Red osier dogwood extract vs. trimethoprim-sulfadiazine (Part 2). Pharmacodynamic effects on ileal and cecal microbiota of broiler chickens challenged orally with *Salmonella* Enteritidis

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ABSTRACT With the subsisting restrictions on the use of antibiotics in poultry production, the use of plant extracts has shown some promising antimicrobial capacity similar to antibiotics; however, such capacity is largely dependent on their total polyphenol concentration and profile. Given the emerging antimicrobial potential of red osier dogwood (**ROD**) extract, the study aimed to investigate the pharmacodynamic effect of ROD extract on the ileal and cecal microbiota of broiler chickens challenged orally with *Salmonella* Enteritidis (**SE**). A 21 d 4×2 factorial experiment was conducted based on 2 main factors, including diets and SE challenge. A total of 384 one-day-old mixed-sex Cobb-500 broiler chicks were randomly allotted to 4 dietary treatments; Negative control (**NC**), NC + 0.075 mg trimethoprim-sulfadiazine (**TMP/SDZ**)/ kg of diet, and NC containing either 0.3 or 0.5% ROD extract. On d 1, half of the birds were orally challenged with 0.5 mL of phosphate-buffered saline (Noninfected group) and the remaining half with 0.5 mL of 3.1×10^5 CFU/mL SE (Infected group). Dietary treatments were randomly assigned to 8 replicate cages at 6 birds/cage. On d 21, 10 birds/treatment were euthanized and eviscerated to collect ileal and cecal digesta for gut microbiota analysis. The ileal and cecal microbiota was dominated by phyla Firmicutes, Proteobacteria, and Actinobacteriota. The SE infection decreased ($P < 0.05$) the relative abundance of Proteobacteria and Actinobacteriota in the ileum and ceca, respectively, however, it increased ($P < 0.05$) Proteobacteria in the ceca. Both 0.3 and 0.5% ROD extracts ($P < 0.05$) depressed the relative abundance of Actinobacteriota in the ileum but marginally improved ($P < 0.05$) it in the ceca compared to the TMP/SDZ treatment. Dietary TMP/SDZ increased ($P < 0.05$) genus *Bifidobacterium* at the ileal and cecal segments compared to other treatments. Dietary 0.3 and 0.5% marginally improved ($P < 0.05$) *Bifidobacterium* in the ceca and depressed ($P < 0.05$) *Weissella* and was comparably similar to TMP/SDZ in the ileum. Regardless of the dietary treatments and SE infection, alpha diversity differed ($P < 0.05$) between ileal and cecal microbiota. Beta diversity was distinct ($P < 0.05$) in both ileal and cecal digesta along the SE infection model. Conclusively, both ROD extract levels yielded a pharmacodynamic effect similar to antibiotics on ileal and cecal microbiota.

Key words: red osier dogwood extract, antibiotic alternative, *Salmonella* Enteritidis, trimethoprim-sulfadiazine, gut microbiota

INTRODUCTION

Among the economically important pathogenic bacteria of poultry birds are the *Salmonella* spp., which has been identified as the causative organism of pullorum and fowl typhoid diseases with severity ranging from high morbidity to mortality depending on the age and strain of the bird, and the strain and concentration of the *Salmonella* inocula. Besides typhoidal *Salmonella*, nontyphoidal *Salmonella enterica* serovar Enteritidis and Typhimurium have been recognized for their epidemiological relevance given their host nonspecificity in animals and humans (Ferrari et al., 2019), as well as plants. According to the World Health Organization (2015) report, *Salmonella enterica* serovars are virulent and capable of triggering intestinal diseases in animals and humans following consumption of contaminated food. Consequently, *Salmonella enterica* has been considered one of the top 3 causes of foodborne diseases. While

there are over 2,600 different nontyphoidal *Salmonella* serovars, Enteritidis is the most commonly isolated among human and nonhuman subjects globally (European Food Safety Authority, 2017; Afshari et al., 2018; Castro-Vargas et al., 2020). Salmonellosis is a common enteric disease of poultry birds. Following oral inoculation in chickens, *Salmonella* Enteritidis (**SE**) colonizes the ileum and ceca when the birds are 14- to 21-days old (Ijaz et al., 2021), thereby tweaking the gut microbiota diversity toward a dysbiotic condition. At less than 21-days posthatch, the gut microbiota of chicks is barely developed, thus increasing their susceptibility and vulnerability to *Salmonella* (Barnes et al., 1972; Yang et al., 2018). Besides nutrient metabolism, the gut microbiota participates in special bodily functions, including protecting the host against pathogens, biosynthesis of certain vitamins, and immunomodulatory functions (Konstantinidis et al., 2020), thus, making it one of the indispensable indices of gut health. As a principle, suitable SE treatment strategies should promote the proliferation of gut-friendly microbes that could resist colonization by SE.

For birds' welfare and food safety concerns, the modern-day poultry industry is constantly stepping up its game in the combat against *Salmonella*. Despite the numerous treatments and preventive strategies to curb SE incidences, for example, the use of antibiotics (Chen et al., 2013), on-farm SE-vaccination interventions (European Commission of the European Parliament, 2006), stringent biosecurity measures, and hazard analysis critical control points (**HACCP**) in feed and water system, little progress seems to have been recorded. For instance, antibiotics may cause perturbation of gut-friendly microbes, thereby giving room for the persistent colonization of the gut by *Salmonella* if present (Bauer-Garland et al., 2006; Sekirov et al., 2008; Bukina et al., 2021). Although, the pharmacokinetic combination of sulfonamides and trimethoprim has reportedly been used for a broad spectrum of pathogenic bacteria infection, especially gram-negative bacteria like SE (Putecova et al., 2021), the increasing resistance of nontyphoidal *Salmonella* to clinically important antibiotics has contributed to the restrictions placed on antibiotic use; thereby putting the poultry industry into a clinical difficulty. Furthermore, under high SE challenge, the counteractive potential of vaccination against SE in flocks has been reported to be insufficient to prevent colonization at the gut levels (Atterbury et al., 2009) or may not protect against bacterial shedding in infected birds (Lim et al., 2012); thus, might encourage further transmission either vertically or horizontally. With the insufficient potency of SE-vaccine and the embargo placed on antibiotic use, the search for more suitable alternatives with exceptional antimicrobial activities has been intensified in poultry nutritional research.

