DIETARY GRAPE POMACE AND RED OSIER DOGWOOD EXTRACT – EFFECTS ON THE GROWTH AND INTESTINAL HEALTH OF BROILER CHICKENS

by

Taiwo J. Erinle

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2022

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

With the relentless search for antibiotic alternatives, phytogenic additives have shown such alternative potential. Three experiments were undertaken to study the effect of grape pomace (GP) and red osier dogwood (ROD) extract on broiler chickens' growth performance, blood profile, and intestinal functions with or without *Salmonella* Enteritidis (SE) or SE-lipopolysaccharides (SE-LPS). In experiment 1, broiler chickens fed 2.5%GP as an antibiotic-alternative had enhanced growth, gut morphology, and cecal *Bacteroides* and *Lactobacillus*. In experiment 2 with SE-LPS challenge, 0.3% and 0.5% ROD extract were fed to challenged and unchallenged broiler chickens with sustained growth performance in the same capacity of antibiotics, improved ileal morphology, and promoted the proliferation of cecal *Lactobacillus* and *Streptococcus*. In experiment 3 involving SE infection, 0.3% and 0.5% ROD extract maintained growth performance and duodenal morphology, and improved leukocytes, monocytes, and immunoglobulin M. In conclusion, GP and ROD extract could possibly replace antibiotics in broiler chickens production.

LIST OF ABBREVIATIONS AND SYMBOLS USED

A:GLB = albumin:globulin ratio

ADG = average daily gain

BW = body weight

COX-2 = cyclooxygenase 2

DMD = dry matter digestibility

GLB = globulin

GSH-Px = glutathione peroxidase

HO-1 = hemeoxygenase-1

ICAM-1 = intercellular adhesion molecule-1

IL-6 = interleukin-6

IL-8 = interleukin-8

MDA = malondialdehyde

Nrf-2 = nuclear factor (erythroid-derived 2)

PUN = plasma urea nitrogen

ROS = reactive oxygen species

SOD = superoxide dismutase

TBARS = Thiobarbituric acid reactive substances

TNF-α = tumor necrosis factor-alpha

VCAM-1 = vascular cell adhesion molecule-1

VFA = volatile fatty acid

VH:CD = villus height to crypt depth ratio

ZO-1 = zonula occludens-1

↑ = Increase

↓ = Decrease

ACKNOWLEDGEMENTS

First and foremost, my profound gratitude goes to my academic father, Professor I. A. Emiola, whose informal and formal recommendations afforded me the opportunity to be supervised by Dr. Deborah Adewole. My sincere appreciation goes to my supervisor, Dr. Deborah Adewole, for accepting me into her laboratory and, most importantly, for her guidance throughout my program. Special thanks go to my supervisory committee members, Dr. Younes Miar and Dr. Martine Boulianne, for their insight, guidance, and assistance on this project and for reviewing my write-ups. Thanks to Janice MacIsaac for her help with my projects, particularly for assisting with the collection, sorting, and cleaning of data, and processing of blood samples. She is very nice, friendly, and always ready to help virtually with everything.

Many thanks to the Atlantic Poultry Research Centre staff for their assistance with the preparation of experimental feed, daily health and safety checks of birds, and most especially during sample collection day. I also want to thank my laboratory co-members, including Taiwo Makinde, Fisayo Oretomiloye, and Samson Oladokun, for their help during the field and lab work. I cannot forget to thank the lab members of Dr. Martine Boulianne, including Lila Maduro, Karine Lamarre, Nicolas Deslauriers, Maude Lanoie, Laura Guerrero, and Laura Franco, for all their assistance during my third study at the Faculty of Veterinary Medicine, University of Montreal.

I also appreciate MITACS and the Canadian Poultry Research Council for providing the funds for the projects. I am also indebted to Chartwells at Dal AC and the Faculty of Graduate Studies for providing me with scholarships and bursaries during my studies.

Overall, my unquantifiable appreciation goes to God Almighty for His unfailing support and for strengthening me to put my best into the program and projects.

CHAPTER 1: Introduction

A section of this introduction has been published elsewhere:

Erinle, T.J. and D.I. Adewole. 2022. Fruit pomaces—their nutrient and bioactive components, effects on growth and health of poultry species, and possible optimization techniques. Animal Nutrition, 9: 357 – 377. https://doi.org/10.1016/j.aninu.2021.11.011.

1. Introduction

Poultry is one of the commonest livestock species in animal husbandry, with chickens being one of the most popularly consumed (Agyare et al., 2018), particularly in Canada and the United States (Bedford, 2020; Economic Research Service/USDA, 2021). Globally, the chicken industry produces more than 9 trillion kilograms of chicken meat annually (Agyare et al., 2018). Current and future projections show that the poultry industry is continuously expanding in meat production (OECD/FAO, 2020) with the need to meet the protein demand of the ever-growing human population. To meet this increasing demand, the livestock feed supply is estimated to increase from 6.0 to 7.3 billion tons of dry matter (Kim et al., 2019). Thus, the sustainability and profitability of the poultry industry could partly and largely be dependent on nutritional manipulation that could afford improved growth and intestinal functionality of birds.

Pathogenic microbes have been identified as the culprit responsible for the prevalent incidence of diseases in livestock production. Consequently, some pathogenic microbes, including *Salmonella spp* could, by bimodal approach, affect chickens and contaminate food products of poultry origin. On an annual basis, the poultry industry is constantly

battling disease occurrence among flocks and has consequently suffered an economic loss of approximately $3 to $6 billion in tackling diseases globally (Chapman, 2014). With the use of antibiotics, the poultry industry has recorded a ground-breaking improvement in feed conversion efficiency, reduction in disease incidence, and to some extent, the improvement in intestinal health of animals (Proctor and Phillips 2019). Despite the positive impacts of antibiotics, preponderant health concerns relating to antibiotic-resistant pathogens in the food chain have been reported as one of the greatest threats to human health (World Health Organization, 2019). Therefore, it becomes imperative to identify antibiotic alternatives that could prevent diseases in poultry farms.

Several reports on the application of grape pomace (GP) and red osier dogwood (ROD) in livestock production have shown that they could provide equivalent effects compared to antibiotics. However, short-chain fatty acids as indicators of gut health and the incidence of breast muscle myopathies in broiler chickens have not been determined in studies with GP. In addition to this, there is a paucity of information on the impact of ROD on broiler chickens challenged with *Salmonella* Enteritidis infection and/or their lipopolysaccharide metabolites. Thus, the present studies were aimed at investigating the effects of the (i) dietary supplementation of GP as an alternative to antibiotics on growth performance, blood parameters, intestinal morphology, cecal microbiota, cecal concentrations of short-chain fatty acids, and incidence of breast muscle myopathies in broiler chickens, and (ii) dietary ROD extract as an alternative to antibiotics on the growth performance, blood profile, intestinal morphology, and intestinal microbiota in broiler chickens infected with live *Salmonella* Enteritidis or its lipopolysaccharides.

CHAPTER 2: Literature Review

A section of this literature review has been published or submitted for publication elsewhere:

Erinle, T.J. and D.I. Adewole 2022. Fruit pomaces—their nutrient and bioactive components, effects on growth and health of poultry species, and possible optimization techniques. Animal Nutrition, 9: 357 – 377. https://doi.org/10.1016/j.aninu.2021.11.011.

Erinle, T. J., Boulianne, M., Miar, Y., Scales, R., and D. Adewole 2022. Red osier dogwood and its use in animal nutrition – A Review. Animal Nutrition, (Submitted)

2. Overview

The presence of phytogenic substances in plants and their by-products makes them a potential antibiotic alternative in animal production. The present literature review summarizes existing knowledge on the antimicrobial, immune-modulatory, and growth improvement potentials of phytogenic feed additives, namely ROD and GP. A better understanding of these key themes is critical to accessing the efficacy of both ROD and GP as viable antibiotics alternatives in animal production.

2.1 Phytogenic feed additives

Phytogenic feed additives (PFA) refer to essential oils, spices, herbs, plant extracts, or combined bioactive ingredients added to animal feed in minute quantities to exert elaborate

effects (Steiner and Syed, 2017). For a long time, plants have been used in fighting infectious diseases in both humans and animals as ethnoveterinary measures. Generally, all plants produce nutrients (e.g., sugars, proteins, and fat), which constitute the primary metabolites (Máthé and Hassan, 2015). However, only a few plants produce secondary metabolites as an innate defensive mechanism against pathogens and predators (Wenk, 2003). The secondary metabolites are also referred to as phytochemicals - the active ingredients of medicinal plants through which their bioactivities are elicited. Phytogenic materials boast of a wide spectrum of biological activities, including antioxidative (Embuscado et al., 2015), selective antimicrobial (Mountzouris *et al*., 2011; Murugesan et al., 2015; Giannenas *et al*., 2013; Steiner and Syed, 2015), immune-modulatory (Giannenas *et al*., 2013), anti-inflammatory (Shen *et al*., 2010), and anti-parasitic (Ryang et al., 2001; Giannenas et al., 2013) bioactivities.

The antimicrobial properties of PFA have been the cornerstone of an argument that they can serve as an alternative to antibiotic growth promoters. Inclusion of plant materials into poultry diets has proven a worthy antibiotics reduction and replacement strategy (den Hartog et al., 2016) given their phenolic compounds, including rutin, gallic acid, catechins, and epicatechins that are known for their strong antimicrobial and antioxidant prowess (Windisch et al., 2008; Pucava et al., 2013). While many potential antibiotics alternatives including probiotics, prebiotics, organic acids, antimicrobial peptides, and many more have been reported (Alfaig et al., 2013; Bednarczyk et al., 2016; Nguyen et al., 2018; Daneshmand et al., 2019), the use of plant materials and/or their extracted compounds have gained much popularity presumably due to their ready availability, relatively cheap or zero price tag, and little or no technical-know-how in their processing.

Two worthy examples of such plant materials include grape pomace and red osier dogwood extract.

2.1.1 Grape (*Vitis vinifera*) pomace

Grape pomace (GP) is an intermediate product derived after pressing or crushing whole fruits to extract their juice, especially in the grape processing industries and wineries (Figure 2.1). Generally, fruits, including but not limited to grape, apple, carrot, orange, and berries, have been employed in the production of juice with large amounts of pomace produced following juice extraction (Kruczek et al., 2016). A measurable amount of the nutrients, including vitamins, minerals, polyphenols, and dietary fibre profiles in fruits, are also found in their by-products, including pomaces (Juśkiewicz et al., 2015; Kruczek et al., 2016). With inadequate processing and valorization, GP can undergo oxidation and fermentation reactions almost immediately after processing in the presence of oxidants, light, and heat (Bhushan et al., 2008; Lou et al., 2014) and may degrade valuable compounds within them (Gowman et al., 2019). Their disposal poses an environmental health risk due to their high volume and moisture content, thus becoming a suitable substrate for obnoxious microbes to thrive. However, appropriate processing, including drying pomace following juice extraction, could solve the disposal predicament. Most of these by-products are underexploited and are thus mostly discarded or used for unproductive purposes like landfills (Gowman et al., 2019). It would be worthwhile to adopt these relatively cheap by-products in a dual-capacity as dietary fibre ingredients and antioxidants in poultry nutrition, which could consequently reduce feed costs (Colombino et al., 2020). Unfortunately, complementary studies on the cost implication of the adoption

of fruit pomaces, including GP, in poultry nutrition and production are lacking or do not exist.

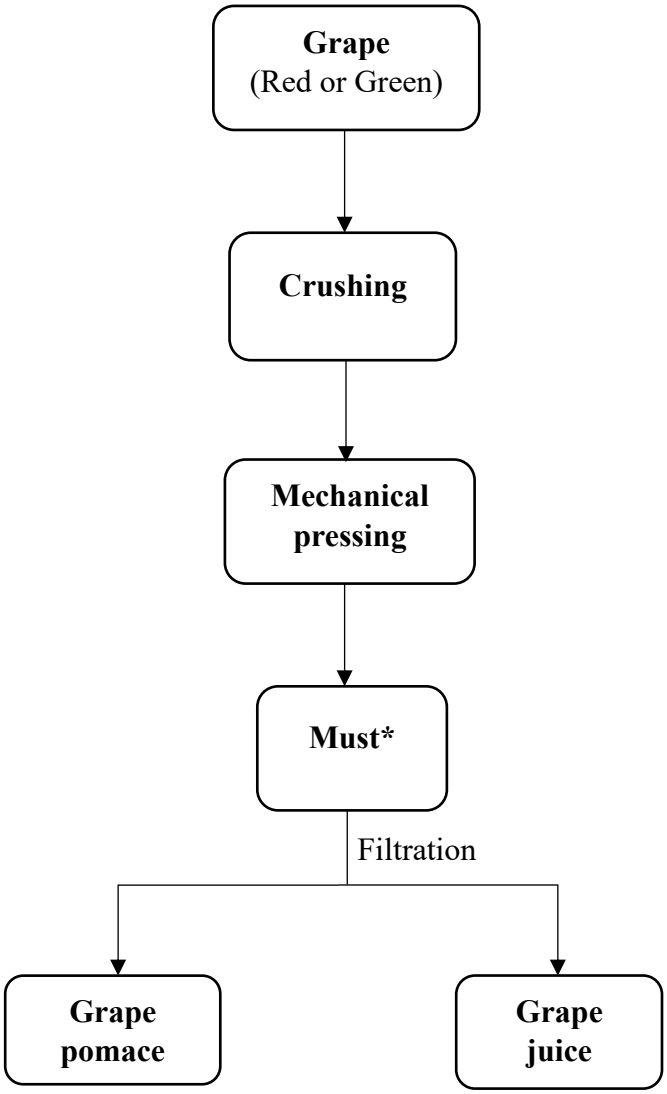


Figure 2.1 Extraction process of GP during grape juice production. Adapted from Granato et al. (2016). *Must is a matrix of juice and remnants of crushed skin, seed, or stalk which are not completely removed following mechanical pressing of grape.

2.1.1.1 Nutrient composition and polyphenolic profile of GP

The amount and type of dietary fibre and phenolics in GP are largely dependent on its varieties and technology employed in the wine-making process. The most abundant polyphenols in GP include phenolic acids (caffeic acid, gallic acid, protocatechuic, 4-

hydroxybenzoic, and syringic acid), phenolic alcohols (hydroxytyrosol), flavonoids (catechin, epicatechin, quercetin-3-O-rhamnoside, and luteolin), stilbenes (resveratrol), and proanthocyanidins (Teixeira et al., 2014; Erinle et al., 2022a). The ability of GP to significantly improve the synthesis of vitamin E in the liver of poultry birds has been reported (Goñi et al., 2007). Increased vitamin E concentration in the body suggests a reinforced antioxidant capacity. In addition, the antioxidant potential of GP has been implicated in increased levels of glutathione peroxidase and superoxide dismutase enzyme activities in the gastrointestinal tract (Kithama et al., 2021). Besides the bioactivities of GP, reports have shown that it contains some nutrients, including protein, fibre, soluble sugar, etc. The nutrient and total phenolic contents and antioxidant capacity of GP are reported in Table 2.1 and 2.2.

Table 2.1. Nutrient composition and fibre fractions of grape pomace reported in literature.

Fruit pomace	Nutrient composition						Fibre fractions					References
	ME (Kcal/kg)	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	TDF (%)	IDF (%)	SDF (%)	NDF (%)	ADF (%)	
Red grape	-	93.3	10.4	10.1	-	-	-	-	-	46.3	48.4	Erinle et al. (2022a)
Grape	-	-	13.8	10.3	32.5	2.4	-	-	-	-	-	Goni et al. (2007)
Grape	-	-	13.9	1.0	15.2	2.4	-	-	-	-	-	Brenes et al. (2008)
Grape	-	-	13.9	1.0	15.2	2.4	-	-	-	-	-	Sayago-Ayerdi et al. (2009)
Grape	-	91.0	9.5	8.7	-	2.7	-	-	-	-	-	Baumgartel et al. (2007)
White grape	4,466	30.5	9.3	4.8	19.9	-	-	-	-	30.6	25.7	Baumgartel et al. (2007)
Red grape	4,968	27.3	15.5	7.0	31.2	-	-	-	-	50.7	36.5	Swanson et al. (2001)
Grape	-	86.8	15.9	7.7	-	-	54.7	50.2	4.5	-	-	Nagarajaiah et al. (2016)
Blue grape	-	85.5	3.6	1.8	-	1.7	-	28.2	12.8	-	-	Nagarajaiah et al. (2016)
Red grape	-	-	13.9	1.0	34.3	2.4	-	-	-	-	-	Chamorro et al. (2015)
Fermented grape	-	-	28.3	3.8	22.2	8.5	-	-	-	-	-	Gungor et al. (2021)
Grape	-	-	12.6	5.9	18.8	4.1	-	-	-	-	-	Gungor et al. (2021)
Red grape	-	96.6	11.4	71.0	-	-	-	-	-	40.9	32.3	Jonathan et al. (2021)
Grape[X]	-	93.9	10.1	9.2	18.2	3.9	-	-	-	38.3	32.5	Hanušovský et al. (2019)
Grape pomace	-	-	13.9	9.1	14.3	23.7	-	-	-	-	-	Alm El-Dein et al. (2017)
Grape pomace	-	89.9	12.3	6.0	35.2	2.8	-	-	-	-	-	Vlaicu et al. (2017)
Grape pomace	4,398	91.5	8.9	7.0	30.2	3.3	-	-	-	-	-	Ebrahimzadeh et al. (2018)
Grape pomace	2,433	-	13.3	8.4	19.3	4.5	-	-	-	-	-	Hosseini-Vashan et al. (2020)

All values are expressed on dry weight basis and in 1 decimal place.

[X] Average nutrient composition of grape pomace obtained in two different locations in Slovakia.

ME = Metabolizable Energy, Kcal/kg; DM = Dry Matter, %; CP = Crude Protein, %; EE = Ether Extract, %; CF = Crude Fibre, %; TDF = Total Dietary Fibre, %; IDF = Insoluble Dietary Fibre, %; SDF = Soluble Dietary Fibre, %; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre

Table 2.2. Total phenolic content and radical scavenging activity of dried grape pomace

Grape pomace	TPC	Total antioxidant activity				References
		DPPH•	RSA	ABTS	ORAC	
Red grape (RG)	12.3	-	-	-	-	Erinle et al. (2022a)
	12.4	-	-	-	-	Kasapidou et al. (2016)
RG cultivars;						
Touriga Nacional	69.3	0.52	-	-	1,054	Tournour et al. (2015)
Touriga Franca	100	0.87	-	-	1,343	Tournour et al. (2015)
Tinta Roriz	132	1.09	-	-	2,337	Tournour et al. (2015)

≃ Approximately.

TPC = Total phenolic content, mg gallic acid equivalent (GAE) per gram dry weight.

[w] Values with unit as mg GAE per gram fresh leaf.

*Methanol-acetone extracted TPC value reported in mg gallic acid equivalent (GAE) per 100 gram dry weight.

DPPH = 1,1 diphenyl-1-picryl hydrazyl assay, µmol trolox equivalent per gram.

[z] DPPH Total antioxidant activity reported as percentage (%).

RSA = radical scavenging activity, mg ascorbic acid equivalent per gram dry weight.

ORAC = oxygen radical absorbance capacity, µmol trolox equivalent per gram.

ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), µmol trolox equivalent per gram.

2.1.1.2 Potential of GP in poultry nutrition

The potential of GP in poultry nutrition, as reported in recent literature, is presented in Table 2.3.

2.1.1.2.1 The use of GP to improve growth performance of poultry birds

Grape pomace has been reported to majorly maintain growth performance in poultry species, mostly chickens. This could be partly due to their dietary fibre constituents and polyphenolic profile, which may limit their inclusion levels in poultry diets. The adoption of GP as a nutraceutical and alternative ingredient in poultry production has gained momentum in the last two decades. The capacity of grape by-products to improve growth performance is mainly dependent on the form of the by-product and the amount incorporated into the diet (Erinle et al., 2022a). The study conducted by Kumanda et al. (2019) was the only study that reported that the addition of 7.5% GP improves the growth performance of broiler chickens. Viveros et al. (2011) demonstrated that feeding 6% GP improved the growth of birds like avoparcin antibiotics did. Goñi et al. (2007) and Sáyago-Ayerdi et al. (2009) submitted that dietary GP could be added up to 6% in broiler chicken diets without impairing growth performance. Contrarily to the reports of Kumanda et al. (2019), Goñi et al. (2007), Brenes et al. (2008), Sáyago-Ayerdi et al. (2009), Chamorro et al. (2015), and Ebrahimzadeh et al. (2018) reported that dietary supplementation of dietary GP in the range of 5 to 10 % did not affect growth performance of broiler chickens. However, a lower dosage of raw or fermented supplemental GP of less than 3% has been demonstrated to improve growth performance in broiler chickens (Pop et al., 2015; Aditya et al., 2018; Erinle et al., 2022a; Gungor et al., 2021; Altop and Erener, 2021). Increasing the inclusion level of GP from 1 to 2 % resulted in the improvement of BW and FCR in

broiler chickens (Pop et al., 2015). However, incorporation of GP at 2.5% was also observed to improve feed intake and FCR in the same magnitude as bacitracin methylene disalicylate antibiotics (Erinle et al., 2022a).

There are a lot of variations when it comes to the significance GP on feed intake, BW, and other growth parameters of poultry birds. As earlier mentioned, the variations in most reports are due to the variation in the inclusion levels and antinutritional factors present in GP. From a critical perspective, GP might improve growth performance; however, the capacity of their polyphenols to reduce abdominal fat is noteworthy and could account for the reduced body weight compared to antibiotics.

2.1.1.2.2 The use of GP to improve gut morphology of poultry birds

The gut performs an indispensable role in digestion and absorption of nutrients and plays a selective barrier function by regulating the passage of metabolites and strengthening its structural integrity against pathogens. There is a constant cross-interaction between gastrointestinal epithelial tissue and its environment. The crucial gut functions may be compromised under certain conditions, such as feeding a low-quality diet. Bioactive substances present in GP have the capacity to improve broiler feed efficiency by increasing nutrient digestibility, motility of the gastrointestinal tract, and bile acid function. In gut-related poultry studies, villus height and crypt depth in small intestinal segments are often considered indicators for nutrient absorption and a slower rate of enterocyte epithelial cell renewal. Although there is a dearth of information on the impacts of GP on gut health, few studies have reported that the supplementation of GP at 6% and 2.5% inclusion levels have been reported to improve villus height to crypt depth ratio in broiler chickens (Viveros et al., 2011; Erinle et al., 2022a). Villus height (VH) and VH:crypt depth (VH:CD) were

reported to decrease when 7.5% and 10% GP was fed to broiler chickens; however, at a 5% inclusion level, there was a significant improvement in the VH:CD at the duodenum and jejunum (Ebrahimzadeh et al., 2018).

In the small intestine, dietary fruit pomaces were found to reduce digesta viscosity and increase the concentration of short-chain fatty acids, mainly acetic and butyric acid, compared to control-fed birds (Colombino et al., 2020). Butyric acid provides the suitable form of energy necessary for the stimulation of growth of intestinal epithelial cells and mucin production, thus, maintaining the tight junction integrity at the intestinal level (Jung et al., 2015; Peng et al., 2009).

2.1.1.2.3 The use of GP to improve gut microbiota of poultry birds

The gut microbiome plays a significant role in the health and metabolism of poultry species (Lin et al., 2016). In a healthy state in the poultry gut, Firmicutes, Bacteroidetes, and Proteobacteria are the three most abundant bacteria phyla; however, phyla Bacteroidetes and Firmicutes are considered the relative most abundant (Qin et al., 2010; Almeida et al., 2019; Forster et al., 2019). The novel application of probiotics, prebiotics, exogenous enzymes, and phytogenic compounds have been shown to modulate the gut microbiome of poultry (Dibner and Richards, 2005; Oakley et al., 2014). Interestingly, dietary fibre has also been reported to induce a beneficial effect on gut health, including serving as a prebiotic to selectively enrich beneficial gut bacteria (Gong and Yang, 2012). This suggests that the phenolic compounds and dietary fibre component of fruit pomaces, including GP, could be adopted to modulate the gut microbial population.

Grape pomace was reported to increase the *Bifidobacteria* counts in chickens and rats (Chacar et al., 2018; Islam et al., 2020, 2019). Viveros et al. (2011) and Islam et al. (2019) also reported a decrease in the abundance of *Enterococcus* bacteria in GP and wild blueberry-fed birds, respectively, compared to the control group. Unfortunately, *Enterococcus species* have been implicated in the incidence of colorectal cancer and damaged eukaryotic cellular DNA in the colon epithelial cell by stimulating the secretion of superoxides and hydroperoxides (Huycke et al., 2002; Balamurugan et al., 2008; Jones et al., 2008). While the gut of chickens houses communities of microbes, *Lactobacillus, Clostridium, Enterococcus,* and *E. coli* are recognized normal residents. The relative abundance of genus *Bacteroides, Bifidobacterium,* and *Faecalibacterium* were reported to be increased following dietary incorporation of wild blueberry and GP in broiler chickens' diets (Islam et al., 2019; Erinle et al., 2022a). *Bacteroides* were suspected of contributing to the degradation of indigestible carbohydrates found in its host. Louis et al. (2010) reported that *Faecalibacterium,* a member of the Ruminococcaceae, contributes to the production of butyrate, which could perform anti-inflammatory functions in the host cell. However, *Bifidobacterium* and *Bacteroides* also contribute to mucin degradation (Hooper et al., 2002; Ruas-Madiedo et al., 2008). The synergistic effect resulting from the combination of different fruit powders has also been reported. An *in vitro* study by Vattem et al. (2005) showed that combined supplementation of blueberry, grape seed, and oregano extract enhanced the antioxidant and anti-*Helicobacter pylori* activity of cranberry powder.

While GP tends to have modulatory effects on the gut microbiota of poultry, their inclusion at higher levels could antagonize its potential beneficial modulatory effects. In GP trials, Chamorro et al. (2017) reported that GP-fed at 5% and 10% did not influence the

population of ileal *Lactobacillus*. The inclusion of GP at 10% was shown to upturn the antimicrobial effect of GP against *Clostridium perfringens* (Chamorro et al., 2017). Viveros et al. (2011) also demonstrated that 6% dietary GP significantly increased the concentration of *E. coli, Lactobacillus, Enterococcus,* and *Clostridium*. A similar result was reported when 0.72% grape seed extract was fed to the birds. At a lower inclusion level, 1 to 4 % GP was reported to significantly increase the relative abundance of *Bacteroides* and *Lactobacillus* bacteria species (Hafsa and Ibrahim, 2018; Erinle et al., 2022a) and a significantly reduced relative abundance of genera *Escherichia-Shigella* and *Clostridia_unclassified* (Erinle et al., 2022a). A reduction in the abundance of *Bacteroides* has been associated with inflammatory bowel disease, Crohn's disease, and ulcerative colitis disease conditions (Zhou and Zhi, 2016). Another mechanism of action of *Lactobacillus* is to secrete antimicrobial peptides known as bacteriocins and lactic acid, which lowers the pH of their immediate environment, thereby inhibiting the proliferation of pathogenic bacteria, including *E. coli*, *Campylobacter jejuni*, and *Clostridium perfringens* (Murry et al., 2004; Neal-McKinney et al., 2012).

2.1.1.2.4 The use of GP to improve oxidative stress in poultry birds

Proanthocyanidins, one of the most reliable antioxidants of plant origin, is reported to possess about 20 times and 50 times higher antioxidant bioactivity compared to vitamins E and C, respectively (Shi et al., 2003). Interestingly, GP is particularly a rich source of these compounds. A chicken study conducted by Goñi et al. (2007) revealed that supplementation of GP at 0.5, 1.5, and 3 % inclusion levels significantly increased vitamin E concentrations in the liver and antioxidant capacity of the chicken meat, especially at the highest inclusion level of the pomace. This suggests that GP could be used as an alternative

to antibiotics and synthetic vitamin E in the poultry diet and, thus, may reduce costs related to the purchase of the vitamin additives.

Lipoxidation reactions have been implicated as one of the leading causes of quality deterioration in lipid-containing substances, including meat and derived meat products in poultry. The possibility of improving the quality and shelf life of meat has been correlated with the enhanced antioxidant capacity in the muscle (Tavárez et al., 2011). Like other fruit pomaces, consumption of dietary GP with or without enzyme was reported to reduce oxidation in chicken meat by reducing MDA concentration upon storage in the exact equivalent of dietary α-tocopheryl acetate (Chamorro et al., 2015). Supplementation of α -tannase into a 10% GP diet was found to achieve a similar protective effect without impairing the growth of birds. Furthermore, the success of grape by-products as anti-lipoxidation in beef patties, pork, turkey and chicken meats, and fish have been extensively reported (Lau and King, 2003; Pazos et al., 2005; Mielnik et al., 2006; Bañón et al., 2007; Carpenter et al., 2007).

Table 2.3. Growth performance and health of poultry birds fed fruit pomaces as reported in recent literature.

Grape pomace	Inclusion levels (%)	Poultry species	Effects	References
1. Red GP	2.5	Broiler chickens	i. Birds' FI was higher when 2.5% RGP was fed and was compared favourably to antibiotic-treated birds. Reduced BW was observed in RGP-birds during the grower phase; however, overall FCR was similar compared to antibiotics. ii. Significant improvement in gut histomorphometric on the RGP-fed birds and was better compared to antibiotic treatments. iii. Significantly decreases Firmicute to Bacteroidetes ratio and improves the population of beneficial microbes, including *Lactobacillus spp*.	Erinle et al. (2022a)
2. Red GP (RGP)	1.5, 3, 4.5, and 6	Cockerels (chickens)	i. The increasing dietary RGP did not affect the overall FI, body WG, FCR and slaughtered weight of cockerels. ii. MCH and GLB increase significantly with increasing inclusion levels of RGP.	Jonathan et al. (2021)
3. GP	0.045, 0.035, and 0.025 % of bird's BW	Broiler chickens	i. Similar BW was reported across the dietary treatments.	Dupak et al. (2021)

Grape pomace	Inclusion levels (%)	Poultry species	Effects	References
			ii. There was a significant reduction in LDL of birds at 450 mg/kg inclusion of GP. iii. Increased SOD at the highest dose of GP while GPx was not affected.	
4. GP ± Fermentation	1.5	Broiler chickens	i. Fermented GP (FGP) improves final BW in the same capacity as the synthetic antioxidant treatment; however, it was better when compared to raw GP. ii. Raw GP at 1.5% significantly increased serum GPx and SOD, while CAT was increased when 1.5% FGP was fed. iii. FGP significantly decimates *Clostridium perfringens* population compared to other treatments; however, other bacterial species, including *Lactobacillus* were not affected. iv. Regardless of fermentation, the GP treatments significantly reduce VH and VH:CD.	Gungor et al. (2021)
5. GP	7.5 and 15	Broiler chickens	i. Dietary GP significantly lowered FI and FCR and higher BW and was compared favourably to birds fed Vitamin C and E, respectively.	Mankola et al. (2021)

Grape pomace	Inclusion levels (%)	Poultry species	Effects	References
			ii. Dietary GP significantly lowered AST, ALT, and TAG and higher TP, GLB, HDL; however, it was similar to the Vitamin C, and E-fed birds. Additionally, 15% GP reduced TC and LDL compared to other treatments. iii. Dietary GP significantly increased IgG, IgM, IgA, and SOD, lower MDA and were comparable to Vitamin C and E.	
6. GP ± Enzyme complex ± Tannase	5 and 10	Broiler chickens	i. 5% dietary GP significantly increased protein and total polyphenol digestibilities. However, supplementation of enzyme complex or tannase or a combination of both reduced the two digestibilities. ii. Significant increase in the plasma α-tocopherol and antioxidant capacity of birds fed 5%GP and Vitamin E, respectively.	Chamorro et al. (2017)
7. RGP	2.5, 4.5, 5.5, and 7.5	Broiler chickens	i. Average weekly FI and FCR significantly reduced when 7.5% RGP was fed compared to other RGP levels and control. However, overall WG was not affected. ii. Blood parameters and carcass characteristics were not affected.	Kumanda et al. (2019)

Grape pomace	Inclusion levels (%)	Poultry species	Effects	References
8. GP	5, 7.5, and 10	Broiler chickens	i. No difference in the performance of birds by the increasing inclusion levels of GP. ii. Blood antioxidants, SOD and GPx, were significantly higher while MDA was reduced among 5 and 7.5% GP-fed birds. iii. All inclusion levels of GP reduced serum TAG and LDL while HDL was increased. iv. Significantly increased antibody titre against NDV among birds fed 5 and 10% GP.	Ebrahimzadeh et al. (2018)
9. GP	5, 10, and 20	Broiler chickens	i. Increasing levels of GP increased FI, particularly at the starter and grower phase; however, BW gain and FCR were not affected. ii. Increasing levels of GP reduced abdominal fat in heat-stressed birds. iii. Increasing levels of GP reduced plasma cholesterol, LDL, AST, MDA, and TAG, while HDL, TP, GPx, and SOD were increased. iv. GP increased the weights of immune organs, bursa and thymus.	Hosseini-Vashan et al. (2020)

Grape pomace	Inclusion levels (%)	Poultry species	Effects	References
10. RGP and White GP (WGP)	20 RGP and 20 WGP	Broiler chickens	i. Dietary WGP did not affect BW, daily WG, FI and FCR, while RGP increased overall FCR. ii. Dietary WGP increased the antioxidant capacity of breast and leg meat compared to the RGP and control treatments.	Reyes et al. (2020)
11. GP	1, 2, 3, and 4	Laying hens	i. Dietary GP at 3 and 4% improved FCR, %EP, EM, SOD, and GPx compared to control treatment. ii. The %EP, EN, and EM were significantly higher among 4% GP-fed birds compared to those fed Vitamin E.	Alm El-Dein et al. (2017)
12. RGP	1.5, 3.5, and 5.5	Quail	i. Overall, FI was significantly improved at 3.5% RGP compared to other treatments. However, overall BW gain, FCE, and final BW were not influenced by the varying inclusion level of RGP. ii. Similarly, the serum biochemical parameters of the birds were not affected.	Mnisi et al. (2021)

FI = Feed intake; BW = Body weight; WG = Weight gain; EM = Egg mass; EW = Egg weight; EN = Egg number; FCR = Feed conversion ratio; FCE = Feed conversion efficiency; FE = Feed efficiency; %HDP = Percentage hen-day production; %EP = Egg production; EPEI = European production efficiency index; SOD = Superoxide dismutase; CAT = Catalase, GST = Glutathione transferase; GPx = Glutathione peroxidase; MDA = Malondialdehyde; IgM = Immunoglobin M; IgG = Immunoglobin G; IgA = Immunoglobin A; TAG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein;

VLDL = Very low density lipoprotein; RBC = Red blood cell, PCV = Packed cell volume, haemoglobin, MCV = Mean corpuscular volume, and MCH = Mean corpuscular haemoglobin; AST = Aspartate transaminase; ALT = Alanine transaminase; TP = serum Total protein; GLB = serum Globulin; TC = Total cholesterol.

2.1.2 Red osier dogwood

Red osier dogwood belongs to the genus *Cornus* (Cornaceae family, usually shrubs) and has several attributable names because of its color, geographical location, or ethnical perceptions. Botanically, ROD is named *Cornus sericea, Cornus stolonifera*, *Swida sericea, Swida stolonifera* or *Thelycrania sericea*. However, some of its common names include red-stemmed dogwood, red osier dogwood, American dogwood, California dogwood, creek dogwood, western dogwood, poison dogwood, waxberry cornel, red twig dogwood, red-osier cornel, red willow, red brush, red rood, and Kinnikinic.

Red osier dogwood prevalently inhabits riparian, wet and boggy areas of the northern-temperate zone; however, it is by no means limited to the Canadian provinces and the United States. It produces white flowers, white to blue berries, and red stalk, sometimes referred to as winter dogwood and often attracts birds and other animals (Gucker, 2012). Generally, plant species belonging to the genus *Cornus* are usually hardy, including ROD. They can withstand unfavourable cold temperatures and thrive in several soil conditions with varying nutrient and pH levels (Hardy, 1989; Pijut, 2004; Gucker, 2012). In addition, they can tolerate flooding because of their non-tap rooting system (Beaudry, 1999), especially when fully established. Although it does well during the warm season, a laboratory study showed that ROD could be withstand extreme cold weather conditions (Gucker, 2012). Propagation of ROD is achieved by seeding or vegetatively by cutting and easily dispersed by animals. However, several methods of cultivating ROD are documented by Swanson (2019). Upon seeding, it takes about 60 days to germinate and attain heights of 15.24 cm and 60.96 cm in the first and second year, respectively. The optimum temperature for germination ranges from 10 – 25 ^{0}C (Acharya et al., 1992).

2.1.2.1 Phenolic and nutrient profiles of ROD

Depending on the season when harvested, ROD could be recognized as one of the most potent phytogenic additives given its rich polyphenolic profile and high total polyphenol concentration. The extraction of polyphenol content in air-dried, spray-dried, or freeze-dried ROD has been demonstrated using methanol with 2% formic acid (Isaak et al., 2013; Scales, 2015) or hydrothermal method at 98 ^{0}C (Apea-Bah et al., 2020). While both methods are commercially used, proponents of the hydrothermal extraction method worry over the health, safety, and flammable concerns of the alcohol extraction model. Without prejudice to the foregoing, there are no comparative studies to determine the best-optimized extraction methods in ROD.

Phytochemical analysis of ROD plant parts, including leaves, stem, fruit, flowers, and root, revealed that ellagic acid, quercetin, and gallic acid and their derivatives were the prominent phenolics (Vareed, 2005; Isaak et al., 2013; Scales, 2015), the combination of which has significant health importance in a living system (Mehrzadi et al., 2020; Patil and Killedar, 2021). In addition, tyrosol, catechin, epicatechin, rutin, kaempferol, cyanidin, caffeic acid, and anthocyanins were reportedly present (Balasundram et al., 2006; Isaak et al., 2013; Scales, 2015; Lee et al., 2018). These phenolic compounds are known for their strong redox buffering and antimicrobial bioactivities (Hagiwara et al., 2010; Isaak et al., 2013; Nair and Nair, 2013; Puvača et al., 2013; Abuelsaad et al., 2014; BenSaad et al., 2017). Quercetin and kaempferol were specifically shown to reduce gastric inflammation in mice infected with *Helicobacter pylori* through the downregulation of *IL*-8 and p38 mitogen-activated protein kinase (Zhang et al., 2017; Yeon et al., 2019). The general molecular structure of quercetin, gallic acid, and anthocyanin are shown in Figure 1.

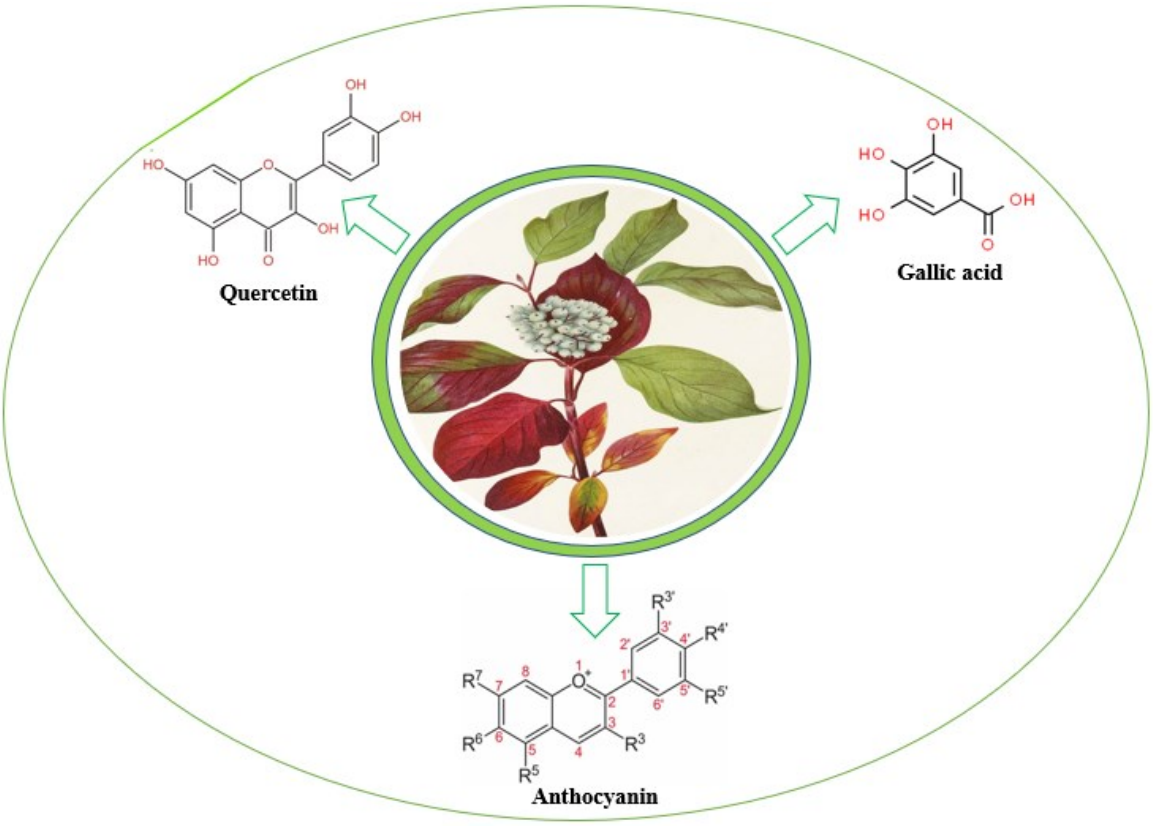


Figure 2.2. Predominant polyphenols in red osier dogwood.

