acid (mM)	49.69	57.67	54.30	2.63	0.471
Propionic acid (mM)	6.75	5.86	7.50	0.31	0.103
Butyric acid (mM)	8.08	7.12	8.86	0.76	0.693
Valeric acid (mM)	1.24	1.11	1.25	0.07	0.578
Lactic acid (mM)	1.90	1.91	2.81	0.42	0.223
Branched chain fatty acids (mM)	1.78	1.36	1.53	0.11	0.228
Volatile fatty acids (mM)	68.34	73.75	72.72	3.36	0.795

[1]Abbreviations: BMD, (bacitracin methylene disalicylate) antibiotic diet; GP, diet containing 2.5% grape pomace; NC, negative control diet.

Table 6. Treatment, sex, and interaction effects of supplemental grape pomace on white stripping and woody breast meat of broiler chickens.

Parameters		Treatment effect[1]			Sex effect		ANOVA P VALUE		
		NC	BMD	GP	M	F	Treatment effect	Sex effect	Interaction effect
White stripping score	1.00	1.00	1.00	1.00	1.00	0.312	0.001		
WS % (n)	Normal	25 (8)	21.88 (7)	34.38 (11)					
	Moderate	40.63 (13)	37.50 (12)	40.63 (13)					
	Severe	34.38 (11)	37.50 (12)	25.00 (8)					
	Extreme	0.00 (0)	3.13 (1)	0.00 (0)					
Woody Breast Score	0.00	0.00	0.00		0.00	0.00	1.000	0.002	
WB % (n)	Normal	84.38 (27)	84.38 (27)	84.38 (27)					
	Wooden breast	15.63 (5)	15.63 (5)	15.63 (5)					
Breast weight (g)		511	567	503	554[a]	500[b]	0.076	0.033	0.257
Body weight (g)		2871	2991	2842	3153[a]	2668[b]	0.263	0.000	0.851
Breast weight (%)		17.62	18.47	17.64	17.26[b]	18.57[a]	0.426	0.032	0.101

[1]Abbreviations: BMD, (bacitracin methylene disalicylate) antibiotic diet; GP, diet containing 2.5% grape pomace; NC, Negative control diet.(n) = Number of birds based on severity of white striping or woody breast.

[a,b]In a row, means assigned different lowercase superscript letters are significantly different, $P < 0.05$ (Tukey's procedure).

not only as a potential alternative to antibiotics but also as a possible portion of composite feed for poultry (Brenes et al., 2016). Studies involving the use of grape by-products have shown inconsistent results basically in terms of growth performance. This might be due to the varying abundance of total polyphenols present in the various varieties of grape by-products including, grape seed extract, grape skin, and grape pomace as dictated by but not limited to edapho-climatic factors (Shi et al., 2003; Rodríguez Montealegre et al., 2006; Hassan et al., 2019). The total polyphenol content in our whole GP is 12.31 mg GAE/g which is lower than the reported 34.1 ± 0.3 mg GAE/g in muscadine GP (Wang et al., 2010), 48.7 mg GAE/g (Viveros et al., 2011), and 33.92 mg GAE/g (Ebrahimzadeh et al., 2018). The polyphenolic profile of whole GP also shows catechin, epicatechin, and gallic acid were observed to be the most abundant. These 3 polyphenols have been considered major catechins with dietary importance for both animals and human health (El Gharras, 2009). The impacts of GP reported in the literature ranges from growth-maintenance to growth-reduction in birds depending on their inclusion levels in chickens' diet. Goñi et al. (2007) and Sáyago-Ayerdi et al. (2009) reported that addition of dietary GP up to 6% could be used in chicken diets without impairing growth performance. Supplementation of 5% or 10% GP was reported to show no significant improvement on growth performance of broiler chickens (Chamorro et al., 2015). Kumanda et al. (2019) also demonstrated that the inclusion of 7.5% dietary red GP reduced the overall feed intake of chickens. However, the study of Pop et al. (2015) reported a nonsignificant improvement in the body weight of broiler chickens which increases as GP inclusion level increases from 1 to 2%. Without overemphasis, there is bewildering evidence that antibiotics improve growth performance parameters of poultry birds (Gadde et al., 2017; Mehdi et al., 2018; Shang et al., 2020). However, based on the antioxidant capacity and the reported safe inclusion levels of GP, it was hypothesized that dietary inclusion of 2.5% GP into broiler chickens' diet would yield an equivalent growth-improvement propensity as antibiotics. The results of our study show that dietary supplementation of 2.5% GP improves AFI with a corresponding increase in average AWG in the first 2 wk of feeding (that is starter phase; d 1 to 14) and favorably compared to the antibiotic diet. This is consistent with the findings of Aditya et al. (2018) who reported that GP supplementation at 0.5% dosage had a beneficial effect on body weight gain during the first 2 wk of life due to the presence of polyphenols. This suggests that the amount of fiber and polyphenols present in 2.5% inclusion level of GP would improve feed intake and growth of broiler chickens at least in the first 2 wk. The benefit of phytogenic additive on body weight and feed conversion ratio is markedly pronounced during the first stage of posthatch life (Toghyani et al., 2011; Abdel-Wareth et al., 2019). During the grower phase, birds fed the antibiotic diet had higher AFI, average AWG, and lower FCR compared to those fed GP and control diets. This is consistent with the report of Kumanda et al. (2019) who submitted that dietary supplementation of 2.5% GP yields similar AFI when compared to control-fed birds. The reduced AWG among the GP-fed birds was due to the reduced AFI during the grower phase. Another plausible factor responsible for similar AWG at d 14 to 28 could be due to the approximately equal amount of dietary polyphenols in the control or 2.5% GP diets which had an impact on gut microbiota profile that is known to reduce body weight. During the finisher phase, AFI, AWG, and FCR in birds that consumed GP diet compared favorably to both the antibiotic and control treatments. Although, the overall AWG of birds fed GP was statistically lower compared to those in the antibiotic treatment; however, the overall FCR was similar to the antibiotic and control treatments. This agrees with the work of Kumanda et al. (2019) who also obtained similar overall weight gain and FCR when broiler chickens were fed 2.5% GP and control diet. The inclusion level of any dietary phytogenic additive in an NC diet that could afford improved performance of birds without side effects is referred to as reasonable doses (Qaid et al., 2021). The sustained overall FCR suggests that supplementation of GP at 2.5% may be the plausible dietary dosage at which the growth performance of broiler chickens is comparable to antibiotics.