While other possible alternatives have been identified, including probiotics, prebiotics, antimicrobial peptides, etc., the technicalities involved in their preparations and storage may deter their ease of adoption in poultry production. It is noteworthy that plants, particularly those with high polyphenol concentrations, possess a wide spectrum of beneficial bioactivities, including selective antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities, and as a result, have gained increased attention as potential substitutes to antibiotics. In addition to their beneficial impacts, polyphenols possess a prebiotic effect at the gut level (Rodríguez-Daza et al., 2021). The antibiotic replacement potential of medicinal plants varies with their polyphenol profile and concentrations. Interestingly, plant species, notably red osier dogwood (**ROD**), contain high total polyphenol content compared to some plants, including olive, tea, parsley, and basil (Isaak et al., 2013; Scales, 2015). In addition to their gallic acid and quercetin constituents, ROD has also been considered a nutritional feed additive (Erinle et al., 2022c), given its appreciable amount of crude protein and ß-carotene (Fashingbauer and Moyle, 1963; Gomaa et al., 2018; Wei et al., 2018; Lee et al., 2018b). Antibiotics and phytogenic additives differ in their precision of action at the gut levels. While antibiotics discriminately reduce and increase gut-friendly and opportunistic gut bacteria, respectively (Costa et al., 2017; Crisol-Martínez et al., 2017; Erinle et al., 2022d), the dietary supplementation of polyphenol-rich additive like ROD extract was reported to upturn the reduction in the relative abundance of cecal *Lactobacillus* caused by bacitracin antibiotics in broiler chickens challenged or unchallenged with SE-lipopolysaccharides (Erinle et al., 2022b). In a nonavian model, Zheng et al. (2021) demonstrated that 0.5% ROD polyphenol extract selectively promotes the relative abundance of *Lactobacillus* in the ileum of matured pigs. In addition to *Lactobacillus*, 0.3% ROD extract was also reported to increase the abundance of *Oscillospira*—a butyrate-producing bacteria that could improve chickens' immunity and intestinal morphology (Mogire, 2020). To the best of our knowledge, the dynamic effects of ROD extract on the gut microbiota of broiler chickens challenged with SE are yet to be reported.

Given the emerging microbial-modulatory potential of ROD polyphenols, we hypothesized that ROD extract, at either 0.3 or 0.5% inclusion level, will improve the gut microbiota of bacterial-infected birds. Thus, the objective of the current study was to investigate the dynamic influence of ROD extract in comparison with the antibiotic trimethoprim-sulfadiazine (**TMP/SDZ**) on the ileal and cecal microbiota of broiler chickens infected with SE.

MATERIALS AND METHODS

The study was conducted in accordance with the guidelines of the University of Montreal Animal Care and Use Committee (Project 20-Rech-2063). The birds were handled following the protocol established by Canadian Council on Animal Care (2009). In addition, the management of birds, diets, experimental design,

and SE infection route and concentrations were described in Part 1 of this report (Erinle et al., 2023a).

Birds and Housing

The SE strain (SNY 04 1540) used in the present study was isolated in 2004 in Dr. Martine Boulianne's Avicole Research Laboratory, University of Montreal, Quebec, Canada. The growth and processing procedures of the SE strain has been described in in Part 1 of this report (Erinle et al., 2023a).

Preparation of Salmonella Enteritidis Inoculum

The SE strain (SNY 04 1540) used in the present study was isolated in 2004 at the Avicole Research Laboratory, University of Montreal, Quebec, Canada. The growth and processing procedures of the SE strain has been described in Part 1 of this report.

Sample Collection

On d 21, 80 birds (i.e., 10 birds per treatment) were randomly selected, weighed, and euthanized by injecting ketamine and xylazine and followed by exsanguination. At exsanguination, digesta content in the ileum (2-cm distal to the ileal mid-length) and ceca were collected and stored in RNAse and DNAse-free microcentrifuge tubes individually, placed in liquid nitrogen, and followed by storage at $-80°$C for subsequent ileal and cecal microbiota analyses.

Ileal and Cecal DNA Extraction, Quality Determination, and Sequencing

The microbiota DNA in the ileal and cecal digesta were extracted separately using QIAamp PowerFecal Pro DNA Kit (Cat. No./ID: 51804) and following the manufacturer's extraction steps. Upon extraction, the concentrations and purity of the extracted DNA were confirmed to meet the sample requirements (concentration: >10 or <200 ng/μL; purity: A260/280 and A260/230 ratios $\geq$1.8 and $\geq$2.0, respectively) of the Integrated Microbiome Resource (http://imr.bio) of Dalhousie University where library preparation and sequencing were carried out. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the Bacteria-specific V3−V4 region (341F = 5'-CCTACGGGNGGCWGCAG-3' and 805R = 3'-GACTACHVGGGTATCTAATCC-5') at the Dalhousie University's Integrated Microbiome Resource.

Bioinformatics and Statistical Analysis

The analysis of ileal and cecal microbiota data was conducted using the Microbiome Helper pipeline (https://github.com/LangilleLab/microbiome_helper/wiki), based on Quantitative Insights Into Microbial Ecology 2 (**QIIME 2**). Amplicon sequence variants created with Deblur. Primer sequences were trimmed from sequencing reads using cut adapt (Martin, 2011), and primer-trimmed files were imported into QIIME2 (Bolyen et al., 2019). The reads from the forward and reverse paired ends were integrated using VSEARCH (Rognes et al., 2016). Following this, the reads were fed into Deblur (Amir et al., 2017) to correct reads and obtain amplicon sequence variants. Taxonomic assignment was done with the SILVA database using a naive Bayes classifier implemented in the scikit learn Python library (Comeau et al., 2017). Rarefaction curves were used to examine the individual alpha diversity for all samples (with the default observed OTUs as the metric). Shannon entropy comparisons for the treatments and infection model groups were explored using boxplots, while the beta diversity was explored and visualized using Bray-Curtis principal coordinate analysis (**PCoA**) plots. The relative abundance at different taxonomic levels (phyla and genera) was visualized using stacked bar charts. The identified microbes present in each sample were respectively summed and ranked in descending order to selected top 10 most abundant bacterial population using Microsoft Excel. This was done on the ileal and cecal microbiota, respectively.

The ileal and cecal microbiota proportions dataset was inputted and subjected to analysis of variance (**ANOVA**) using a General Linear Model of Minitab LLC (2019) software, and the error terms of the dataset were tested to confirm conformation to 3 basic assumptions. Non-normal data were transformed for parametric analysis. The nonparametric Kruskal-Wallis' median test was used where normality failed upon transformation. Analyzed data were graphically presented as means and probability values. Statistical differences were considered at $P < 0.05$. P values were not reported where statistical differences were greater than 0.05.