Although these compounds could be found in many other plants, including olive (Serreli and Deiana 2018), however, in the most recent comparative studies conducted by Isaak et al. (2013), methanol-extract of air-dried ROD sourced in Manitoba contains a very high amount of total phenolic content (TPC) with the peak value at 220 mg gallic acid equivalence (GAE) g^{-1} dry weight (DW) and was higher than the 40.27 mg GAE g^{-1} DW reported by Makris et al. (2000) for olive extract. Another medicinally important plant is garlic which has been consistently noted for its powerful antioxidant capacity due to the phenolic and organosulfur compounds. Studies that evaluated total polyphenolic content in some garlic cultivars and extracts reported that their TPC ranges from 21.27 to 33.96 mg GAE g^{-1} fresh weight (Chen et al. 2013) and 6.95 to 19.69 mg GAE g^{-1} fresh weight (Lu et al. 2011), respectively. Wei et

al. (2019) also reported that the TPC of ROD was higher when compared to lemongrass, lavender, rosemary, and calendula. The peak TPC value in ROD may range from 220 to 265 mg GAE g^{-1} (Isaak et al., 2013; Wei et al., 2019; Yang et al., 2019; Erinle et al., 2022b). The amount of phenolic content in a plant material has been considered an important determinant of its redox buffering capacity (Isaak et al., 2013). This suggests that ROD is a better plant material that is yet to receive the necessary attention it deserves.

In-feed polyphenols have been extensively reported to interfere with nutrient digestion due to their enzyme-inhibition tendency. Interestingly, such interference could be associated with the concentrations of polyphenols, the plant type from which they are derived, and the constituent food matrix in which they are incorporated. A complete understanding of the pharmacokinetics and pharmacodynamics of plant bioactive substances, from their digestion in the mouth to metabolism at organ-specific sites, is essential in determining their beneficial health effect in animal production. Unfortunately, both concepts are yet to be comprehensively unraveled in research. However, many research hypotheses have speculated such metabolic pathways of polyphenols in humans and animals using *in vitro* simulation methods (Tarko et al., 2013; Smeriglio et al., 2016). Recent studies reported that phytochemicals, including anthocyanins, are rapidly absorbed during digestive processes in the stomach and small intestine of rats which is characterized by the intense red coloration of the stomach and intestine (Talavéra et al., 2003; He et al., 2009; Mullen et al., 2010). In a study mimicking the absorption of ROD polyphenols in humans using an *in vitro* co-culture model, rapid absorption and transportation of quercetin-3-glucoside, quercetin-glucuronide, rutin, quercetin-3-*O*-malonylglucoside, and kaempferol-glucoside without any hindrance (Jiang et al., 2019). Furthermore, anthocyanins, including cyanidin-3-*O*-galactoside,

pelargonidin-3-*O*-glucoside, and pelargonidin-3-*O*-rutinoside, which are predominantly found in some *Cornus,* were reported to be high when exposed to an acidic condition of the stomach (David et al., 2019). This is an indication of the extent of bioavailability of ROD polyphenols; as a result, it could afford a high concentration of ROD bioactive substances in the blood and eventually at some target organs, given its rapid absorption and transportation.

In addition to the polyphenolic profile, ROD plant, usually the shoot, has been shown to contain a considerable amount of nutrients, as shown in Table 2.4. These include 6.7 % crude protein, 4.2 % ether extract, 29.2 % crude fibre, 56.8 % nitrogen-free extract, 1.13 % calcium, 0.33 % phosphorus, and 1586 IU/lb carotene (Fashingbauer and Moyle, 1963). Meanwhile, Lee et al. (2018) reported 88.6 % dry matter, 9.61 % crude protein, 13.25 % neutral detergent fibre, 10.10 % acid detergent fibre, 4.40 % ether extract, 4.93 % total starch, and 3,881 kcal kg^{-1} gross energy. Thus, ROD plant material could be considered both a phytogenic and nutritional feed additive; however, its high phenolic concentration could deter its high inclusion level as a composite feed ingredient for poultry species. Given the nature of the gastrointestinal tract (GIT) of ruminants with high ruminal microbial populations that specialize in the fermentation of forages, high inclusion levels of ROD plant materials could be more tolerable. It has been estimated that approximately 90% of polyphenols are digested in the GIT sections with highly populated microbial communities (Tarko et al., 2013).

Table 2.4. The nutrient profile of red osier dogwood (*Cornus sericea*).

Nutrient composition	Fashingbauer and Moyle (1963)	Lee et al. (2018)	Wei et al. (2018)	Gomaa et al. (2018)
Dry matter (%)	-	88.6	93.3	-
Crude protein (%)	6.70	9.61	10.8	8.8
Ether extract (%)	4.20	4.40	-	-
Crude fibre (%)	29.2	-	-	-
Nitrogen-free extract (%)	56.8	-	-	-
Neutral detergent fibre (%)	-	13.3	23.6	37.3
Acid detergent fibre (%)	-	10.1	31.7	-
Total starch (%)	-	4.93	-	-
Organic matter (%)	-	-	92.1	92.1
Gross energy (kcal kg^{-1})	-	3881	-	-
Calcium (%)	1.13	-	-	-
Phosphorus (%)	0.33	-	-	-
Carotene (IU/lb)	1586	-	-	-

2.1.2.2 Seasonal variation of phenolic component of ROD

Red osier dogwood plant contains varying concentrations of total polyphenolic compounds depending on seasons (Isaak et al. 2013), as shown in Figures 2. Seasonal variations have been reported to influence the phytochemical profile in medicinal plants due to varying duration of sunlight rather than temperature (Anesini et al. 2008; Isaak et al. 2013). According to Harbowy et al. (1997), sunlight plays a significant role in the biosynthesis of phenolic compounds in plants and usually, plants exposed to more sunlight contain more phenolics. The concentration of total phenolics in ROD has been consistently highest during the summer (Isaak et al., 2013; Scales, 2015), while anthocyanin peaked in autumn and winter seasons (Isaak et al., 2013). The antioxidant bioactivity of ROD is not affected by the varying temperature. The plant protects itself from light-induced oxidative damage by increasing anthocyanin production in autumn (Feild et al., 2001). From the extrapolated and analyzed data from the Environment Canada's National Climate Data and Information Archive (2012), which showed normal ranged temperatures during the summer and fall seasons, but low precipitation in the summer of 2011, Isaak et al. (2013) concluded that precipitation could be one of the confounding factors contributing to the variation in phenolic compounds in *C. stolonifera*. Besides temperature or precipitation, sunlight was considered another factor influencing the variation in total polyphenol content in amaranth cultivars, with plants exposed to more sunlight having the highest TPC and lowest in shaded plants (Khandaker et al., 2008). In addition to this, Popović et al. (2018) advocated that geographical location may also be responsible for the variability in phenolic compounds in two species of Cornus, namely, *C. mas* and *C. sanguinea*. Besides possible interaction between phenolics and food matrix, integration of these factors may be

responsible for the inconsistent results obtained in many *in vivo* phytoadditive studies. While it cannot yet be affirmatively concluded that one factor is primely responsible for the variation, there could be a need for more environmental studies to validate these speculations.

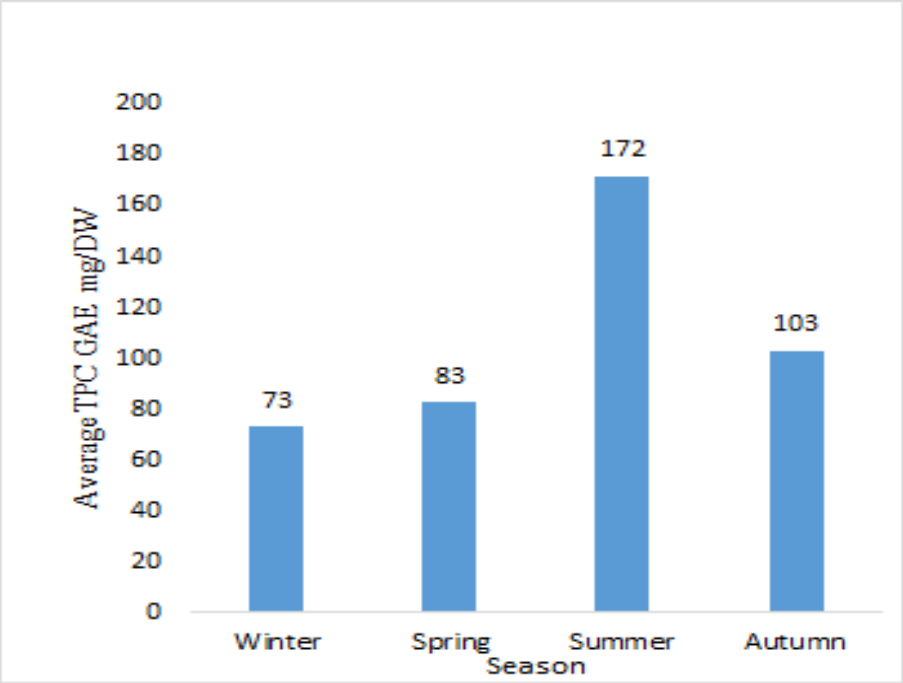


Figure 2.3. A bar chart showing an approximate total phenolic concentration (TPC) gallic acid equivalent mg/dry weight per season. Adapted from Isaak et al. (2013) by finding average of TPC per season in span of three years; 2010, 2011, and 2012.

2.1.2.3 Antioxidant capacity of ROD

Given that various plants, including ROD, contain varying concentration levels in their phenolic constituents, oxygen radical absorbance capacity (ORAC) would therefore be sufficient to compare the efficacy of ROD with some selected medicinal plants used in animal nutritional studies. The ORAC assay is one of the standard methods of evaluating the total antioxidant activity of polyphenols and has also been applied in some ROD

studies. Red osier dogwood was reported to have a peak ORAC value of 1,632 µmol trolox equivalents (TE) g^{-1} DW in the summer (Isaak et al., 2013) compared to the ORAC values of 627.14µmol TE g^{-1} DW, 744.95 µmol TE g^{-1} DW, 524.76 µmol TE g^{-1} DW, and 1,280 µmol TE g^{-1} DW in methanol-extract of leaves of tea, parsley, basil, and olive plants, respectively as reported by Wojcikowski et al. (2007). The ORAC of these plants is graphically presented in Figure 3. Comparing the red and green leaves of ROD, Scale (2015) reported that the ORAC was higher in dark green ROD leaves, which could be obtained during the summer. This is not surprising as a higher concentration of ellagic and gallic acids was recorded in the dark green leaves. Generally, red-colored leaves are rich in anthocyanins. Anthocyanins, including cyanidin 3-*O*-galactoside, pelargonidin 3-*O*-glucoside, and pelargonidin 3-*O*-rutinoside, are effective antioxidants, inhibitors of gram-negative bacteria, anticancer, and anti-inflammatory agents (Bruce et al., 2000; Kang et al., 2003; Cooke et al., 2005; Smeriglio et al., 2016). The higher ORAC values reported in ROD suggested it could be more effective and efficient at abating oxidative stress caused by microbial contamination and free radicals in the gut. Scales (2015) found that ellagic acid and gallic acid concentration levels in green leaves of ROD were higher during the summer months.

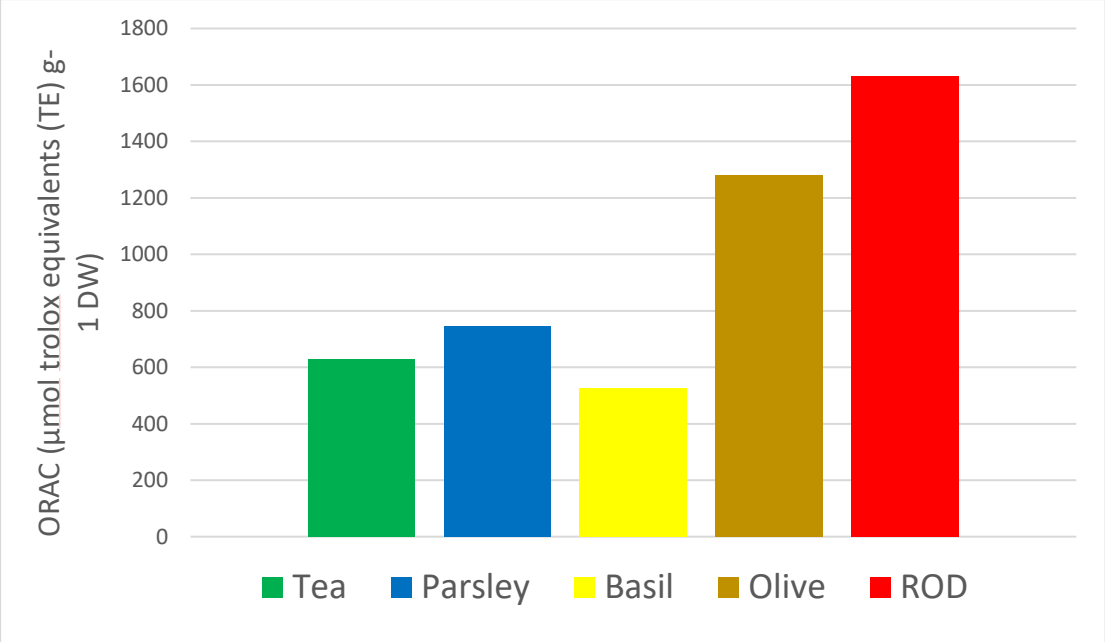


Figure 2.4. Oxygen reactive absorbance capacity of ROD and some plants

A strong antioxidative prowess of gallic acid has been demonstrated in mice (Palafox-Carlos et al., 2012; Nair and Nair, 2013). In the reports of Hagiwara et al. (2010) and BenSaad et al. (2017), ellagic acid has shown the capacity to prompt the occurrence of death of cancer cells and exert their antimicrobial and immunomodulatory activity. Both ellagic and gallic acids are predominantly present in ROD, as shown in Figure 4. The rutin present in ROD could be easily hydrolyzed to quercetin and rutinose in the presence of HCl at 100 ^{0}C. The antioxidant capacity of individual phenolics using ABTS$^{\bullet}$+ revealed that gallic acid and quercetin have higher antioxidative activity compared to catechin (Kopjar et al., 2016).

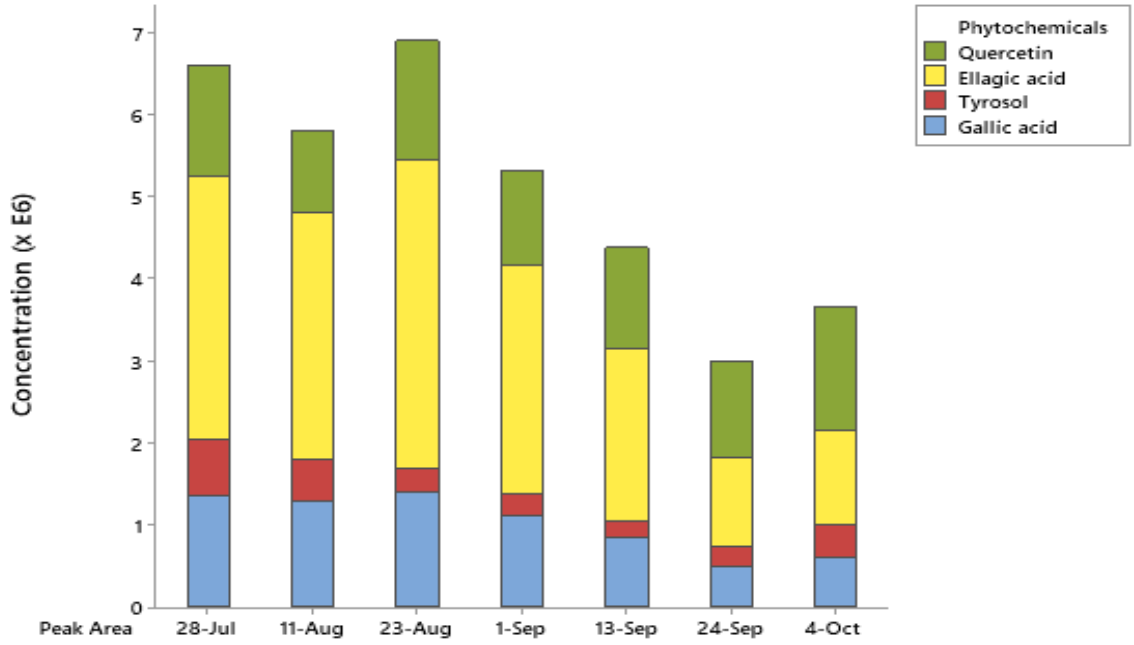


Figure 2.5. A stacked bar chart showing varying compositions of phenolics (10^6 Trolox Equivalent/100g samples) in red osier dogwood at different months of summer and autumn seasons. Adapted from Scale (2015)

Anthocyanin is an important group of flavonoids, a sub-class of phenolic compounds characterizing the bright coloration (other than green) in response to stress conditions in plants (Steyn et al., 2002; Hatier and Gould, 2008). The concentration of anthocyanins in the ROD plant was highest during winter but had a correspondingly lower ORAC (Isaak et al., 2013). The complementary findings reported by Scales (2015) and Isaak et al. (2013) showed that the ORAC value of ROD was higher when the leaves were dark green, which is obtainable in the summer compared to the red ROD leaves during the autumn and winter. During summer periods, there is high irradiance from the sun, which excites chlorophyll in plants, thus increasing the rate of biosynthesis of metabolites (Buttery and Buzzell, 1977). The increased rate of photosynthesis of primary metabolites could facilitate an increased synthesis of secondary metabolites; this might be explained by the higher antioxidative

capacity in green ROD leaves obtainable during summer compared to during winter and autumn. There is a higher possibility that blending a fixed proportion of ROD leaves at the summer (higher in ellagic acid, gallic acid, quercetin, etc.) and winter (higher in anthocyanins) could potentiate higher antioxidative properties of ROD than when singly used. Possible synergistic effects from a combination of polyphenols have been reported (Palafox-Carlos et al., 2012; Kopjar et al., 2016). The beneficial health effects of *Cornus* polyphenols have been extensively reported by Vareed (2005).

In vitro studies have demonstrated ROD's antioxidant capacity in the upregulation of hemeoxygenase-1, superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Yang et al., 2019). In addition, both Jiang et al., (2019) and Yang et al. (2019) reported that polyphenols in ROD extracts also prevented pro-inflammatory responses in Caco-2 cells by repressing gene expression of inflammatory cytokines. This was not surprising as ROD has shown to be a potent redox buffer due to its high ORAC values.

2.1.2.4 Potentials of ROD in monogastric animal production

2.1.2.4.1 Effects of ROD on the growth performance and gut morphology of monogastric animal

Determination of growth performance is one of the important parameters considered in assessing the efficacy of feed or feed additives in animal production. In recent times, the use of bioactive substances from plants has shown the potential to influence the growth and health of animals (Salobir et al., 2012). The effects of air-dried raw or extract of ROD reported in the literature is presented in Table 2.5. In studies using air-dried raw ROD, the ROD is usually a mixture of the plant parts, including 75% leaves and 25% bark (Koo et al., 2020), 75% leaves, 10% bark, and 15% stem (Gomaa et al., 2018; Wei et al. 2018,

2019) or 60% leaves and 40% bark (Amarakoon, 2017). It is not unexpected that animals would respond differently to the varying dietary supplements. This raises some knowledge-based questions as to what the growth response of animals fed different ROD compositions would be like.

The application of ROD in swine and poultry research is gaining momentum as their antibiotic-replacement potentials have been currently reported in some studies. Koo et al. (2018, 2020) and Jayaraman et al. (2018) reported that dietary supplementation of 4% air-dried raw ROD (containing 75% leaves and 25% bark) helped to maintain the growth performance of weaned piglets challenged with *E. coli* in the same capacity as those receiving 0.025% in-feed bacitracin methylated disalicylate (BMD). In a study demonstrated by Lee et al. (2018), dietary inclusion of 0.2% and 0.4% ROD extract were reported to reduce average daily gain in weaned piglets; however, specifically at the 0.4% ROD extract inclusion level, the body weight of the piglets was observed to marginally improve body weight compared to antibiotic and control fed piglets. In another study where 0.5% ROD extract was applied in matured pigs, growth performance and volatile fatty acids (VFA) were not affected; however, the average daily gain was numerically higher among pigs fed dietary 0.5% ROD extract compared to control (Zheng et al., 2021). Given the above, it could be assumed that the best inclusion levels of either air-dried raw ROD or ROD extract are yet to be attained. Similarly, in broiler chickens, Mogire et al. (2021) established that incorporation of ROD extract did not influence growth performance, feed conversion ratio, and relative organ weight in broiler chickens; however, it favourably matched up with the expectations when compared with the broiler chickens fed avilamycin antibiotic. Regarding ROD-antibiotic comparison, Mogire et al. (2021) reported that ROD

improved birds' livability by their mortality-reductive capacity. Furthermore, Erinle et al. (2022b) demonstrated that in broiler chickens challenged with *Salmonella* Enteritidis lipopolysaccharides (*SE*-LPS), feeding ROD extract at 0.3% and 0.5% maintained average feed intake, feed efficiency, and mortality compared to feeding bacitracin diet. This suggests the prospect that comes with the use of ROD, particularly in the pursuit of reducing antibiotic use in animal production. The inclusion of ROD into the diets of broiler chickens and rabbits have proven to reduce mortality (Scales, 2015; Mogire et al., 2021). The stepwise biochemical mechanism with which the phytogenic compounds in ROD influence growth performance has not been fully unravelled; however, the selective antimicrobial action of ROD polyphenols could be related to the reduced mortality.

On gut morphology, there are significant impacts of ROD extract on the architecture of the small intestinal section. In studies comparing the efficacy of ROD with antibiotics, Mogire et al. (2021) and Erinle et al. (2022b) reported that ROD improved villus height to crypt depth ratio (VH:CD) in the jejunal and ileal section of the GIT of broiler chickens fed diets supplemented with 0.1 and 0.3 % ROD extract. Meanwhile, in chickens challenged with *SE*-LPS, a deeper crypt depth was reported in chickens fed 0.3 and 0.5 % ROD extract, which was perceived to be ROD's ameliorative mechanism in improving the gut (Erinle et al., 2022b). In weaned piglets, higher inclusion levels of air-dried raw ROD at 2 - 4% were demonstrated to enhance VH:CD in the jejunal and ileal section of the GIT (Jayaraman et al., 2018; Koo et al., 2020). In the small intestine, high VH:CD indicates more surface area for nutrient digestibility and absorption (da Silva et al., 2009). It could be perceived that ROD polyphenols do not impede nutrient digestion and absorption in monogastric animals. According to Mogire et al. (2021), supplementation of ROD extract increased ileal

digestibility of amino acids (AA) and crude fat in broiler chickens, while crude fat, protein, and dry matter digestibilities were not affected. Contrary to the existing belief that polyphenols may negatively impact nutrients, the polyphenol profiles of ROD extract were shown to upregulate AA transporter genes, including B^0AT1 and CAT1 (Mogire et al., 2021). Although there are only a few existing studies that have applied ROD extract in broiler chickens and swine, the promising research outcome with ROD begs for more studies to reinforce the existing beneficial effects of ROD and determine the optimum inclusion levels, which are yet to be reported in swine and poultry research.

2.1.2.4.2 Effects of ROD on oxidative and immune-related stress in monogastric animal

Oxidation is a common metabolic chemical process involved in the release of energy in living systems; however, it may also produce free radicals which are unstable, capable of independent existence and highly reactive due to the presence of their unpaired electrons. Drawing on the bodily-produced antioxidants causes their downregulation, thus, distorting the oxidative and immune status of animals.

In swine farming, piglets are reported to be highly susceptible to oxidative stress at weaning age, especially when infected with *E. coli* (Amarakoon, 2017). This is ascribable to the piglets' immature immune system during their early life. Immaturity of the immune system in newborns and infants has been related to their increased susceptibility to virulent pathogens (Russell et al., 2012). In most monogastric animals, particularly pigs, the weaning phase has been dubbed a critical and stress-inciting phase due to the increased susceptibility to pathogenic microbes and endotoxins and gastrointestinal disorders (Pié et al., 2007) in the young ones. In *E. coli*-challenged weaned piglets, Koo et al. (2020) and

Amarakoon (2017) demonstrated that a 4% inclusion level of ROD in weaners' diet did not only depress levels of thiobarbituric acid reactive substances (TBARS) and malondialdehyde MDA but also caused upregulation of SOD when compared to both unchallenged and antibiotic-treated birds thereby neutralizing the oxidative stress induced by the bacteria. The antioxidant potential of polyphenol-rich plant materials cannot be overemphasized. Polyphenols of GP, apple, leaves of green teas and olive were reported to increase the levels of plasma GPx and total antioxidant capacity in weaned piglets (Jiang et al., 2014). It is incredible that ROD polyphenols do not only directly improve body antioxidant enzymes but also have been reported to increase blood parameters, including white blood cells, lymphocytes, monocytes, and granulocytes which is an indication of improved lymphocyte function and cell-mediated immunity following the attainment of active immunization in beef heifers (Wei et al., 2019). In broiler chickens, the body's antioxidant enzyme system and immune organs do not seem to have benefited from the antioxidant benevolence of ROD extract. Erinle et al. (2022b) reported that supplementation of 0.3% and 0.5% ROD extract did not improve serum SOD and total antioxidant power in broiler chickens challenged or unchallenged with *SE*-LPS. Both Mogire et al. (2021) and Erinle et al. (2022b) reported that the incorporation of 0.1 – 0.5 % dietary ROD extract did not have an effect on the weights of immune-related organs, particularly the liver and spleen.

2.1.2.4.3 Effect of ROD on gut microbiota of monogastric animal

Microbial community in the gut of animals, including bacteria, fungi, archaea, protozoans and viruses, impact nutrient utilization (Stanley et al., 2012; Park et al., 2017), immune system (Schokker et al., 2017; Lazar et al., 2018; Akinyemi et al., 2020), hormonal action

(Zhenping et al., 2013), maturation of the gastrointestinal tract (Kelly and Conway, 2005) and general well-being of the animal. Bacteria species are the most reckoned microbe in the GIT, with approximately 10^{11} – 10^{12}/g organism of bacteria species colonizing the caecum compared to the 10^{8} – 10^{9}/ g organisms in the ileum of chickens (Witzig et al., 2015; Thomas et al., 2019). The five most abundant bacteria phyla reported in the literature are the Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Spirochaetes (Thomas et al., 2019; Akinyemi et al., 2020; Mogire et al., 2021).

In broiler chickens, dietary supplementation of 0.1% and 0.3% ROD extract were reported to have no influence on the community diversity of both ileal and cecal microbiota; however, an increase in the population of Firmicutes and a decrease in Bacteroidetes phyla were observed (Mogire, 2020). This suggests an increased Firmicutes to Bacteroidetes ratio (FBR). Increased FBR has been positively correlated with increased body weight and energy utilization efficiency in broiler chickens (Singh et al., 2008). On the contrary, Erinle et al. (2022b) reported that Firmicutes, Actinobacteria, and Proteobacteria were the only phyla found in the cecal microbiota of broiler chickens fed 0.3% and 0.5% ROD extract; however, the relative abundance of genera *Lactobacillus* and *Streptococcus* were greater than what was obtained among the antibiotic-fed birds. In matured pigs, dietary supplementation of 0.5% ROD polyphenol extract was reported to increase alpha diversity of the ileal microbiota and commensal bacteria counts, which are mostly Firmicutes and Proteobacteria phyla (Zheng et al., 2021). Remarkably, ROD polyphenol exhibited prebiotic effects on the gut microbiota by tremendously increasing the microbial population of *Lactobacilli*, particularly *Lactobacillus delbrueckii* and *Lactobacillus mucosae*, *Sharpea* and *Dialister*, and *Lachnospiraceae _bacterium_DJF_LS97k1* (Zheng et al., 2021). Many

strains of *Lactobacillus species* are gut-friendly and have been shown to be capable of maintaining intestinal barrier function, especially in a diseased condition, by modulating the expression of heat shock protein or tight junction proteins or by restricting adhesion of pathogens (Yu et al., 2012; Liu et al., 2015a; Sun et al., 2015; Zheng et al., 2021). Although there is no sufficient research evidence on the selective antimicrobial action of air-dried raw or extract of ROD on the dynamics of gut microbiota, their capacity to consistently improve the population of gut-friendly *Lactobacillus* is noteworthy.

Table 2.5. Summary of the potential application of ROD.

Research topics	Application rates	Findings	References
IN VITRO			
Red dogwood extract (RDE) prevents inflammatory responses in Caco-2 Cells and Caco-2 BBe1/EA.hy926 Cell co-culture	100 µg/mL	i. ↑ Rate of polyphenols absorption. ii. Prevention of IL-8 production. iii. ↓ Gene expression of IL-8, TNF-α, IL-6, ICAM-1, VCAM 1, and COX-2 in TNF-α inflamed Caco-2 cell.	Jiang et al. (2019)
Effect of Manitoba-grown RDE on recovering Caco-2 cells from H_2O_2-induced oxidative damage.	100 µg/mL	i. ↑ Cell viability. ii. Prevention of IL-8 and ROS production. iii. ↑ Gene expression of HO-1, SOD, GSH-Px, and Nrf-2. iv. ↓ Diffusion of fluorescein. v. ↑ Transepithelial resistance. vi. ↑ Relative mRNA concentration of tight junction claudin-1, claudin-3, and occludin. vii. Repaired structural integrity of ZO-1. viii. ↑ Protein expression of ZO-1 and claudin-3.	Yang et al. (2019)

Research topics	Application rates	Findings	References
Inclusion of red osier dogwood (ROD) in high-forage and high-grain diets affected *in vitro* fermentation.	0, 3, 6, or 12 %	i. ↑ ROD levels ↓ total VFA concentration. ii. ↑ Acetate to propionate ratio. iii. ↓ DMD and fermentation pattern at lower pH. iv. ↓ Rumen acidosis caused by high grain diets.	Wei et al. (2018)
Effect of dried distillers grains with solubles and RDE on fermentation pattern and microbial profiles of a high-grain diet in an artificial rumen system.	0 and 1 %	i. ↑ Acetate to propionate ratio. ii. ↓ Starch disappearance and possible reduce rumen acidosis. iii. Little variation in Shannon diversity index. iv. ↑ Rumen *Treponema*.	Gomaa et al. (2021)
***IN VIVO* (Swine)**			
Dietary supplementation of ROD polyphenol extract changes the ileal microbiota structure and increases *Lactobacillus* in a pig model.	0 and 0.5 %	i. ↑ *Lactobacillus delbrueckii* and *Lactobacillus mucus* suggesting a prebiotic-effect of ROD polyphenol extract.	Zheng et al. (2021)
Effects of dietary ROD on growth	2 and 4 %	i. No effect on growth performance.	Koo et al. (2020)

Research topics	Application rates	Findings	References
performance, blood profile, ileal morphology, and oxidative status in weaned pigs challenged with *E. coli* $K88^+$.		ii. ↑ SOD and ↓ MDA concentrations in the serum in equal capacity of an antibiotics. iii. ↓ Ileal crypt depth and ↑ ileal VH:CD.	
Effects of dietary supplementation with ground ROD on oxidative status in weaned pigs challenged with *E. coli* $K88^+$.	2 and 4 %	i. ↓ Serum and ileal MDA with 4% ROD supplementation which is comparable to antibiotics. ii. Both 4% ROD and antibiotics ↓ serum and ileal SOD. iii. Serum and ileal MDA and SOD were not affected by 2% ROD.	Koo et al. (2018)
PSIV-30 Effects of dietary supplementation with ground ROD on growth performance, blood profile, and ileal histomorphology in weaned pigs challenged with *E. coli* $K88^+$.	2 and 4 %	i. No effect on growth performance. ii. No effect on PUN and plasma glucose. iii. ↓ Ileal crypt depth and marginally ↑ ileal VH:CD.	Jayaraman et al. (2018)
PSI-31 Effects of ROD extract on	0.2 and 0.4 %	i. ↓ BW in 0.2% fed weaned piglets compared to those fed antibiotics diet.	Lee et al. (2018)

Research topics	Application rates	Findings	References
nutrient digestibility and growth performance of weaned piglets.		ii. ↓ ADG in ROD treatments; however, the ADG was similar to weaned piglets fed basal diet. iii. No effect on fecal score, apparent total tract digestibility of dry matter and energy.	
Regulation of oxidative stress in weaned piglets challenged with *E. coli*.	2 and 4 %	i. No effect on BW. ii. ↓TBARS among weaned pigs fed 4% ROD and antibiotics similar to the unchallenged pigs. iii. ↑ SOD activity in the serum and ileum.	Amarakoon (2017)
***IN VIVO* (Poultry)**			
Effects of ROD extracts on growth performance, intestinal digestive and absorptive functions, and meat quality of broiler chickens	0.1 and 0.3 %	i. No effect on growth performance. ii. ↑ Birds livability. iii. ↓ Jejunal crypt depth among chickens fed 0.1% and ↑ VH:CD in both 0.1% and 0.3% ROD treatments. iv. ↓ mRNA abundance for cationic amino acid transporter in ROD and antibiotic treatments.	Mogire et al. (2021)

Research topics	Application rates	Findings	References
		v. ↑ mRNA abundance for neutral amino acid transporter. vi. 4% ROD ↑ apparent ileal digestibility of crude fat. vii. All the ROD and PC treatments ↑ amino acid digestibility.	
Effect of ROD extract on growth performance, blood biochemical parameters, and gut functionality of broiler chickens challenged or unchallenged with *Salmonella Enteritidis* lipopolysaccharides.	0.3 and 0.5%	i. Both levels of ROD extract marginally improved AWG of broiler chickens compared to antibiotic-treated birds. ii. No effect on cecal SCFA, relative immune organs, and serum antioxidants. iii. ↑ Relative abundance of cecal *Lactobacillus* and *Streptococcus* genera. iv. ↑ crypt depth and VH:CD in both unchallenged and challenged group of birds. v. ↑ Plasma GLB and ↓ A:GLB among birds receiving 0.3% ROD extract.	Erinle et al. (2022b)

2.1.2.5 Improving the efficiency of ROD for use in poultry nutrition

Some plant extracts have been reported to have no effect on the growth performance and health of an animal, particularly poultry species (Hernández et al., 2004; Aydin et al., 2008; Al-Kassie et al., 2011), with an implication on their polyphenol content. The impact of polyphenols on digestive enzymes is controversial in the literature, with many studies suggesting polyphenols reduce enzymatic activities. Considerable research shreds of evidence have also been presented that polyphenol derived from some plants, including green tea and chameleon plants, increased pepsin activity (Tagliazucchi et al., 2005; Garg et al., 2019). Ironically, the enzyme-inhibition tendency of ROD polyphenols could be of interest in ruminant production, given its capacity to improve protein efficiency and reduce rumen acidosis. In fact, ROD extract polyphenols were shown to improve digestibility and absorption of AA and crude fat, while other nutrient digestibilities remain unaffected in chickens (Mogire et al., 2021). Therefore, enhancing ROD for animal use should be targeted toward further enrichment of their polyphenol profile rather than the polyphenol-enzyme inhibition concerns.

Based on the available reports, ROD used in most current studies is made up of a varying proportion of plant parts, including 75% leaves, 10% bark, and 15% stem (Gomaa et al., 2018; Wei et al., 2018, 2019), 60% leaves and 40% bark (Amarakoon, 2017) or 75% leaves and 25% bark (Koo et al., 2020) obtained from immature ROD plant. Improving the use of ROD for animal use could be through the inclusion of their flower/fruit/seed in the preparation protocol prior to its incorporation in the animal feed. Biosynthesis of plant polyphenols is a systematic and dynamic process that is dependent on the plant, plant parts, and seasonal variation. According to Feduraev et al. (2019), there is a significant spike in the

phenolic compounds and antioxidant activity in flowers/seeds compared to the roots, stems, and leaves. In fact, the authors reported a decreasing antioxidant activity in the following order: flowers/seed > leaves > root > stem. Although, this ordering pattern is not static and would greatly depend on the specific storage organ of the resident plant. In little bur-clover, leaf and seed extracts were reported to accumulate more phenolic and flavonoid compounds; thus, the two structures contain the highest TPC compared to the root and stem (Kabtni et al., 2020). Vareed et al. (2005) highlighted that the flower/fruit of most *Cornus* species harbours arrays of phytochemicals compared to their leaf, stem, and root. Given the above information, we suspect that TPC could be higher in ROD flower/fruits/seeds compared to other plant parts. Thus, the inclusion of ROD flower/fruit/seed in either raw or extract of ROD would afford a broader spectrum of polyphenol profiles and possibly a more comprehensive range of beneficial bioactivities. However, future *in vitro* and *in vivo* studies would be needed to confirm this speculation.

CHAPTER 3: Dietary grape pomace – Effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens

This work has been published elsewhere:

Erinle TJ, Oladokun, S, MacIsaac J, Rathgeber B, Adewole D. 2022. Dietary grape pomace – Effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens. Poult. Sci.; 101519.
https://doi.org/10.1016/j.psj.2021.101519

The work has also been presented at:

2021 PSA Annual Meeting - Poultry Science Association

3. Abstract

The search for alternatives to antibiotics in poultry production is still ongoing and has been directed towards the 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, cecal microbiota, cecal 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 three dietary treatments – Negative control (**NC**, a corn-wheat soybean-based diet), NC + 0.05% bacitracin methylene disalicylate (**BMD**), and NC + 2.5% grape pomace (**GP**). Each treatment was assigned to eight replicate pens with 25 birds per pen. Body weight (**BW**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined weekly. On d 36, two chickens/pen were euthanized for measuring blood

biochemical parameters, cecal SCFA, and cecal 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 comparedto other treatments. The level of cecal 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.

Keywords: grape pomace, broiler chickens, growth performance, gut morphology, cecal microbiota.

3.1 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 has ushered strict restrictions placed on the use of antibiotics as growth

promoters in livestock production in 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 useof 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 apotent 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 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 - 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, insoluble dietary fibre, 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 the profit margin for both chicken farmers and wineries. Although previous studies have investigated the effect of

GP on the 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; Ebrahimzadeh et al., 2018; Aditya 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 5-10% has been reported to show no significant improvement in the 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 a 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 in 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, nostudy has investigated the effect of dietary GP on the incidence of WS and WB and cecal 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.

3.2 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).

3.2.1 Experimental birds and management

A total of 576 one-day-old mixed-sex Cobb-500 broiler chicks were obtained from AtlanticPoultry Incorporated, Port Williams, Nova Scotia, and were raised on floor pens. Roomtemperature was monitored daily and was gradually reduced from 30 to 22.6 ^{0}C from d 0 to 42.The lighting program was set to produce 18 hours of light and 6 hours of darkness throughout theexperimental period, and illumination was gradually reduced from 20 1x on d 0 to 5 1x on d 39.