In our study, the dietary treatments affected the histomorphometric structure in the gut. The gut plays an important role in the digestion and absorption of nutrients and plays a selective barrier function by allowing passage of nutrients and blockage of pathogenic microbes and their metabolites. These crucial gut functions could be compromised in the presence of some factors, such as low-quality diets. Villus height and CD are considered indicators of gut health for better nutrient absorption and a slower rate of enterocyte epithelial cell renewal. A healthy gut presents a higher VH and VH:CD and shallow crypt (Oliveira et al., 2008; Laudadio et al., 2012). Conversely, lower VH and deeper crypts are associated with poor digestion, less nutrient absorption, and consequently poor growth performance (Qaisrani et al., 2014). The use of bacitracin has reported to improve gut morphology in broiler chickens (Adewole and Akinyemi, 2021). However, it is interesting that dietary inclusion of 2.5% GP for broiler chickens significantly increased VH and VH:CD in the duodenum and jejunum compared to the control and antibiotic groups. This is the opposite of results reported by Ebrahimzadeh et al. (2018) when 5% and 7.5% dietary GP was fed to broiler chickens. This could be due to the higher inclusion levels of GP. When a lower inclusion of GP at 60 mg/kg was employed in the study of Viveros et al. (2011), an increase in the VH:CD was observed, and this was comparable to birds fed dietary antibiotics. Crypt depth was observed to be shallower in the jejunum and ileum of birds receiving 2.5% GP and antibiotics compared to those fed the control diet; however, duodenal CD was not affected. Shallower crypt indicates a lower rate of enterocyte cell renewal and tissue turnover (Berrocoso et al., 2017; Ząbek et al., 2020), thus reducing the amount of nutrients needed for maintenance of the gut and consequently improve bird performance. Thus, supplementation of GP at a 2.5% inclusion level might be sufficient to maintain and improve healthy gut architecture in the absence of an antibiotic.