RESULTS

Ileal and Cecal Microbial Composition

The effect of dietary supplementation of 0.3 and 0.5% ROD extract on the ileal and cecal microbiota composition of broiler chickens infected or uninfected with SE is presented in Figures 1 to 4. The aggregate of the operational taxonomic unit into each taxonomic rank, as well as the relative abundance of predominant phyla and genera-based treatment and infection model effects are shown in Figures 1 and 3 for ileal bacterial phyla and genera, respectively, and Figures 2 and 4 for cecal bacterial phyla and genera, respectively. In the ileal microbiota, the bacteria phyla were dominated by Firmicutes (96.9−98.5%) followed by Proteobacteria (0.7−2.9%) and Actinobacteriota (0.1−0.9%). However, in the cecal microbiota, the dominant phyla include Firmicutes (74.2−96.4%), Actinobacteriota (2.6−24.7%), and Proteobacteria (1.0−2.1%). Inclusion of TMP/SDZ antibiotic was observed to numerically lower the relative abundance of phylum Firmicutes in the ceca, compared to ROD and basal treatments.

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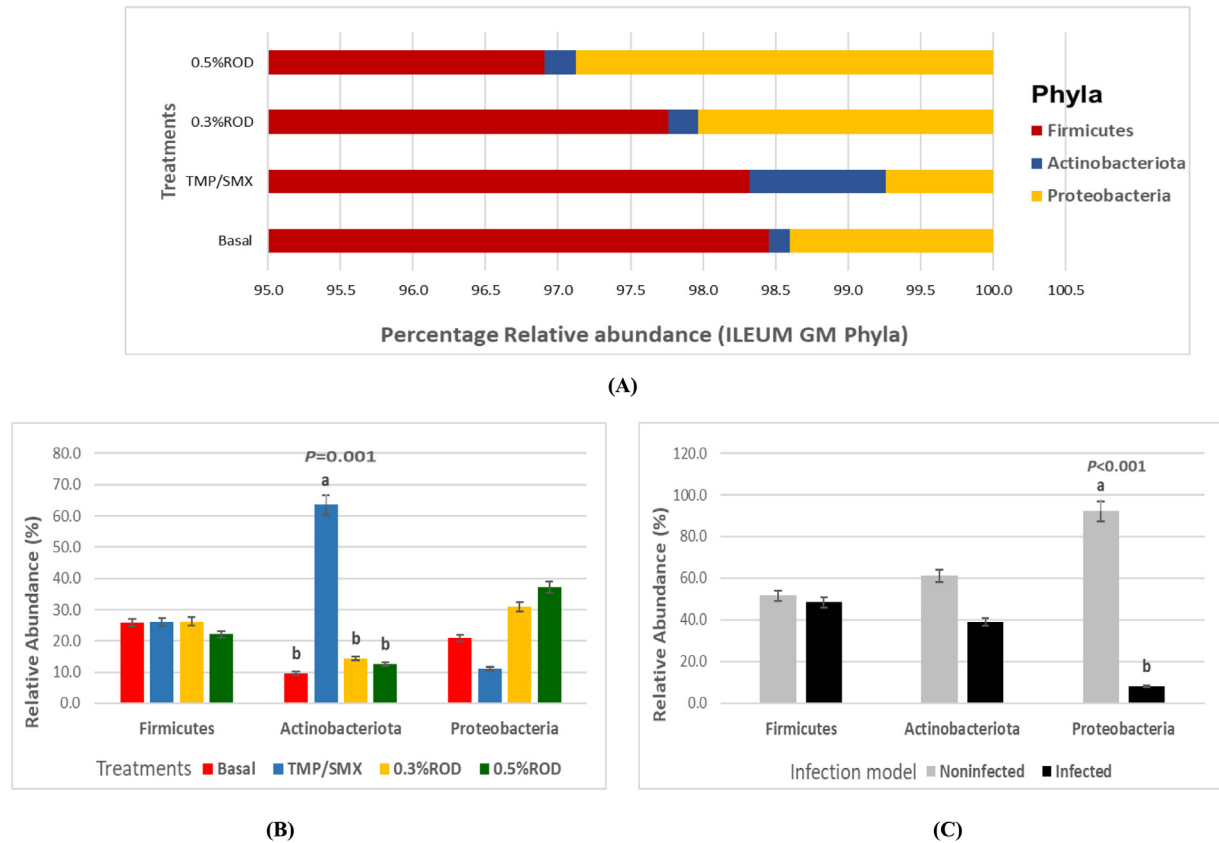
Figure 1. (A) Profile, (B) descriptive treatment effect, (C) descriptive infection model effect on the percentage relative abundance of ileal bacterial phyla of broiler chickens orally gavaged with or without *SE* and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: Basal = negative control; TMP/SDZ = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; 0.3%ROD = diet containing 0.3% red osier dogwood extract; and 0.5%ROD = diet containing 0.5% red osier dogwood extract. Phyla and infection model without a mean separation have their *P* value greater than 0.05.