3.2.2 Diets and experimental design

The GP used in this study was obtained from Gaspereau Vineyards, Nova Scotia. The productwas freeze-dried using a Supermodulyo freeze-dryer (Model:220 Thermo Savant;

Holbrook, New York, USA) and ground using a coffee grinder. The birds were randomly allotted to three treatments groups containing eight 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 tomeet the nutrient requirements of broiler chickens as recommended by NRC (1994), and birds were fed on a phase-feeding program as follows: starter (1 to 14 d of age), grower (14 to 24 d of age) and finisher (24 to 42 d of age). The ingredient and nutrient compositions of the diets for the three 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 (1990) 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.

Table 3.1. Gross and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated)[1]

Ingredients	Starter phase (1-14 days)			Grower phase (14-28 days)			Finisher phase (28-42 days)		
	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[v]	--	--	2.50	--	--	2.50	--	--	2.50
BMD 110 G [W]	--	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 [X]	0.61	0.61	0.63	0.53	0.53	0.55	0.49	0.49	0.51
Starter Vitamin/mineral premix[Y]	0.50	0.50	0.50	--	--	--	--	--	--
Grower/Finisher Vitamin/mineral premix[Z]	--	--	--	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

Ingredients	Starter phase (1-14 days)			Grower phase (14-28 days)			Finisher phase (28-42 days)		
	NC	BMD	GP	NC	BMD	GP	NC	BMD	GP
Crude Fat	4.50	5.03	6.53	7.22	6.98	7.42	5.23	4.74	6.90

[1]NC, Negative control diet, BMD, antibiotic diet, GP, diet containing 2.5% grape pomace.

[v] 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; 12.40ppm copper; 11.73ppm zinc.

[W] Bacitracin methylene disalicylate (providing 55 mg/kg mixed feed); Alpharma, Inc., Fort Lee, NJ, US.

[X] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[Y]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. [z] 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.

3.2.3 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, Nova Scotia Department of Agriculture for post- mortem.

3.2.4 Blood biochemistry analysis

On d 36, two birds were randomly selected from each pen, individually weighed, and euthanized by electrical stunning and exsanguination. Blood samples were collected from each bird into 5mL heparinized tubes and were centrifuged at 5000 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 manufacturerinstructions.

3.2.5 Short-chain fatty acid concentrations and total eubacteria count

Digesta from the pair of ceca were mixed and divided into two sub-samples; 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 byAlimetrics Diagnostics Ltd.

3.2.6 Gut morphology

One centimeter of the duodenal, jejunal, and ileal midpoints was removed from each euthanized bird, and preserved in formalin for three days. 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, i.e., 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.

3.2.7 Gut microbiota

The second portion of the mixed cecal digesta was stored in plastic RNAse and DNAse-freetubes, 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 2020). Bioinformatics analyses were conducted in the R statistical environment.

3.2.8 Breast muscle myopathy

Breast muscle samples were collected on four birds (two males and two 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 by Kuttappan et al. (2012).

3.2.9 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. The parametric dataset was analyzed by one-way ANOVA, while non-parametric 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 $P<0.05$.

3.3 Results

3.3.1 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 3.1. The TPC of diets supplemented with 2.5% GP at the starter, grower, and finisher diets were 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 3.2. Epicatechin, catechin, and gallic acid were observed to be the most abundantpolyphenols in whole GP.

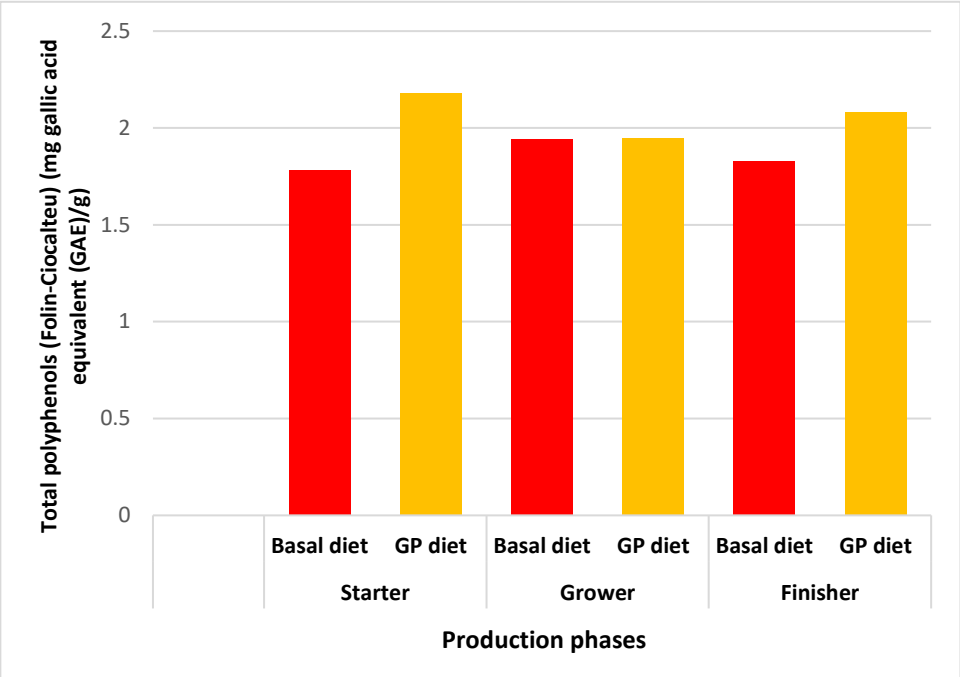


Figure 3.1. Total polyphenols content (mg gallic acid equivalent GAE/g) in treatments offered to broiler chicken according to production phases. NC, BMD, and GP diets per production phase. NC = Negative control diet (corn-wheat-soybean meal diet), NC + 0.05% in-feed bacitracin methylene disalicylate diet = BMD and GP = diet containing 2.5% grape pomace.

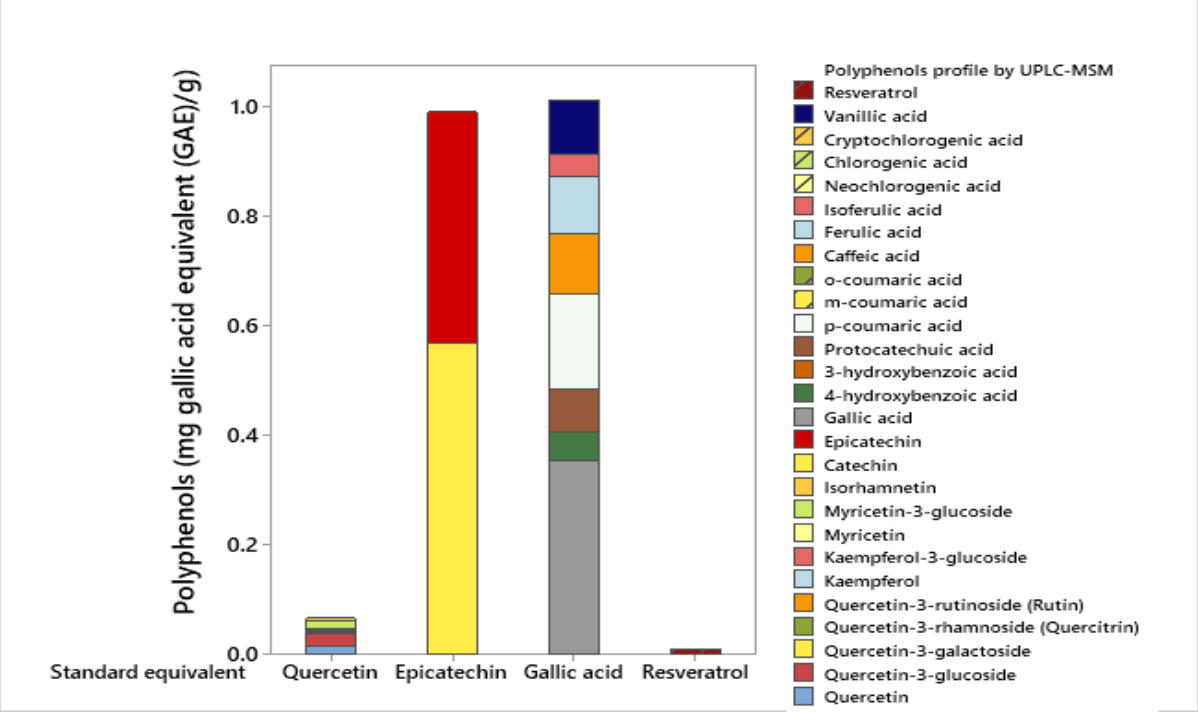


Figure 3.2. Polyphenols profile of whole grape pomace by UPLC-MSMS (mg standard equivalent/g)

3.3.2 Growth Performance

The growth performance of broiler chickens fed GP as an in-feed antibiotic replacement is presented in Table 3.2. 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, the average AWG was significantly higher (P<0.05) in birds fed the antibiotic diet.

Table 3.2. 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[2]	*P*-value
		NC	BMD	GP		
Starter (d 1 - 14)	Average feed intake (g/bird)	942[b]	1017[a]	1008[a]	10.5	0.002
	Average weight gain (g/bird)	296[b]	342[a]	333[a]	5.72	<0.001
	FCR	1.58	1.48	1.5	0.02	0.116
Grower (d 14 - 28)	Average feed intake (g/bird)	2,811[b]	3,086[a]	2,930[ab]	38.6	0.004
	Average weight gain (g/bird)	907[b]	1028[a]	894[b]	15.3	<0.001
	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]	40.8	0.012
	Average weight gain (g/bird)	1,422	1,455	1,418	12.4	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]	45.7	<0.001
	Average weight gain (g/bird)	2,629[b]	2,828[a]	2,648[b]	25.8	<0.001
	FCR	1.72	1.65	1.69	0.02	0.358

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

[2]SEM = standard error of the mean.

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

3.3.3 Gut morphology

The effects of the dietary GP on the morphology of intestinal segments of broiler chickens is presented in Table 3.3. 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 highest (P<0.05) in bird fedGP 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.

Table 3.3. Effect of dietary grape pomace on intestinal morphology of broiler chickens

Parameters (mm)	Treatment effect[1]			SEM[2]	*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[3]	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]NC, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, GP, diet containing 2.5% grape pomace.

[2]SEM = standard error of the mean.

[3]VH:CD = Villus height:crypt depth ratio.

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

3.3.4 Plasma biochemistry and serum immunoglobulins

The effect of dietary GP supplementation on blood biochemical indices of broiler chickens is shown in Table 3.4. 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 3.4. Effect of dietary grape pomace on blood biochemistry and immunoglobulin profiles of broiler chickens

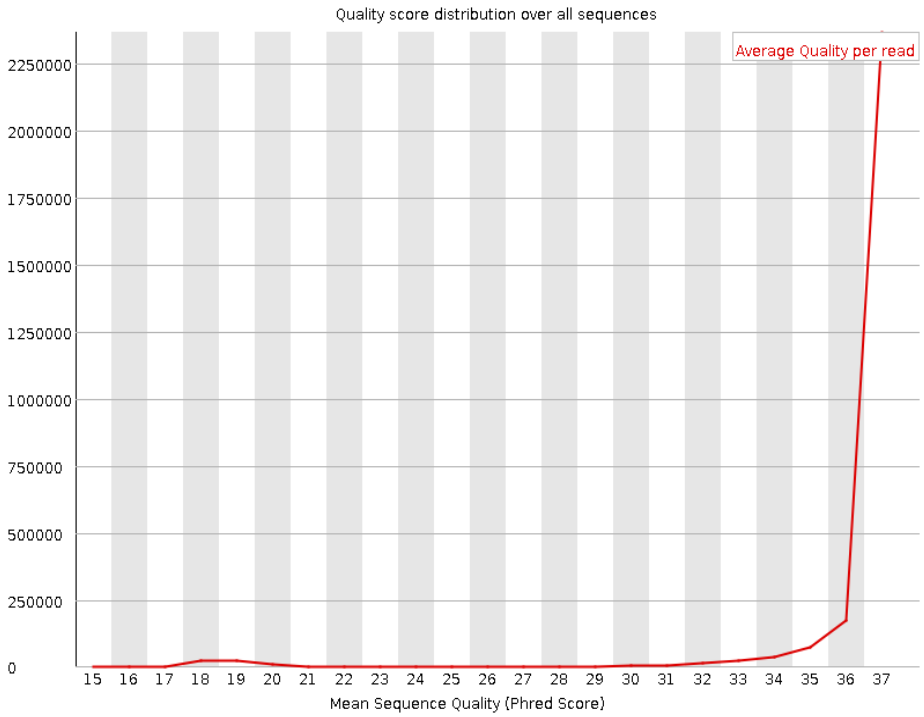
Parameters	Treatment effect[1]				
	NC	BMD	GP	SEM[2]	*P*-value
Sodium (mmol/L)	150	150	151	0.90	0.797
Potassium (mmol/L)	4.78	4.95	4.54	0.19	0.443
Na:K ratio[3]	31.3	29.4	33.4	0.98	0.178
Chloride (mmol/L)	111	110	111	0.75	0.929
Calcium (mmol/L)	2.66^{ab}	2.48^{b}	2.76^{a}	0.04	0.007
Phosphorus (mmol/L)	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)	13.9	13.7	14.6	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)[4]	3,063^{a}	1,866^{b}	3,222^{a}	222	0.001
ALT (U/L)[5]	4.64	4.30	4.16	0.32	0.690
AST (U/L)[6]	252^{ab}	305^{a}	230^{b}	31.5	0.010
CK (U/L)[7]	16,304	21,057	10,890	4,733	0.095
GGT (U/L)[8]	13.4	11.6	14.6	0.71	0.198
Lipase (U/L)	25.6	25.1	23.7	1.29	0.825
T.Protein (g/L)[9]	27.9	27.0	29.2	0.47	0.122
Albumin (g/L)	10.6	10.8	11.1	0.16	0.368
Globulin (g/L)	17.2	16.1	17.8	0.36	0.116
A:G[10]	0.67	0.69	0.61	0.02	0.075
Iron (umol/L)	16.7	16.5	16.9	0.44	0.934
Uric Acid (umol/L)	333	285	314	11.0	0.213
Bile Acids (umol/L)	14.4	13.6	12.9	0.56	0.576
Creatinine (umol/L)	0.00	0.00	0.00	0.07	0.319
T.Bilirubin (umol/L)[11]	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]NC, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, GP, diet containing 2.5% grape pomace.

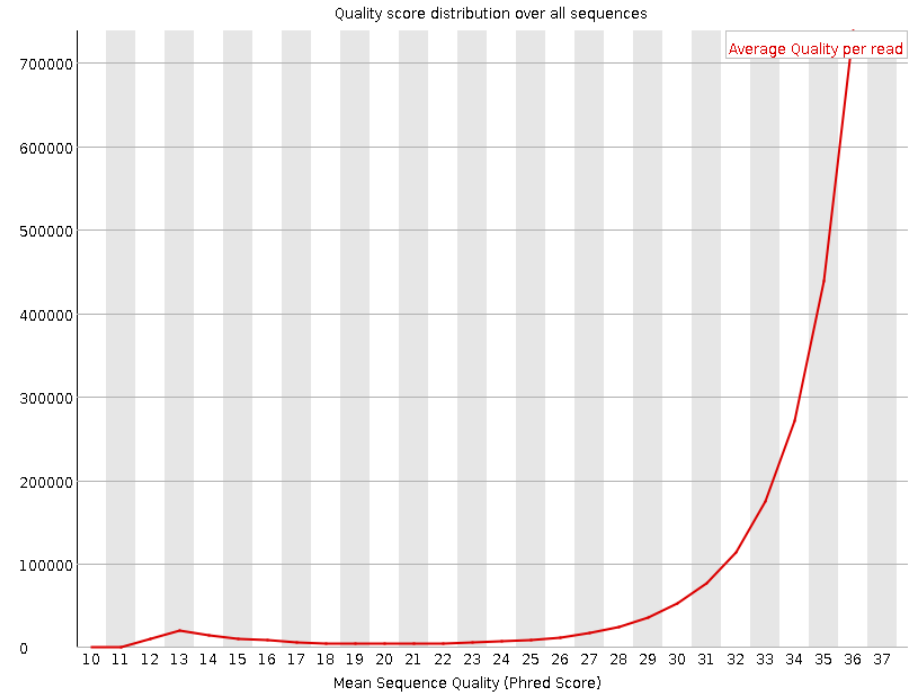
[2]SEM = standard error of the mean. [3]Na:K = Sodium:Potassium ratio. [4]ALP = Alkaline Phosphatas. [5]ALT = Alanine aminotransferase. [6]AST = Aspartate aminotransferase. [7]CK = Creatine kinase. [8]GGT = Gamma-glutamyl transferase. [9]T. Protein = Total Protein. [10]A:G = Albumin Globulin ratio. [11]T.Bilirubin = Total Bilirubin. In a row, means assigned different lowercase letters are significantly different, $P < 0.05$ (Tukey's procedure).

3.3.5 Cecal microbiota

A total of 6,169 OTUs 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 Figure 3.3. There was an effect of dietary treatment on the totalnumber of quality filtered read counts, as illustrated in Figure 3.4. Aggregation of OTU into each taxonomic rank and the relative abundance of the most abundant phyla, genera (classified and unclassified) are presented in Figures 3.5, 3.6, 3.7, and 3.8. 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 knowns as Bacteroidetes). Compared to the antibiotic treatment, the percentage relative abundance of most abundant bacterial genera was significantly higher (P<0.05) in the GP and NC treatments as shown in Figure 3.6. 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 *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 3.9). Permutational analysisof 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 3.10.



(A)



(B)

Figure 3.3. Quality score distribution over all sequences. Per-sequence averaged raw Q30 (Phred32) scores of; A) forward sequencing read and B) reverse sequencing read.

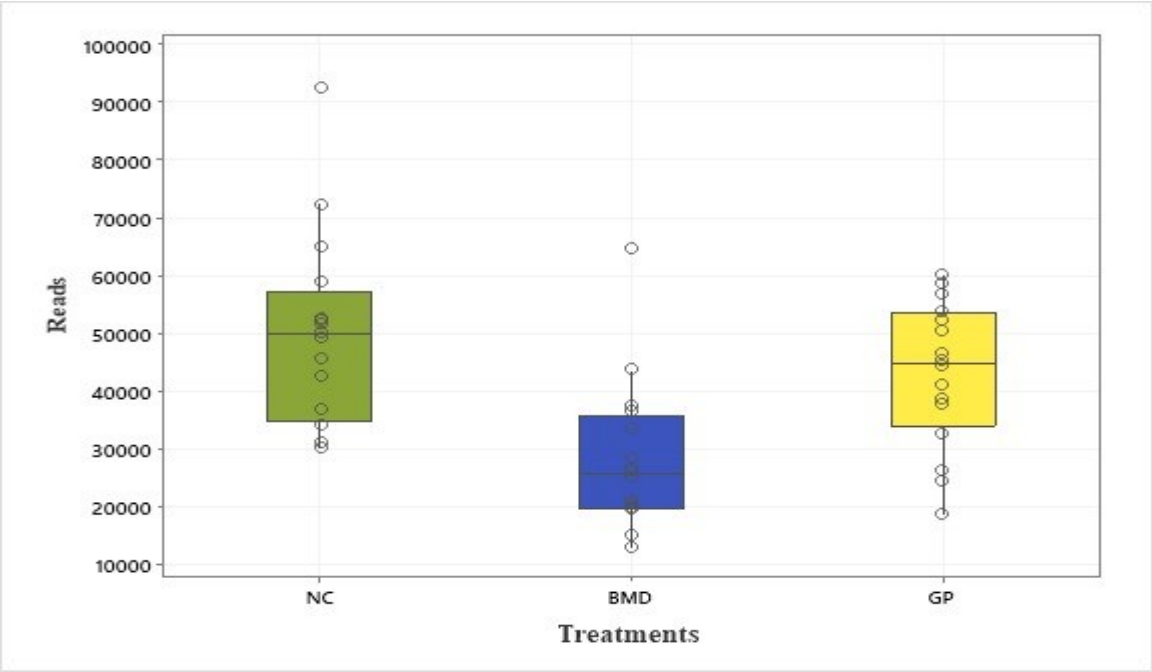


Figure 3.4. Box-and-whisker plot showing the total number of quality filtered read counts per treatment. Treatments included- Negative control diet (NC), BMD (bacitracin methylene disalicylate) antibiotic diet, and GP, diet containing 2.5% grape pomace.

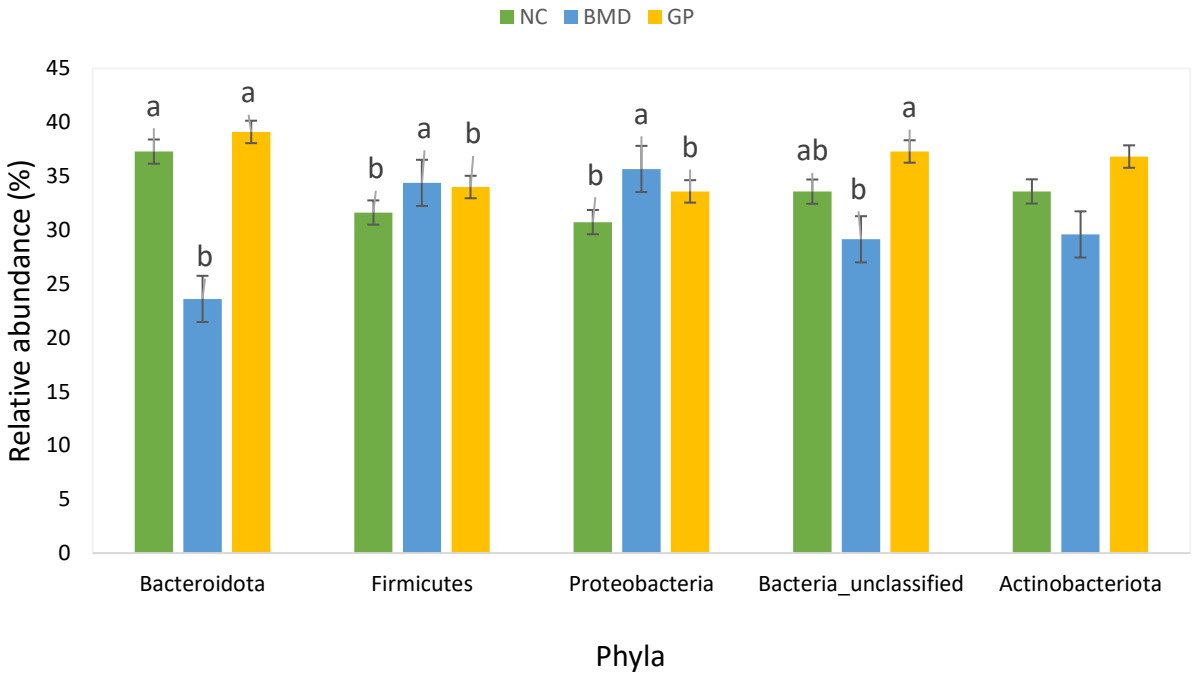


Figure 3.5. Percentage relative abundance of the most abundance bacteria Phyla in the ceca of broiler chickens fed grape pomace as substitute to in-feed antibiotics. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

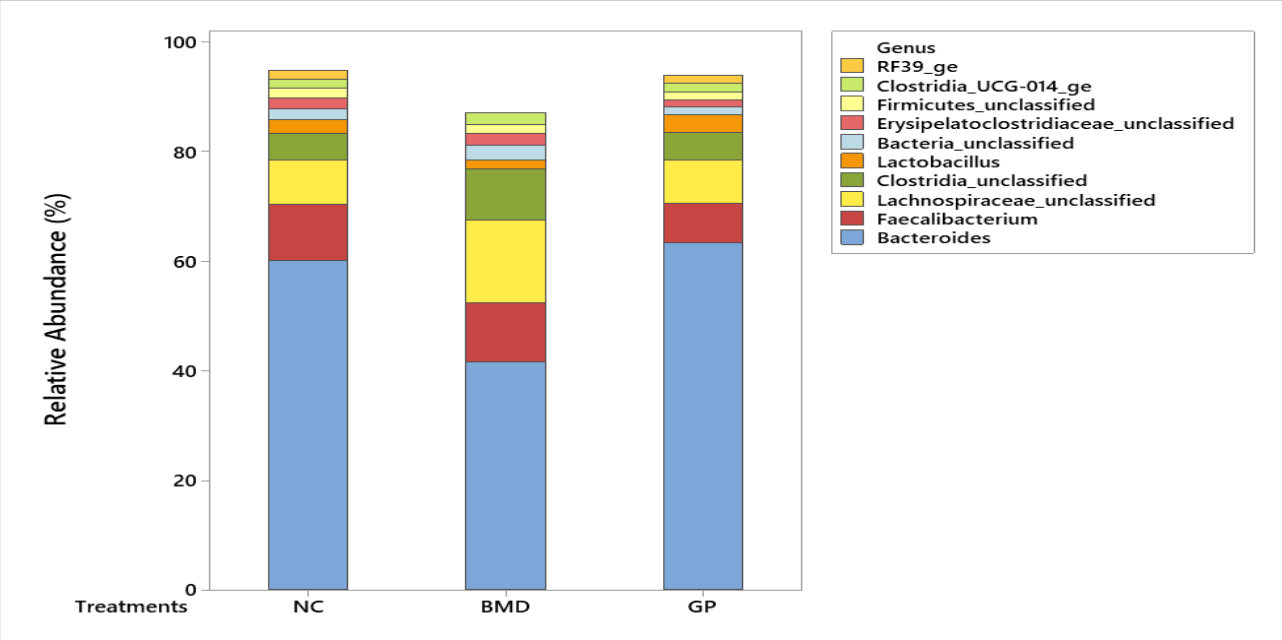


Figure 3.6. Percentage relative abundance of the most abundant bacteria genera in the ceca samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

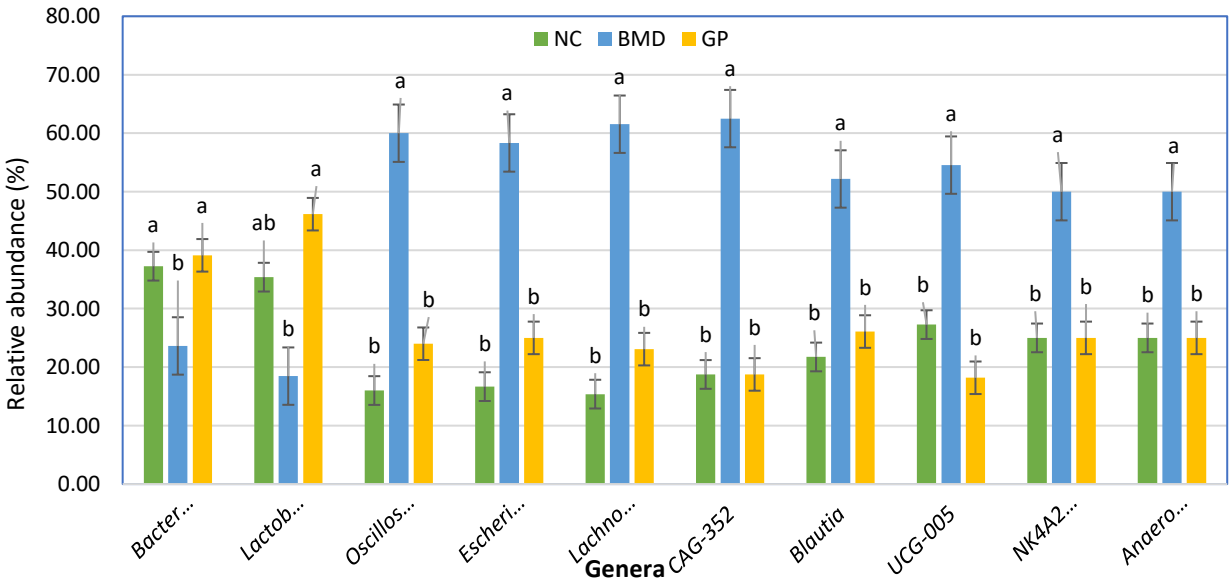


Figure 3.7. Percentage relative abundance of the most abundant classified genera of bacteria in the ceca samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

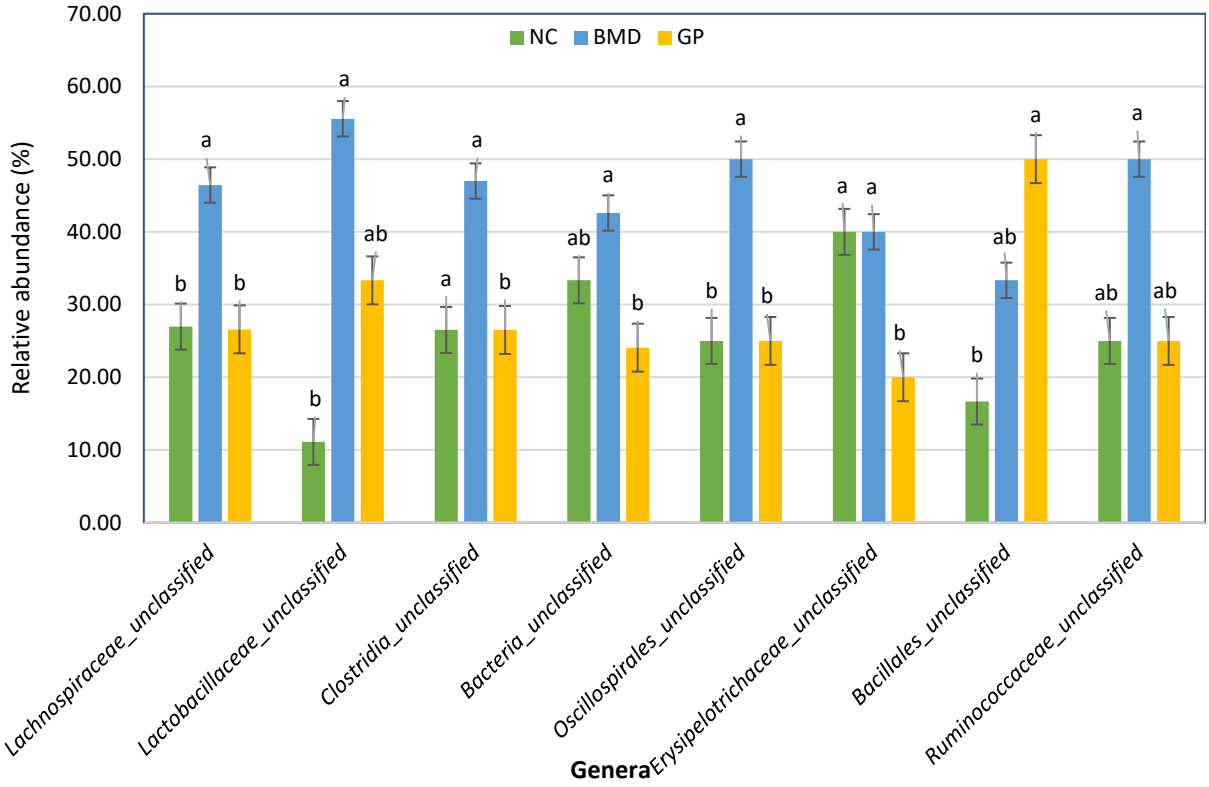


Figure 3.8. Percentage relative abundance of the most abundant unclassified genera of bacteria in the cecal samples obtained from broiler chickens fed 3 different treatments. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

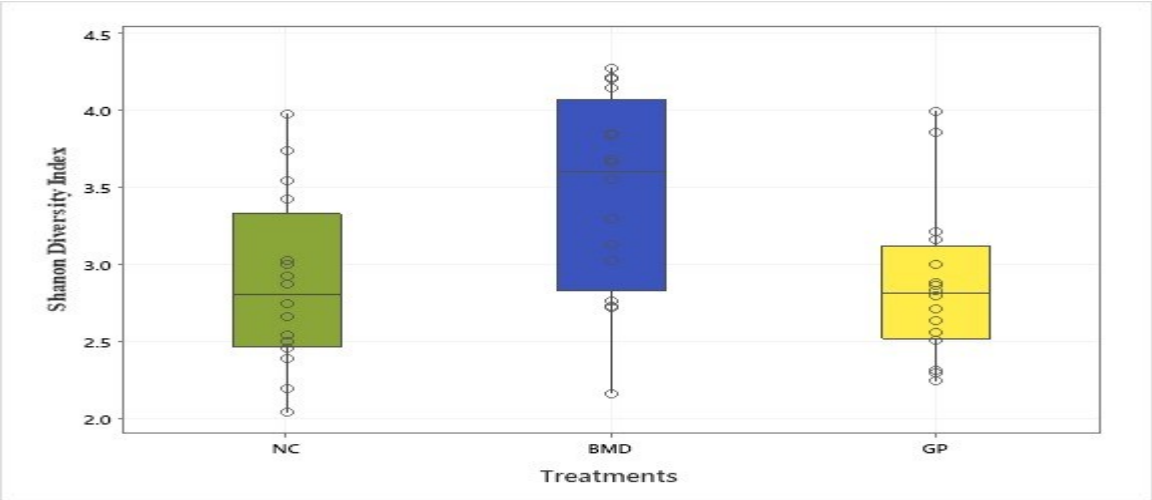


Figure 3.9. Box-and-whisker plot showing significant differences in the Shannon diversity index (Alpha diversity) (P > 0.05; F Value- 0.723). Ceca content was collected from 36-day-old broiler chickens offered three different dietary treatments. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

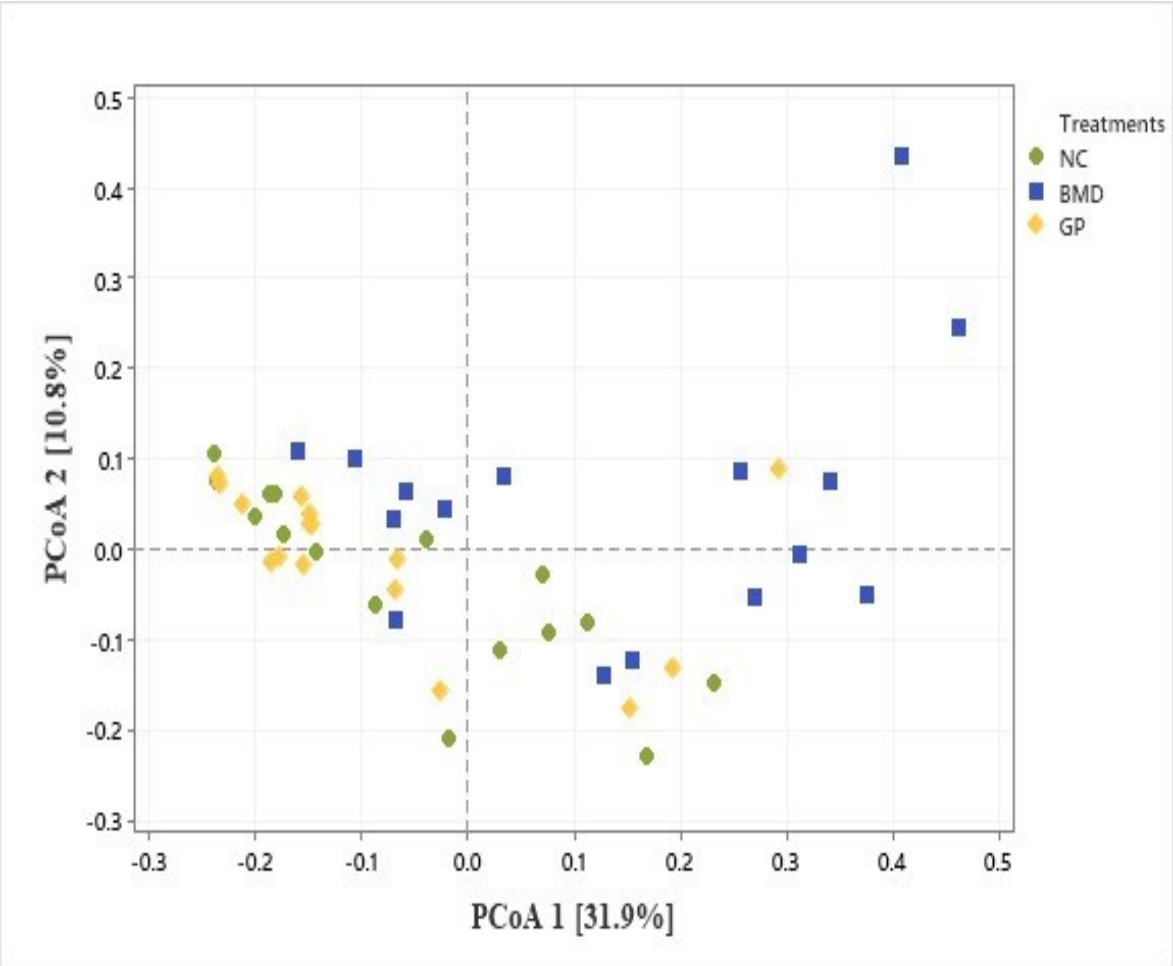


Figure 3.10. Multivariance analysis determined significant differences ($P < 0.05$) in beta-diversity among treatments. Treatment groups: NC = Negative control diet, BMD = bacitracin methylene disalicylate (antibiotic diet), and GP = diet containing 2.5% grape pomace.

3.3.6 Cecal 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 3.5. Compared to antibiotic and control diets,supplementation of 2.5% GP did not have a significant effect on the total eubacteria count, SCFA, AA, PA, BA, VA, LA, BCFA, and VFA.

Table 3.5. 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]**				
	NC	**BMD**	**GP**	**SEM[2]**	***P*-value**
Total eubacteria x 10^{12} (16S rDNA copies/g)	2.58	2.26	2.33	0.49	0.819
Short chain fatty acids (mM)	72.5	78.8	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]NC, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, GP, diet containing 2.5% grape pomace.

[2]SEM = standard error of the mean.

3.3.7 Breast muscle myopathy

White striping and WB scores of broiler chickens fed dietary GP are presented in Table 3.6. 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 a few birds having WB incidence.

Table 3.6. 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		*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	25.0 (8)	21.9 (7)	34.4 (11)					
	Moderate	40.6 (13)	37.5 (12)	40.6 (13)					
	Severe	34.4 (11)	37.5 (12)	25.0 (8)					
	Extreme	0.00 (0)	3.13 (1)	0.00 (0)					
Wooden Breast Score		0.00	0.00	0.00	0.00	0.00	1.000	0.002	
WB % (n)	Normal	84.4 (27)	84.4 (27)	84.4 (27)					
	Wooden breast	15.6 (5)	15.6 (5)	15.6 (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 (%)		17.6	18.5	17.6	17.3[b]	18.6[a]	0.426	0.032	0.101

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

(n) = Number of birds based on the severity of white striping or woody breast. $P < 0.05$ (Tukey's procedure).

3.4 Discussion

Grape pomace contains bioactive substances which have been recognized to possess antioxidative and antimicrobial properties. In this regard, GP has been sought 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 33.92mg 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 three 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 in the 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 non-significant improvement in the body weight of broiler chickens, which increases as GP inclusion level increases from 1 to 2%. Without overemphasis, there are bewildering evidence that antibiotics improve the 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 two weeks 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 2weeks 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 two weeks. The benefit of phytogenic additives on bodyweight and feed conversion ratio is markedly pronounced during the first stage of post-hatch 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 14-28 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 antibioticand 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 theantibiotic 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 the 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 been 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. A shallower crypt indicates a lower rate of enterocyte cell renewal and tissue turnover (Berrocoso et al., 2017; Ząbek et al., 2020), reduces the amount of nutrients needed for the maintenance of the gut and consequently

improves 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 non-significant effects reported by Kumanda et al. (2019) could be a result of the higher inclusion levels of dietary GP used. ALT and AST are important indicators of the health status of the liver (Zhang, 2011) as they play a 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 non-medicated, 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 five major phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia (Lozupone et al., 2012). It is important to mention that information on the impacts of GP on gut microbiota is very scanty. However, a considerable number of *in vitro* demonstrations have 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 cecal microbiota and were observed to be dominated by five major phyla, namely, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and others unclassified (Bacteria_unclassified). Similar to our findings, Firmicutes and Bacteroidetes remain the largest phyla (Almeida et al., 2019; Qin et al., 2010; Forster et al., 2019). The ratio of the microbial population in these two 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, a higher proportion of Bacteroidetes and lower Firmicutes was reported among individuals who consumed fibre-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 perceptions, 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 thegut microbial community. Additionally, the suppressive effect of polyphenols on the F:B ratio has been implicated in the loss of body weight (Xue et al., 2016). This could be responsible for the reduced AWG among the 2.5% GP-fed birds compared 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 and inflammatory bowel disease and is sought as a 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 – 40 g/kg of grape seed was fed to broiler chickens, as reported by Abu 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 affect 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 the adhesion of pathogens (Liu et al., 2015). In humans, a 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 the proliferation of gut-friendly microbes like *Lactobacillus* and *Bacteroides*. Unfortunately, antibiotics have 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. The bacteria genus *Escherichia-shigella,* has been dubbed opportunistic pathogenic microbes (Elbere et al., 2018), often created by antibiotic use (Dudek-Wicher et al., 2018). *Lachnospiraceae_ge* and *Blautia* are members of Lachnospiraceae, which are known to be a partof 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 a reduction in the Lactobacillus while increasing Clostridales in broiler chickens (Crisol-Martinez et al., 2017; Costa 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 cecal microbiota, the composition of cecal short-chain fatty acids was not affected. This could be a result of the similar total eubacteria present in the ceca across the dietary treatment.