Blood is a noteworthy medium for a reliable assessment of the physiological and health status of animals. Literature reports on the impacts of supplemental GP on the plasma biochemical indices of broiler chickens are limited. Our study found that dietary GP supplementation at 2.5% had significant effects on plasma Ca, P, ALP, and AST, while plasma glucose and serum immunoglobulins (G and M) were not influenced. Unfortunately, the mode of action through which dietary GP influences plasma metabolites is not fully understood. Unlike our study, Kumanda et al. (2019) demonstrated that varying inclusion levels of GP from 0% to 7.5% had no significant effect on serum phosphorus, calcium, alkaline phosphatase, and other serum biochemical parameters. The nonsignificant effects reported by Kumanda et al. (2019) could be as a result of the higher inclusion levels of dietary GP used. ALT and AST are important indicators of healthy status of the liver (Zhang, 2011) as they play critical function in protein and amino acid metabolism in the liver cells. The reduced plasma AST in broiler chickens fed dietary 2.5% GP could be an indication of improved hepatic enzyme activity. In the findings of Ebrahimzadeh et al. (2018), AST was found to be similar among birds fed diets containing nonmedicated, supplemental vitamin E, and GP up to 7.5% inclusion levels, respectively. Compared to the antibiotic diet, birds fed 2.5% GP and control diets had elevated ALP. However, the value of ALP obtained in the present study was higher than the ALP reported by Kumanda et al. (2019). Elevated ALP indicates damaged liver or increased bone cell activity (Meyer-Sabellek et al., 1988; Lala et al., 2020). In the presence of high AST, ALT, and bilirubin, an increased level of ALP is triggered by liver damage (Lala et al., 2020). This suggests that the elevated ALP in this study is not due to liver damage. The dietary treatments, namely GP, antibiotic, and control diets, did not have any effect on Serum IgG and IgM. This is in variance with the result of Ebrahimzadeh et al. (2018) who observed a significantly increased concentration of serum IgG with increasing dietary levels of GP from 5% to 10%.

The gut microbiota has been recognized to contribute to bodily functions such as digestion and metabolism of nutrients, protection from pathogenic microbes, synthesis of certain vitamins, and modulation of the immune system (Konstantinidis et al., 2020). The most prevalent microbes that colonize the gut belong to 5 major phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia (Lozupone et al., 2012). It is important to mention that information of the impacts of GP on gut microbiota is very scanty. However, considerable number of in vitro demonstrations has reported that incorporation of grape by-products could selectively inhibit the proliferation of some intestinal microorganisms (Özkan et al., 2004). In the current study, the dietary treatments had significant effects on ceca microbiota and was observed to be dominated by 5 major phyla, namely, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and others unclassified (Bacteria_unclassified). Similar to our findings, Firmicutes and Bacteroidetes remain the largest phyla (Qin et al., 2010; Almeida et al., 2019; Forster et al., 2019). The ratio of the microbial population in these 2 dominant phyla plays a significant role in the regulation of intestinal homeostasis. In contrast to the antibiotic birds, it is surprising that the relative abundance of Bacteroidetes was greater than that of Firmicutes in the ceca of birds fed 2.5% dietary GP and control; thus, suggesting a lower Firmicutes-to-Bacteroidetes ratio (F:B). Plant bioactive substances, namely catechin and quercetin were reported to down-regulate F:B ratio (Xue et al., 2016). Interestingly, phytochemical analysis of our GP shows it is rich in both phenolic compounds. In humans, higher proportion of Bacteroidetes and lower Firmicutes was reported among individuals who consumed fiber-rich diets (De Filippo et al., 2010). This could be the reason for the lower F:B ratio reported in the current study. Contrary to most perception, antibiotics do not have fixed effect on the relative abundance of firmicutes and Bacteroidetes (Zhang et al., 2014); however, are mostly found to increase F:B ratio (Zhang et al., 2014; Dudek-Wicher et al., 2018). While there is lack of consensus as to the relevance of higher F:B in improving capacity of energy harvesting and performance in animals (Ley et al., 2005; Singh et al., 2012; Stanley et al., 2013; Jami et al., 2014), multiple studies have correlated higher F:B to incidence of obesity and dysbiotic microbiome in animals (Ley et al., 2006; Mariat et al., 2009; Razavi et al., 2019; Magne et al., 2020). This indicates that dietary inclusion of 2.5% GP has positive modulatory effects on the gut microbial community. Additionally, the suppressive effect of polyphenols on the F:B ratio have been implicated in loss of body weight (Xue et al., 2016). This could be responsible for the reduced AWG among the 2.5% GP-fed birds compare with the antibiotic-fed birds particularly during the grower phase and for the overall period. In addition, compared to the antibiotic treatment, both dietary 2.5% GP and the control diets significantly had a lower relative abundance of Proteobacteria. An increase in the population of Proteobacteria has been implicated in the incidence of metabolic syndrome, inflammatory bowel disease and sought as microbial signature of dysbiosis (Carvalho et al., 2012; Shin et al., 2015; Bradley and Pollard, 2017). At the genus level, dietary GP significantly increased the relative abundance of *Bacteroides* and *Lactobacillus* bacteria. This is similar to the increased ileal count of *Lactobacillus* when 10 to 40 g/kg of grape seed was fed to broiler chickens, as reported by Hafsa and Ibrahim (2018). However, the result of our study was in variance with the study of Chamorro et al. (2017) where dietary grape pomace at 50 g/kg of feed did not influence *Lactobacillus* count. Viveros et al. (2011) reported that the inclusion of GP concentrate at 7.2 g/kg did not show any effect on the ileal count of *Lactobacillus*. Many strains of *Lactobacillus species* have the capacity to maintain the intestinal barrier function, particularly during a disease condition, by modulating the expression of heat shock protein or tight junction proteins or by restricting adhesion of pathogens (Liu et al., 2015). In humans, reduced abundance of *Bacteroides* has been associated with inflammatory bowel disease, Crohn's disease, and ulcerative colitis disease conditions (Zhou and Zhi, 2016). Dietary GP at 2.5% could be the optimum inclusion level that would selectively enhance proliferation of gut-friendly microbes like *Lactobacillus* and *Bacteroides*. Unfortunately, antibiotics has been reported to deplete the population of microbes in the Lactobacillaceae family (Wise and Siragusa, 2007; Neumann and Suen, 2015). In contrast to the GP and control treatments, there was a significantly higher relative abundance of *Oscillospirales_ge, Escherichia-shigella, Lachnospiraceae_ge, CAG-352, Blautia, UCG-005, NK4A214_group,* and *Anaerovoracaceae_ge* in birds fed the antibiotic diet. This may be due to the higher Shannon diversity index in the antibiotic treatment compared to the GP and control treatments. Bacteria genus *Escherichia-shigella,* have been dubbed opportunistic pathogenic microbes (Elbere et al., 2018) often created by the antibiotic use (Dudek-Wicher et al., 2018). *Lachnospiraceae_ge* and *Blautia* are members of Lachnospiraceae, which are known to be a part of the main producers of short-chain fatty acids (Vacca et al., 2020) and have also been positively correlated with good performance in birds (Stanley et al., 2016). Beta diversity, which measures similarity between multiple microbial communities, indicates it was significantly different in the antibiotic treatment compared to other treatments. Most studies that use 16S RNA genes reported altered diversity following antibiotic application (Orlewska et al., 2018a, b). This is no surprise as bacitracin has been reported to cause reduction in the Lactobacillus while increasing Clostridales in broiler chickens (Costa et al., 2017; Crisol-Martínez et al., 2017). This implies that dietary supplementation of 2.5% GP could help to stabilize gut microbiota in broiler chickens compared to antibiotics. Despite the significant effect of dietary treatments on ceca microbiota, the composition of ceca short chain fatty acids was not affected. This could be as a result of the similar total eubacteria present in the ceca across the dietary treatment.