Regardless of SE infection, there was a specific pattern of dietary treatment effects ($P < 0.05$) on the phylum Actinobacteriota in both ileum and ceca and it was observed to be significantly higher among the birds fed TMP/SDZ antibiotics compared to the ROD extract and control treatments. Furthermore, ileal Proteobacteria was significantly higher ($P < 0.05$) among the noninfected birds compared to the infected birds. Meanwhile, Actinobacteriota and Proteobacteria phyla in the ceca were significantly higher and lower ($P < 0.05$) among the noninfected and infected birds, respectively. At the ileal genera taxa, the relative abundance of the top 10 most abundant bacteria genera in a decreasing order includes Enterococcus, Streptococcus, Lactobacillus, Romboutsia *Escherichia-Shigella, Preptostretococcaceae, Bifidobacterium, Lactococcus, Weissella,* and *Lachnospiraceae.* Contrary to the TMP/SDZ antibiotic treatment, the relative abundance of ileal *Bifidobacterium* genus was significantly decreased ($P < 0.05$) among birds fed dietary supplementation of 0.3 and 0.5% ROD extract and control. Unlike the control treatment, the relative abundance of *Weissella* in the ileum was also observed to be depressed ($P < 0.05$) among birds fed both levels of ROD extract compared to the TMP/SDZ antibiotic treatment. In the ceca, the top 10 dominant bacteria genera in a decreasing order include *Bifidobacterium, Corynebacterium, Curtobacterium, Sanguibacter, Saccharopolyspora, Eggerthella, Bacillus, Kurthia, Erysipelatoclostridium,* and *Clostridium_innocuum.* Compared to the TMP/SDZ
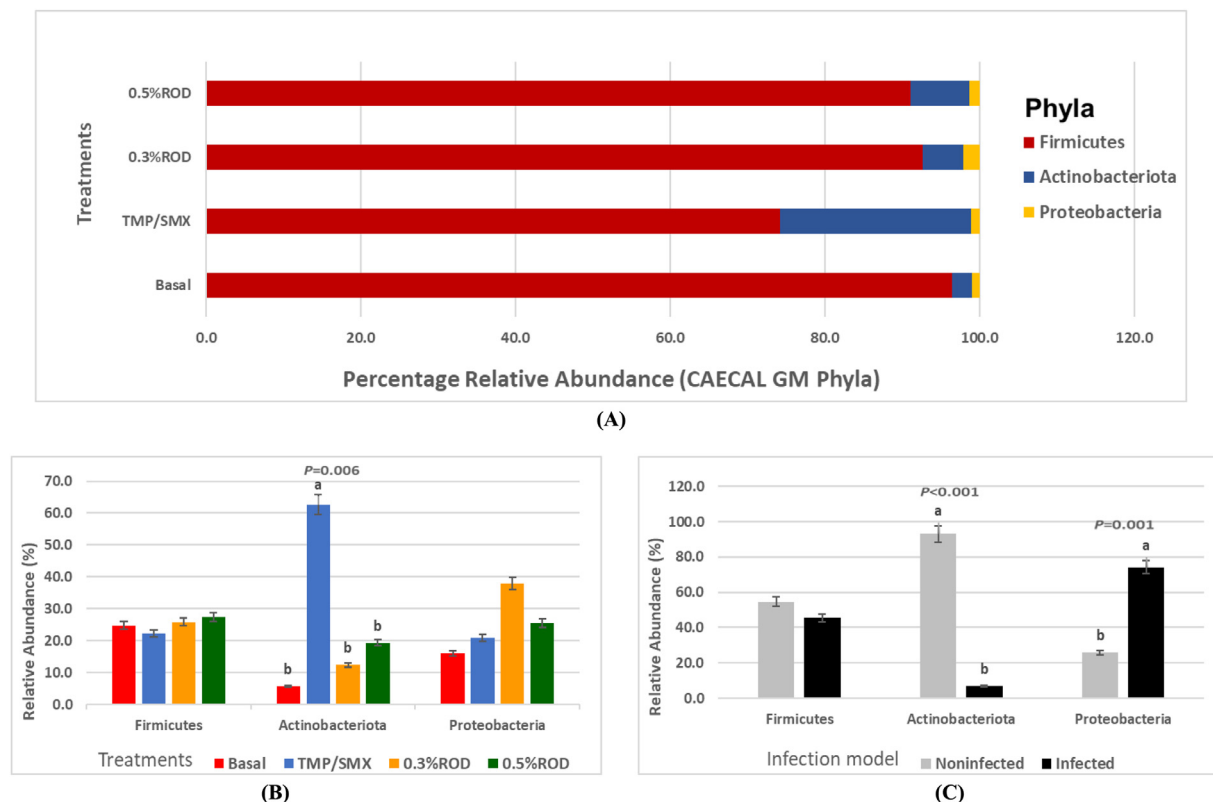
treatment, the relative abundance cecal *Bifidobacterium* was marginally improved ($P < 0.05$) compared to the control treatment.

Ileal and Cecal Microbial Diversity

The effect of dietary supplementation of 0.3 and 0.5% ROD extract on the ileal and cecal microbiota diversity of broiler chickens infected or uninfected with SE is shown in Figures 4 to 7. Shannon diversity (i.e., specie richness) was not affected ($P > 0.05$) either by the dietary treatments or the SE challenge as presented in Figure 5; however, the Shannon diversity differed ($P < 0.05$) between the ileal and cecal microbiota and was higher ($P < 0.05$) in the latter compare to the former. Based on Bray-Curtis dissimilarity PCoA shown in Figure 6, the dietary treatments did not alter ($P > 0.05$) the beta diversity of ileal and cecal microbiota. In Figures 7 and 8, there were distinct clusters representing a significant difference ($P < 0.05$) in the beta diversity of the ileal and cecal microbiota vis-à-vis the infection model.

DISCUSSION

The results of fecal excretion of SE and other parameters were reported in the Part 1 of this study
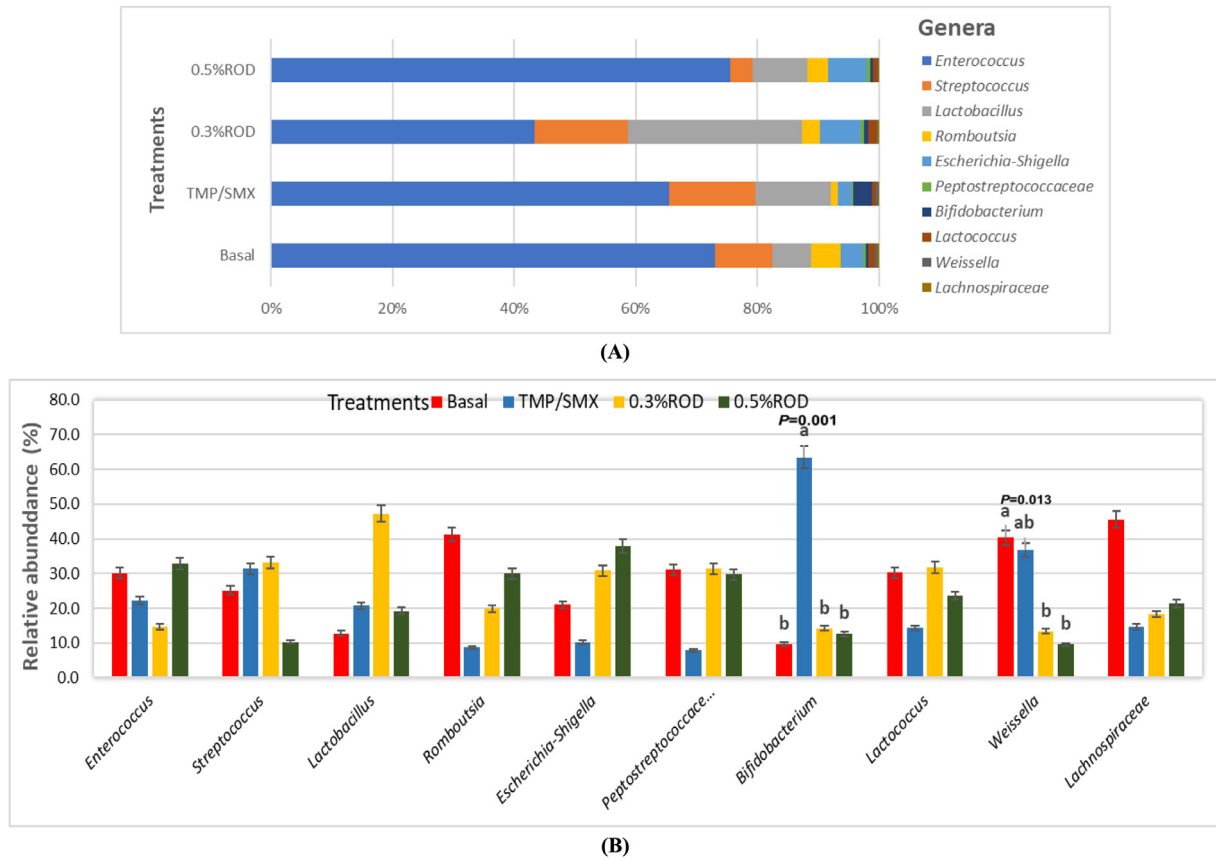
Figure 2. (A) Profile, (B) descriptive treatment effect, (C) descriptive infection model effect on the percentage relative abundance of cecal bacteria phyla of broiler chickens orally gavaged with or without *SE* and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: Basal = negative control; TMP/SDZ = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; 0.3%ROD = diet containing 0.3% red osier dogwood extract; and 0.5%ROD = diet containing 0.5% red osier dogwood extract. Phyla and infection model without a mean separation have their *P* value greater than 0.05.