White striping and woody breast are the two 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; Lopez 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.

3.5 Conclusion

Dietary incorporation of grape pomace at the inclusion level of 2.5% had beneficial effects onthe growth performance of broiler chickens during the starter phase; however, it had slight negative effects from 14-28d, and no difference in feed efficiency throughout the overall period. Furthermore, there was an improvement in the villus height and villus height crypt depth ratioand modulation of gut microbiota, while the cecal 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.

CHAPTER 4: Effect of red osier dogwood extract on growth performance, blood biochemical parameters, and gut functionality of broiler chickens challenged or unchallenged intraperitoneally with *Salmonella* Enteritidis lipopolysaccharide

This work has been published elsewhere:

Erinle, T., J. MacIsaac, C. Yang, and D. Adewole. 2022. Effect of red osier dogwood extract on growth performance, blood biochemical parameters, and gut functionality of broiler chickens challenged or unchallenged intraperitoneally with *Salmonella* Enteritidis lipopolysaccharide. Poult. Sci.:101861.
https://doi.org/10.1016/j.psj.2022.101861

4. Abstract

As we advance in the search for antibiotic-alternatives, harnessing plant materials with high total polyphenol concentration (TPC) would be quintessential. Given the high TPC in red osier dogwood (ROD) extract, the current study aimed to determine its efficacy on the growth performance, intestinal health, blood biochemistry, and antioxidant capacity of broiler chickens. A 21-day 4x2 factorial feeding trial was conducted based on two main factors, namely dietary treatments and *Salmonella Enteritidis* Lipopolysaccharides (***SE*-LPS**) challenge. A total of 384 one-day-old mixed-sex Cobb-500 broiler chicks were randomly allotted to four dietary treatments – Negative control (**NC**), NC+0.05% bacitracin methylene disalicylate (**BMD**), NC+0.3%**ROD**, and NC+0.5% **ROD**. Each treatment was assigned to eight replicates with six birds/replicate. On d 13 and 20, half of the birds were intraperitoneally injected with 1mL phosphate-buffered-saline /kg BW of birds (Unchallenged-group) and the remaining half with 1mg *SE*-LPS /kg BW of birds (Challenged-group). Average weight gain (**AWG**), average feed intake (**AFI**), feed

conversion ratio (**FCR**), and mortality were determined weekly. On d 21, ten chickens/treatment were euthanized for measuring blood biochemical parameters, immune organ weights, cecal SCFA, and ceca microbiota. The *SE*-LPS decreased (P<0.05) AWG and FCR on d 14 and 21, respectively. On d 14, 21, and overall basis, both ROD extract levels marginally improved (P<0.05) the AWG of unchallenged birds compared to other treatments in the unchallenged-group. Challenged and unchallenged birds fed ROD extract had deeper (P<0.05) crypt depth (CD) and higher (P<0.05) villus height:CD, respectively, in the ileum. Globulin (GLB) and albumin:GLB were increased and reduced (P<0.05), respectively, among birds fed 0.3%ROD compared to other treatments. There was no treatment effect on ceca SCFA, relative weights of immune organs, and serum antioxidants. Birds fed ROD extract had a higher (P<0.05) relative abundance of cecal *Lactobacillus* and *Streptococcus* genera compared to the antibiotic treatment. Conclusively, incorporating 0.3% and 0.5%ROD extract into broiler chickens' nutrition improved growth performance and ileal morphology, and modified cecal microbiota of broiler chickens, regardless of the intraperitoneal *SE*-LPS challenge.

Keywords: red osier dogwood extract, broiler chickens, *Salmonella* enteritidis lipopolysaccharides, cecal microbiota, total antioxidant power.

4.1 Introduction

The global poultry industry is constantly embattled by the prevalence of disease-causing pathogens and their metabolites, undermining its performance, profitability, and survivability. On a global scale, the average annual economic burden these diseases pose on the poultry industry is $3 to $6 billion (Chapman and Jeffers, 2014). The current antibiotics restriction has consequentially contributed to the proliferation of intestinal pathogenic bacteria species that are known to impair chicken's health and cause food-borne

infections in humans, including salmonellosis (Huyghebaert et al., 2011) and endotoxaemia (Acharya and Bajaj, 2017). Both disease conditions are often caused by pathogenic *Salmonella* or their lipopolysaccharides. While the liveability and growth improvement potentials of antibiotic drugs on poultry species can not be contested, the iron-fisted restriction to their use is quite understandable and would further prevent the menace of antibiotic-resistant bacteria resulting from the overwhelming use of critical and high priority antibiotics. There have been continuous efforts directed at identifying suitable alternatives to antibiotic use in poultry production. Suitable antibiotic alternatives could be referred to as feed additives, such as plant extracts, beneficial microbial culture, special plant fibre, antimicrobials, or other metabolites usually of natural origin that could particularly improve growth and gut health, in either a marginal, equivalent, and/or better capacity compared to antibiotic effect given the same condition.

Of the ubiquitous bacteria species, the gram-negative ones are the most economically significant group recognized for their appreciable resistance to some antimicrobials due to the extra unique structure in their outer membrane. The implicated bacteria in this category, include *Salmonella spp*, which produces lipopolysaccharides (LPS; an endotoxin containing virulence factors and proteins), contributing to the structural integrity of the bacteria. Interestingly, LPS comprises O antigen, oligosaccharides, and lipid A, thus, accounting for about 75 – 80% of the outer membrane of gram-negative bacteria (Klein and Raina, 2019; Avila-Calderón et al., 2021). Lipopolysaccharides are powerful immune-stimulants that trigger the innate immune response of the host (Klein and Raina, 2019; Valdez, 2021) upon recognition by Toll-like receptors in cells, including monocytes, beta cells, and macrophages, thus promoting the secretion of pro-inflammatory cytokines. The gastrointestinal tract hosts trillions of gram-positive or gram-negative bacteria. Lipopolysaccharides have been allegedly reported to be present sometimes in a healthy gut

(Reisinger et al., 2020); however, at a certain threshold, they cause inflammation, fever, diarrhea, septic shock, and potential death (Wassenaar and Zimmermann, 2018; Farhana and Khan, 2021) provided they are from pathogenic bacteria like *Salmonella*. The secretion of LPS is not limited to the host's gut, as a reasonable amount of LPS concentration could also be detected in food/feed (Wassenaar and Zimmermann, 2018). In broiler chicken studies, LPS have been implicated in the impairment of performance characteristics (Zheng et al., 2016; Zhang et al., 2020; Chen et al., 2020), inducement of oxidative stress (Li et al., 2015; Zhang et al., 2020), causing intestinal inflammation (Zhang et al., 2020) and disruption of the structural integrity of the intestinal wall, gut barrier functions and nutrient absorption (Zhang et al., 2020). Farhana and Khan (2021) reported that LPS is often used in serotyping gram-negative bacteria, and their early detection in the serum could be a diagnostic marker for infection. Thus, the severity of LPS on the host's immune system is expected to vary depending on the chemical structure of LPS and the bacteria-type producing the LPS. Responses of broiler chickens to LPS derived from *E. coli* and *Salmonella* Typhimurium are the most reported. However, there is a paucity of ample information on broiler chickens' responses when challenged with *Salmonella* Enteritidis LPS.

Remarkably, some studies have demonstrated that oxidative stress caused by LPS could be improved by dietary supplementation of exogenous antioxidants (Wu et al., 2013; Jang et al., 2014). Interestingly, bioactive substances in plants have been well-documented to possess antioxidant prowess in an equivalent capacity of some vitamins, such as vitamin E (Mazur-Kuśnirek et al., 2019). An example of such plants with a high concentration of bioactive substances is the red osier dogwood (ROD) plant.

Red osier dogwood (*Cornus stolonifera*) is a naturally growing ornamental shrub in all provincial areas of Canada (Scales, 2015). Red osier dogwood is a phytogenic additive that has been reported to contain a high concentration of phenolic compounds, primarily gallic acids, quercetin, rutin, and anthocyanins (Isaak et al., 2013; Scales, 2015). Dietary Supplementation of ROD reduced antibiotics usage in weaned pigs and limited the occurrence of diarrhea and death in rabbits (Scale, 2015); it afforded protection to weanling pigs against oxidative stress induced by *E. coli* infection (Amarakoon, 2017), and maintained growth performance and improved nutrient digestibility and absorption, and livability in broiler chickens (Mogire et al., 2021). In fact, *in vitro* demonstrations mimicking a real-time intestinal absorption of ROD extract bioactive substances using Caco-2 cells confirmed that the phenolic compounds in ROD are capable of mitigating inflammatory responses by stepping down the production of interleukin-8 secretion and reactive oxygen species and stepping up the production of body antioxidant enzymes including superoxide dismutase and glutathione peroxidase (Jiang et al., 2019; Yang et al., 2019). A considerable number of studies involving dietary application of ROD extract have been demonstrated on swine, rabbits, equine, bovine, and poultry. The study conducted by Mogire et al. (2021) was the first to investigate the use of ROD at 0.1% and 0.3% inclusion rate in broiler chickens but lacked an immune-response challenge model. To the best of our knowledge, there is currently no study on the effect of ROD extract on blood parameters, antioxidants, and gut health of broiler chickens challenged with LPS. Thus, there is a need for more research to establish the antioxidant and antimicrobial activities of ROD extract in broiler chickens with a stressed immune system.

Given the improved gut health and maintained growth performance in broiler chickens fed 0.1% and 0.3% dietary ROD extract, we hypothesized that a higher inclusion level of 0.3% and 0.5% might be better treatment combinations that will attenuate the triggered immune

response of broiler chickens challenged intraperitoneally with *SE*-LPS, without compromising their growth performance. Thus, this study aimed to examine the ameliorative potential of dietary ROD extract at 0.3% and 0.5% as an alternative to in-feed antibiotics on growth performance, blood biochemical parameters, gut health, and antioxidant status of broiler chickens challenged intraperitoneally with *SE*-LPS.

4.2 Materials and Methods

The experimental protocol was approved by Dalhousie University Animal Care and Use Committee (Animal Care Certification Number 2020-043). The birds were handled following the guidelines established by the Canadian Council on Animal Care (2009).

4.2.1 Birds and housing

A total of 384 one-day-old mixed-sex Cobb-500 broiler chicks were obtained from Atlantic Poultry Incorporated, Port Williams, Nova Scotia, and were raised in a two-tier battery cage system (0.93 m × 2.14 m) at a stocking density of 0.076 m^2/birds for 21 d. Upon arrival, the mixed-sex birds were weighed in groups of 6 and randomly allocated to each cage. The room temperature was monitored daily and was gradually reduced from 32 to 24 ^{0}C from d 0 to 21. The lighting program was set to produce 18 hours of light and 6 hours of darkness throughout the experimental period, and illumination was gradually reduced from 20 1× on d 0 to 5 1× on d 21.

4.2.2 Diets and experimental design

The ROD extract used in this study was obtained from Red Dogwood Enterprise, MB, Canada. The birds were randomly assigned to eight treatment groups containing eight replicate cages of 6 birds each. The experiment was designed as a 4 × 2 factorial arrangement based on 2 main factors, as shown in Table 4.1. The main factors were: 1) 4 dietary treatments: corn-wheat-soybean meal based diet negative control (**NC**), NC with

0.05% bacitracin methylene disalicylate (**BMD**) per kilogram of diet; and NC supplemented with 0.3% or 0.5% ROD extract and 2) 2 intraperitoneal injections: 1 mL sterile 1×phosphate buffered saline (**PBS**) per kg BW of birds (AVL82762, HyClone Laboratories, Inc., Logan, UT) as the unchallenged group (**U**), or 1 mg *SE*-LPS per kg BW of birds (ATCC 13076; Sigma-Aldrich, St. Louis, MO) as the challenged group (**C**). The intraperitoneal injection was carried out on d 13 and 20. The basal diet was formulated as isocaloric and isonitrogenous to meet the nutrient requirements of broiler chickens as recommended by National Research Council (1994). The compositions of the experimental diets are presented in Table 4.2. Experimental diets containing BMD and ROD were mixed from a single basal diet; thus, the reason for reporting analyzed nutrient contents of the basal diets only. The proximate composition of the control diets was determined following A.O.A.C. (1990) procedure. The phenolic profiling of the ROD extract used in our study is presented in Figure 4.1. Total polyphenols in the ROD and diets at the starter and grower phases and polyphenols profile of ROD (Folin-Coicalteu) were determined using an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) at the Institute of Nutrition and Functional Foods, Quebec, Canada.

Table 4.1. Experimental design

	Challenge model	
Dietary treatment (number of replicates; n)	**Unchallenged (U)**	**Challenged (C)**
(a) Basal (NC)	1. NC + PBS (n=8)	2. NC + *SE*-LPS (n=8)
(b) + Antibiotics	3. NC + BMD + PBS (n=8)	4. NC + BMD + *SE*-LPS (n=8)
(c) + 0.3% ROD extract	5. NC + 0.3% ROD + PBS (n=8)	6. NC + 0.3% ROD + *SE*-LPS (n=8)
(d) +0.5% ROD extract	7. NC + 0.5% ROD + PBS (n=8)	8. NC + 0.5% ROD + *SE*-LPS (n=8)

Basal, Negative control (NC) diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract.

U = Unchallenged group; C = Challenged group; PBS = Phosphate buffered saline; *SE*-LPS = *Salmonella* Enteritidis lipopolysaccharides

Table 4.2. Gross and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated)

Ingredients	Starter phase (1-14 days)				Grower phase (14-21 days)			
	Basal	BMD	0.3% ROD	0.5% ROD	Basal	BMD	0.3% ROD	0.5% ROD
Corn	42.37	42.27	41.83	41.48	45.99	45.65	45.22	44.86
Soybean meal (47.5 %CP)	40.13	40.15	40.17	40.2	36.15	36.21	36.24	36.26
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Vegetable oil	2.82	2.85	3.01	3.14	3.74	3.85	4.01	4.14
Dicalcium phosphate	1.57	1.57	1.57	1.57	1.39	1.39	1.39	1.39
Limestone	1.45	1.45	1.45	1.45	1.32	1.32	1.32	1.32
DL Methionine Premix [W]	0.61	0.61	0.61	0.61	0.53	0.53	0.53	0.53
Starter Vitamin/Mineral premix [X]	0.50	0.50	0.50	0.50	-	-	-	-
Grower/Finisher Vitamin/Mineral premix [Y]	-	-	-	-	0.50	0.50	0.50	0.50
Sodium chloride	0.40	0.40	0.40	0.40	0.38	0.38	0.38	0.38
Red dogwood extract	-	-	0.30	0.50	-	-	0.30	0.50
BMD 110 G [Z]	-	0.05	-	-	-	0.05	-	-
Lysine HCL	0.16	0.16	0.16	0.16	-	0.12	0.12	0.12
Formulated Composition								
Crude Protein	23	23	23	23	21.5	21.5	21.5	21.5
Metabolizable Energy (kcal kg^{-1})	3,000	3,000	3,000	3,000	3,100	3,100	3,100	3,100
Calcium	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87
Available Phosphorus	0.48	0.48	0.48	0.48	0.44	0.44	0.44	0.44
Digestible Lysine	1.28	1.28	1.28	1.28	1.15	1.15	1.15	1.15
Digestible Methionine + Cystine	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87
Sodium	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18
Analyzed Composition								
Crude Protein	24.1				22.1			

Ingredients	Starter phase (1-14 days)				Grower phase (14-21 days)			
	Basal	BMD	0.3% ROD	0.5% ROD	Basal	BMD	0.3% ROD	0.5% ROD
Calcium	0.81				0.75			
Total Phosphorus	0.68				0.62			
Sodium	0.15				0.12			
Crude Fat	3.22				4.40			

[1] Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[W] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[X] 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.

[Y] 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.

[Z] Bacitracin methylene disalicylate (providing 55 mg/kg mixed feed); Alpharma, Inc., Fort Lee, NJ, US.

4.2.3 Growth performance and sample collection

Average body weight (**ABW**) and average feed intake (**AFI**) were determined weekly on a cage 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, Nova Scotia Department of Agriculture Dalhousie University for post-mortem examinations.

4.2.4 Blood biochemistry and antioxidant assay

On d 21, 80 birds (ten birds from each treatment) were randomly selected, individually weighed, and euthanized by electrical stunning and exsanguination. At exsanguination, approximately 8 mL of blood samples were collected from the jugular vein of each bird and were divided into 2 aliquots (4 mL each) in 5 mL heparinized tube and 5 mL serum tube for the determination of plasma biochemistry and serum enzyme-link immunosorbent assay (ELISA), respectively. Samples for blood biochemical analysis were centrifuged at 5000 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 Y (**IgY**) and immunoglobulin M (**IgM**) were analyzed using respective ELISA kits from Bethyl Laboratories, Inc. (catalog number E33-104-200218 and E33-102-180410) following manufacturer instructions. Superoxide dismutase (**SOD**) and total antioxidant power (**TAP**) were analyzed using a SOD assay kit (Item Number 706002; Cayman Chemical, USA) and Oxiselect total antioxidant capacity assay kit (MAK187-1KT; Sigma-Aldrich), respectively following the manufacturers' instructions.

4.2.5 Gut morphology

A 1.5 cm segment at the middle of the duodenum jejunum, and ileum was collected and preserved in 10% buffered formalin (Sigma-Aldrich, St. Louis, MO) for three days. The formalin-preserved intestinal segments were immersed in paraffin and cross-sectioned.

Each cross-sectioned segment was mounted on a glass slide (n = 10 per treatment) and stained with Alcian blue and periodic acid-Schiff (PAS) reagents. The morphological slides were examined under 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, i.e., 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 ratio (**VH:CD)** was subsequently estimated.

4.2.6 Short-chain fatty acid concentrations and total eubacteria count

Digesta from the pair of ceca were mixed and divided into two sub-samples. The cecal samples for SCFA and total eubacteria determination were immediately preserved using BioFreeze™ sampling kits (Alimetrics Diagnostics Ltd., Espoo, Finland) following the manufacturer's recommended protocol. In addition to the cecal SCFA concentration, the analysis of the most prevalent bacterial species was performed by Alimetrics Diagnostics Ltd.

4.2.7 Gut microbiota

The second portion of the mixed cecal digesta were stored in plastic RNAse and DNAse-free tubes, placed in liquid nitrogen, and followed by storage at -80°C for further gut microbiota analysis. 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-V5 region (515FB 5'-GTGYCAGCMGCCGCGGTAA-3' and 926R 3' CCGYCAATTYMTTTRAGTTT-5'). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3) at the Integrated Microbiome Resource (http://imr.bio) of Dalhousie University. 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 standard operating procedure on MicrobiomeHelper which is based in Quantitative Insights Into Microbial Ecology 2 (QIIME2) (https://github.com/LangilleLab/microbiome_helper/wiki/Amplicon-SOP-v2-(qiime2-2019.7).

4.2.8 Relative weight of immune organs

Two lymphoid organs (spleen and liver) were collected from each bird, and the relative weight was expressed as a percentage of the individual BW.

4.2.9 Statistical analysis

Datasets were subjected to 4 × 2 factorial analysis of variance (ANOVA) using the General Linear Model of Minitab LLC, (2019) software. Error terms of individual response variable were confirmed for the validity of three basic assumptions including, normality, constant variance, and independence. A normal probability plot of residuals was done to verify the normality of error terms using the Anderson Darling test in the same statistical package. Where error terms of datasets were found to be non-normal or non-constant, the respective original datasets were subjected to various transformation functions. If the error terms' normality and homoscedasticity were still violated upon transformations, then such datasets were analyzed using the Kruskal-Wallis test. Following ANOVA, differences between significant means were separated using Tukey's honest significant difference (**HSD**) test and Mann Whitney for the parametric and non-parametric datasets, respectively, in the same statistical package. Analyzed datasets were presented as means, standard error of the mean (**SEM**), and probability values. Statistically different values were considered at $P<0.05$.

4.3 RESULTS

4.3.1 Total polyphenol content (TPC)

The result of the polyphenol profile of ROD extract (mg standard equivalent/g) and the TPC of the dietary treatments are presented in Figures 1 and 2, respectively. The measured total polyphenol in the ROD extract was 238.81 mg gallic acid equivalent (GAE)/g. From the polyphenol profile of ROD extract, gallic acid and quercetin were observed to be the most abundant phenolic compounds. The TPC (Folin-Ciocalteu) (mg GAE/g) in the starter diets namely, A, C, and D, respectively, were 1.55, 2.1, and 2.56, respectively. However, in the grower phase, TPC in A, C, and D were 1.16, 1.73, and 2.12 mg GAE/g.

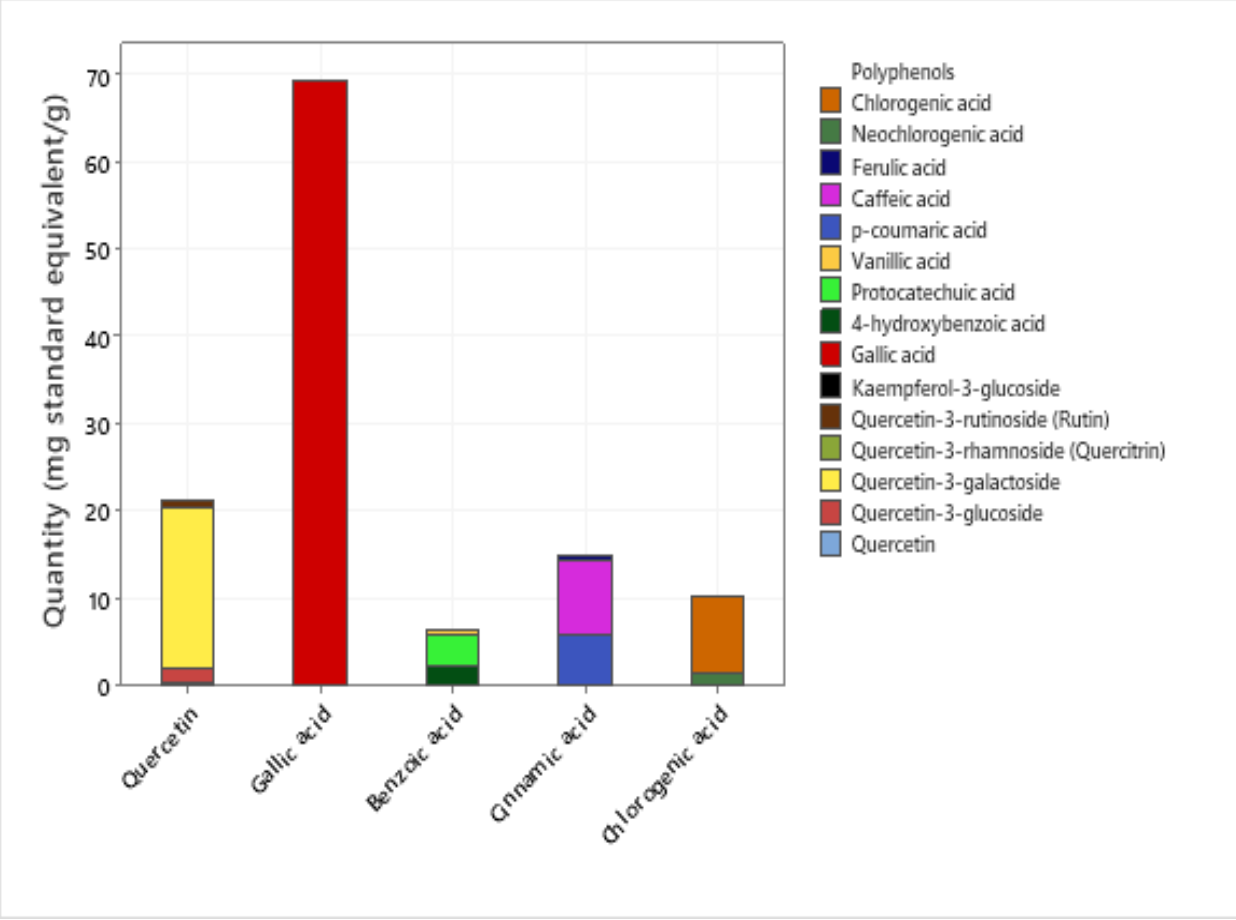


Figure 4.1. Polyphenols profile of red osier dogwood extract by UPLC-MSMS (mg standard equivalent/g).

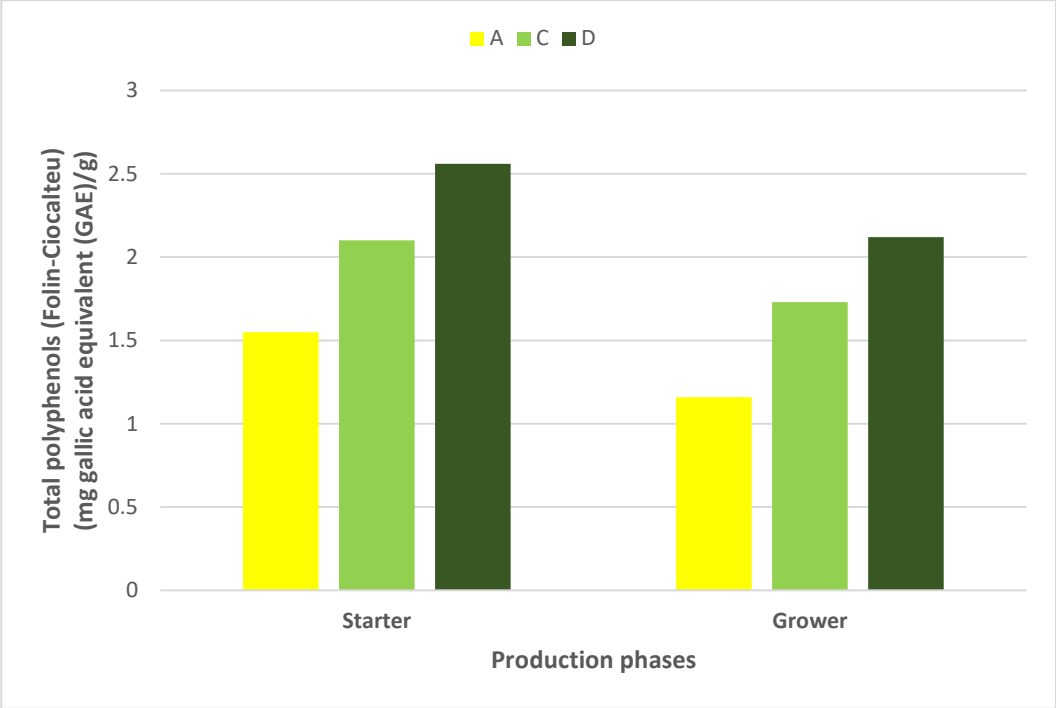


Figure 4.2. Total polyphenols content (mg gallic acid equivalent GAE/g) in treatments fed to broiler chicken according to production phases. A, B, C, and D diets per production phase. Treatment: A = Negative control, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

4.3.2 Growth performance

The effects of ROD extract as an antibiotic-alternative on growth performance of broiler chickens challenged or unchallenged with *SE*-LPS are presented in Table 4.3. No interaction effect was observed; thus, the results are reported based on the main effects. No treatment effects ($p>0.05$) were observed on the growth response of the unchallenged birds on d 7 and the challenged birds throughout the entire experimental period. However, on d 14 and d 21, and overall basis, AWG of the unchallenged group of birds followed a specific pattern of variation and was observed to be marginally improved ($p<0.05$) among birds fed dietary 0.3% and 0.5% ROD extract compared with those fed antibiotic and control diets. On d 14, there was a significant model effect ($p<0.05$) on AWG and was seen to be significantly higher ($p<0.05$) among the unchallenged group of birds compared to the challenged birds. On d 21, there was a significant treatment*challenge model interaction

effect ($p<0.05$) on AFI of birds. Meanwhile, the FCR was significantly influenced ($p<0.05$) by the dietary challenge model and was observed to be lower among the unchallenged birds compared with the challenged birds. The dietary treatments and model did not affect mortality.

Table 4.3. Effect of red osier dogwood extract on growth performance of broiler chickens challenged intraperitoneally with *Salmonella* Enteritidis Lipopolysaccharide examined at weekly levels.

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Day 0-7												
Average feed intake (g/bird)	U	128	117	119	119	2.110	120	124	1.450	0.217	0.189	0.226
	C	126	128	121	119	1.980						
Average weight gain (g/bird)	U	97.3	98.8	95.4	91.1	1.920	95.7	98.0	1.300	0.612	0.379	0.835
	C	98.6	99.3	96.4	97.7	1.750						
FCR	U	1.33	1.19	1.27	1.31	0.031	1.27	1.28	0.020	0.828	0.931	0.287
	C	1.29	1.33	1.26	1.23	0.030						
Mortality(%)	U	0.00	0.00	0.00	0.00	0.080	0.00	0.00	0.060	0.822	1.000	-
	C	0.00	0.00	0.00	0.00	0.080						
Day 7-14												
Average feed intake (g/bird)	U	288	329	304	301	6.500	304	294	4.300	0.127	0.240	0.481
	C	284	300	285	305	5.600						
Average weight gain (g/bird)	U	209[b]	251[a]	243[ab]	228[ab]	5.580	235[a]	223[b]	3.330	0.013	0.048	0.197
	C	219	235	216	219	3.480						
FCR	U	1.38	1.31	1.24	1.31	0.022	1.30	1.32	0.020	0.261	0.695	0.076
	C	1.30	1.28	1.32	1.40	0.022						

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Mortality(%)	U	0.00	0.00	0.00	0.00	0.040	0.00	0.00	0.030	0.552	1.000	-
	C	0.00	0.00	0.00	0.00	0.040						
Day 14-21												
Average feed intake (g/bird)	U	681	723	750	694	9.360	712	717	7.190	0.208	0.710	0.047
	C	748	725	708	687	11.10						
Average weight gain (g/bird)	U	347[b]	416[a]	404[ab]	379[ab]	8.680	386	367	5.630	0.019	0.063	0.081
	C	373	388	359	349	6.880						
FCR	U	2.00	1.75	1.87	1.84	0.040	1.86[b]	1.96[a]	0.030	0.045	0.036	0.818
	C	2.02	1.88	1.99	1.98	0.030						
Mortality(%)	U	0.00	0.00	0.00	0.00	0.030	0.00	0.00	0.020	0.566	1.000	
	C	0.00	0.00	0.00	0.00	0.030						
Overall (0-21)												
Average feed intake (g/bird)	U	1,096	1,169	1,173	1,114	11.80	1,138	1,136	8.420	0.201	0.923	0.069
	C	1,162	1,155	1,115	1,114	12.20						
Average weight gain (g/bird)	U	653[b]	766[a]	743[ab]	698[ab]	15.10	715	687	9.210	0.018	0.108	0.140
	C	690	722	671	666	10.30						
FCR	U	1.70	1.53	1.59	1.60	0.030	1.61	1.66	0.016	0.053	0.096	0.716
	C	1.69	1.61	1.67	1.68	0.020						

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Mortality(%)	U	0.00	0.00	0.00	0.00	0.030	0.00	0.00	0.019	0.422	0.793	-
	C	0.00	0.00	0.00	0.00	0.025						

[1]Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract;

[2]U = Unchallenged group; C = Challenged group;

[3]SEM = standard error of the mean.

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

4.3.3 Gut morphology

The effects of ROD extract on the intestinal morphology of broiler chickens challenged or unchallenged intraperitoneally with *SE*-LPS are presented in Table 4.4. There were no interaction and model effects; therefore, the results were interpreted based on the treatment effects. With the exception of ileal CD and VH:CD, dietary supplementation of 0.3% and 0.5% ROD extract did not have a significant effect on gut morphology variables of the broiler chickens. In the ileal section, both levels of ROD extract significantly deepened ($p<0.05$) CD among the challenged birds compared with the antibiotic and control birds. Dietary supplementation of 0.3% ROD extract significantly improved ($p<0.05$) VH:CD compared with other treatments.

Table 4.4. Effect of red osier dogwood extract on gut morphology of broiler chickens challenged intraperitoneally with *Salmonella* Enteritidis Lipopolysaccharide.

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Duodenum												
Villus height (mm)	U	1.32	1.42	1.32	1.35	0.021	1.35	1.31	0.016	0.820	0.228	0.476
	C	1.33	1.30	1.33	1.29	0.025						
Villus width (mm)	U	0.17	0.15	0.15	0.16	0.007	0.16	0.15	0.004	0.684	0.487	0.192
	C	0.14	0.15	0.15	0.14	0.004						
Crypt depth (mm)	U	0.11	0.10	0.10	0.10	0.002	0.10	0.11	0.002	0.218	0.868	0.891
	C	0.12	0.10	0.10	0.10	0.003						
VH:CD[4]	U	13.1	16.0	13.4	14.1	0.550	14.1	13.2	0.334	0.224	0.152	0.767
	C	12.4	13.5	13.3	13.7	0.371						
Jejunum												
Villus height (mm)	U	0.72	0.76	0.72	0.75	0.017	0.73	0.71	0.013	0.874	0.476	0.441
	C	0.75	0.72	0.73	0.67	0.019						
Villus width (mm)	U	0.15	0.15	0.17	0.17	0.005	0.15	0.15	0.003	0.373	0.884	0.486
	C	0.16	0.16	0.16	0.16	0.005						
Crypt depth (mm)	U	0.07	0.08	0.06	0.06	0.003	0.06	0.06	0.002	0.102	0.486	0.797
	C	0.07	0.07	0.06	0.06	0.002						
VH:CD[4]	U	13.2	11.6	12.3	12.5	0.493	11.6	11.5	0.286	0.567	0.691	0.917
	C	12.4	11.5	12.4	11.7	0.292						
Ileum												
Villus height (mm)	U	0.36	0.35	0.36	0.38	0.010	0.36	0.38	0.008	0.460	0.151	0.939
	C	0.38	0.37	0.37	0.41	0.012						
Villus width (mm)	U	0.18	0.16	0.17	0.17	0.006	0.14	0.14	0.843	0.740	0.114	0.359
	C	0.15	0.16	0.16	0.14	1.690						

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Crypt depth (mm)	U	0.09	0.09	0.08	0.10	0.003	0.09	0.09	0.002	0.006	0.443	0.208
	C	0.09^{ab}	0.09^{ab}	0.10^{a}	0.10^{a}	0.004						
VH:CD[4]	U	3.96^{b}	3.95^{b}	5.0^{a}	3.92^{b}	0.146	4.06	4.37	0.108	0.228	0.082	0.178
	C	4.62	4.40	4.54	4.61	0.158						

[1]Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract.

[2]U = Unchallenged group; C = Challenged group.

[3]SEM = standard error of the mean.

[4]VH:CD = Villus height:crypt depth ratio.

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

4.3.4 Serum biochemistry

The effect of ROD extract on serum biochemical indices of broiler chickens challenged or unchallenged intraperitoneally with *SE*-LPS is shown in Table 4.5. No interaction was detected on the measured blood parameters. Similarly, no treatment effect was observed on the plasma biochemical indices of birds in the challenged group. Dietary Supplementation of 0.3% ROD extract significantly increased and reduced ($p<0.05$) globulin (**GLB**) and albumin:globulin (**A:G**), respectively, in the unchallenged birds compared with the antibiotic treatment; however, they were marginally similar to those receiving dietary 0.5% ROD extract and control treatments. With respect to the challenge model effects, calcium, iron, total protein (**TP**), cholesterol (**CHOL**), albumin (**ALB**), GLB, and gamma-glutamyl transferase (**GGT)** were significantly higher ($p<0.05$) among the challenged birds, while lipase and creatine kinase (**CK**) were significantly higher ($p<0.05$) among the unchallenged group.

Table 4.5. Effect of red osier dogwood extract on plasma biochemical indices of broiler chickens challenged intraperitoneally with *Salmonella* Enteritidis Lipopolysaccharide

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Calcium (mmol/L)	U	2.58	2.41	2.70	2.54	0.048	2.56[b]	2.71[a]	0.031	0.099	0.014	0.836
	C	2.71	2.64	2.78	2.69	0.037						
Phosphorus (mmol/L)	U	1.97	2.18	1.99	1.82	0.062	1.99	1.98	0.043	0.812	0.922	0.152
	C	1.89	1.92	2.01	2.11	0.061						
Magnesium (mmol/L)	U	0.75	0.80	0.73	0.75	0.023	0.74	0.77	0.016	0.875	0.263	0.228
	C	0.82	0.72	0.80	0.84	0.022						
Sodium (mmol/L)	U	140	141	141	140	1.290	140	142	0.884	0.772	0.404	0.912
	C	139	142	144	142	1.210						
Potassium (mmol/L)	U	6.83	6.81	6.76	6.48	0.220	6.72	7.07	0.154	0.953	0.685	0.536
	C	7.15	6.70	6.94	7.50	0.220						
Na:K[4]	U	20.9	22.4	20.9	21.7	0.814	21.5	20.8	0.491	0.977	0.878	0.510
	C	20.5	21.8	21.3	19.4	0.568						
Chloride (mmol/L)	U	104	108	107	106	0.920	106	105	0.622	0.725	0.778	0.885
	C	104	106	107	105	0.851						
Iron (umol/L)	U	17.9	17.8	15.9	18.2	0.519	17.5[b]	22.6[a]	0.520	0.091	<0.001	0.629
	C	22.2	21.8	21.1	25.1	0.699						
Amylase (U/L)	U	899	470	808	617	115.0	699	539	63.10	0.424	0.063	0.575
	C	556	519	574	509	47.40						
Lipase (U/L)	U	26.9	20.5	18.8	24.4	3.690	22.2[a]	19.4[b]	2.020	0.616	0.011	0.837
	C	21.4	19.0	17.8	19.5	1.350						

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Bile Acids (mmol/L)	U	17.8	19.6	22.2	19.7	0.934	19.8	21.9	0.662	0.188	0.502	0.679
	C	21.0	20.8	22.6	23.1	0.942						
Glucose (mmol/L)	U	13.4	12.9	13.6	13.3	0.366	13.3	13.9	0.212	0.694	0.739	0.729
	C	13.6	13.8	13.9	14.3	0.222						
T.Protein (g/L)[5]	U	22.8	21.5	25.3	21.8	0.574	23.2[b]	26.3[a]	0.463	0.079	<0.001	0.608
	C	27.0	24.5	27.0	26.7	0.639						
Cholesterol (mmol/L)	U	2.61	2.60	2.78	2.79	0.083	2.70[b]	3.05[a]	0.061	0.658	0.005	0.962
	C	3.04	2.96	3.03	3.16	0.082						
Uric Acid (umol/L)	U	393	298	321	326	16.10	335	358	10.30	0.334	0.724	0.131
	C	354	379	380	320	13.10						
Urea (mmol/L)	U	0.33	0.32	0.32	0.36	0.016	0.33	0.33	0.011	0.834	0.815	0.127
	C	0.33	0.35	0.36	0.27	0.015						
CK (U/L)[6]	U	2,629	3,246	2,393	2,024	311.0	2,573[a]	2,088[b]	179.0	0.218	0.003	0.714
	C	2,186	2,226	2,188	1,754	145.0						
Creatinine (umol/L)	U	0.00	0.00	0.00	0.00	0.136	0.00	0.00	0.118	0.387	0.112	-
	C	0.00	0.00	0.50	0.00	0.186						
Albumin (g/L)	U	9.32	9.12	9.27	9.03	0.193	9.22[b]	10.20[a]	0.151	0.908	0.002	0.769
	C	10.2	9.90	10.1	10.6	0.209						
Globulin (g/L)	U	13.4[ab]	12.4[b]	15.9[a]	12.7[ab]	0.468	13.8[b]	15.9[a]	0.377	0.035	0.003	0.521
	C	17.5	15.0	15.5	16.5	0.538						
A:G[7]	U	0.69[ab]	0.74[a]	0.59[b]	0.71[ab]	0.021	0.67	0.66	0.015	0.044	0.298	0.585
	C	0.62	0.70	0.64	0.67	0.022						
ALP (U/L)[8]	U	5,619	5,464	6,598	6,828	587.0	6,127	7,393	380.0	0.887	0.246	0.698

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
	C	7,434	7,682	7,875	6,582	484.0						
ALT (U/L)[9]	U	5.76	5.79	6.17	8.16	0.574	6.47	5.93	0.411	0.825	0.189	0.071
	C	6.50	7.80	5.10	4.30	0.583						
AST (U/L)[10]	U	170	181	174	171	4.110	174	172	2.540	0.571	0.900	0.746
	C	165	174	171	178	3.080						
GGT (U/L)[11]	U	8.85	10.1	9.70	9.79	0.428	9.43 [b]	11.5 [a]	0.438	0.633	0.005	0.536
	C	12.1	11.9	10.8	13.8	0.710						
T. Bilirubin (umol/L)[12]	U	0.00	0.00	0.00	0.00	0.066	0.00	0.00	0.059	0.908	0.831	-
	C	0.00	0.00	0.00	0.00	0.098	0.00	0.00				

[1]Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract; [2]U = Unchallenged group; C = Challenged group.