White striping and woody breast are the 2 main types of breast muscle myopathies associated with broiler chickens. It has been speculated that localized hypoxia, oxidative stress, high levels of calcium in the intracellular tissue, and muscle fiber type switching could be likely causes of myopathies in broiler chickens (Mutryn et al., 2015). The appearance of such anomaly on fillets reduces their acceptability by consumers (Kuttappan et al., 2016). The incidences of WS and WB were not affected by the dietary treatments and the incidence of these myopathies was generally low in the current study. It was noted that male chickens had higher slaughter and breast weights than the females, while the females showed a higher breast weight percent of body weight compared to the males. Some previous studies (Ojedapo et al., 2008; López et al., 2011; Benyi et al., 2015) have reported a similar situation. Studies (Benyi et al., 2015; Ikusika et al., 2020) have also shown that the differences in body weight between male and female chickens are strain-dependent.

CONCLUSION

Dietary incorporation of grape pomace at the inclusion level of 2.5% had beneficial effects on the growth performance of broiler chickens during the starter phase; however, it had slight negative effects from 14 to 28 d, and no difference in feed efficiency throughout the overall period. Furthermore, there was an improvement in the villus height and villus height crypt depth ratio and modulation of gut microbiota while the ceca short-chain fatty acid concentrations were not affected in birds fed grape pomace. The present study suggests that the inclusion of grape pomace at 2.5% in the diet of broiler chickens is favorable for the optimization of intestinal health without affecting their blood biochemical and immune profiles.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101519.

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