(Erinle et al., 2023a). SE was not detected in all the cloacal swabs sampled on d 0, 1, 5, 12, and 18 postinfection. However, SE infected birds had reduced feed conversion ratio, reduced concentration of serum IgM and lymphocytes, and higher concentrations heterophils, heterophils:lymphocyte, and monocytes, compared to the noninfected birds. These results suggest the systemic presence of SE among the SE infected birds. The possibility of birds carrying *Salmonella* despite a failed detection in their fecal samples has been previously established by Van Immerseel et al. (2004). The gut microbiota remains a key component of the body systems involved in the metabolism of ingested food materials and immunomodulation and consequently acts as a reliable indicator of disease origination and development in humans and animals, including poultry birds. A tweak in the gut microbiota composition, diversity, and specie richness is caused by multifactorial reasons, including diets, exposure to antibiotics, infection, and environmental stressors (Burkholder et al., 2008; Martinez et al., 2021; Strain et al., 2022), which could compromise the vital roles of a healthy gut microbiota. *Salmonella* has been reported as one of the virulent pathogens capable of causing diarrhea, appetite loss, and other prognostic symptoms among poultry species (Oh et al., 2017). SE thrives, proliferates, and colonizes, and promotes the growth of other opportunistic pathogens in the gut, thus, reducing the host's resistance to pathogen colonization. While the menace of *Salmonella*

spp. on the gut microbiota of poultry birds is not uncommon in research, no studies have investigated the effects of ROD extract as an alternative to antibiotics on the gut microbiota of broiler chickens infected orally with SE.

Firmicutes, proteobacteria, and actinobacteria are part of the top 5 bacterial phyla reported in most poultry studies regardless of the type of dietary treatment or stress conditions (Oakley et al., 2014; Díaz Carrasco et al., 2018; Mandal et al., 2020; Zheng et al., 2021; Erinle et al., 2022c). In the current study, the ileal and cecal microbiota is dominated by phyla Firmicutes, Proteobacteria, and Actinobacteriota, however, Firmicutes was heavily abundant, accounting for up to 98.5% of the total gut bacteria. This is quite understandable as gut bacteria species richness and diversity, and taxonomic classification swiftly change almost exclusively to Firmicutes as age increases in chickens (Oakley and Kogut, 2016; Shang et al., 2018). Without prejudice to the preceding, Firmicutes and Proteobacteria were the top 2 phyla in the ileum, while Firmicutes and Actinobacteria were the top in the ceca in the ceca regardless of the dietary treatments and SE infection. In a recent study where dietary ROD polyphenol extract was fed to swine, Firmicutes and Proteobacteria were reported to be the top 2 bacterial phyla in the ileal digesta (Zheng et al., 2021). It is noteworthy that SE infection in the current study altered the composition of both ileal and cecal microbiota. We observed a reduction and an
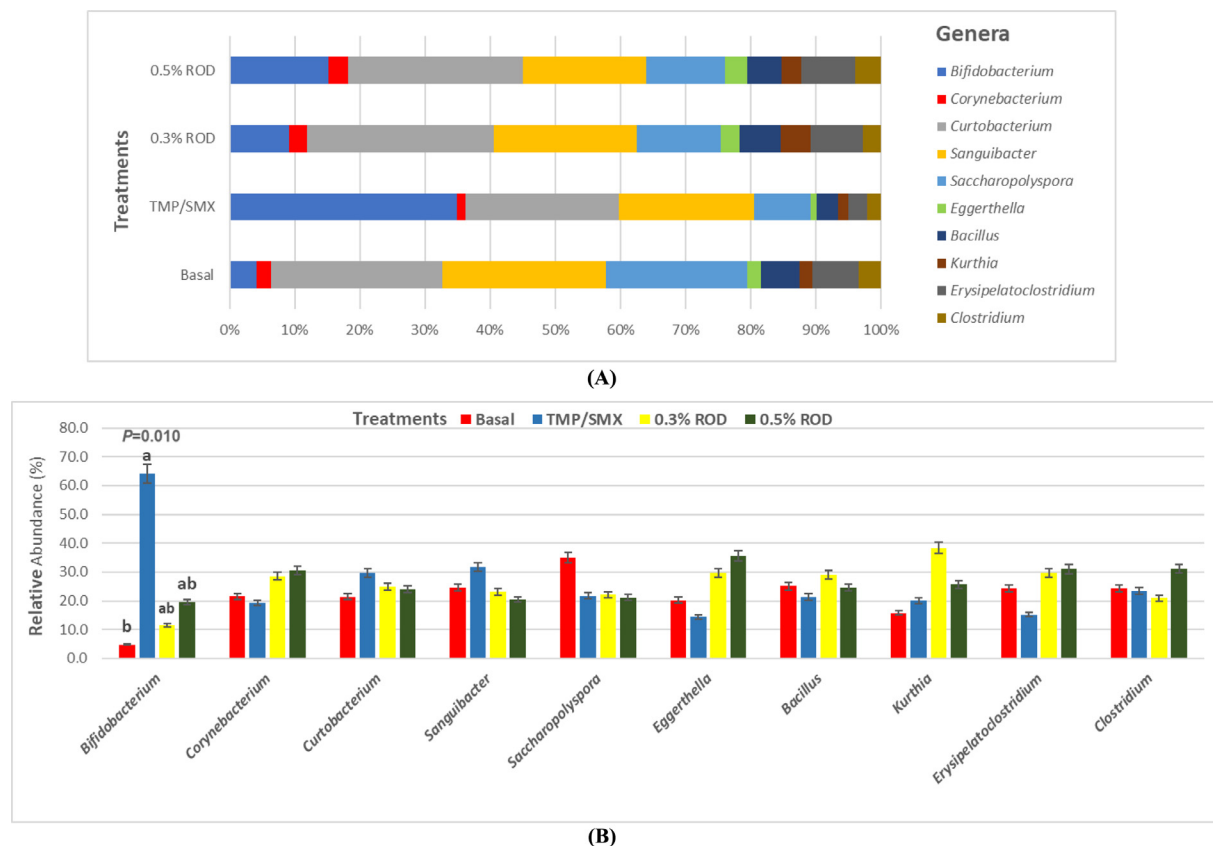
Figure 3. (A) Profile and (B) descriptive treatment effect on the percentage relative abundance of top 10 most abundant ileal bacterial genera of broiler chickens orally gavaged with or without *SE* and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: Basal = negative control; TMP/SDZ = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; 0.3%ROD = diet containing 0.3% red osier dogwood extract; and 0.5%ROD = diet containing 0.5% red osier dogwood extract. Genera without a mean separation have their *P* value greater than 0.05.