[3]SEM = standard error of the mean.

[4]Na:K = Sodium:Potassium ratio

[5]T. Protein = Total Protein

[6]CK = Creatine kinase

[7]A:G = Albumin Globulin ratio

[8]ALP = Alkaline Phosphatase

[9]ALT = Alanine aminotransferase

[10]AST = Aspartate aminotransferase

[11]GGT = Gamma-glutamyl transferase

[12]T.Bilirubin = Total Bilirubin.

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

4.3.5 Serum immunoglobulins, antioxidant status, and relative weight of immune organs

The effect of ROD extract on serum immunoglobulins Y and M, antioxidant status, and relative weights of immune organs of broiler chickens challenged or unchallenged intraperitoneally with *SE*-LPS is shown in Table 4.6. No interaction was observed. Also, serum IgY and IgM, SOD, and TAP were not significantly affected ($p>0.05$) by the dietary inclusion of 0.3% and 0.5% ROD extract. A significant model effect ($p<0.05$) was observed on serum IgM and was seen to be higher among challenged birds compared to the unchallenged birds. The dietary treatments did not influence ($p>0.05$) the relative weight of immune organs. However, the challenge model had a significant effect ($p<0.05$) on relative spleen weight, which was higher among the challenged birds compared to the unchallenged ones. In addition, no interaction between the dietary treatment and challenge model was noticed.

Table 4.6. Effect of red osier dogwood extract on serum immunoglobulin Y and M, antioxidant status, and relative weight of immune organs of broiler chickens challenged intraperitoneally with *Salmonella* Enteritidis Lipopolysaccharide.

		Treatment[1]					Challenge model[2]			*P*-Value		
Parameters	**Challenge model[2]**	**Basal**	**BMD**	**0.3% ROD**	**0.5% ROD**	**SEM[3]**	**U**	**C**	**SEM[3]**	**Treatment Effect**	**Model Effect**	**Interaction Effect**
Serum IgY (mg/mL)	U	4.92	4.36	7.72	4.74	1.370	6.20	7.41	1.120	0.349	0.352	0.790
	C	6.27	6.41	8.71	8.60	1.780						
Serum IgM (mg/mL)	U	0.42	0.37	0.34	0.34	0.034	0.33[b]	0.43[a]	0.030	0.493	0.012	0.199
	C	0.49	0.35	0.54	0.58	0.047						
SOD (U/ml)[4]	U	1.61	1.79	1.62	1.59	0.050	1.62	1.59	0.041	0.409	0.232	-
	C	1.61	1.52	1.62	1.53	0.063						
TAP (uM copper reducing equivalents)[5]	U	1750	1638	1642	1787	47.50	1705	1739	31.50	0.823	0.601	0.630
	C	1699	1756	1765	1736	41.90						
Relative liver weight (% of BW of birds) [6]	U	2.70	2.55	2.83	2.59	0.050	2.67	2.78	0.030	0.219	0.091	0.101
	C	2.84	2.66	2.69	2.91	0.040						
Relative spleen weight (% of BW of birds) [6]	U	0.08	0.07	0.08	0.07	0.003	0.07[b]	0.09[a]	0.002	0.867	<0.005	0.151
	C	0.09	0.09	0.08	0.10	0.003						

[1] Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract.

[2] U = Unchallenged group; C = Challenged group; [3]SEM = standard error of the mean; [4] SOD = Superoxide dismutase; [5]TAP = Total antioxidant power; [6]Relative weight of liver or spleen = (weight of liver or spleen (in grams) * 100) / bodyweight of bird (in grams)

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

4.3.6 Cecal short-chain fatty acid concentration

The ceca SCFA concentration and total eubacteria counts of ROD-extract-fed-broiler chickens challenged or unchallenged intraperitoneally with *SE*-LPS is presented in Table 4.7. Compared to antibiotic and control treatments, dietary supplementation of 0.3% and 0.5% ROD extract did not affect ($p>0.05$) total eubacteria count, short chain fatty acid (**SCFA**), acetic acid (**AA**), propionic acid (**PA**), butyric acid (**BA**), valeric acid (**VA**), lactic acid (**LA**), branched chain fatty acid (**BCFA**), and volatile (**VFA)**. In addition, no significant difference ($p>0.05$) existed between challenge and unchallenged groups. No interaction was observed on the ceca short-chain fatty acid concentration; however, there was a significant interaction effect ($p<0.05$) on the total eubacteria count.

Table 4.7. Effect of red osier dogwood extract on total eubacteria count and short-chain fatty acids concentration in the ceca of broiler chickens challenged with *Salmonella* Enteritidis Lipopolysaccharide.

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
Total eubacteria x 10^{12} (16S rRNA gene copies/gram of sample)	U	4.41	4.29	11.6	7.05	1.650	6.27	7.71	1.071	0.290	0.246	0.028
	C	11.6	10.8	8.56	5.86	1.380						
SCFA (mmol/kg)[4]	U	37.1	65.0	92.6	88.0	19.20	66.6	74.6	11.00	0.152	0.535	0.241
	C	76.0	75.3	67.7	86.1	10.70						
Acetic acid (mmol/kg)	U	26.2	46.6	70.0	65.0	13.40	48.5	53.1	7.840	0.104	0.608	0.163
	C	56.7	49.1	48.2	90.6	8.020						
Propionic acid (mmol/kg)	U	1.86	3.20	1.80	2.07	0.856	2.63	3.37	0.475	0.551	0.135	-
	C	3.95	3.13	2.54	4.74	0.410						
Butyric acid (mmol/kg)	U	10.3	12.4	18.6	16.1	4.230	14.3	15.2	2.350	0.445	0.796	0.716
	C	16.9	13.1	15.0	23.4	1.970						
Valeric acid (mmol/kg)	U	0.82	0.77	1.04	0.90	0.183	0.89	0.79	0.122	0.314	0.701	-
	C	0.88	0.64	0.59	0.85	0.162						
Lactic acid (mmol/kg)	U	0.00	0.00	0.23	0.00	0.979	0.00	0.06	0.547	0.057	0.323	-
	C	0.00	0.00	0.70	1.01	0.481						

Parameters	Challenge model[2]	Treatment[1]				SEM[3]	Challenge model[2]		SEM[3]	*P*-Value		
		Basal	BMD	0.3% ROD	0.5% ROD		U	C		Treatment Effect	Model Effect	Interaction Effect
BCFAs (mmol/kg)[5]	U	0.29	0.74	0.44	0.54	0.108	0.43	0.43	0.133	0.667	0.460	-
	C	1.20	0.36	0.94	1.39	0.244						
VFAs (mmol/kg)[6]	U	36.7	65.0	91.3	86.5	18.60	65.9	73.3	10.70	0.167	0.557	0.222
	C	79.7	66.4	67.2	122	10.40						

[1]Basal, Negative control diet, BMD (bacitracin methylene disalicylate) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract.

[2]U = Unchallenged group; C = Challenged group.

[3]SEM = standard error of the mean.

[4]SCFA = Short chain fatty acid

[5]BCFA = Branch chain fatty acid

[6]VFA = Volatile fatty acid

4.3.7 Cecal microbiota

The effect of 0.3% and 0.5% ROD extract on the cecal microbiota of broiler chickens challenged or unchallenged intraperitoneally with *SE*-LPS is shown in Figures 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, and 4.11, and Figures 4.12 and 4.13. The aggregation of OTU into each taxonomic rank, as well as the relative abundance of the most abundant phyla, and genera based on treatments, group, and treatment/group effects are presented in Figures 4.3, 4.4, and 4.5, respectively. The percentage relative abundance of the three phyla, namely Actinobacteriota, Proteobacteria, and Firmicutes were not influenced by the dietary treatments; however, Firmicutes was the most abundant phylum. Unlike other genera, supplementation of 0.3% and 0.5% ROD extract significantly increased ($p<0.05$) the percentage relative abundance of genera *Lactobacillus* and *Streptococcus* compared to the antibiotic treatments; however, they were similar to the control treatment regardless of the *SE*-LPS challenge (Figure 4.6, 4.7, and 4.8). Furthermore, the Shannon diversity (i.e., specie richness) was not affected either by the dietary treatments or the challenge, as shown in Figures 4.9 and 4.10. In addition, a principal coordinate analysis showed a significant difference ($p<0.05$) in the beta diversity in the cecal microbiota, with more diversity observed among the birds fed 0.3%, 0.5% ROD extract, and control treatments as shown in Figure 4.11. There was no difference in the alpha and beta diversity between the challenged and unchallenged groups, as presented in Figures 4.12 and 4.13.

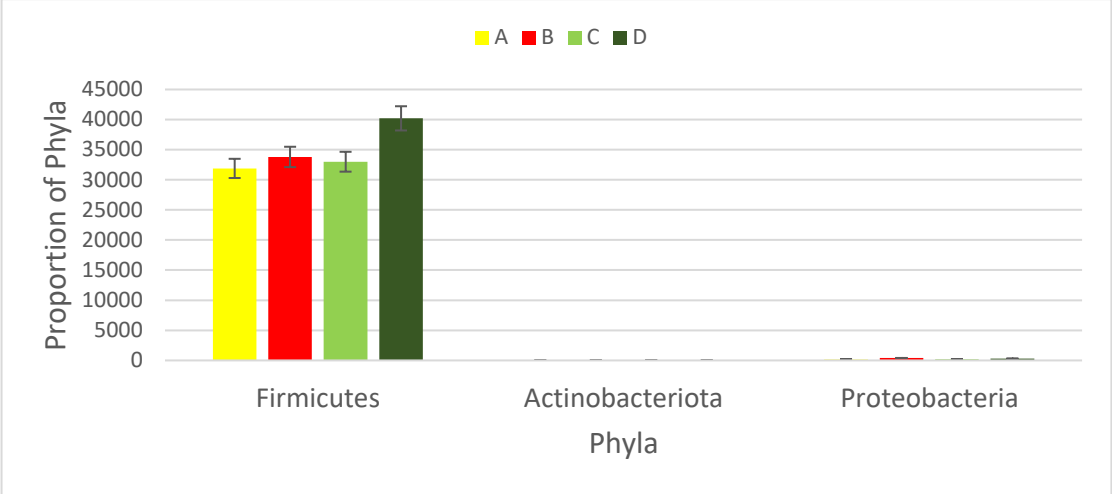


Figure 4.3. Proportion of the most abundant bacteria phyla in the ceca of broiler chickens challenged intraperitoneally with or without *SE*-LPS and fed red osier dogwood extract as a substitute for in-feed antibiotics. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

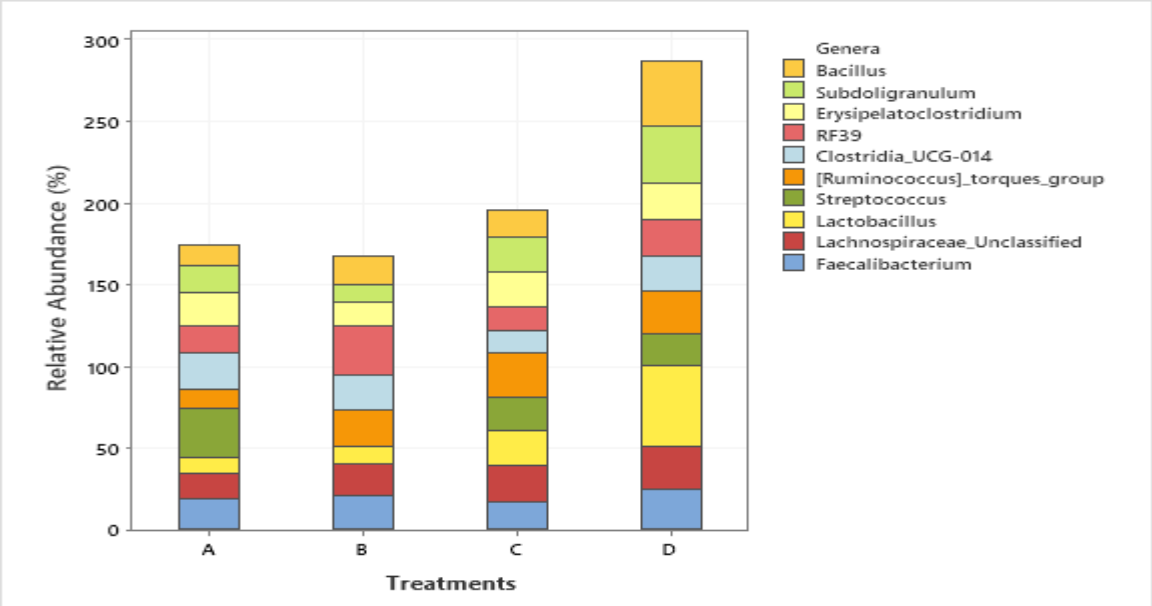


Figure 4.4. Percentage relative abundance of the most abundant bacteria genera in the ceca of broiler chickens challenged intraperitoneally with or without *SE*-LPS and fed 4 different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

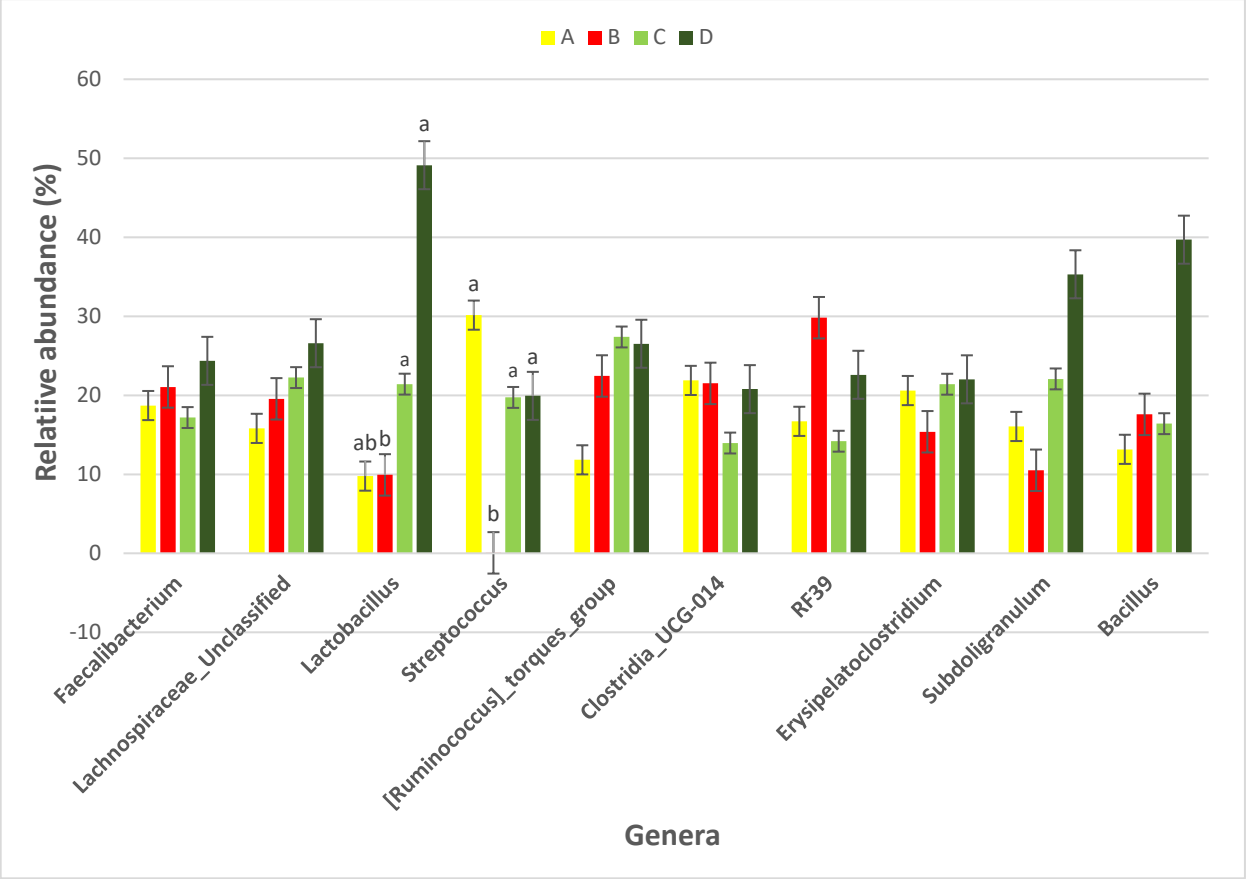


Figure 4.5. Percentage relative abundance of the top 10 most abundant bacteria genera in the ceca of broiler chickens challenged intraperitoneally with or without *SE*-LPS and fed 4 different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

Note: Genera without a mean separation have their p-value greater than 0.05

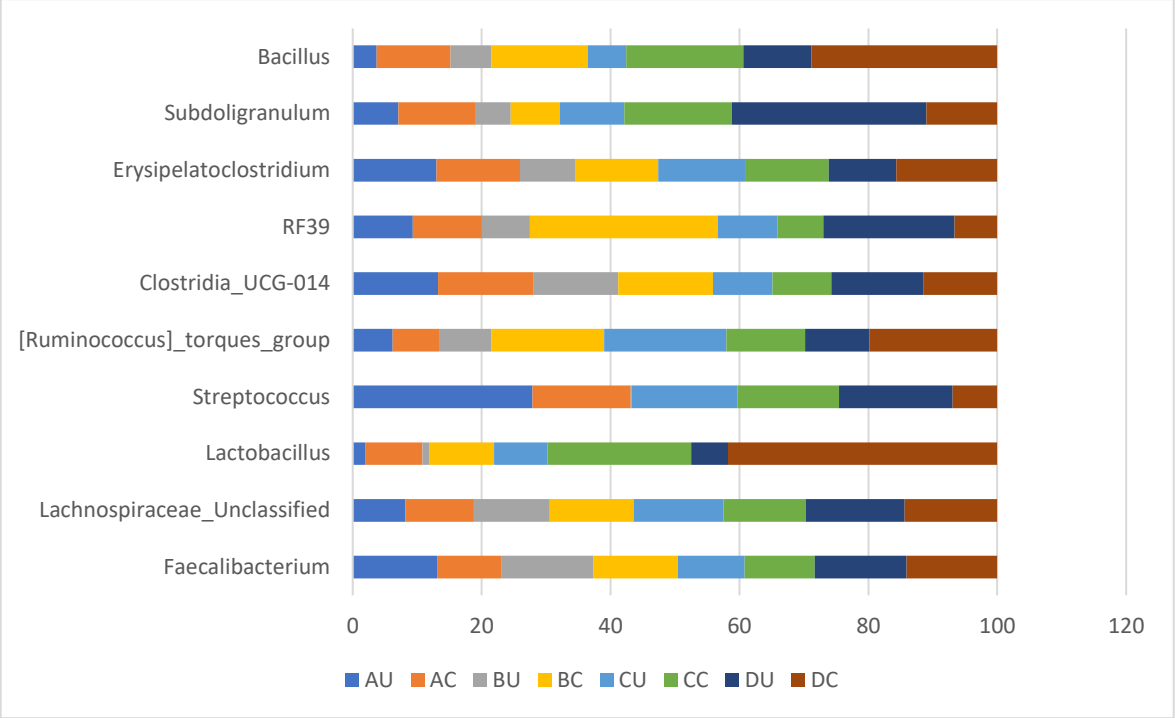


Figure 4.6. Percentage relative abundance of the top 10 most abundant bacteria genera in the ceca of broiler chickens challenged intraperitoneally with or without *SE*-LPS and fed 4 different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

Challenge groups: U = group of birds that were not challenged with *SE*-LPS, C = group of birds that were challenged with SE-LPS.

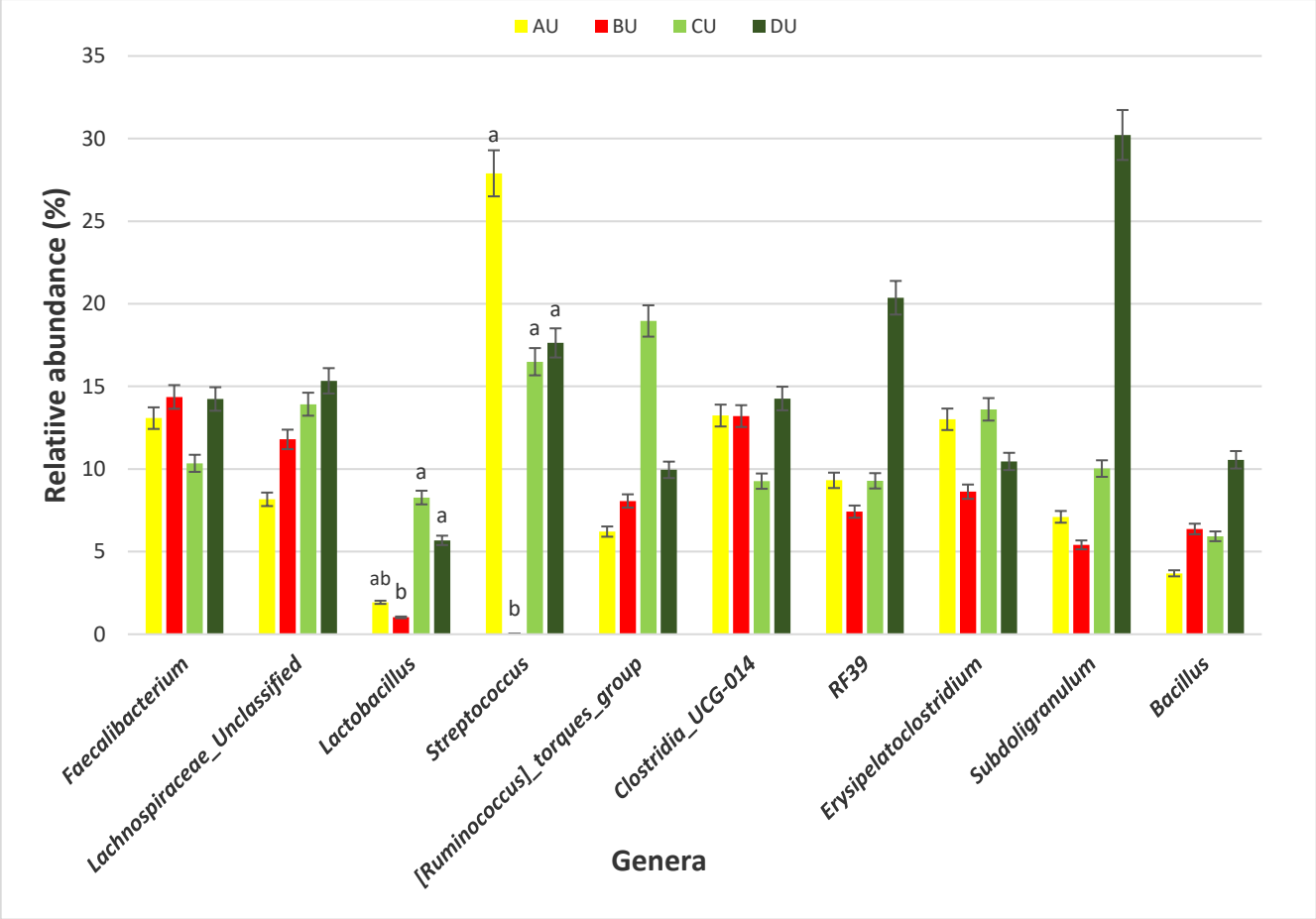


Figure 4.7. Percentage relative abundance of the top 10 most abundant bacteria genera in the ceca of *SE*-LPS-unchallenged broiler chickens fed 4 different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract. Challenge groups: U = group of birds that were not challenged with *SE*-LPS, C = group of birds that were challenged with *SE*-LPS.

Note: Genera without a mean separation have their p-value greater than 0.05

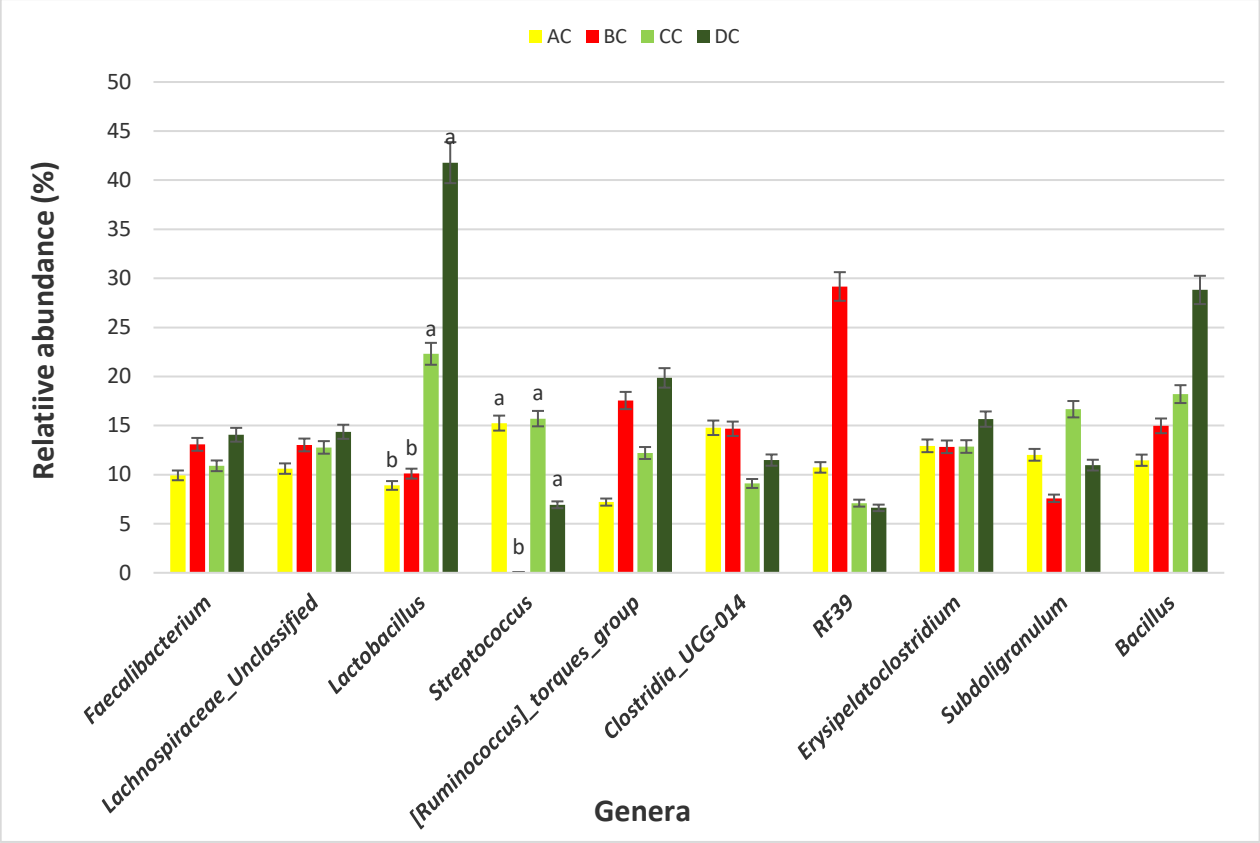


Figure 4.8. Percentage relative abundance of the top 10 most abundant bacteria genera in the ceca of *SE*-LPS-challenged broiler chickens fed 4 different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract. Challenge groups: U = group of birds that were not challenged with *SE*-LPS, C = group of birds that were challenged with *SE*-LPS.

Note: Genera without a mean separation have their p-value greater than 0.05

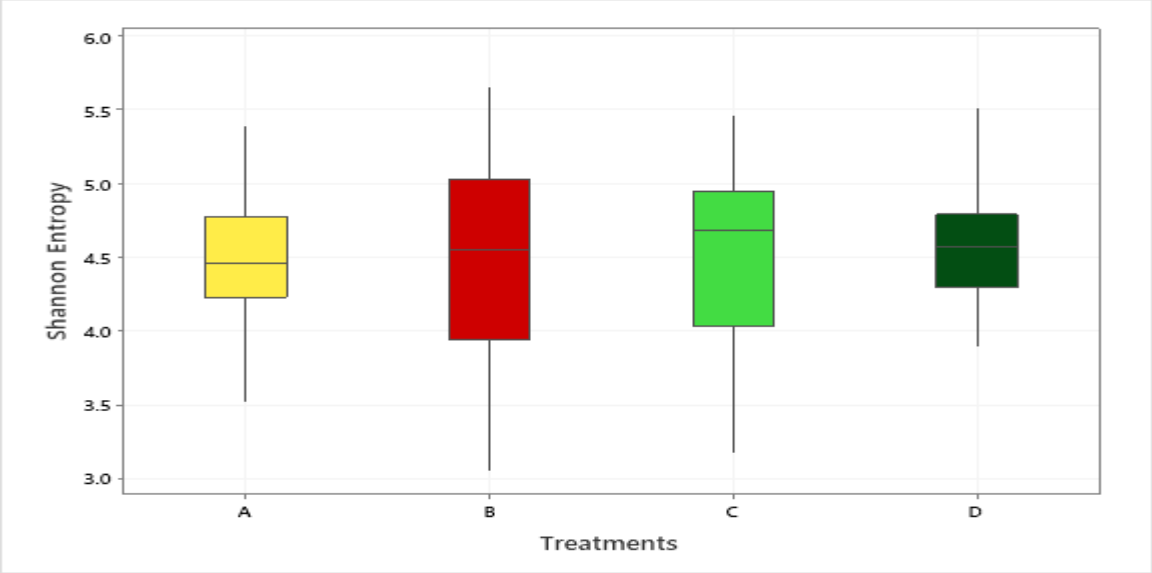


Figure 4.9. Box-and-whisker plot showing non-significant differences in the Shannon entropy (Alpha diversity) ($p>0.05$). Ceca content was collected from 21-day-old broiler chickens fed four different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

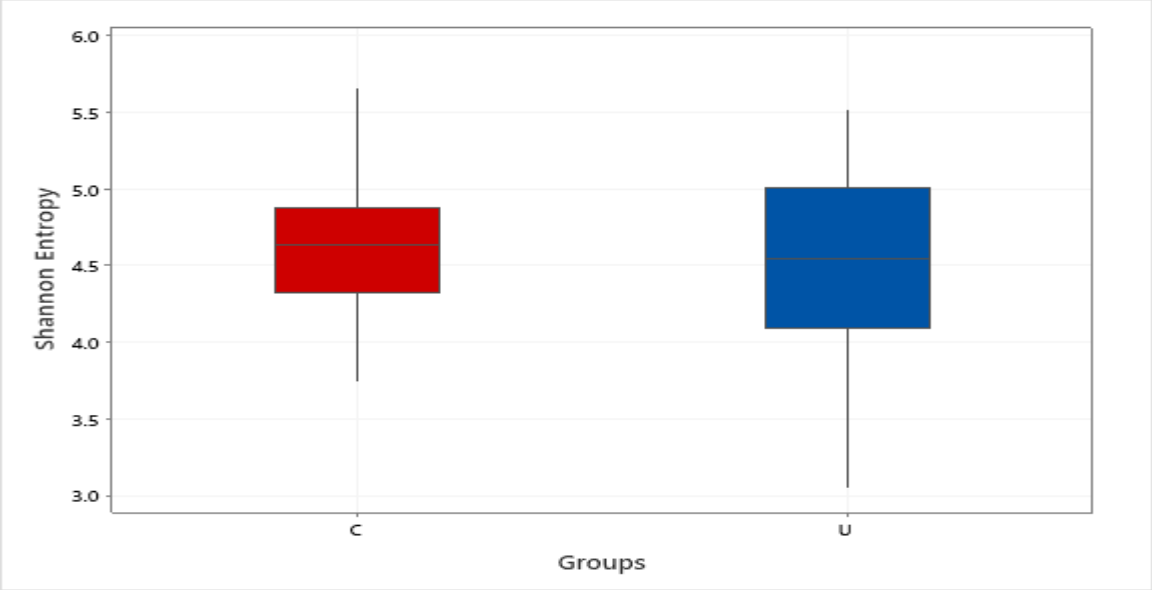


Figure 4.10. Box-and-whisker plot showing non-significant differences in the Shannon entropy (Alpha diversity) ($p>0.05$). Ceca content was collected from 21-day-old broiler chickens challenged with *SE*-LPS and fed four different dietary treatments. Treatment: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

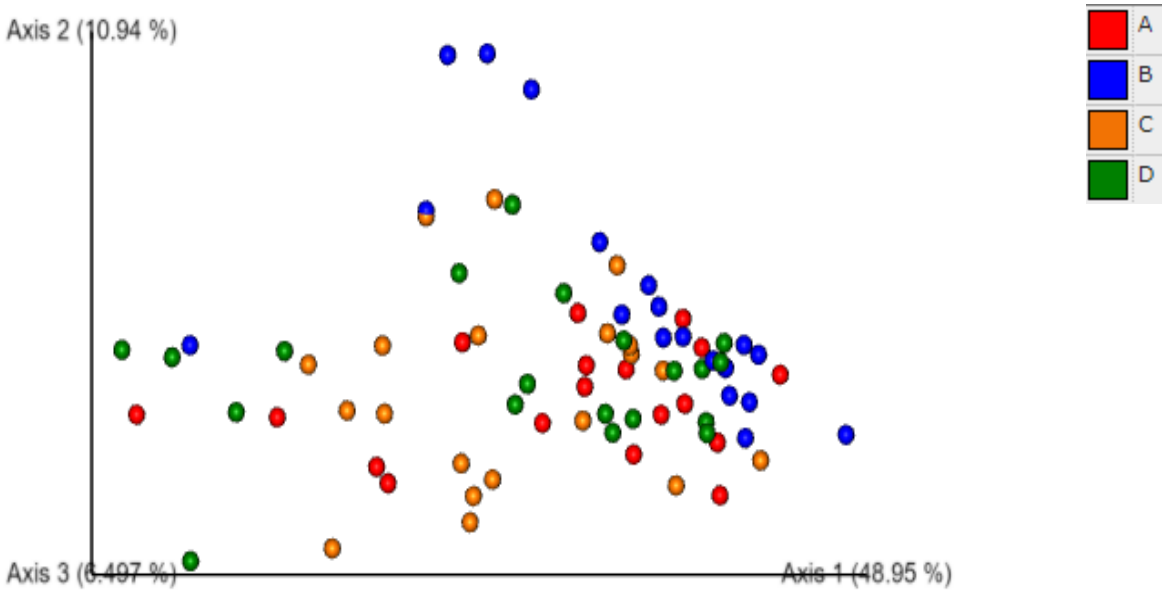


Figure 4.11. Multivariance analysis determined differences in beta-diversity among treatments. Treatment groups: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

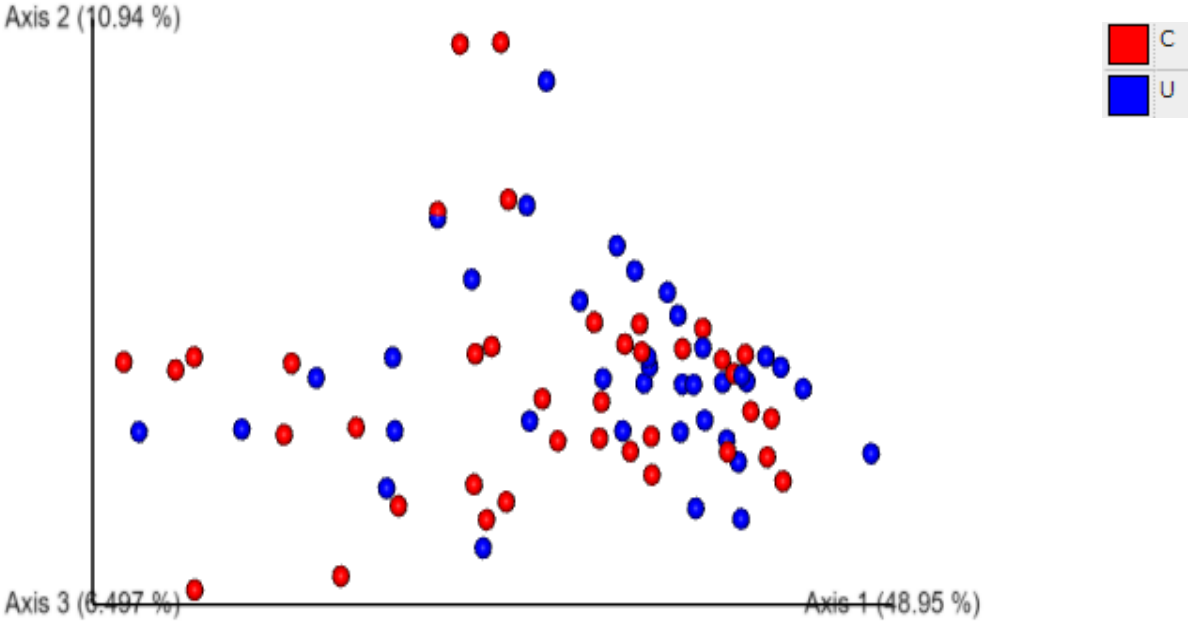


Figure 4.12. Multivariance analysis determined differences in beta-diversity between the challenge groups. Challenge groups: U = group of birds that were not challenged with *SE*-LPS, C = group of birds that were challenged with *SE*-LPS.

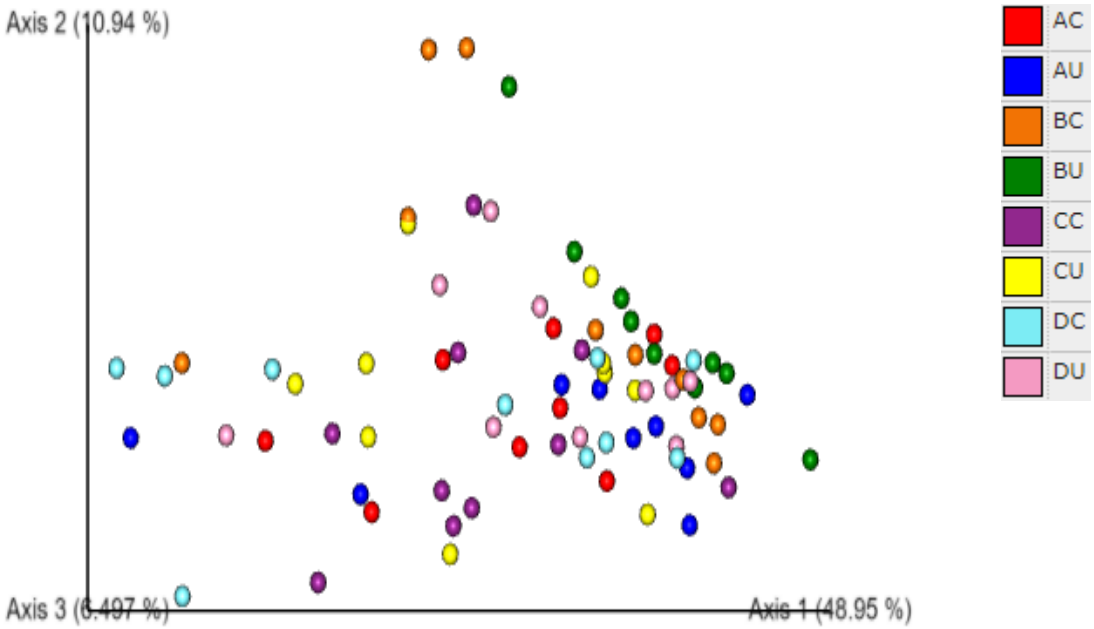


Figure 4.13. Multivariance analysis determined differences in beta-diversity among treatments and groups. Treatment groups: A = Negative control, B = Antibiotic (bacitracin methylene disalicylate) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract. Challenge groups: U = group of birds that were not challenged with *SE*-LPS, C = group of birds that were challenged with *SE*-LPS.

4.4 Discussion

Phenolic compounds are known for their array of beneficial bioactivities, including antioxidant and selective antimicrobial capacities. Red osier dogwood extracts have been reported to contain a high amount of phenolic compounds of about 220 mg gallic acid equivalents g^{-1} dry weight (Isaak et al., 2013). In our study, the TPC of diets was observed to increase with an increasing amount of ROD extract inclusion. The TPC in the ROD extract used in the current study was 238.81 mg gallic acid equivalent (GAE)/g, with gallic acid and quercetin being the most prevalent phenolic compounds. The combination of gallic acid and quercetin has commendable health benefits. Both polyphenols have been reported as a potential treatment for colorectal cancer in Wistar rats, as well as, causing an upregulation of bodily antioxidant enzymes in bleomycin-induced pulmonary fibrosis in rats (Mehrzadi et al., 2020; Patil and Killedar, 2021).

In all of the growth response variables evaluated, no interactions were observed; thus, the results presented are discussed with respect to the main effects. The use of antibiotics for the acceleration of growth, improved feed conversion efficiency, and treating diseases has been affirmed and reaffirmed in literature (Sarmah et al., 2006; Mehdi et al., 2018; Shang et al., 2020). In the current study, AFI, AWG, FCR, and mortality were similar among broiler chickens fed dietary 0.3% and 0.5% ROD extract on d 7 compared to the antibiotic and control birds. This is similar to the findings of Mogire et al. (2021), who reported that the dietary inclusion of 0.1% and 0.3% ROD extract did not influence the growth performance of broiler chickens but were favorable to birds fed avilamycin diet. Furthermore, throughout the entire experimental period, no treatment effect was observed on the growth response of broiler chickens challenged with *SE*-LPS. Despite the higher concentration of *SE*-LPS injected intraperitoneally at 2 mg/kg, Shang et al. (2015) reported a similar growth performance of broiler chickens even in the presence of dietary antibiotics. According to Xie et al. (2000), clinical signs, including reduced feed intake, water intake, body weight, etc., were associated with broiler chickens receiving 5 mg of *Salmonella* Thyphimurium LPS per kg of BW. In addition, Rauber et al. (2014) and Guaiume (2005) also obtained a similar growth performance in broiler chickens injected intraperitoneally with 200 or 400 µg *E. coli* LPS. This suggests that the dose of *SE*-LPS used in the current study is within the maximum tolerable limit. Unlike Mogire et al. (2021), on d 14, 21, and at the overall basis, both 0.3% and 0.5% inclusion levels of ROD extract were found to marginally improve AWG of unchallenged birds compared to the same group of birds receiving antibiotics. This is attributable to the impact of the gallic acid and quercetin which are richly available in ROD extract. Samuel et al. (2017) and Zhang and Kim (2020) reported that gallic acid and quercetin, respectively, enhanced the growth performance of broiler chickens. The challenge model significantly affected AWG on d 14 and FCR on d 21 and was found to be improved among the unchallenged birds compared to the

challenged group. This is in line with the findings of Yang et al. (2008) and Hu et al. (2011), where reduced daily gain was reported in broiler chickens exposed to 1 mg/kg *SE*-LPS and 0.5 mg/kg *E.coli* LPS, respectively.

There was neither dietary treatment nor challenge model effect on the duodenal and jejunal VH, VW, CD, and VH:CD and ileal VH and VW. The non-significant effect of ROD extract on the duodenal morphology is in line with the findings of Mogire et al. (2021). However, unlike our findings, Mogire et al. (2021) reported that 0.1% and 0.3% ROD extracts significantly increased VH:CD in the jejunum compared to the birds receiving antibiotics. In contrast to the antibiotic and control treatments, dietary supplementation of both 0.3% and 0.5% ROD extract significantly deepened ileal CD in the challenged group of birds, whereas ileal VH:CD was significantly highest among the unchallenged birds fed 0.3% ROD extract. In nursery pigs challenged with *E. coli* K88+, Jayaraman et al. (2018) reported that ROD extract supplementation increased VH:CD and reduced CD in the ileum. The inconsistent impact of ROD extract on gut morphology might be due to the absence or presence of different challenge models, animal species, and ROD inclusion rates as reported in most literature. The population and diversity of gut microbiota have been reported to increase down the GIT (Ursell et al. 2012); suggesting a higher abundance of bacteria population in the ileum compared to the duodenum and jejunum. An increase in VH and a decrease in CD are considered desirable indicators for large surface area for absorption and improved gut morphology; however, deeper CD could also be considered a desirable trait as it permits renewal of the villus epithelia in response to inflammation caused by the pathogens (Yason et al., 1987; Adeleye et al., 2018) or their metabolites. This suggests that the increased ileal CD among the *SE*-LPS challenged broiler chickens receiving ROD extracts could be a result of the ameliorative mechanism of ROD extract in rejuvenating the ileal gut architecture.

Blood contains important biomarkers that could be used in the assessment of the physiological and health status of animals. It is notable that information on the effects of dietary ROD extract on the blood biochemistry of broiler chickens does not exist in the literature. Plasma proteins, including ALB and GLB, are produced in the liver and perform complex physiological roles. A decrease in plasma ALB is purportedly associated with the incidence of malnutrition and renal impairment, while an increase in GLB level is related to chronic inflammation (Li et al., 2018). In the current study, GLB and A:G were higher and lower among the unchallenged birds that consumed 0.3% ROD extract compared to the antibiotic-treated birds. The effect of plant extracts on the blood parameters of broiler chickens is sometimes controversial in the literature. In some studies, plant extracts were reported to reduce serum GLB (Soltan et al., 2008) and increase ALB and A:G in infection-free broiler chickens (Soltan et al., 2008; Sharma et al., 2015). In contrast, an increase in serum GLB concentration was also reported in unchallenged broiler chickens fed dietary plant extract (Ismail et al., 2020). Since the 0.3% and 0.5% ROD extract did not negatively affect liver enzymes, growth parameters, and gut morphology compared to the antibiotic and control treatments, the reduced ALB and increased GLB cannot be associated with malnutrition or chronic inflammation in birds. Besides this, the GLB reported in our study is within normal range of 5 – 18.0 g/L reported by Thrall (2007). This suggests that ROD extract supplementation did not adversely affect the plasma biochemical indices of broiler chickens. Comparing the challenge model, calcium, iron, TP, CHOL, ALB, GLB, and GGT were significantly higher among the challenged birds, while lipase and CK were significantly higher among the unchallenged group. Elevated calcium, iron, TP, CHOL, GLB, GGT, lipase, and CK have been associated with immune-related diseases, and kidney or intestinal disease in animals (Williams et al., 2021). While calcium ions are known to play a key role in the regulation of the circulatory system and cell-to-cell communication, their increased accumulation is noteworthily associated with hemolytic anaemic diseases

including sickle cell, ß-thalassemia, and familial phosphofructokinase deficiency (Stafford et al. 2017). This further suggests that elevated calcium ions in the body could impair glycolytic ATP formation – an essentially important cellular energy in the body. Xie et al. (2000) demonstrated that total plasma protein concentration increases between 24 – 48 hours after LPS challenge. Furthermore, in support of our findings, Sharma et al. (2015) reported an increase in GLB when broiler chickens were challenged with *E. coli*.

There were no interaction effects and challenge model effects on TAP and SOD. Like the antibiotic and control treatments, dietary supplementation of ROD extract did not influence the serum TAP and SOD of broiler chickens challenged or unchallenged with *SE*-LPS. Antimicrobial growth promoters have been used to improve the antioxidant status of weaned pigs (Koo et al., 2020). The result obtained in the current study suggests that supplementation of ROD extract-maintained TAP and SOD in the same capacity of antibiotics. Contrary to our findings, there was a significant increase in the serum SOD fed 4% ROD plant product compared to weaned piglets fed antibiotics (Amarakoon et al., 2019; Koo et al., 2020). This could be due to the difference in the ROD plant product and its higher inclusion level at 4% used in the studies. Furthermore, ROD supplementation at 0.3% and 0.5% did not affect serum IgY and IgM of broiler chickens; however, IgM was significantly higher among the challenged group of birds compared to the unchallenged. According to Larsson et al. (1993) and Rathnapraba et al. (2007), serum IgM is the first antibody produced during the first week post-infection. Thus, the higher IgM among the *SE*-LPS challenged chickens is not unexpected given the presence of *SE*-LPS - an immune stressor.

The gut microbiota performs an indispensable role in influencing the health and performance of poultry birds. The gut microbiota of poultry is mostly reported to be dominated by bacteria species from the phylum Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria (Ali et al., 2021), and Verrucomicrobia; however, Firmicutes

and Bacteroidetes are the largest phyla (Almeida et al., 2019; Qin et al., 2010; Forster et al., 2019). Given the novelty of the ROD extract, their impact on the intestinal microbiota of broiler chickens is very scanty. Regardless of the challenge model, the cecal microbiota of broiler chickens fed the dietary treatments did not significantly influence the bacteria phyla, namely, Firmicutes, Actinobacteria, and Proteobacteria and were dominated by Firmicutes. It is surprising that Bacteroidetes were not detected in the cecal content of broiler chickens in our study. However, gut microbiota studies with no Bacteroidetes have been reported in the literature, particularly with the use of broad-spectrum antibiotics (Carvalho et al., 2012; Dubourg et al., 2013; Stanley et al., 2013; Oladokun et al., 2021). The hypervariable region V4-V5 targeted using the 16S rRNA gene sequencing could be responsible for the absence of phylum Bacteroidetes in our study. Bukin et al. (2019) demonstrated that hypervariable regions play a significant role in the precision and resolution for taxa, particularly among genera and species, with V2-V3 reported to have the highest resolution. In another study by García-López et al. (2020), a more diverse gut microbiota is detectable using V3-V4 hypervariable region. The hypervariable region V4-V5 region has reportedly been used for a wider microbial domain, including archaea and bacteria domains (Fadeev et al., 2021). For future studies, we would recommend either V2-V3 or V3-V4 for chicken's gut microbiota analysis because they are more specific for bacteria alone. With or without the *SE*-LPS challenge, 0.3% and 0.5% ROD extract conferred a more beneficial effect by significantly increasing the abundance of genera *Lactobacillus* and *Streptococcus* compared to the antibiotic treatment. In contrast to antibiotic use, plant materials rich in polyphenols have been consistently shown to increase the population of gut-friendly *Lactobacillus spp* (Giannenas et al., 2018; Abolfathi et al., 2019; Erinle et al., 2022a). Crisol-Martínez et al. (2017) reported that the use of bacitracin diminishes the abundance of Lactobacilli in the gut. *Lactobacillus* plays a beneficial role in the gut by producing lactate, maintaining intestinal barrier function, particularly in

immune-related diseased conditions and regulating the expression of heat shock proteins and tight junction proteins (Liu et al., 2015; Honda and Littman, 2016). Like most plant extracts, ROD extract exerted a better gut improvement influence than antibiotics. Although ROD extract increased Streptococcus in the chickens, compared to antibiotic treatment, however, it was similar to the control-fed birds. Some *Streptococcus spp* are known for their pathogenic virulence; however, many streptococcal species, including *S. salivarius, S. dentisani, S. oligofermentans*, and *S.* A12, have been reported to possess antimicrobial properties by producing bacteriocins, proteases, or hydrogen peroxide (Huang et al., 2016; Llena et al., 2019; Ferrer et al., 2020). This suggests that the higher abundance of genus Streptococcus does not always imply opportunistic pathogens. Dietary supplementation of 0.3% and 0.5% ROD extract did not influence species richness and diversity in the gut ecosystem. The dietary treatments had significantly different beta diversity of the microbial population. This could be explained by the consistently higher abundance of *Lactobacillus* and *Streptococcus* in the ROD extract and control treatment compared to the antibiotic treatment. Orlewska et al. (2018a; b) reported an altered diversity in soil microbial communities following antibiotic application. Despite the significant effect of ROD extract on the cecal microbiota, there was no corresponding effect on the SCFA profile at the cecum. This is not unexpected as the number of total eubacteria was not altered by the dietary treatments and *SE*-LPS challenge. Erinle et al. (2022b) speculated that uniform copies of total eubacteria in cecal content of birds often give rise to unaltered cecal SCFA concentrations. However, there was a significant interaction effect between treatment and challenge model on total eubacteria, which did not, in turn, influence the concentration of SCFA profile of the birds.

The relative weight of immune organs was not affected by the dietary supplementation of ROD extract. This corroborates the report of Mogire et al. (2021), where there was no difference in the relative weight of liver and spleen of broiler chickens fed either ROD

extract, antibiotics, or control diets. The spleen is one of the most critical immune organs in poultry species. Immune cells in the spleen were reported to help in the fight against pathogenic microbes through specific immune response mechanisms (Dailey, 2002). In another study involving 500 µg/mL *Salmonella Typhimurium* LPS challenge, (Rauber et al., 2014) reported that the relative weight of liver remained unaffected. Comparing unchallenged versus challenged groups, the relative spleen weight was observed to be significantly higher among birds in the latter compared to the former. According to Ahiwe et al. (2019), increased spleen weight was reported in broiler chickens challenged with *Salmonella* Typhimurium LPS. In the presence of LPS antigen, there is a high propensity of hyperplasia, which causes inflammation by activating inflammatory cells. An increase in the size of immune organs could be associated with increased immune activities to counteract the effect of stressors, including pathogens or their metabolites. Thus, the increased spleen size among the challenged group of birds could be a part of the birds' innate defense mechanism against the *SE*-LPS.

4.5 Conclusions

Based on the results obtained, the *SE*-LPS depressed AWG and FCR during d 14 and 21, respectively. However, dietary supplementation of ROD extract at 0.3% and 0.5% maintained the growth performance of broiler chicken throughout the production phase in the equal capacity of the antibiotic, regardless of the *SE*-LPS challenge. Additionally, CD and VH:CD of the birds were improved in the ileum when both levels of ROD extract were supplemented into broiler chicken's diets compared to the antibiotics-fed birds, however, it was best at the 0.3% inclusion level. Furthermore, dietary supplementation of 0.3% and 0.5% ROD extract improved in the *Lactobacillus* while the blood biochemical indices, cecal SCFA concentrations, and innate antioxidant and immune systems were not compromised. This study, therefore, suggests that dietary supplementation of ROD extract

at 0.3% or 0.5% could be a potential consideration for replacing antibiotics in broiler chicken nutrition.

CHAPTER 5: Red osier dogwood extract versus Trimethoprim-sulfamethoxazole (Part 1). Effects on the growth performance, blood parameters, gut histomorphometry, and *Salmonella* excretion of broiler chickens orally challenged with *Salmonella* Enteritidis

5. Abstract

The poultry industry has not been spared from the prevalent incidence of diseases caused by invasive pathogens, especially *Salmonella*. Due to the pressing need to identify a suitable antibiotic alternative for use in poultry production, this study investigated the efficacy of red osier dogwood (**ROD**) extract on the growth, blood parameters, gut morphology and *Salmonella* excretion in broiler chickens orally challenged with *Salmonella* Enteritidis. A 4×2 factorial experiment was conducted based on two main factors, namely dietary treatments and *Salmonella* Enteritidis (**SE**) challenge. A total of 404 one-day-old male Ross broiler chicks were randomly assigned to four dietary treatments; 1)Negative control (**NC**), 2)NC+0.075ppm of Trimethoprim-sulfadiazine (**TMP/SDZ**) /kg of diet, 3)NC+0.3%ROD extract, and 4)NC+0.5%ROD extract. The absence of SE in the fecal samples obtained from chick delivery boxes was confirmed on d 0. On d 1, half of the birds were orally gavaged with 0.5mL of phosphate-buffered saline each (non-infected group) and the remaining with 0.5mL of 3.1×10^5CFU/mL SE (infected group) in all treatment groups. Dietary treatments were randomly assigned to eight replicate cages at six birds/cage. On 1, 5, 12, and 18 day post-infection (**DPI**), cloacal fecal samples were collected on the six birds/cage to assess SE excretion. Average weight gain (**AWG**), average feed intake (**AFI**), feed conversion ratio (**FCR**), and mortality were determined weekly. On d 21, ten chickens/treatment were euthanized to perform hematology, gut histomorphometry, serum immunoglobulins G and M (**IgG** and **IgM**) and superoxide dismutase measurements. Both ROD extract levels did

not affect (P>0.05) growth performance; however, the SE-infected birds showed increased (P<0.05) AFI and FCR throughout the experimental period. Regardless of the SE-infection, both ROD extract levels improved (P<0.05) duodenal villus height (**VH**), crypt depth (**CD**), and VH:CD compared to other treatments. Ileal villus width and CD of the noninfected and infected birds, were improved (P<0.05) by both ROD extract levels; however, only 0.3%ROD extract increased VH:CD compared to other treatments. The SE-infected birds showed lower (P<0.05) lymphocytes (**L**) but increased (P<0.05) heterophils (**H**), H:L, and monocytes (**MON**). Both ROD extract levels did not affect (P>0.05) white blood cell differential, while dietary 0.3%ROD extract increased (P<0.05) leukocytes and MON of the infected birds. Similar to the control, the IgM of SE-infected birds was highest (P<0.05) in birds fed 0.3%ROD extract compared to other treatments. Conclusively, both ROD extract levels sustained growth performance indices, duodenal histomorphology, and body defence against SE infection in broiler chickens; however, the 0.3%ROD extract was better.

Keywords: red osier dogwood extract, antibiotic-replacement, *Salmonella* Enteritidis, oral gavage, white blood cell differentials

5.1 Introduction

Salmonella – a gram-negative, facultative anaerobic bacteria, is unarguably one of the most economically important causes of foodborne diseases following the consumption of *Salmonella*-contaminated food materials. In Canada, human non-typhoidal salmonellosis is notifiable, with an average case of approximately 6,500 reported annually between 2009 to 2013 (Public Health Agency of Canada, 2008; 2009; Government of Canada, 2007; 2016). Given the available statistics, the United States seems to be most impacted by Salmonellosis incidence and has recorded approximately 1.4 million gastroenteritis cases, 26,500

hospitalizations, and 420 deaths caused by the non-typhoidal *Salmonella* (Cao, 2014; Centers for Disease Control and Prevention (CDC), 2018); and as a result, it has caused an annual loss of $1.4 – 3.0 billion (McGruder, 1995; WHO, 2018). On a global scale, non-typhoidal *Salmonella* causes a yearly estimated illness and death of over 93.8 million and 39,000 – 303,000 deaths, respectively (Majowicz et al., 2010); as a result, the severity of these bacterial species cannot be treated with contempt. While there are over 2,600 unique non-typhoidal *Salmonella* serovars, *Salmonella enterica* ssp. Enteritidis is the most typical culprit among human and nonhuman isolates in Europe, Africa, Latin America, and the Caribbean (Galanis et al., 2006; Afshari et al., 2018; Castro-Vargas et al., 2020).

It is notable that the epidemiological prevalence of *Salmonella* Enteritidis (*SE*) has been closely associated with edible poultry products and, consequently, has exclusively been reported to contribute to the increased incidence of salmonellosis (Antunes et al., 2016; Centers for Disease Control and Prevention, 2020; Boubendir et al., 2021). The menace of *Salmonella* infections in poultry is not limited to the host, as vertical transmission of the bacteria from the maternal into their eggs is (Berchieri Jr et al., 2001). For decades, antibiotics, including chloramphenicol, neomycin, ampicillin, ciprofloxacin, ceftriaxone, fluoroquinolones, and trimethoprim/sulfamethoxazole (TMP/SMX) have been heavily relied upon in the combat of salmonellosis in animals. Despite the remarkable collaborative efforts, including critical biosecurity measures, medications, and vaccination, to control *SE* infection in poultry and prevent contamination of poultry products, the poultry industry is still constantly embattled by antibiotic-resistant *SE*. According to the Centre for Disease Control (2020) and White et al. (2001), *Salmonella* strains resistant to medically-important antibiotics, including ceftriaxone, ciprofloxacin, and ceftiofur, have been isolated in chickens and turkeys. Thus, identifying a more suitable antibiotic alternative has become a

desirable goal to address animal health and improve economic return for poultry farmers, and assure food safety concerns for the public.

Some phytogenic additives have undoubtedly proven worthy antibiotic substitutes due to their high concentrations of diverse polyphenols. Among the identified numerous phytogenic additives, red osier dogwood (ROD; *Cornus stolonifera*) has been reckoned to contain a high concentration of total polyphenol, with ranges between 220 – 234 mg gallic acid equivalence (GAE) g-1 dry weight (Isaak et al., 2013; Scales, 2015; Erinle et al., 2022b), values higher than the 40.27 mg GAE g-1 dry weight obtained in an olive plant (Isaak et al. 2013). In addition, ROD extract boasts of a considerable antioxidant capacity given its higher oxygen reactive absorbance capacity when compared to that of methanol extract of tea, parsley, basil, and olive leaves (Isaak et al., 2013). Some virulent pathogens like *Salmonella* when invading gut epithelial tissue and barrier provoke inflammatory cytokines production (Ferenczi et al., 2016; Song et al., 2020; Thiam et al., 2021). *In vitro* studies by Jiang et al. (2019) and Yang et al. (2019) reported that polyphenols in ROD extracts exert their antioxidant prowess and prevent pro-inflammatory responses in Caco-2 cells by repressing gene expression of inflammatory cytokines.

Incorporation of ROD plant material into the diets of *Escherichia coli*-challenged pigs (Jayaraman et al., 2018; Koo et al., 2021) was reported to improve gut morphology, which has been associated with optimized nutrient digestion and absorption. Furthermore, dietary supplementation of ROD was also reported to suppress blood thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) in weaned piglets infected with *E. coli* (Amarakoon, 2017), thereby reducing bacteria-induced oxidative stress. In the absence of antibiotics, dietary supplementation of ROD extract has been demonstrated to sustain growth performance and intestinal health of unchallenged broiler chickens (Mogire et al., 2021) and

when challenged with SE lipopolysaccharide (LPS) (Erinle et al., 2022b). Because the two studies lacked an actual Salmonella infection challenge it was deemed necessary to investigate the impact of ROD in poultry birds under disease-challenged conditions. In addition to the LPS, pathogenic bacteria produce several highly toxic exotoxins to attack their host. Until now, no *in vivo* study has been conducted to examine the effects of dietary ROD extract as a potential antibiotic alternative on broiler chickens infected with *SE*. This study investigates the effects of ROD extract on the growth performance, blood parameters, gut morphology, and *Salmonella* excretion of broiler chickens challenged with *Salmonella* Enteritidis.

5.2 Material and Methods

The experimental protocol was approved by the University of Montreal Animal Care and Use Committee (Project 20-Rech-2063). The birds were handled following the guidelines established by the Canadian Council on Animal Care (2009).

5.2.1 Birds and housing

A total of 404, less than one-day-old, male Ross 308 broiler chicks were obtained from a commercial hatchery in Quebec. They were raised in a 4Tier Poultry Super Brooder (from Alternate design website): (38” Wide × 31” Deep × 15” high for 21 d. Upon arrival, the mixed-sex birds were weighed into a group of 6 birds and allocated to cages in two different rooms to house the noninfected and about-to-be infected birds separately. The room temperature was monitored daily and was gradually reduced from 32 to 24 ^{0}C from d 0 to 21. The lighting program was set to produce 24 hours, 6 hours, and 10 hours of light during d 1 – 3, d 4 – 14, and d 15 – 21, respectively, and illumination was gradually reduced from 20 1x on d 0 to 5 1x on d 21.

5.2.2 Preparation of *Salmonella* Enteritidis inoculum

The *Salmonella* Enteritidis (SHY 04 1540) used in the current study was isolated in 2004 from a clinical case and used in previous infection studies by Dr. Martine Boulianne's Avicole Research Laboratory, University of Montreal, Quebec, Canada. The strain was grown overnight on blood agar at 37 ^{0}C. Three colonies of *SE* strain were transferred into 10 mL of Luria-Bertani (LB) broth culture. Following this, 1 mL from the *SE*-LB broth mixture was added to another 100 mL of pre-warmed LB and shook at 150 rpm in an incubator at 37 ^{0}C for about 3 hours. The inoculum concentration was periodically confirmed using a spectrophotometer at a wavelength 600 nm. The inoculum was further serially diluted to achieve the appropriate bacteria coliform-forming unit (CFU). The SE concentration in this study was 3.1×10^5 CFU/mL.

5.2.3 Diets and experimental design

The ROD extract used in this study was obtained from Red Dogwood Enterprise Ltd., Swan River, Manitoba. The birds were randomly assigned to eight treatment groups containing eight replicate pens of 6 birds each. The experiment was designed as a 4 × 2 factorial arrangement based on two main factors, as shown in Table 5.1. The main factors were: 1) 4 dietary treatments: corn-wheat-soybean meal-based diet negative control (**NC**), negative control with 0.075 ppm of Trimethoprim-sulfadiazine antibiotic (**TMP/SDZ**) per kilogram of diet; and negative control supplemented with 0.3% or 0.5% ROD extract and 2) 2 infection model groups: the noninfected groups (**N**) were challenged orally with 0.5 mL of sterile 1×phosphate buffered saline (**PBS**) per bird (AVL82762, HyClone Laboratories, Inc., Logan, UT), and the infected groups (**I**) were challenged orally with 0.5 mL/bird of 3.1×10^5 CFU/mL of *SE*. The individual oral gavage challenge was done on d 1. The basal diet was formulated as isocaloric and isonitrogenous to meet the nutrient requirements of broiler

chickens as recommended by NRC (1994). The gross and proximate compositions of the experimental diets are presented in Table 5.2. The experimental diets containing TMP/SDZ and ROD extract were mixed from the negative control diets, and as a result, only the nutrient content of the negative control diet was reported. The proximate composition of the experimental diets was determined following the procedure of AOAC (1990). The polyphenols profile of ROD extract and the total polyphenols in the starter and grower ROD and control treatments have been reported in our previous study (Erinle et al., 2022b).

Table 5.1. Experimental design

	Challenge model	
Dietary treatment (number of replicates; n)	**Unchallenged (U)**	**Challenged (C)**
a) Negative control	1. Basal (NC) + PBS (n=8)	2. NC + *SE* (n=8)
b) + Antibiotics	3. NC + 0.075ppm TMP/SDZ + PBS (n=8)	4. NC + 0.075ppm TMP/SDZ + *SE* (n=8)
c) + 0.3% ROD extract	5. NC + 0.3% ROD + PBS (n=8)	6. NC + 0.3% ROD + *SE* (n=8)
d) +0.5% ROD extract	7. NC + 0.5% ROD + PBS (n=8)	8. NC + 0.5% ROD + *SE* (n=8)

[1]Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract
N = Non-infected group; I = Infected group; PBS = Phosphate buffered saline; *SE* = *Salmonella* Enteritidis.

Table 5.2. Gross and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated)

Ingredients	Starter phase (1-14 days)				Grower phase (14-21 days)			
	Basal	**TMP/ SDZ**	**0.3% ROD**	**0.5% ROD**	**Basal**	**TMP/ SDZ**	**0.3% ROD**	**0.5% ROD**
Corn	42.37	42.27	41.83	41.48	45.99	45.65	45.22	44.86
Soybean meal	40.13	40.15	40.17	40.2	36.15	36.21	36.24	36.26
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Vegetable oil	2.82	2.85	3.01	3.14	3.74	3.85	4.01	4.14
Dicalcium phosphate	1.57	1.57	1.57	1.57	1.39	1.39	1.39	1.39
Limestone	1.45	1.45	1.45	1.45	1.32	1.32	1.32	1.32
DL Methionine Premix [W]	0.61	0.61	0.61	0.61	0.53	0.53	0.53	0.53
Starter Vitamin/Mineral premix [X]	0.50	0.50	0.50	0.50	-	-	-	-
Grower/Finisher Vitamin/Mineral premix [Y]	-	-	-	-	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40	0.38	0.38	0.38	0.38
Red dogwood extract	-	-	0.30	0.50	-	-	0.30	0.50
TMP/SDZ (g) [Z]	-	75.0	-	-	-	75.0	-	-
Lysine HCL	0.16	0.16	0.16	0.16	-	0.12	0.12	0.12
Calculated Analysis								
Crude Protein	23	23	23	23	21.5	21.5	21.5	21.5
Metabolizable Energy (kcal kg^{-1})	3,000	3,000	3,000	3,000	3,100	3,100	3,100	3,100
Calcium	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87
Available Phosphorus	0.48	0.48	0.48	0.48	0.44	0.44	0.44	0.44
Digestible Lysine	1.28	1.28	1.28	1.28	1.15	1.15	1.15	1.15
Digestible Methionine + Cystine	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87
Sodium	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18
Analyzed Composition								
Crude Protein	24.1				22.1			
Calcium	0.81				0.75			

Ingredients	Starter phase (1-14 days)				Grower phase (14-21 days)			
	Basal	TMP/ SDZ	0.3% ROD	0.5% ROD	Basal	TMP/ SDZ	0.3% ROD	0.5% ROD
Total Phosphorus	0.68				0.62			
Sodium	0.15				0.12			
Crude Fat	3.22				4.40			

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[W] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[X] 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.

[Y] 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.

[Z] Trimethoprim-sulfadiazine (providing 0.75 g/kg mixed feed).

5.2.4 Fecal excretion of *Salmonella* Enteritidis

To confirm for the absence of *SE* in purchased day-old chicks four papers containing fecal samples collected from chick delivery boxes bottom were put into four separate whirl-paks to which was added a LB broth (Fisher BP1426-500) at a 1:10 ratio depending on the weight of paper+fecal samples. LB-enriched fecal samples were incubated overnight at 37 ^{0}C. 100 µL of LB-enriched fecal samples were plated onto xylose-lysine-tergitol 4 (XLT4)agar (Item No:223420, Bacto BBL Difco Microbiology, USA) then incubated for 24 hrs at 37 ^{0}C. To assess excreted *Salmonella*, cloacal swabs were collected individually on all birds (n= 404) per cage at 1, 5, 12, and 18 day post-infection (**DPI**) and diluted in 6 ml of buffered peptone water before streaking onto XLT4 agar.

5.2.5 Growth performance

Individual body weight and feed intake (on a pen basis) were determined weekly. Feed intake and body weight were used to estimate average feed intake (**AFI**) and average weight gain (**AWG**). Mortality was recorded daily to correct for AFI and feed conversion ratio (**FCR**). Birds that died were necropsied by a poultry pathologist to determine cause of death.

5.2.6 Gut histomorphometry

A 1.5 cm segment at the mid-length of the duodenum, jejunum, and ileum was collected and preserved in 10% buffered formalin (Sigma-Aldrich, St. Louis, MO) for three days. The formalin-preserved intestinal segments were cross-sectioned and placed in cassettes and were then immersed in 4% paraformaldehyde tissue fixation solution and later into paraffin. Each of the cross-sectioned segments was mounted on a glass slide (n = 10 per treatment) and stained with HPS (COLHPS1). The morphological slides were examined under 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**) as described by Shang et al., 2020. The villus height:crypt depth ratio (**VH:CD)** was subsequently estimated.

5.2.7 Hematology, serum immunoglobulins, and superoxide dismutase

On d 21, 80 birds (ten birds from each treatment) were randomly selected for blood sampling and individually weighed Approximately 5 mL of blood was collected from the brachial vein and divided in; (i) EDTA tubes for hematology and (ii) serum tubes for serum immunoglobulins and superoxide dismutase analyses. The hematological parameters include hematocrit (**HCT**), total protein (**TP**), leukocytes (**LEU**), heterophils (**HET**), lymphocytes (**LYM**), HET:LYM (**H:L**), monocytes (**MON**), eosinophils (**EP**), and basophils (**BP**) were assayed at the Centre de diagnostic veterinaire de l'Université de Montréal. Samples for serum immunoglobulins G (**IgG**) and immunoglobulin M (**IgM**) were assayed using an enzyme-link immunosorbent assay (ELISA) kit from Bethyl Laboratories Inc. (catalogue number E33-104-200218 and E33-102-180410, respectively) following manufacturer protocols. Superoxide dismutase (**SOD**) was analyzed using a SOD assay kit (Item Number 706002; Cayman Chemical, USA), following the manufacturer's protocol.

5.2.8 Statistical analysis

Datasets were subjected to 4 × 2 factorial analysis of variance (ANOVA) using the General Linear Model of Minitab LLC, (2019) software. Error terms of individual response variables were confirmed for the validity of three basic assumptions, including normality, constant variance, and independence. A normal probability plot of residuals was done to verify the normality of error terms using the Anderson Darling test in the same statistical package. Where error terms of datasets were found to be non-normal or non-constant, the respective original datasets were subjected to various transformation functions. If the normality and homoscedasticity of error terms were still violated upon transformations, then such datasets

were analyzed using the Kruskal-Wallis test. Following ANOVA, differences between significant means were separated using Tukey's honest significant difference (**HSD**) test and Mann Whitney for the parametric and non-parametric datasets, respectively, in the same statistical package. Analyzed datasets were presented as means, standard error of the mean (**SEM**), and probability values. Statistically different values were considered at $P<0.05$.

5.3 Results

5.3.1 Fecal excretion of *Salmonella* Enteritidis

The effect of ROD extract on the fecal excretion of SE is shown in Table 5.3. There was no presence of *Salmonella* Enteritidis in all the cloacal swab samples collected on d 0, 1, 5, 12, and 18 DPI.

Table 5.3. Effect of ROD extract *Salmonella* count in excreta from SE-infected broiler chickens treated with ROD extract.

		Treatment[1]					Infection model[2]			*P*-value		
Day post-infection	**Infection model[2]**	**Basal**	**TMP/ SDZ**	**0.3% ROD**	**0.5% ROD**	**SEM[3]**	**N**	**I**	**SEM[3]**	**Treatment Effect**	**Model Effect**	**Interaction Effect**
D 1	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000
	I	0.00	0.00	0.00	0.00	0.00						
D 5	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000
	I	0.00	0.00	0.00	0.00	0.00						
D 12	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000
	I	0.00	0.00	0.00	0.00	0.00						
D 18	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000
	I	0.00	0.00	0.00	0.00	0.00						

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[2] N = Non-infected group; I = Infected group

[3] SEM = standard error of the mean.

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

5.3.2 Growth performance

The effect of ROD extract as an antibiotic alternative on the growth performance of broiler chickens infected with or without *SE* is presented in Table 5.4. There were no interactions; thus, the results are reported based on dietary treatments and the challenge model's main effects. Throughout the experimental period, dietary supplementation of 0.3% and 0.5% ROD extract did not affect the growth performance of birds with or without *SE* infection. AFI and AWG at week 1, and AFI and FCR at week 2, 3 and on overall were higher ($p<0.05$) among the infected birds compared to the noninfected group of birds.

Table 5.4. Effect of red osier dogwood extract on growth performance of broiler chickens challenged orally with *Salmonella* Enteritidis.

Week / Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-Value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Model Effect	Interaction Effect
Week 1												
Average feed intake (g/bird)	N	122	122	123	117	1.77	120[b]	125[a]	1.20	0.305	0.025	0.305
	I	127	127	124	122	1.53						
Average weight gain (g/bird)	N	96.8	97.5	102	90.2	2.68	96.5[b]	105[a]	1.96	0.238	0.035	0.238
	I	112	110	98.9	98.5	2.70						
FCR	N	1.26	1.25	1.20	1.28	0.02	1.24	1.19	0.02	0.487	0.103	0.487
	I	1.14	1.16	1.28	1.25	0.02						
Mortality	N	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.793	0.557	-
	I	0.00	0.00	0.00	0.00	0.04						
Week 2												
Average feed intake (g/bird)	N	299	305	305	299	4.29	302[b]	315[a]	3.13	0.252	0.032	0.252
	I	322	329	309	300	4.32						
Average weight gain (g/bird)	N	243	240	248	229	6.09	240	241	4.13	0.167	0.963	0.167
	I	254	259	222	227	5.67						
FCR	N	1.23	1.27	1.23	1.33	0.03	1.25[b]	1.30[a]	0.02	0.393	0.031	0.393
	I	1.25	1.28	1.34	1.27	0.02						
Mortality	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.107	0.154	-
	I	0.00	0.00	0.00	0.00	0.00						

Week / Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-Value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Model Effect	Interaction Effect
Week 3												
Average feed intake (g/bird)	N	539	533	538	509	10.5	530[b]	667[a]	11.0	0.592	<0.001	0.592
	I	641	669	688	669	9.07						
Average weight gain (g/bird)	N	397	377	405	395	9.02	394	374	5.90	0.979	0.102	0.979
	I	370	385	371	369	7.32						
FCR	N	1.37	1.43	1.33	1.29	0.04	1.34[b]	1.74[a]	0.04	0.916	<0.001	0.916
	I	1.77	1.75	1.88	1.84	0.02						
Mortality	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.107	0.154	-
	I	0.00	0.00	0.00	0.00	0.00						
Overall (Week 1-3)												
Average feed intake (g/bird)	N	959	960	965	923	12.9	952[b]	1,106[a]	13.6	0.513	<0.001	0.513
	I	1,090	1,125	1,121	1,091	13.9						
Average weight gain (g/bird)	N	737	715	755	715	15.7	730	719	10.3	0.690	0.588	0.690
	I	736	754	692	694	13.6						
FCR	N	1.31	1.35	1.28	1.29	0.03	1.29[b]	1.50[a]	0.02	0.899	<0.001	0.899
	I	1.50	1.50	1.64	1.59	0.01						
Mortality	N	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.03	0.255	0.644	-
	I	0.00	0.00	0.00	0.00	0.04						

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[2]N = Non-infected group; I = Infected group

[3] SEM = standard error of the mean.

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

5.3.3 Gut morphology

The effect of ROD extract on gut histomorphometry of broiler chickens challenged orally with *SE* is presented in Table 5.5. In the duodenum, VH of noninfected birds was significantly increased ($p<0.05$) among fed 0.3% and 0.5% ROD extract compared to other treatments. However, 0.3% ROD extract increased ($p<0.05$) VH among the infected birds compared to other treatments. Unlike the antibiotic and control treatments, CD in noninfected and infected groups of birds significantly depressed ($p<0.05$) among 0.5% ROD extract treated birds; however, it was marginally improved ($p<0.05$) in the 0.3% ROD extract. Comparing between infection model, duodenal VH and CD were significantly higher ($p<0.05$) among the infected group of birds. Regardless of the challenge, duodenal VH:CD was significantly increased ($p<0.05$) among fed 0.3% and 0.5% ROD extract compared to other treatments. There was a significant interaction effect ($p<0.05$) on VW. In the jejunum, there was neither treatment nor infection model effect on the gut morphology; however, both VH and VW were significantly influenced ($p<0.05$) by the dietary treatment*infection model interaction effect. In the ileum, VH was not significantly influenced ($p>0.05$) by the dietary treatments. In addition, there was a significant interaction effect ($p<0.05$) on VW and CD. Compared to other treatments, VW and CD among the noninfected and infected birds, respectively, were significantly increased ($p<0.05$) among the birds that consumed ROD extract at either 0.3% or 0.5%. VH:CD was increased ($p<0.05$) in birds fed 0.3% ROD extract diet compared to the antibiotic and 0.5% ROD extract treatments, however, the VH:CD was marginally similar to the control treatment.