increase in the relative abundance of Proteobacteria in the ileum and ceca of infected birds, respectively, compared to the noninfected birds. SE infection influences the gut microbiota in chickens (Videnska et al., 2013). In chickens with dysbiosis caused by *Salmonella* infection, phylum Proteobacteria was reported to be increased, while phylum Firmicutes was decreased in ceca of chickens (Oh et al., 2017; Chang et al., 2020). While a significant abundance of Proteobacteria has been positively correlated with a high heterophil:lymphocyte ratio—an important biomarker of stress and innate immune status (Thiam et al., 2022). Interestingly, in Part 1 of the present study, we established that birds infected with SE had a higher H:L (Erinle et al., 2023a). Despite the SE infection, no *Salmonella* genera were found in the ceca digesta, which houses more bacteria population compared to the other gastrointestinal sections. This could be due to the relatively abundant Proteobacteria in the ceca of SE-infected birds, concentration of SE inoculum used in the model, and a possible reduction of *Salmonella* as postinfection days increase. Some members of Proteobacteria, particularly Enterobacteriaceae, have been recognized for their protection against the *Salmonella* colonization in chickens by competitive exclusion (Deriu et al., 2013; Videnska et al., 2013; Litvak et al., 2019). In contrast to the TMP/SDZ antibiotics, the dietary supplementation of 0.3 and 0.5%

ROD extract decreased the relative abundance of phyla Actinobacteria in the ileum. However, in the ceca, both levels of ROD extract marginally improved the relative abundance of Actinobacteria compared to the antibiotic treatment. Meanwhile, Actinobacteria was more abundant in the ceca of noninfected birds. Some members of Actinobacteria, notably *Streptomyces*, are capable of synthesizing peptide antibiotics called actinomycin which has inhibitory action against multiresistant *Staphylococcus aureus*, malignant tumors, and cancerous activities (Farber, 1966; Lewis Jr., 1973). Thus, there is a possible potentiation effect between dietary antibiotic supplements and antibiotic-producing microbes rather than an inhibitory impact. Although the mode of action of antibiotics differs from ROD's, however, the marginal improvement in the abundance of Actinobacteriota among the ROD-treated birds compared to antibiotics is noteworthy.

The cecum houses a more stable bacterial population of about 10^{10} to 10^{11}/g than the 10^8 to 10^9/g in the ileum digesta (Shang et al., 2018); thus, suggesting that a more complex microbial metabolism would be taking place in the cecum. At the ileal genera level, dietary supplementation of 0.3 and 0.5% ROD extract significantly repressed the relative abundance of *Bifidobacterium* and *Weissella* compared to the TMP/SDZ antibiotic and control treatment. Whereas in the ceca, 0.3 and 0.5%

Figure 4. (A) Profile and (B) descriptive treatment effect on the percentage relative abundance of top 10 most abundant cecal bacterial genera of broiler chickens orally gavaged with or without *SE* and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: Basal = negative control; TMP/SDZ = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; 0.3%ROD = diet containing 0.3% red osier dogwood extract; and 0.5%ROD = diet containing 0.5% red osier dogwood extract. Genera without a mean separation have their *P* value greater than 0.05.

ROD extract marginally increased *Bifidobacterium*. This could be traced to the suppressive influence of ROD extract on phylum Actinobacteriota compared to antibiotics. Similar to TMP/SDZ antibiotic effect in the current study, bacitracin methylene disalicylate was reported to consistently improve cecal *Bifidobacterium* and *Lactobacillus* count and pathogenic *E. coli* and *Clostridium perfrigens* counts in broiler chickens at d 14, 21, and 42 of age (Dev et al., 2020). In in vitro studies conducted by Shah and Dave (2002), Touré et al. (2003), and Cheikhyoussef et al. (2010), considerable strains of *Bifidobacteria* presented a probiotic effect through their production and deployment of bacteriocins (a notable antimicrobials) and some short-chain fatty acids, namely acetate and lactate, against obnoxious bacteria including but not limited to *Listeria monocytogenes*; and could consequentially improve growth, thyroid hormonal functions, and ileal architecture (Abdel-Moneim et al., 2020). Quercetin and gallic acid are the 2 most prevalent polyphenols found in ROD extract (Scales, 2015; Erinle et al., 2022c); however, they could be responsible for the reduction in the relative abundance of *Bifidobacterium*. Although polyphenols have selective modulatory antimicrobial action and have been reported to stimulate the proliferation of some bacterial species like *Bifidobacteria, Lactobacilli,* and *Faecalibacterium* (Rodríguez-Daza et al., 2021),

however, Firrman et al. (2016) and Zheng et al. (2017) demonstrated that polyphenol quercetin suppressed the growth of *Bifidobacterium*. Furthermore, gallic acid and 3-*O*-methyl gallic acid found in tea plant were reported to affect the growth of *Bifidobacterium* but in a less severe magnitude (Lee et al., 2006). Feed nutrients that escape enzymatic digestion in the fore gut are often subjected to degradation by microbes, including *Bifidobacterium*, at the hind gut to produce short chain fatty acids, ammonia, vitamin B, toxic metabolites, and many more. Notwithstanding the preceding, the functions of *Bifidobacterium* would be more relevant in the ceca than ileum. Hence, the pharmacodynamic effect of ROD extract on ileal and cecal *Bifidobacterium*. Pathogenic intestinal bacteria species, including *Weissella confusa* and *Escherichia coli*, were reported to reduce the antioxidant capacity of quercetin by degrading quercetin in plant extract to produce 3.4-dihydroxyphenylacetic acid (Zhang et al., 2014; Duda-Chodak et al., 2015). Contrary to TMP/SDZ antibiotic and control, 0.3 and 0.5% ROD extract exerted a potent and precise antioxidant and antimicrobial force, which depressed the relative abundance of genus *Weissella* in broiler chickens infected with SE. Thus, suggesting that the genus *Weissella* does not have a degradation effect on the quercetin polyphenol in ROD extract. However, it is noteworthy that ROD polyphenols have a pharmacodynamic effect