Table 5.5. Effect of red osier dogwood extract on gut morphology of broiler chickens challenged orally with *Salmonella* Enteritidis.

Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-Value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Mode Effect	Interaction Effect
Duodenum												
Villus height (mm)	N	1.58^{ab}	1.47^{b}	1.69^{a}	1.79^{a}	0.036	1.63^{b}	1.79^{a}	0.034	0.002	0.001	-
	I	1.67^{b}	1.77^{b}	2.08^{a}	1.78^{b}	0.030						
Villus width (mm)	N	0.15^{b}	0.20^{a}	0.14^{b}	0.15^{b}	0.007	0.16	0.17	0.005	0.067	0.204	0.003
	I	0.16	0.17	0.16	0.18	0.004						
Crypt depth (mm)	N	0.19^{ab}	0.21^{a}	0.18^{ab}	0.16^{b}	0.005	0.19^{b}	0.20^{a}	0.005	<0.001	0.036	0.345
	I	0.21^{a}	0.21^{ab}	0.21^{ab}	0.18^{b}	0.010						
VH:CD[4]	N	6.97^{b}	7.26^{b}	10.2^{a}	9.92^{a}	0.398	8.37	8.76	0.346	<0.001	0.346	0.690
	I	8.13^{ab}	7.48^{b}	9.89^{a}	9.95^{a}	0.290						
Jejunum												
Villus height (mm)	N	1.08	0.82	0.76	0.85	0.058	0.90	0.89	0.046	0.728	0.703	0.019
	I	0.86	0.96	0.92	0.82	0.034						
Villus width (mm)	N	0.22	0.18	0.18	0.21	0.011	0.20	0.20	0.008	0.844	0.998	0.001
	I	0.19	0.22	0.22	0.19	0.005						
Crypt depth (mm)	N	0.15	0.14	0.13	0.14	0.004	0.13	0.13	0.004	0.955	0.368	0.282
	I	0.12	0.14	0.14	0.13	0.004						
VH:CD[4]	N	7.49	6.16	6.62	8.30	0.340	7.12	6.95	0.284	0.486	0.661	0.089
	I	7.00	7.13	6.92	6.47	0.228						

Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-Value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Mode Effect	Interaction Effect
Ileum												
Villus height (mm)	N	0.39	0.34	0.38	0.38	0.010	0.37	0.38	0.010	0.165	0.399	0.786
	I	0.38	0.36	0.39	0.41	0.011						
Villus width (mm)	N	0.15^{b}	0.15^{b}	0.17^{ab}	0.18^{a}	0.004	0.16	0.16	0.005	0.042	0.742	0.018
	I	0.15	0.17	0.18	0.14	0.006						
Crypt depth (mm)	N	0.10	0.09	0.09	0.10	0.003	0.09	0.10	0.003	<0.001	0.273	0.047
	I	0.09^{bc}	0.08^{c}	0.11^{ab}	0.10^{a}	0.004						
VH:CD[4]	N	4.19^{ab}	3.98^{b}	5.03^{a}	3.82^{b}	0.144	4.10	4.30	0.139	0.245	0.218	0.098
	I	4.31	4.35	4.22	4.32	0.133						

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[2] N = Non-infected group; I = Infected group

[3] SEM = standard error of the mean.

[4] VH:CD = Villus height : crypt depth ratio

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

5.3.4 Hematology parameters

The effect of ROD extract on hematology, and serum immunoglobulins and superoxide dismutase of broiler chickens infected with *SE* is shown in Table 5.6. The dietary treatments did not significantly affect ($p>0.05$) HCT, TP, HET, LYM, H:L, EP, and BP of birds with or without *SE* challenge. A marginal increase in LEU and MON was observed in the 0.3% ROD infected birds only ($p<0.05$) while LEU and MON were significantly lower among infected birds fed 0.5% ROD extract. Comparing the infection model, HET, H:L, and MON were significantly higher ($p<0.05$) among the infected birds, while LYM was higher ($p<0.05$) among the noninfected birds compared to their counterpart.

Table 5.6. Effect of red osier dogwood extract on differential white blood cell count of broiler chickens challenged orally with *Salmonella* Enteritidis.

Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-Value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Model Effect	Interaction Effect
Hematocrit (L/L)	N	0.26	0.27	0.26	0.25	0.004	0.26	0.26	0.003	0.228	0.896	0.440
	I	0.25	0.27	0.25	0.27	0.005						
Total Protein (g/L)	N	40.0	40.0	37.0	39.6	0.614	39.15	39.65	0.455	0.200	0.604	0.536
	I	40.3	41.4	39.0	37.9	0.688						
Leukocytes ($\times 10^9$ /L)	N	14.6	14.3	14.8	13.0	0.832	14.44	15.54	0.775	0.236	0.115	-
	I	15.3^{ab}	15.0^{ab}	20.4^{a}	14.9^{b}	1.350						
Heterophils (%)	N	35.1	40.9	34.5	34.4	2.060	35.1^{b}	44.0^{a}	1.560	0.929	0.008	0.866
	I	41.0	43.2	47.8	43.9	2.060						
Lymphocytes (%)	N	55.4	48.3	56.8	50.6	2.200	52.8^{a}	43.6^{b}	1.860	0.748	0.022	0.600
	I	43.6	40.8	36.7	44.3	2.890						
H:L[4]	N	0.66	0.78	0.61	0.52	0.090	0.64^{b}	1.12^{a}	0.063	0.599	<0.001	0.700
	I	1.01	1.12	1.33	1.01	0.061						
Monocytes (%)	N	2.57	2.00	6.25	3.25	0.581	3.52^{b}	5.68^{a}	0.434	0.003	0.007	0.596
	I	4.71^{ab}	5.40^{ab}	8.80^{a}	3.80^{b}	0.547						
Eosinophils (%)	N	3.57	2.89	1.00	1.80	0.450	2.00	2.00	0.291	0.047	0.623	-
	I	2.00	4.00	1.00	2.00	0.376						
Basophils (%)	N	1.00	2.00	2.00	2.00	0.671	2.00	2.00	0.613	0.950	0.941	-
	I	3.00	5.00	3.00	2.00	1.060						

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[2]N = Non-infected group; I = Infected group

[3] SEM = standard error of the mean.

[4] H:L = Heterophils : Lymphocytes ratio

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

152

5.3.5 Serum immunoglobulins and superoxide dismutase

In Table 5.7, no interaction was observed. However, both the serum IgG and SOD were not affected ($p>0.05$) by the dietary treatments and the challenge model. Serum IgM was similar across treatments for the noninfected birds; however, it was significantly higher ($p<0.05$) among the infected birds treated with 0.3% ROD extract compared to the 0.5% ROD extract and antibiotic treatments. Serum IgM was higher ($p<0.05$) in the noninfected group than the infected group.

Table 5.7. Effect of red osier dogwood extract on serum immunoglobulins and superoxide dismutase of broiler chickens challenged orally with *Salmonella* Enteritidis.

Parameters	Infection model[2]	Treatment[1]				SEM[3]	Infection model[2]		SEM[3]	*P*-value		
		Basal	TMP/ SDZ	0.3% ROD	0.5% ROD		N	I		Treatment Effect	Model Effect	Interaction Effect
IgG	N	4.12	3.60	3.66	4.32	0.416	3.62	3.61	0.240	0.928	0.972	0.842
(mg/mL)[4]	I	3.66	3.55	3.75	3.48	0.243						
IgM	N	1.84	1.27	1.52	1.47	0.074	1.51[a]	1.21[b]	0.061	0.006	0.024	0.570
(mg/mL)[5]	I	1.53[ab]	1.10[c]	1.59[a]	1.15[bc]	0.094						
SOD	N	1.71	1.91	1.96	1.73	0.103	3.48	3.94	0.071	0.576	0.300	0.783
(U/mL)[6]	I	1.77	1.75	1.96	2.04	0.097						

[1] Basal, Negative control diet, TMP/SDZ (Trimethoprim-sulfadiazine) antibiotic diet, 0.3% ROD, diet containing 0.3% red osier dogwood extract, 0.5% ROD, diet containing 0.5% red osier dogwood extract

[2] N = Non-infected group; I = Infected group

[3] SEM = standard error of the mean.

[4] IgG = Immunoglobulin G

[5] IgM = Immunoglobulin M

[6] SOD = Superoxide dismutase

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

5.4 Discussion

Chicks are the most susceptible to *Salmonella* infection. This is partly attributable to their new but developing immune system. In the current study, oral inoculation of day-old broiler chicks with 0.5 mL/bird of 3.1×10^5 CFU/mL of *SE* did not result in the presence of *SE* in their cloacal fecal samples at 1 DPI. However, this does not suggest the systemic absence of *SE* among the *SE*-infected birds. According to the report of Van Immerseel et al. (2004), it is possible that birds could be carriers of *Salmonella* regardless of its failed detection in their fecal sample. In the research demonstration by Borsoi et al. (2011), *SE* was detected in the fecal samples of broiler chickens following the oral gavage of 1×10^5 CFU/mL of *SE*. This conflicts in with the current research finding. Such conflict could be due to the variation in the *SE* inoculum strain and volume, which was not reported by Borsoi et al. (2011). Furthermore, in some *SE*-challenge studies using a lower concentration of *Salmonella* inoculum (usually $<10^5$ CFU/mL), the presence of *Salmonella* infection was confirmed using a non-fecal assessment method, particularly with the use cecal tonsil (Higgins et al., 2008, 2010; Prado-Rebolledo et al., 2017). This suggests that the challenged birds in the current study could be *SE* carriers even though *SE* was not detected by cloacal fecal samples. On 5, 12, and 18 DPI, no dietary treatment effect was observed on the *SE* shedding in the cloacal fecal samples of birds. This is not surprising as *SE* presence was not confirmed 24 hours after the *SE* challenge.

No interaction effects were observed on all the evaluated growth performance parameters. As a result, the results discussed are based on the main effects of dietary treatments and the challenge model.Antibiotics have a proven capacity to reliably improve feed conversion efficiency and, consequently, accelerate the growth performance of birds (Mehdi et al., 2018; Ali et al., 2019; Saleh et al., 2020; Shang et al., 2020). According to Gaucher et al. (2015), absence of antibiotics use in poultry production has been associated with

consequential production losses. It would therefore be interesting to verify if ROD extract could match up the positive impact of antibiotics on the growth performance indices of broiler chickens. In the present study, neither the supplementation of ROD extract (0.3% and 0.5%) nor TMP/SDZ have influenced AFI, AWG, FCR, and mortality of birds with or without *SE* infection. This corroborates the findings of Mogire et al. (2021), who reported an insignificant impact of 0.1% and 0.3% ROD extract on the growth performance of birds, although the study lacked a disease challenged model. In contrast to the present findings, Erinle et al. (2022b) demonstrated that supplementation of 0.3% and 0.5% ROD extract significantly sustained average feed intake, feed efficiency, and mortality of birds challenged with *SE* lipopolysaccharides compared to the birds fed in-feed bacitracin. Unlike the impact of antibiotic-free diet on poultry production as reported by Gaucher et al. (2015), our present results suggest that dietary supplementation of ROD extract at either 0.3% or 0.5% does not have negative impact on the growth response of broiler chickens and as a result, will not cause consequential production losses. Comparing the infection model, AFI and AWG at week 1 were significantly higher among the infected birds, whereas AFI and FCR at week 2 and 3 and overall were significantly higher among the noninfected group of birds. *Salmonella* infection has a dynamic impact on birds' growth performance. The severity of the infection largely depends on the age and strain of birds and the concentration of *Salmonella* inoculum. In some studies where *Salmonella* infection was established in poultry birds older than 21 days, poorer growth performance indices of birds were reported (Marcq et al., 2011; El-Shall et al., 2020). Meanwhile, at d 9 to 11 post-hatch, Zhen et al. (2018) reported that oral challenge of birds with 1 mL of 1×10^9 CFU of *Salmonella* Enteritidis did not significantly affect feed intake, body weight gain, FCR of chicks at different periods. In theory, given its virulence, *Salmonella* is expected to have a drastic draconian impact on the growth of poultry birds. However, in the current study, the unexpected significant discrepancies which partly favoured AFI in *SE*-challenged birds but

with a depressed FCR could be due to the elongated time elapse during the weekly weighing of left-over feed and bodyweight of birds in the pen containing the noninfected birds before moving to the infected birds. Mortality was not affected by the dietary treatments and infection model. Ribeiro et al. (2007) reported a no significant difference in the livability of birds challenged with *SE*.

The gut is involved in the digestion and absorption of nutrients and serves as a selective barrier which permits the transportation of nutrients while blocking intestinal pathogens and their metabolic products. As a result, the gut structure, including the height and width of villi, and crypt depth, has been considered one of the critical indicators of a healthy gut (Laudadio et al., 2012). In the presence or absence of disease or immune stressors, it is pertinent that the gut architectural integrity must be maintained. In the duodenum, there was a significant increase in VH of noninfected birds fed either 0.3% or 0.5% ROD extract diets, while the VH of infected birds were significantly increased among those fed only 0.3% ROD extract. Both ROD levels were also observed to substantially increase duodenal VH:CD compared to control and antibiotic treatments. This is deviance from Mogire et al. (2021) report, where 0.1% and 0.3% ROD extract did not influence the duodenal and ileal histomorphometry. In addition, the present study was also different from our previous findings (Erinle et al., 2022b) in which 0.3% and 0.5% ROD extract did not affect duodenal and jejunal morphology, which could be due to the different challenge models. Laudadio et al. (2012) reported that intestinal morphology from healthy and well performing birds is characterized by higher VH and VW, suggesting a large nutrient absorption surface area. Thus, the two ROD levels improved duodenal morphology. The duodenum interacts with accessory organs, including the pancreas and liver, which are critical in the digestion process of feed material. With the exception to VW, dietary TMP/SDZ antibiotic decreases duodenal morphology. Still, in the duodenum, the SE infection model presents a

significantly higher VH and deeper CD among the infected birds than the noninfected birds. The higher VH is quite surprising and could reflect the increase in AFI throughout the experimental period. In the jejunum, there was a significant interaction effect between the dietary treatment and infection model on VH and VW. In the ileum, there was a significant interaction effect on CD; however, 0.3% and 0.5% ROD extract significantly deepened CD of infected birds compared to the antibiotic and control-fed infected birds. This was similar to previous findings where 0.3% and 0.5% ROD extract was reported to increase ileal CD of birds challenged with *SE* lipopolysaccharides (Erinle et al., 2022b). *Salmonella* Typhimurium was reported to increase the incidence of intestinal epithelial exfoliation and, consequently, increase the goblet cell density (Fasina et al., 2010). According to Conrad and Stocker (2013), the number of goblet cells is highest in the crypts. Thus, a higher CD among the infected birds could be due to the detrimental effect of *SE* on gut morphology. Besides the possible detrimental effects of *SE* in the gut, we suspect that the increased CD could also be due to the gut architectural modulation by ROD extract in response to disease or immune stressors. However, there was a significant interaction effect on the ileal VW. An increase in VW has also been associated with increased villus surface area for nutrient absorption.

The reliability of blood as a predictable barometer of the physiological and health status of animals cannot be contested. White blood cells (WBC) component of the blood serves as the military defence system of the body against infections. WBC components, including HET, LYM, and H:L are reliable signals that indicate the severity of stressors (Zulkifli et al., 2000; Ghareeb et al., 2008). For example, an increased HET, decreased LYM, and consequently increased H:L have been reported in *Salmonella* infection (Jazi et al., 2019). Heterophils are the preponderant granulated leukocytes which increase birds' response to acute inflammation caused by pathogens. Therefore, in the presence of SE, the body

defence system deploys HET as the first counter defensive mechanism. Such heterophil response increases HET counts and is considered a worthy indicator of infection severity. In addition to HET, LYM, on the other hand, constitute the B immune cells which are responsible for synthesis of antibodies (Blumenreich, 1990). A reduction in the LYM components of the WBC is an indication of immunosuppression in poultry birds. Thiam et al. (2021) correlated a low H:L with an improved gut barrier and immune response and could be used as a biomarker for *Salmonella* resistance in chickens. As expected, the SE infection in the current study significantly increased HET, MON, and H:L and decreased LYM of SE-challenged birds. Although ROD extract did not affect the differential WBC in the present study, 0.3% ROD extract inclusion level stimulated the production of LEU and MON in SE-infected birds. Unfortunately, a similar feat was not achieved at 0.5% ROD extract inclusion level as it lowered LEU and MON but was comparable to antibiotic and control treatments. In humans, antibiotics like beta-lactam and vancomycin have been reported to decrease WBC and MON count (Shuman et al., 2012). A lower count of LEU is a common indicator of immunosuppression and consequently increases susceptibility to infections. As a part of the crucial component of the bodily immune system, MON travels around the body to scavenge pathogenic microbes and dead and damaged cells and contribute to immune responses during infection (Yáñez et al., 2017). This suggests that 0.3% ROD extract possesses the capacity to stimulate the inflammatory response by promoting the production of LEU and MON.

Serum immunoglobulins, including IgM and IgG, are products of the WBC sought as indicators of humoral immunity. The IgM antibodies have been reported to exhibit a greater affinity for the antigen and, as a result, are potent pathogen neutralizers better than the IgG's (Keyt et al., 2020). In the present study, IgM was significantly highest among SE-infected birds fed 0.3% ROD extract and lowest among those fed TMP/SDZ treatment.

This is different from our previous reports where both 0.3% and 0.5% ROD extract did not influence the IgG and IgM of birds challenged with SE-LPS (Erinle et al., 2022b). This could be due to variation in the challenge models. According to Heyman and Shulman (2016), more than 80% of human patients with recurrent infections have a deficiency in IgM. In MOPC 104E plasma cells, antibiotics was reported to inhibit the secretion of serum immunoglobulins, notably IgM, by inhibiting the glycosylation of carbohydrate component of immunoglobulins (Hickman and Kornfeld, 1978). The desirable impacts 0.3% ROD extract on the WBC and IgM over the TMP/SDZ antibiotics suggest that it promotes antibody production and the amount of circulating leukocytes in the blood. Comparing the infection model groups, SE infection had significant effects on IgM and was significantly lower among the infected group of birds. The serum IgM is reported to be the first antibody produced during the early period post-infection (Larsson et al., 1993; Rathnapraba et al., 2007) and declines gradually with age (Khare et al., 1976; Holodick et al., 2016). Regardless of the SE infection, the range of IgM concentrations obtained in the current study was 1.1 – 1.84 mg/mL, however, was within the range of mean concentrations 0.71 - 2.55 mg/ml reported by (Lebacq-Verheyden et al., 1974; Chhabra and Goel, 1980; Davis, 1985).

5.5 Conclusions

From the results obtained, SE infection increased AFI and FCR throughout the experimental period. However, dietary supplementation of ROD extract at 0.3% and 0.5% maintained the growth performance of broiler chickens throughout the feeding phases in the same magnitude as antibiotics. In addition, SE-infected birds had higher VH and deeper CD. Regardless of the presence of SE infection, both 0.3 and 0.5% ROD extract improved duodenal VH and VH:CD and marginally lowered CD compared to the antibiotic-fed broiler chickens. Meanwhile, the dietary treatment and infection model had an interaction

effect on the ileal VW and CD. Furthermore, Leukocytes, MON, and IgM were increased among infected chickens fed 0.3% ROD extract but decreased among those fed 0.5% ROD extract. However, SE infection adversely affected the hematology of infected birds compared to the noninfected birds. Given the sustained growth performance, improved duodenal histomorphology, and leukocytes percentage in both infected and noninfected broilers, both 0.3% and 0.5% ROD extract could be a suitable antibiotic replacement; however, 0.3% ROD extract had the best experimental outcome over the antibiotics.

CHAPTER 6: Red osier dogwood extract versus Trimethoprim-sulfadiazine(Part 2). Pharmacodynamic effects on ileal and cecal microbiota of broiler chickens challenged orally with *Salmonella* Enteritidis

6. 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 two main factors, including diets and *Salmonella* Enteritidis (**SE**) challenge. A total of 384 one-day-old mixed-sex Cobb-500 broiler chicks were randomly allotted to four dietary treatments; Negative control (**NC**), NC+0.075mg 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 (Non-infected 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 eight replicate cages at six birds/cage. On d 21, ten 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.

Keywords: redo osier dogwood extract, trimethoprim-sulfamethoxazole, antibiotic substitute, *Salmonella* enteritidis, gut microbiota

6.1 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*, non-typhoidal *Salmonella enterica* serovar Enteritidis and Typhimurium have been recognized for their epidemiological relevance given their host non-specificity 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 three causes of foodborne diseases. While there are over 2,600 different non-typhoidal *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 towards a dysbiotic condition. At less than 21 days post-hatch, 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 non-typhoidal *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; Lee et al., 2018b; Wei et al., 2018). 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., 2022a), 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 non-avian 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 on the ileal and cecal microbiota of broiler chickens infected with SE.

6.2 Material 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 Chapter 5.

6.2.1 Preparation of *Salmonella* Enteritidis inoculum

The *Salmonella* Enteritidis 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 *Salmonella* Enteritidis strain has been described in Chapter 5.

6.2.2 Sample collection

On d 21, 80 birds (i.e., ten 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 ^{0}C for subsequent ileal and cecal microbiota analyses.

6.2.3 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 ≥1.8 and ≥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.

6.2.4 Bioinformatics and statistical analyses

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 three basic assumptions. Non-normal data were transformed for parametric analysis. The non-parametric 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.

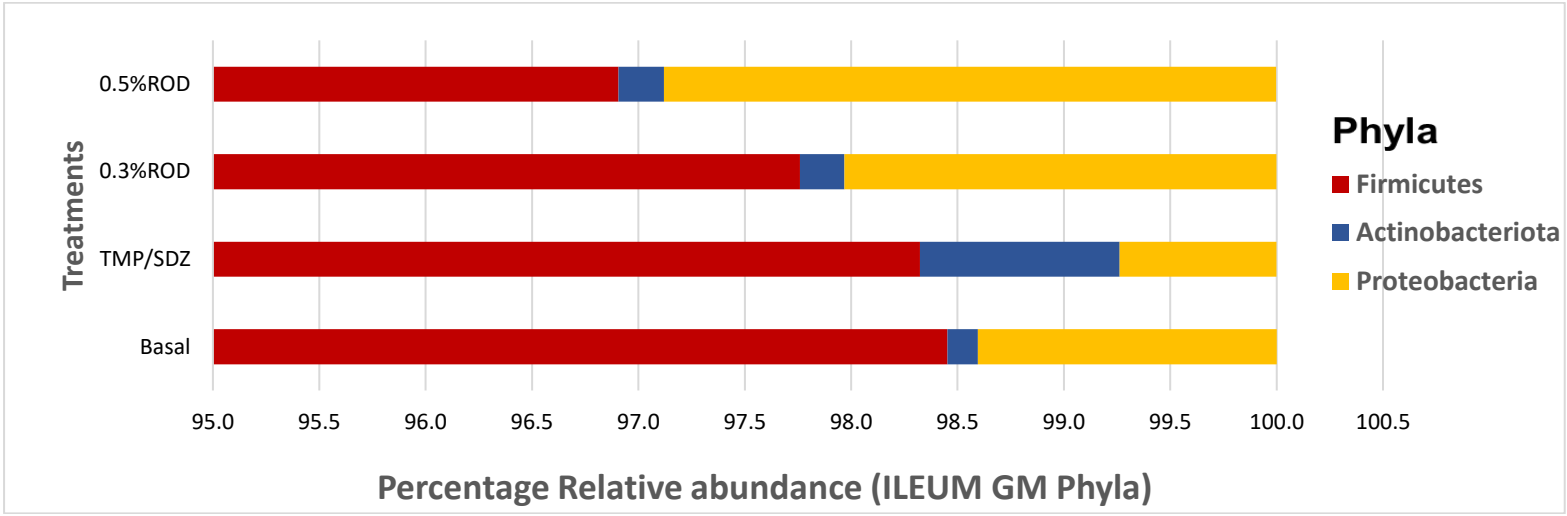
6.3 Results

6.3.1 Ileal and cecal microbial composition

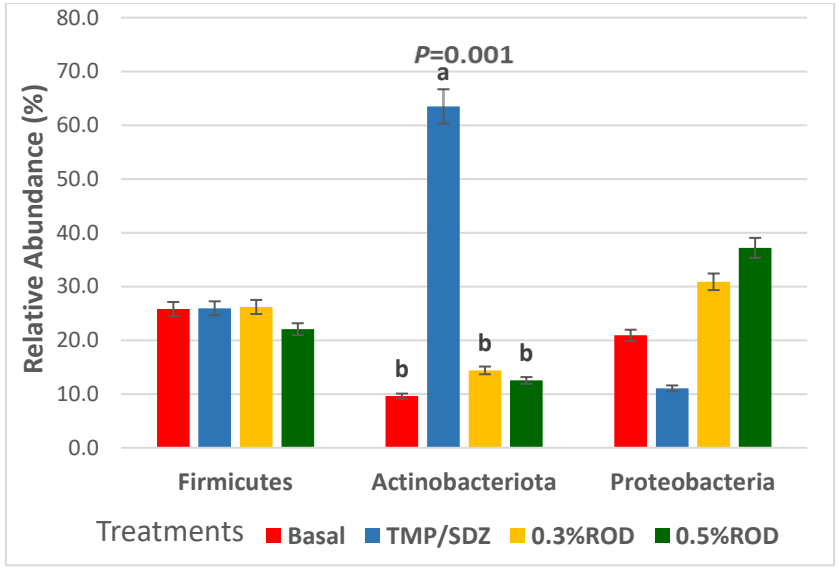
The effect of dietary supplementation of 0.3% and 0.5% ROD extract on the ileal and cecal microbiota composition of broiler chickens infected or uninfected with SE is presented in Figures 6.1, 6.2, 6.3, and 6.4. The aggregate of the operational taxonomic unit into each taxonomic rank, as well as the relative abundance of predominant phyla and genera-based

treatment and infection model effects are shown in Figures 6.1 and 6.3 for ileal bacterial phyla and genera, respectively and Figures 6.2 and 6.4 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 antibiotics was observed to numerically lower the relative abundance of phylum Firmicutes in the ceca. 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 non-infected birds compared to the infected birds. Meanwhile, Actinobacteriota and Proteobacteria phyla in the ceca were significantly higher and lower ($p<0.05$) among the non-infected and infected birds, respectively. At the ileal genera taxa, the relative abundance of the top 10 most abundant bacteria genera in a decreasing order include *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 ceca, the top 10 dominant bacteria genera in a decreasing order include *Bifidobacterium, Corynebacterium, Curtobacterium, Sanguibacter, Saccharopolyspora, Eggerthella, Bacillus, Kurthia, Erysipelatoclostridium,* and *Clostridium_innocuum*.

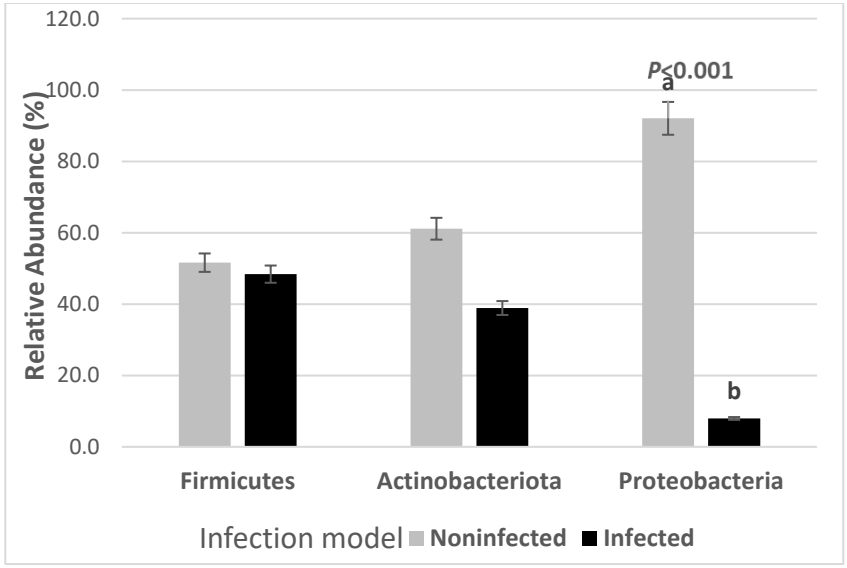
Compared to the TMP/SDZ treatment, the relative abundance cecal *Bifidobacterium* was marginally improved ($p<0.05$) compared to the control treatment.


0.5%ROD
0.3%ROD
TMP/SDZ
Basal
Treatments
95.0
95.5
96.0
96.5
97.0
97.5
98.0
98.5
99.0
99.5
100.0
100.5
Percentage Relative abundance (ILEUM GM Phyla)
Phyla
Firmicutes
Actinobacteriota
Proteobacteria

(a)


Relative Abundance (%)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
P=0.001
a
b
b
b
Firmicutes
Actinobacteriota
Proteobacteria
Treatments
Basal
TMP/SDZ
0.3%ROD
0.5%ROD

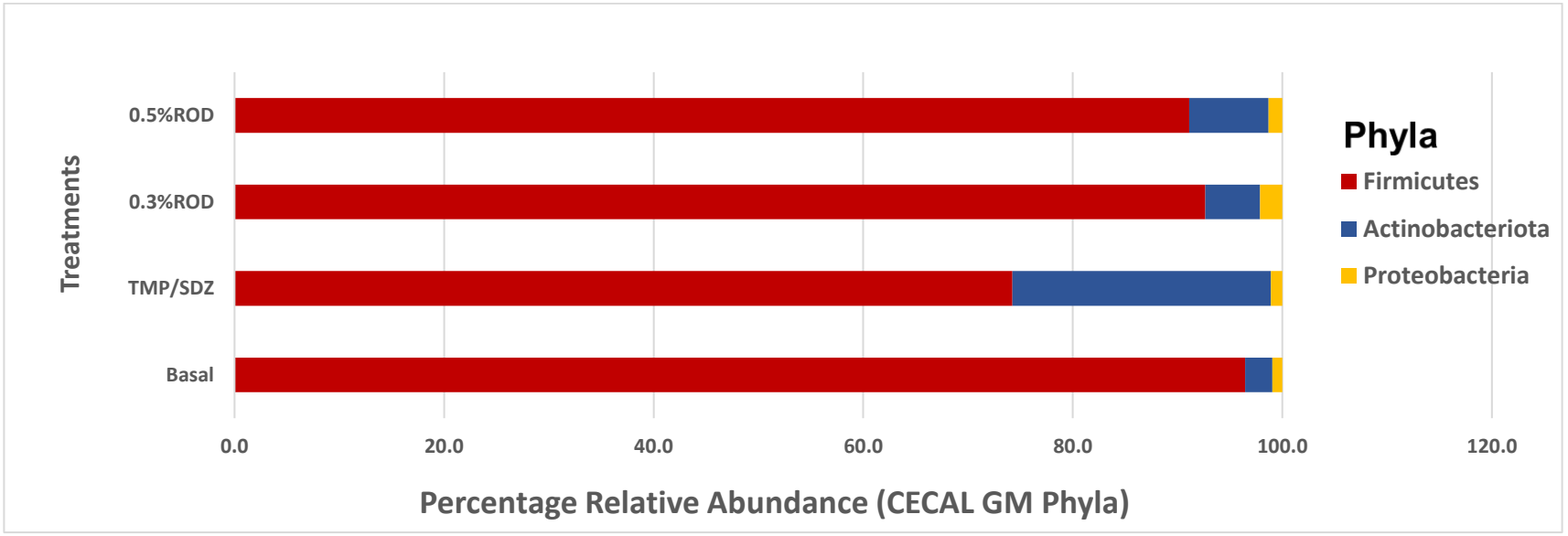
(b)


Relative Abundance (%)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
P≤0.001
a
b
Firmicutes
Actinobacteriota
Proteobacteria
Infection model
Noninfected
Infected

(c)

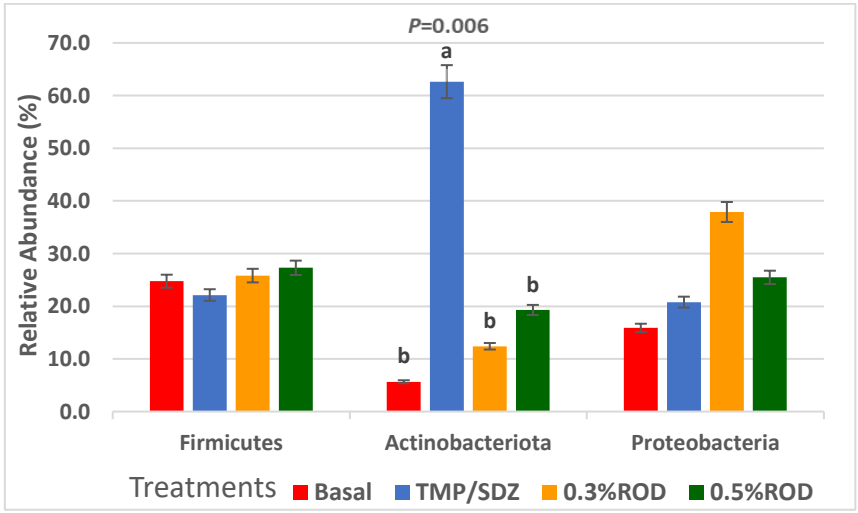
171

Figure 6.1. (**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 0.075mg 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.
Note: Phyla and infection model without a mean separation have their p-value greater than 0.05

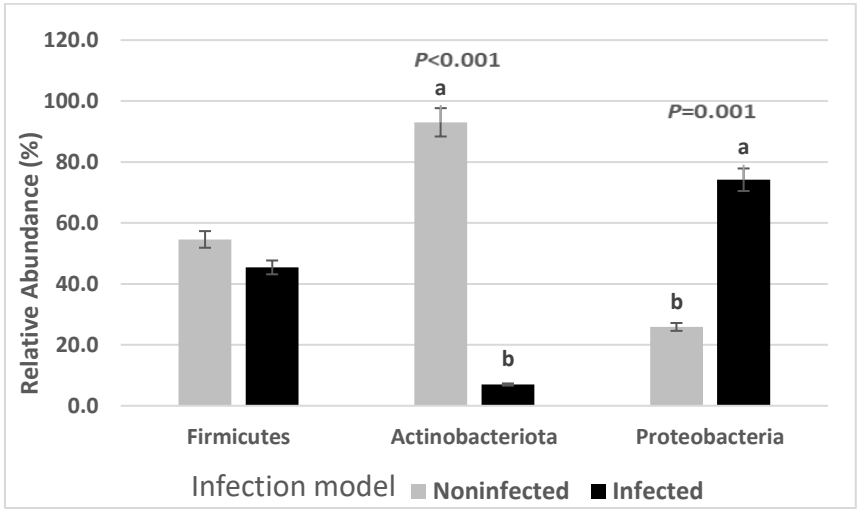

Treatments
0.5%ROD
0.3%ROD
TMP/SDZ
Basal
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Percentage Relative Abundance (CECAL GM Phyla)
Phyla
Firmicutes
Actinobacteriota
Proteobacteria

173

(a)


P=0.006
Relative Abundance (%)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
a
b
b
b
Firmicutes
Actinobacteriota
Proteobacteria
Treatments
Basal
TMP/SDZ
0.3%ROD
0.5%ROD

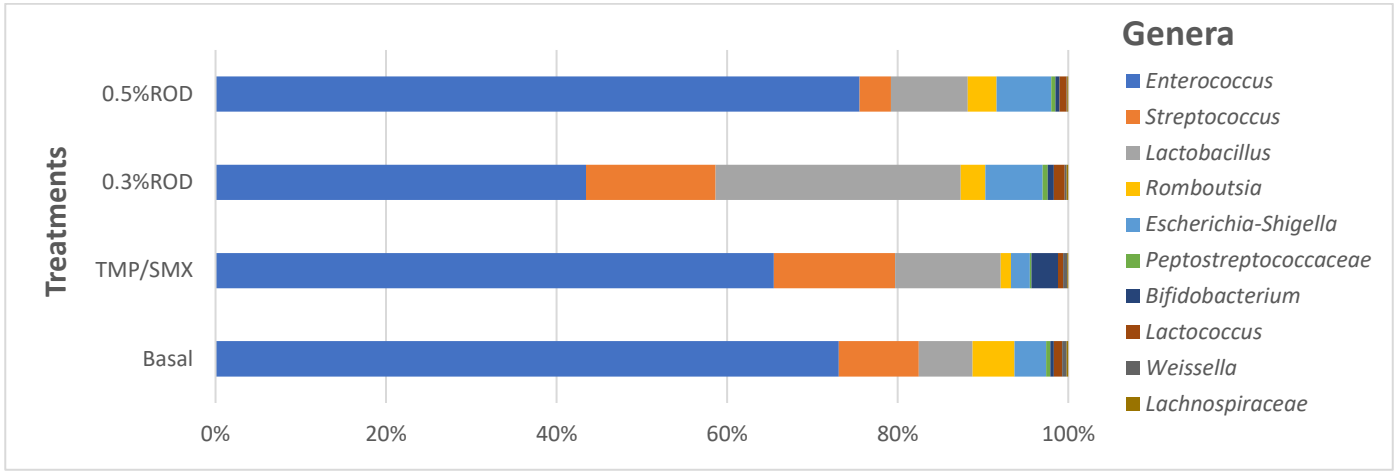
(b)


P<0.001
P=0.001
Relative Abundance (%)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
a
b
b
a
Firmicutes
Actinobacteriota
Proteobacteria
Infection model
Noninfected
Infected

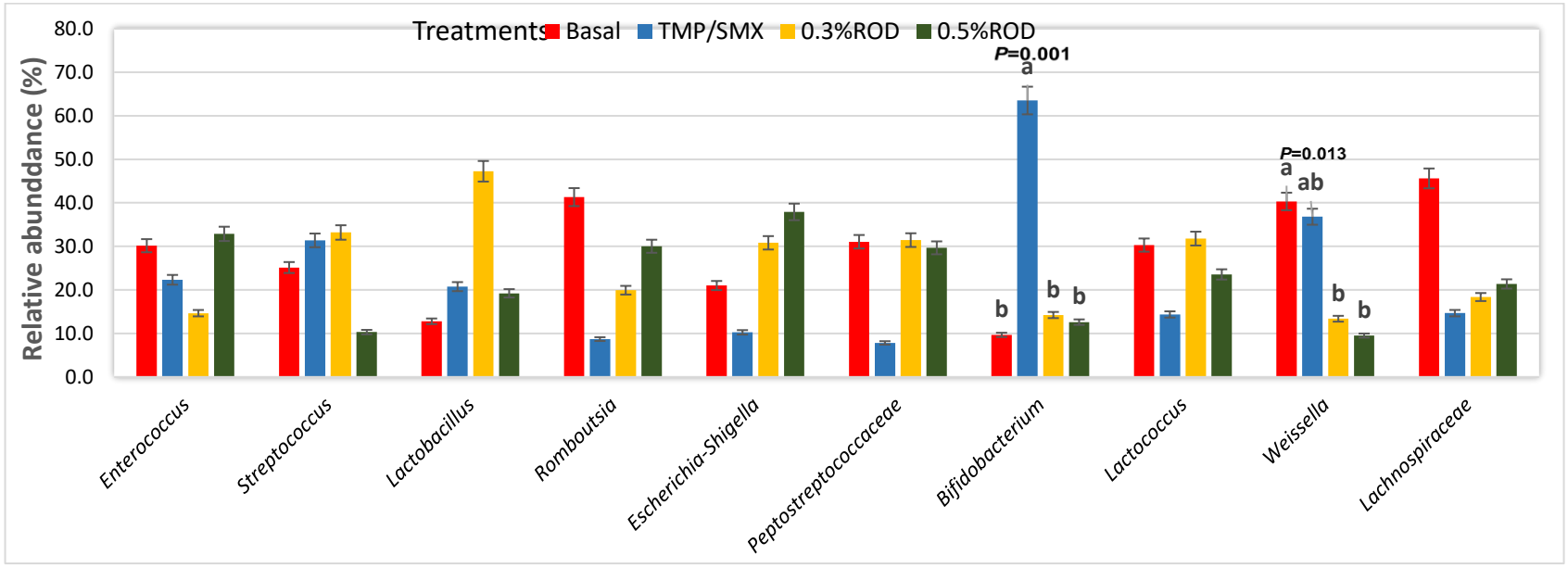
(c)

Figure 6.2. (**a**) Profile, (**b**) descriptive treatment effect, (**c**) descriptive infection model effect on the percentage relative abundance of cecal 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 0.075mg 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.