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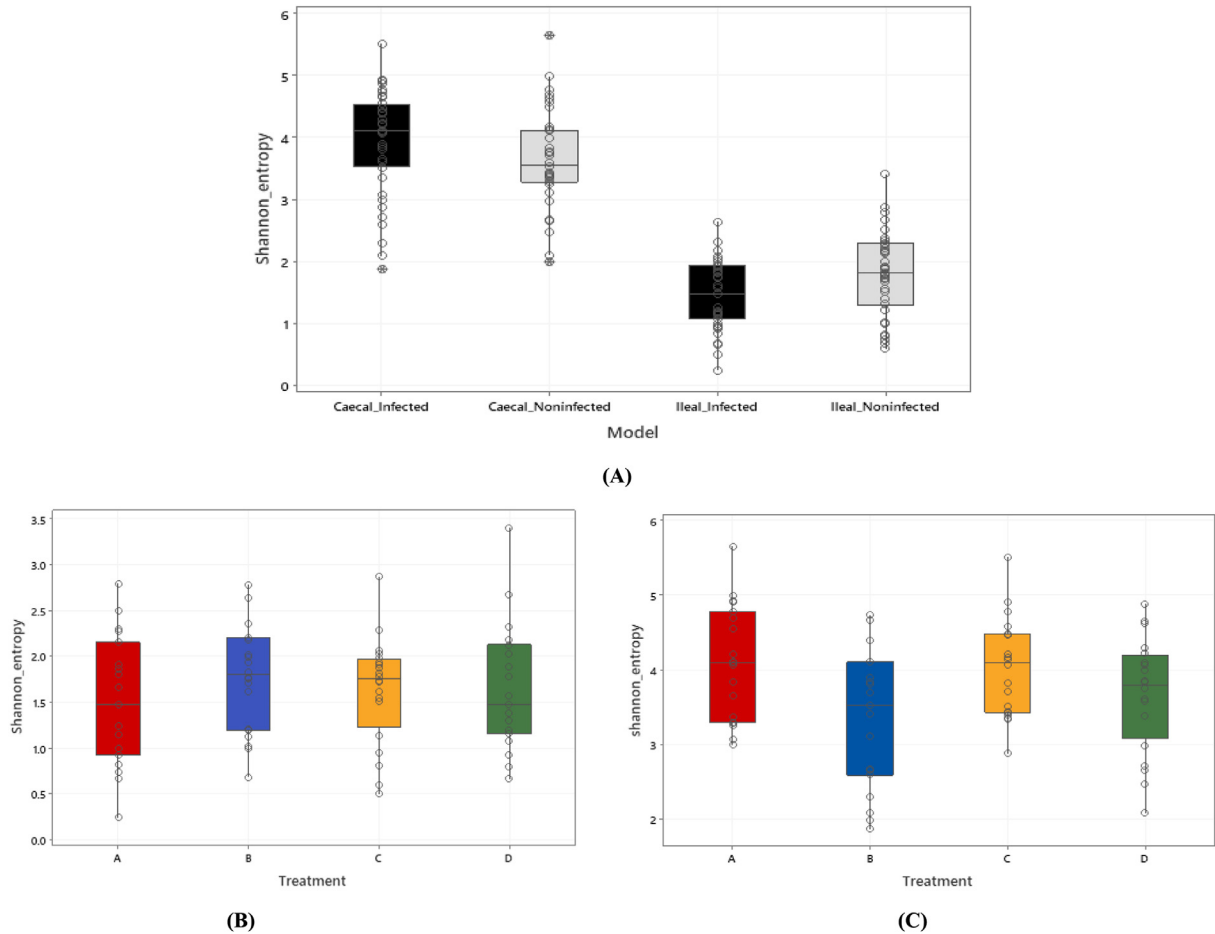
Figure 5. Box- and whisker plot showing (A) significant difference between ileal and cecal microbiota (GLM, $P < 0.001$), (B) insignificant treatment effects on the ileal microbiota ($P > 0.05$), and (C) insignificant treatment effect on the cecal microbiota ($P > 0.05$) of broiler chickens orally gavaged with or without *SE* and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: A = negative control; B = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; C = diet containing 0.3% red osier dogwood extract; and D = diet containing 0.5% red osier dogwood extract.
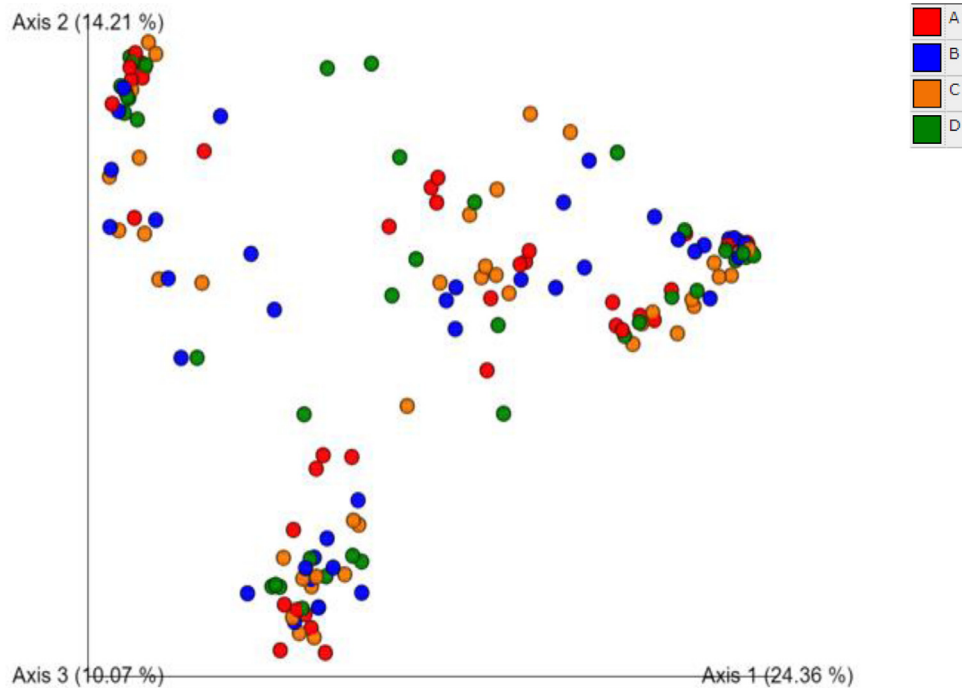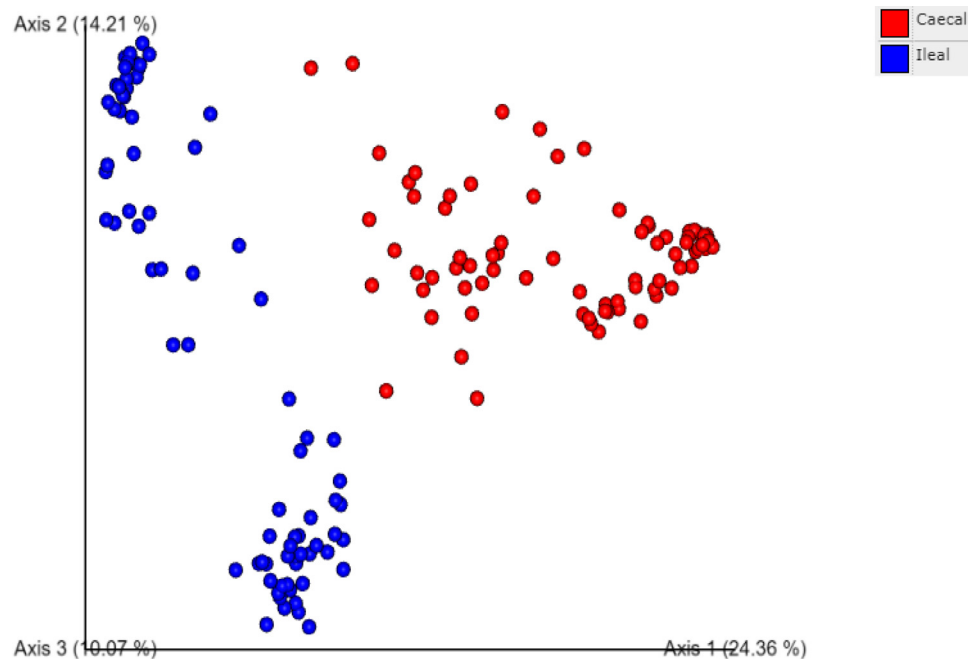

Figure 6. Bray-Curtis principal coordinate analysis determined differences in beta-diversity among treatments. Treatment: A = negative control; B = diet containing 0.075 mg antibiotic (trimethoprim-sulfadiazine; TMP/SDZ) diet; C = diet containing 0.3% red osier dogwood extract; and D = diet containing 0.5% red osier dogwood extract.
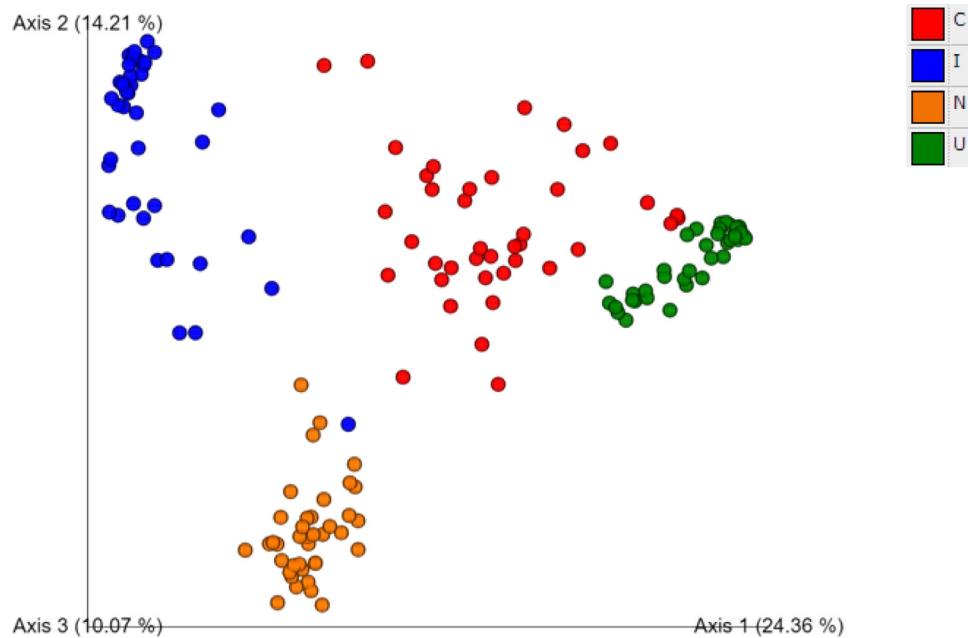
Figure 7. Bray-Curtis principal coordinate analysis determined significant differences ($P < 0.05$) in beta-diversity between the infection model. Challenge groups: U = ceca microbiota of birds that were not challenged with SE; C = ceca microbiota of birds that were challenged with SE; I = ileal microbiota of birds that were challenged with SE group; and N = ileal microbiota of group of birds that were not challenged with SE.

Figure 8. Bray-Curtis principal coordinate analysis determined significant differences ($P < 0.05$) in beta-diversity between the ileum and ceca microbiota.

on the ileal and cecal microbiota. As stated earlier, such pharmacodynamic effects could be due to the variation in bacterial population in the ileum and cecum and consequently a dynamism in polyphenol metabolism in these gastrointestinal sections. Dietary polyphenols, particularly the nonabsorbable ones, are better metabolized where the gut microbial population tends to be highest, usually in the cecum. Depending on the polyphenol biochemical structures and bond with their sugar component (Catalkaya et al., 2020), approximately 5 to 10% of total dietary polyphenols ingested were reported

to be metabolized and absorbed in the small intestinal segments (Gowd et al., 2019).

With respect to the ileal and cecal microbiota diversity, neither the dietary treatments nor SE infection affected the alpha diversity, as shown by the Shannon diversity index. However, the alpha diversity was higher in the ceca than in the ileum, thus, indicating more species richness and evenness in the ceca. Many ileal and ceca microbiota comparative studies have reported that alpha diversity of microbiota composition is usually higher in the cecum than in the ileum of chickens

(Kollarcikova et al., 2019; Bindari et al., 2021; Hemetsberger et al., 2022), including rats (Lee et al., 2018a). The SE infection model gave rise to distinct clustering in the Bray-Curtis dissimilarity between the ileal and cecal microbiota vis-à-vis infection model; thus, suggesting a change in species diversity not only between the ileal and cecal environments but also by SE influence in the gut environments.

CONCLUSIONS

From the results obtained, SE infection influenced the ileal and cecal microbiota with a distinct beta diversity among the infection model groups. The SE infection model had a dynamic effect on the phylum Proteobacteria which was increased and decreased at the ileal and cecal of infected birds, respectively, compared to noninfected counterparts. Actinobacteriota was significantly increased in the cecal of noninfected birds compared to the infected birds. Supplemental trimethoprim-sulfamethoxazole consistently increased the relative abundance of phyla Actinobacteriota and genus *Bifidobacterium* in the ileum and ceca. Meanwhile, dietary supplementation of 0.3 and 0.5% ROD extract showed a similar effect but only on the relative abundance of cecal Actinobacteriota and *Bifidobacterium,* plus a beneficial microbial reductive effect on the relative abundance of genus *Weissella*. The present study suggests that the inclusion of ROD extract at 0.3 and 0.5% inclusion levels had antimicrobial capacity similar to antibiotics, particularly on the ileal and cecal microbiota of SE-infected broiler chickens.

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DISCLOSURES

The authors declare no conflicts of interest(s).

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