Note: Phyla and infection model without a mean separation have their p-value greater than 0.05


Genera
Enterococcus
Streptococcus
Lactobacillus
Romboutsia
Escherichia-Shigella
Peptostreptococcaceae
Bifidobacterium
Lactococcus
Weissella
Lachnospiraceae
Treatments
0.5%ROD
0.3%ROD
TMP/SMX
Basal
0%
20%
40%
60%
80%
100%

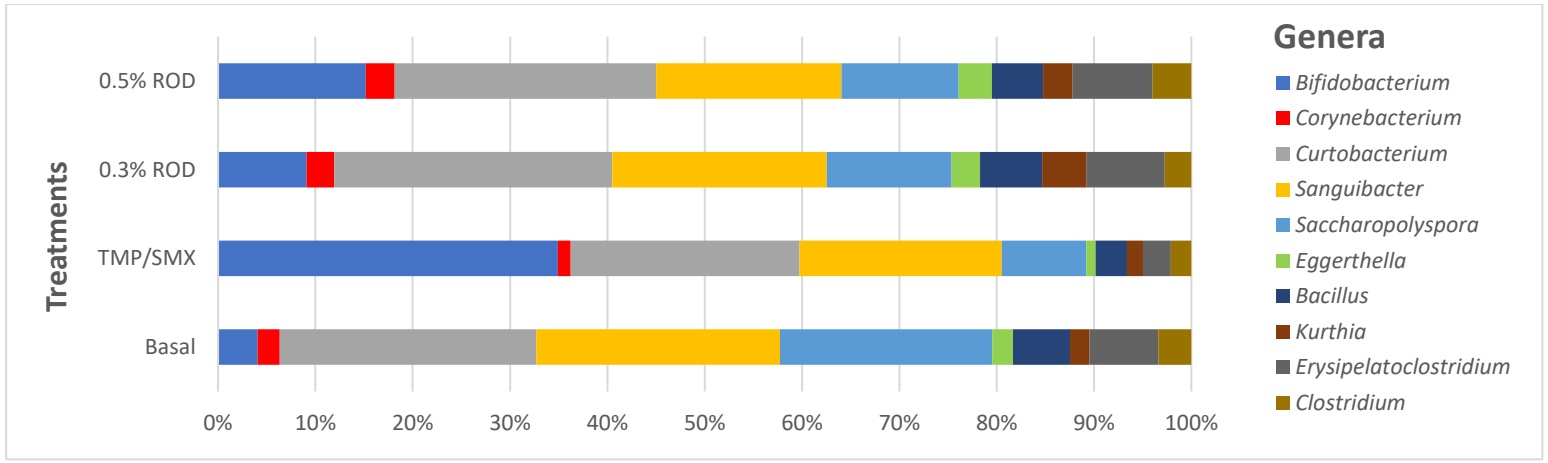
(a)


Treatments Basal TMP/SMX 0.3%ROD 0.5%ROD
Relative abundance (%)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
P=0.001
P=0.013
a
b
ab
Enterococcus
Streptococcus
Lactobacillus
Romboutsia
Escherichia-Shigella
Peptostreptococcaceae
Bifidobacterium
Lactococcus
Weissella
Lachnospiraceae

(b)

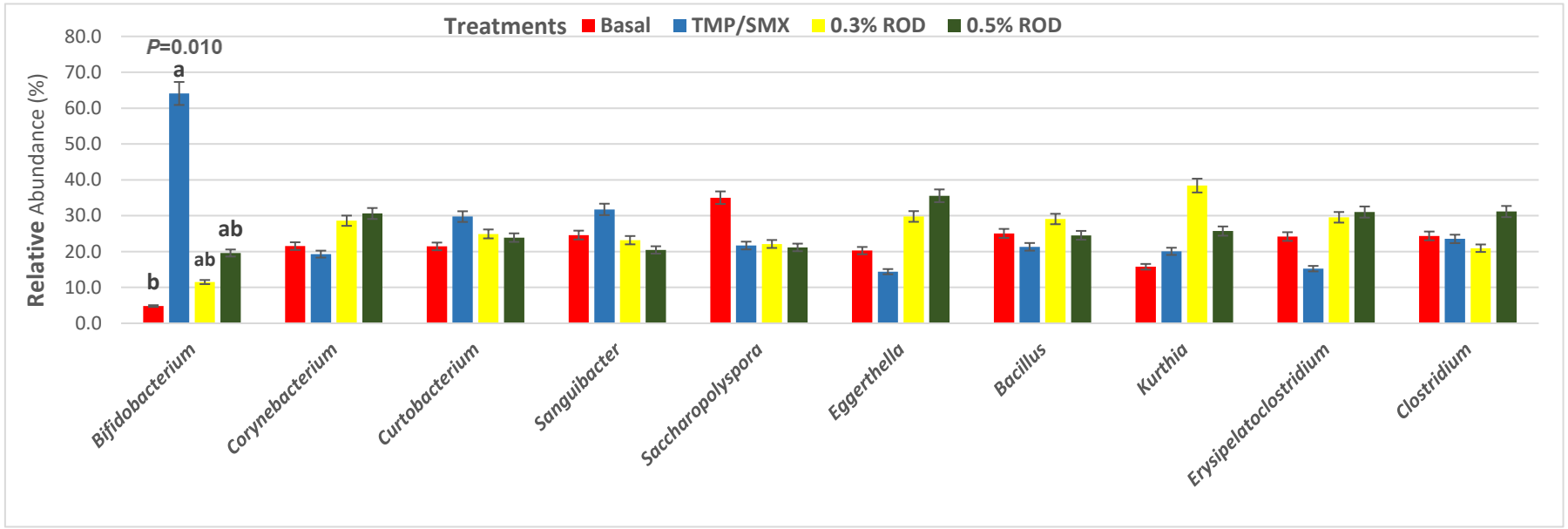
175

Figure 6.3. (**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 0.075mg 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. Note: Genera without a mean separation have their p-value greater than 0.05.


Treatments
0.5% ROD
0.3% ROD
TMP/SMX
Basal
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Genera
Bifidobacterium
Corynebacterium
Curtobacterium
Sanguibacter
Saccharopolyspora
Eggerthella
Bacillus
Kurthia
Erysipelatoclostridium
Clostridium

(a)

177


Treatments
Basal
TMP/SMX
0.3% ROD
0.5% ROD
Relative Abundance (%)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
P=0.010
a
b
ab
ab
Bifidobacterium
Corynebacterium
Curtobacterium
Sanguibacter
Saccharopolyspora
Eggerthella
Bacillus
Kurthia
Erysipelatoclostridium
Clostridium

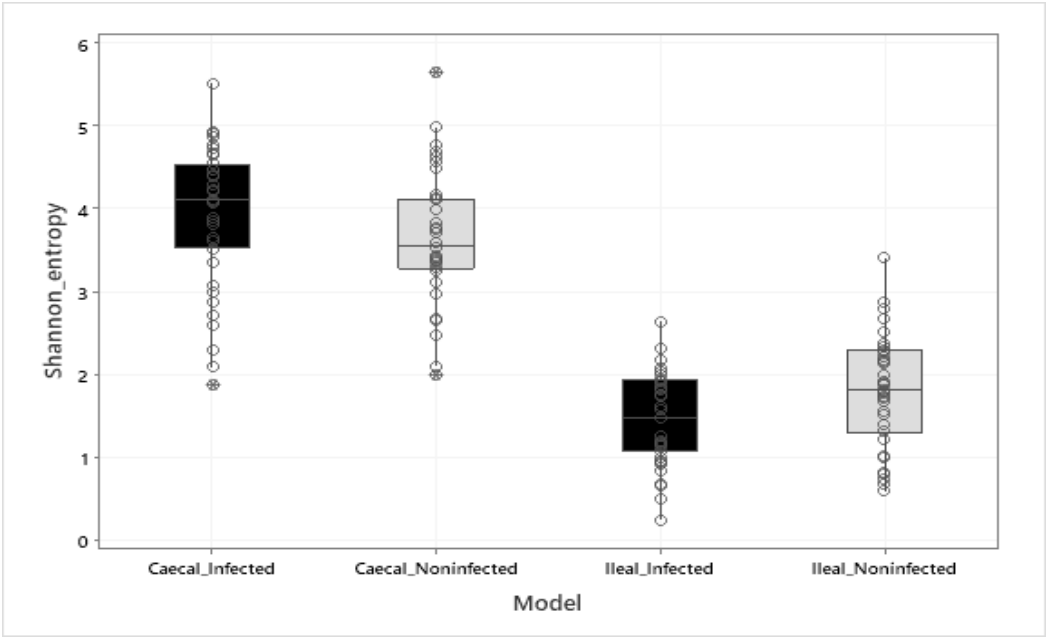
(b)

Figure 6.4. (**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 0.075mg 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.

Note: Genera without a mean separation have their p-value greater than 0.05

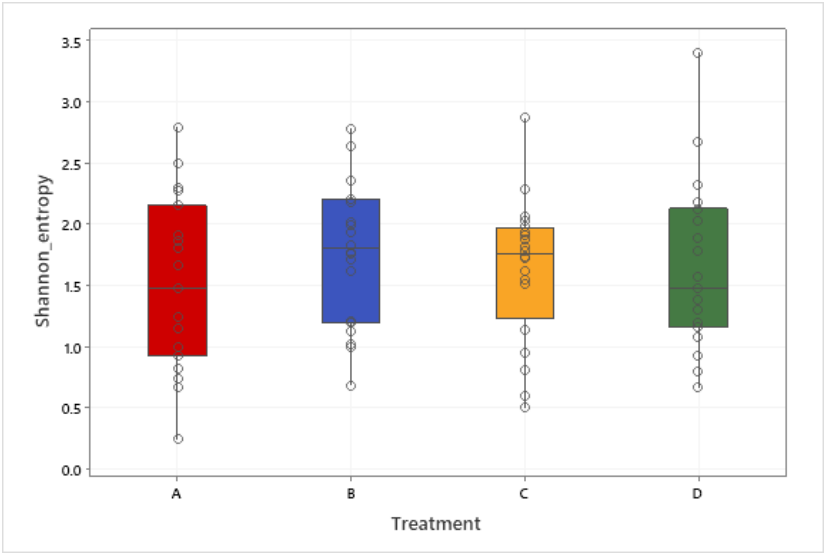
6.3.2 Ileal and cecal microbial diversity

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 6.5, 6.6, 6.7, and 6.8. 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 6.5; 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 6.6, the dietary treatments did not alter ($p>0.05$) the beta diversity of ileal and cecal microbiota. In Figures 6.7 and 6.8, 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.

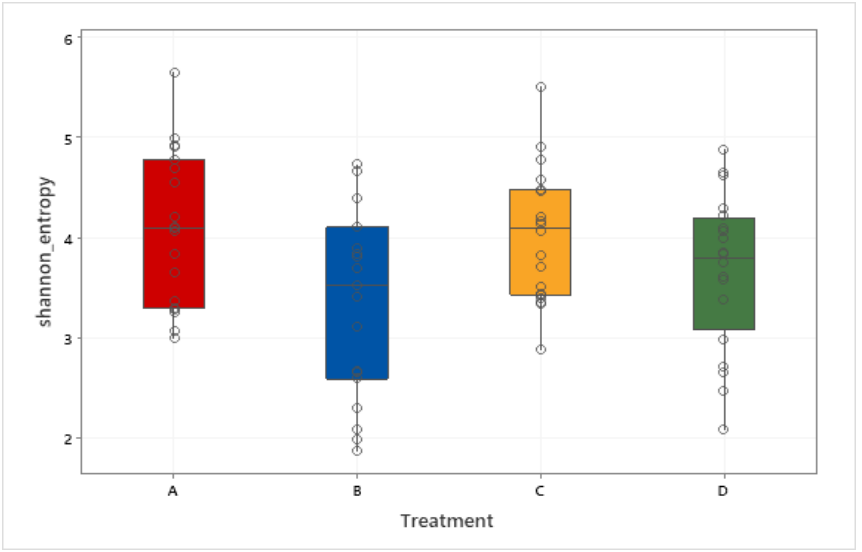

Shannon_entropy
0
1
2
3
4
5
6
Caecal_Infected
Caecal_Noninfected
Ileal_Infected
Ileal_Noninfected
Model

180

(a)


Shannon_entropy
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
A
B
C
D
Treatment

(b)


shannon_entropy
2
3
4
5
6
A
B
C
D
Treatment

(c)

Figure 6.5. 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 0.075mg 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.

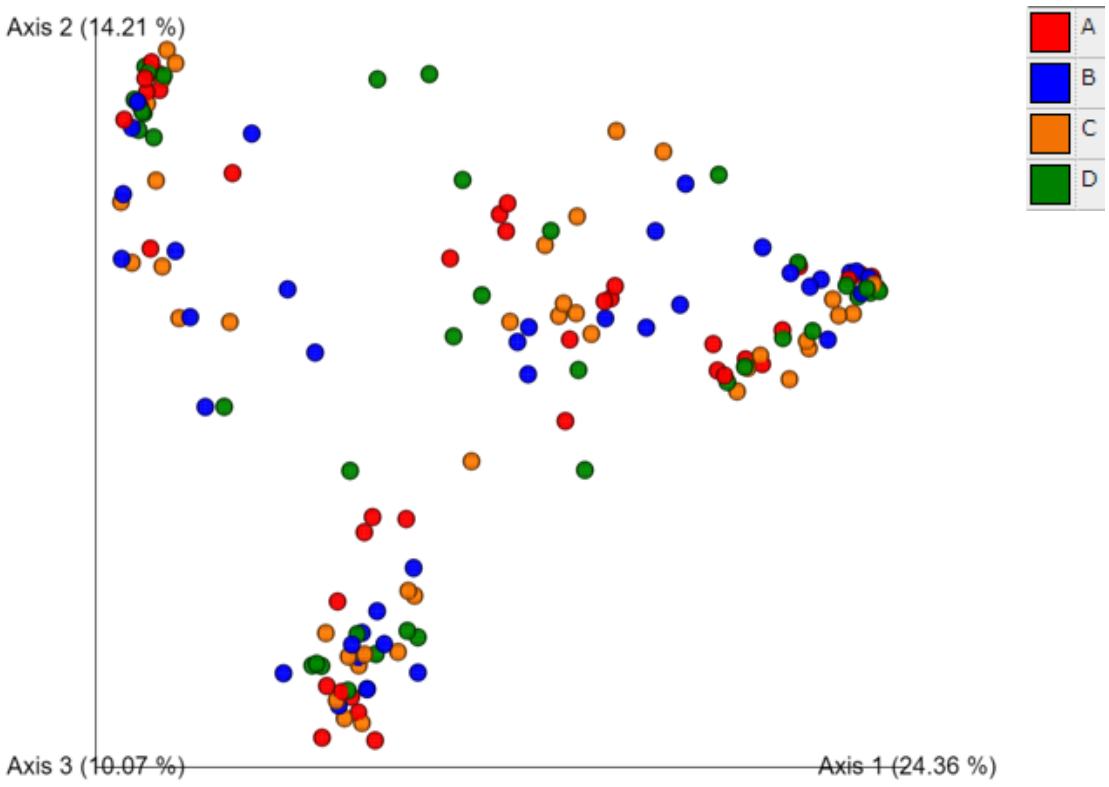


Figure 6.6. Bray-Curtis principal coordinates analysis determined differences in beta-diversity among treatments. Treatment: A = Negative control, B = diet containing 0.075mg antibiotic (Trimethoprim-sulfadiazine; TMP/SDZ) diet, C = diet containing 0.3% red osier dogwood extract, and D = diet containing 0.5% red osier dogwood extract.

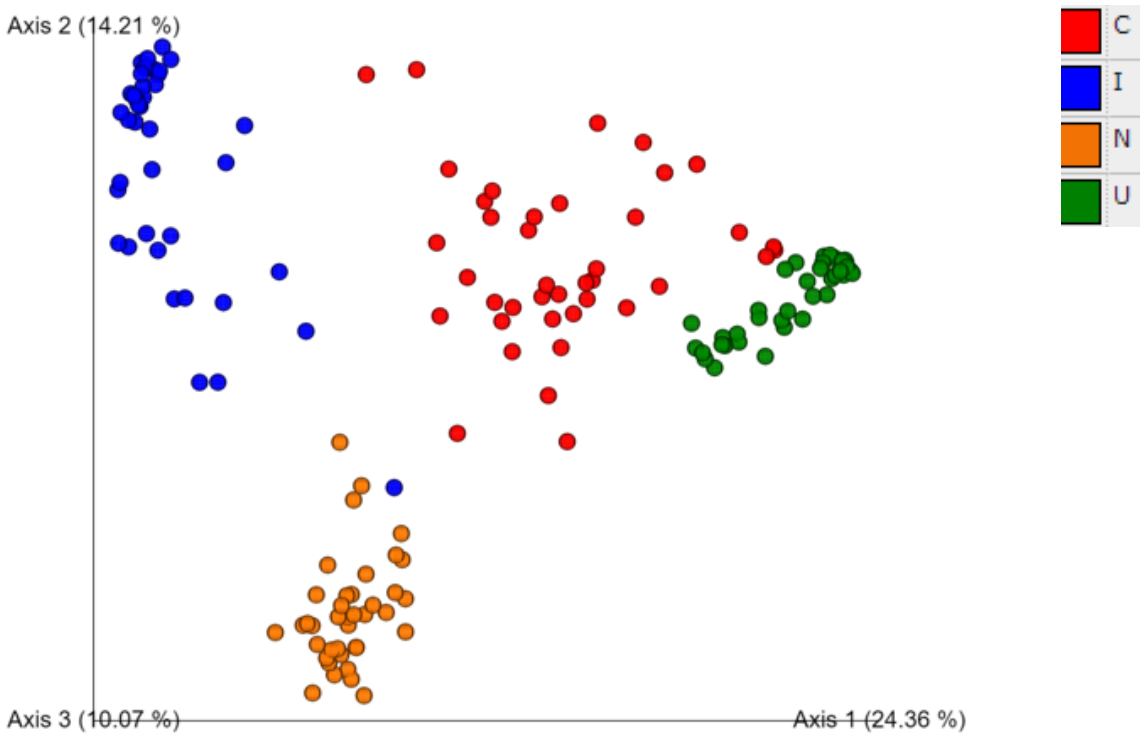


Figure 6.7. Bray-Curtis principal coordinates analysis determined significant differences (P<0.05) in beta-diversity between the infection model. Challenge groups: U = ceca microbiota of group of birds that were not challenged with SE, C = ceca microbiota of group of birds that were challenged with SE, I = group. N = ileal microbiota of group of birds that were not challenged with SE, C = ileal microbiota of group of birds that were challenged with SE.

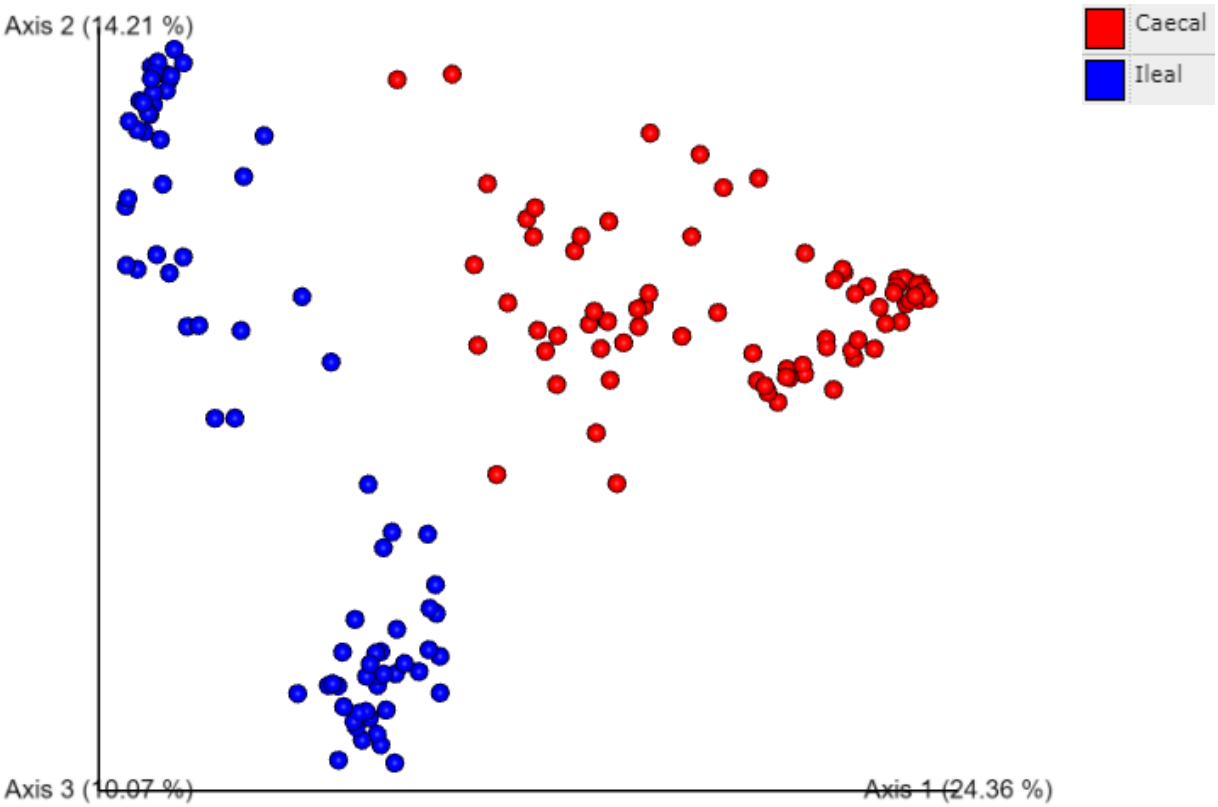


Figure 6.8. Bray-Curtis principal coordinates analysis determined significant differences (P<0.05) in beta-diversity between the ileum and ceca. Challenge groups: U = ceca microbiota of group of birds that were not challenged with SE, C = ceca microbiota of group of birds that were challenged with SE, I = group. N = ileal microbiota of group of birds that were not challenged with SE, C = ileal microbiota of group of birds that were challenged with SE.

6.4 Discussion

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 the 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). *Salmonella* Enteritidis thrives, proliferates, 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 five 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., 2022b;c). In the current study, the ileal and cecal microbiota is dominated by phyla Firmicutes, Proteobacteria, and Actinobacteriota, however, Firmicutes were 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 two 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, Firmicute and Proteobacteria were reported to be the top two 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 increase in the relative abundance of Proteobacteria in the ileum and ceca of infected birds, respectively, compared to the non-infected birds. *Salmonella* Enteritidis 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), some members of Proteobacteria, particularly Enterobacteriaceae, have been recognized for their protection against the *Salmonella* colonization in chickens by competitive exclusion (Videnska et al., 2013; Deriu et al., 2013; Litvak et al., 2019). 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 and the possible reduction of *Salmonella* as post-infection days increase. 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 non-infected birds. Some members of Actinobacteria, notably *Streptomyces,* are capable of synthesizing peptide antibiotics called actinomycin which has inhibitory action against multi-resistant *Staphylococcus aureus*, malignant tumours 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 is noteworthy.

The caecum houses a more stable bacterial population of about $10^{10} – 10^{11}$ / gram than the $10^{8} – 10^{9}$ / gram in the ileum digesta (Shang et al., 2018), thus, suggesting that a more complex microbial metabolism would be taking place in the caecum. 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% 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 two most prevalent polyphenols found in ROD extract (Scales, 2015; Erinle et al., 2022b); 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). 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 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 caecum and consequently a dynamism in polyphenol metabolism in these gastrointestinal sections. Dietary polyphenols, particularly the non-absorbable ones, are better metabolized where the gut microbial population tends to be highest, usually in the caecum. Depending on the polyphenol biochemical structures and bond with their sugar component (Catalkaya et al., 2020), approximately 5 – 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.

6.5 Conclusions

From the results obtained, *Salmonella* Enteritidis 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 non-infected counterparts. Actinobacteriota was significantly increased in the cecal of non-infected 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.

CHAPTER 7: Conclusions

7.1 Conclusions

Phytogenic additives, namely GP and ROD extract, demonstrated considerable antibiotic-replacement potentials. A dietary supplementation of 2.5% GP into broiler chickens' diet would afford an improved growth performance of broiler chickens given the similar overall feed conversion efficiency compared to bacitracin antibiotic. Similarly, incorporation of ROD extract into broiler chickens diets also maintained the growth performance of birds even when challenged with *Salmonella* Enteritidis infection or *Salmonella* Enteritidis lipopolysaccharides. Both GP and ROD extract improved the microbial population of beneficial microbes, notably *Lactobacillus*, which was depressed by bacitracin antibiotic; however, such beneficial impact did not translate to enhanced production of cecal short-chain fatty acids. However, in birds with *Salmonella* Enteritidis infection, ROD exhibited a decisive pharmacodynamic effect on the ileal and cecal microbiota by promoting the proliferation of cecal *Bifidobacterium* while decreasing *Weissella*. While GP improved the duodenal and jejunal morphology, ROD extract displayed a gut morphology improvement capacity at the duodenum and ileum of broiler chickens infected with *Salmonella* Enteritidis or their lipopolysaccharide metabolites, respectively. Furthermore, 0.3% ROD extract reverse-engineered the detrimental effects of *Salmonella* Enteritidis infection in birds by stimulating the production of white blood cells, monocytes, and immunoglobulins M.

The results highlighted above will enhance competitiveness and innovation in Canada as it; 1) indicates a productive alternative method of converting GP from its worthless to worthwhile status by supplementing them into poultry diets; 2) addresses the increasing public concerns about food security and safety through the possible means of producing chickens without antibiotic use. Routine use of these products, namely GP and ROD extract

especially at 2.5% and 0.3% inclusion levels, respectively, in poultry nutrition will not only improve the profitability of chicken farmers but also will create a value-added market opportunity for grape processors and serve as possible alternatives to antibiotic use.

Bibliography

A.O.A.C. 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemist, Washington DC.

A.O.A.C. 1994. Official Methods of Analysis. Assoc. Off. Anal. Chem.,Arlington, VA.

Abdel-Moneim, A. M. E., A. M. Elbaz, R. E. S. Khidr, and F. B. Badri. 2020. Effect of in Ovo Inoculation of Bifidobacterium spp. on Growth Performance, Thyroid Activity, Ileum Histomorphometry, and Microbial Enumeration of Broilers. Probiotics Antimicrob. Proteins 12:873–882 Available at https://link.springer.com/article /10.1007/s12602-019-09613-x (verified 18 May 2022).

Abdel-Wareth, A. A. A., S. Kehraus, and K.-H. Su€dekum. 2019. Pep- permint and its respective active component in diets of broiler chickens: growth performance, viability, economics, meat physico- chemical properties, and carcass characteristics. Poult. Sci. 98:3850–3859.

Abolfathi, M.-E., S. A. Tabeidian, A. D. Foroozandeh Shahraki, S. N. Tabatabaei, and M. Habibian. 2019. Comparative effects of n-hexane and methanol extracts of elecampane (*Inula helenium* L.) rhizome on growth performance, carcass traits, feed digestibility, intestinal antioxidant status and ileal microbiota in broiler chickens. Arch. Anim. Nutr. 73:88–110.

Abuelsaad, A.S.A., G. Allam, and A.A.A. Al-Solumani. 2014. Hesperidin inhibits inflammatory response induced by Aeromonas hydrophila infection and alters CD4+/CD8+ T cell ratio. Mediators Inflamm. Hindawi. doi:https://doi.org/10.1155/2014/393217.

Acharya, C., and J. S. Bajaj. 2017. Gut Microbiota and Complications of Liver Disease. Gastroenterol. Clin. 46:155–169.

Acharya, S.N., C.B. Chu, R. Hermesh, and G.B. Schaalje. 1992. Factors affecting red-osier dogwood seed germination. Can. J. Bot. 70: 1012–1016. NRC Research Press. doi:https://doi.org/10.1139/b92-125.

Adeleye, O. O., I. O. Otakoya, A. O. Fafiolu, J. O. Alabi, L. T. Egbeyale, and O. M. O. Idowu. 2018. Serum chemistry and gut morphology of two strains of broiler chickens to varying interval of post hatch feeding. Vet. Anim. Sci. 5:20–25.

Adewole, D., and F. Akinyemi. 2021. Gut microbiota dynamics, growth performance, and gut morphology in broiler chickens fed diets varying in energy density with or without Bacitracin Methylene Disalicylate (BMD). Microorg 9:787.

Aditya, S., S. J. Ohh, M. Ahammed, and J. Lohakare. 2018. Supple- mentation of grape pomace (Vitis vinifera) in broiler diets and its effect on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality. Anim. Nutr. 4:210–214.

Afshari, A., A. Baratpour, S. Khanzade, and A. Jamshidi. 2018. *Salmonella* Enteritidis and *Salmonella* Typhimorium identification in poultry carcasses. Iran. J. Microbiol. 10:45 Available at /pmc/articles/PMC6004630/ (verified 11 August 2021).

Agyare, C., V.E. Boamah, C.N. Zumbi, C.N. and F.B. Osei .2018. Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrob. Resist. Glob. Threat 1–20. https://doi.org/10.5772/intechopen.79371

Ahiwe, E. U., M. E. Abdallh, E. P. Chang'a, M. Al-Qahtani, A. A. Omede, H. Graham, and P. A. Iji. 2019. Influence of autolyzed whole yeast and yeast components on broiler chickens challenged with *Salmonella* lipopolysaccharide. Poult. Sci. 98:7129–7138.

Ahsan, U., E. Kuter, I. Raza, B.H. Köksal, Ö. Cengiz, M. Yıldız, P.K. Kızanlık, M. Kaya, O. Tatlı, and Ö. Sevim. 2018. Dietary supplementation of different levels of phytogenic feed additive in broiler diets: The dynamics of growth performance, caecal microbiota, and intestinal morphometry. Brazilian J. Poult. Sci. 20: 737–746. SciELO Brasil. doi:https://doi.org/10.1590/1806-9061-2017-0698.

Aiello, R.J., L.E. Armentano, S.J. Bertics, and A.T. Murphy. 1989. Volatile fatty acid uptake and propionate metabolism in ruminant hepatocytes. J. Dairy Sci. 72: 942–949. J Dairy Sci. doi:10.3168/JDS.S0022-0302(89)79187-6.

Akanmu, A.M. 2018. Effect of medicinal plant extracts from West Africa on rumen fermentation parameters enteric methane emission and growth performance in Merino sheep. University of Pretoria.

Akinyemi, F.T., J. Ding, H. Zhou, K. Xu, C. He, C. Han, Y. Zheng, H. Luo, K. Yang and C. Gu. 2020. Dynamic distribution of gut microbiota during embryonic development in chicken. Poult. Sci. 99: 5079–5090. Elsevier. doi:https://doi.org/10.1016/j.psj.2020.06.016.

Alfaig, E, M. Angelovicova, M. Kral, V. Vietoris, and R. Zidek. 2013. Effect of probiotics and thyme essential oil on the texture of cooked chicken breast meat. Acta Sci. Pol. Technol. Aliment. 12: 379–384.

Ali, A., E. N. Ponnampalam, G. Pushpakumara, J. J. Cottrell, H. A. R. Suleria, and F. R. Dunshea. 2021. Cinnamon: A natural feed additive for poultry health and production—A review. Animals 11:2026 Available at https://www.mdpi.com/2076-2615/11/7/2026 (verified 30 November 2021).

Ali, M., N. Chand, R. U. Khan, S. Naz, and S. Gul. 2019. Anticoccidial effect of garlic (Allium sativum) and ginger (Zingiber officinale) against experimentally induced coccidiosis in broiler chickens. J. Appl. Anim. Res. 47:79–84 Available at https://www.tandfonline.com/doi/abs/10.1080/09712119.2019.1573731 (verified 28 April 2022).

Al-Kassie, G.A.M., M.A.M. Al-Nasrawi, and S.J. Ajeena. 2011. The effects of using hot red pepper as a diet supplement on some performance traits in broiler. Pakistan J. Nutr. 10: 842–845.

Allen, M.S. 2000. Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. J. Dairy Sci. 83: 1598–1624. Elsevier. doi:10.3168/JDS.S0022-0302(00)75030-2.

Alm El-Dein, A.K., O.S. Rashed, M.M.M. Ouda, N.B. Awaden, I.I. Ismail, M.S. Mady. 2017. Comparative study between dietary supplementation of Grape Pomace and Vitamin E as antioxidant on some Productive, Reproductive and Physiological Performance of Male and Female aged Inshas strain chickens. Egypt Poult Sci. 37:855–72.

Almeida, A., A. L. Mitchell, M. Boland, S. C. Forster, G. B. Gloor, A. Tarkowska, T. D. Lawley, and R. D. Finn. 2019. A new genomic blueprint of the human gut microbiota. Nature 568:499–504.

Almeida, A., A.L. Mitchell, M. Boland, S.C. Forster, G.B. Gloor, A. Tarkowska, T.D. Lawley and R.D. Finn. 2019. A new genomic blueprint of the human gut microbiota. Nature. 568:499–504. https://doi.org/10.1038/s41586-019-0965-1

Amarakoon, A.M.S.B.K. 2017. Regulation of oxidative stress in weaned piglets challenged with Escherichia coli. MSc. Thesis, University of Manitoba. Available online: http://hdl.handle.net/1993/32492

Amir, A., D. McDonald, J. A. Navas-Molina, E. Kopylova, J. T. Morton, Z. Zech Xu, E. P. Kightley, L. R. Thompson, E. R. Hyde, A. Gonzalez, and R. Knight. 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems 2:e00191-16 Available at https://journals.asm.org/doi/full/10.1128/mSys tems.00191-16 (verified 13 May 2022).

Anesini, C., G.E. Ferraro and R. Filip. 2008. Total polyphenol content and antioxidant capacity of commercially available tea (Camellia sinensis) in Argentina. J Agric Food Chem . 56: 9225–9. doi:doi: 10.1021/jf8022782.

Antunes, P., J. Mourão, J. Campos, and L. Peixe. 2016. Salmonellosis: the role of poultry meat. Clin. Microbiol. Infect. 22:110–121.

Apea-Bah, F.B., D. Head, R. Scales, R. Bazylo, and T. Beta. 2020. Hydrothermal extraction, a promising method for concentrating phenolic antioxidants from red osier dogwood (Cornus stolonifera) leaves and stems. Heliyon, 6(10): e05158. doi:10.1016/j.heliyon.2020.e05158

Atterbury, R. J., J. J. Carrique-Mas, R. H. Davies, and V. M. Allen. 2009. *Salmonella* colonisation of laying hens following vaccination with killed and live attenuated commercial *Salmonella* vaccines. Vet. Rec. 165:493–496 Available at https://onlinelibrary.wiley.com/doi/full/10.1136/vr.165.17.493 (verified 9 May 2022).

Attia, Y.A., H.S. Zeweil, A.A. Alsaffar, and A.S. El-Shafy. 2011. Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. Arch. Geflügelk 75: 40–48.

Avila-Calderón, E. D., M. del S. Ruiz-Palma, M. G. Aguilera-Arreola, N. Velázquez-Guadarrama, E. A. Ruiz, Z. Gomez-Lunar, S. Witonsky, and A. Contreras-Rodríguez. 2021. Outer Membrane Vesicles of Gram-Negative Bacteria: An Outlook on Biogenesis. Front. Microbiol. 12:557902.

Aydin, R., M. Karaman, T. Cicek, and H. Yardibi. 2008. Black Cumin (Nigella sativa L.) Supplementation into the Diet of the Laying Hen Positively Influences Egg Yield Parameters, Shell Quality, and Decreases Egg Cholesterol. Poult. Sci. 87: 2590–2595. Elsevier. doi:10.3382/PS.2008-00097.

Balamurugan, R., E. Rajendiran, S. George, G.V. Samuel, B.S. Ramakrishna. 2008. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. J. Gastroenterol. Hepatol. 23:1298–1303. https://doi.org/10.1111/J.1440-1746.2008.05490.X

Balasundram, N., K. Sundram, and S. Samman. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99: 191–203. Elsevier. doi:https://doi.org/10.1016/j.foodchem.2005.07.042.

Bañón, S, P. Díaz, M. Rodríguez, M.D. Garrido, A. Price. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. Meat Sci. 2007; 77:626–633. https://doi.org/10.1016/J.MEATSCI.2007.05.015

Barnes, E. M., G. C. Mead, D. A. Barnuml, and E. G. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311–326 Available at https://www.tandfonline.com/doi/abs/10.1080/00071667208415953 (verified 8 May 2022).

Bauer-Garland, J., J. G. Frye, J. T. Gray, M. E. Berrang, M. A. Harrison, and P. J. Fedorka-Cray. 2006. Transmission of *Salmonella* enterica serotype Typhimurium in poultry with and without antimicrobial selective pressure. J. Appl. Microbiol. 101:1301–1308 Available at https://onlinelibrary.wiley.com/doi/full/10.1111/j.136 5-2672.2006.03036.x (verified 8 May 2022).

Baumgärtel, T, H. Kluth, K. Epperlein, M. Rodehutscord. 2007. A note on digestibility and energy value for sheep of different grape pomace. Small Rumin. Res. 67:302–306. https://doi.org/10.1016/j.smallrumres.2005.11.002

Beaudry, L.J. 1999. Plant indicator guide for Northern British Columbia: boreal, sub-boreal, and subalpine biogeoclimatic zones (BWBS, SBS, SBPS, and Northern ESSF). Ministry of Forests, Research Program.

Bedford, E. 2020. Per capita meat consumption in Canada by type 1998-2019. https://statista.com/statistics/442461/per-capita-meat-consumption-by-type-canada (accessed 3.17.21).

Bednarczyk, M., K. Stadnicka, I. Kozłowska, C. Abiuso, S. Tavaniello, A. Dankowiakowska. and G. Maiorano. 2016. Influence of different prebiotics and mode of their administration on broiler chicken performance. Animal. 10: 1271-1279.

BenSaad, L.A., K.H. Kim, C.C. Quah, W.R. Kim, and M Shahimi. 2017. Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum. BMC Complement. Altern. Med. 17: 47. BioMed Central. doi:https://doi.org/10.1186/s12906-017-1555-0.

Benyi, K., T. S. Tshilate, A. J. Netshipale, and K. T. Mahlako. 2015. Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. Trop. Anim. Heal. Prod. 47:1225–1231.

Berchieri Jr, A., C. K. Murphy, K. Marston, and P. A. Barrow. 2001. Observations on the persistence and vertical transmission of *Salmonella* enterica serovars Pullorum and Gallinarum in chickens: Effect of bacterial and host genetic background. Avian Pathol. 30:221–231 Available at https://www.tandfonline.com/doi/abs/10.1080/03079450120054631 (verified 26 April 2022).

Berrocoso, J. D., R. Kida, A. K. Singh, Y. S. Kim, and R. Jha. 2017. Effect of in ovo injection of raffinose on growth performance and gut health parameters of broiler chicken. Poult. Sci. 96:1573–1580.

Bhushan, S., K. Kalia, M. Sharma, B. Singh, and P.S. Ahuja. 2008. Processing of Apple Pomace for Bioactive Molecules. Crit. Rev. Biotechnol. 28:285–296. https://doi.org/10.1080/07388550802368895.

Bindari, Y. R., R. J. Moore, T. T. H. Van, M. Hilliar, S. B. Wu, S. W. Walkden-Brown, and P. F. Gerber. 2021. Microbial communities of poultry house dust, excreta and litter are partially representative of microbiota of chicken caecum and ileum. PLoS One 16:e0255633 Available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0255633 (verified 20 May 2022).

Blumenreich, M. S. 1990. The White Blood Cell and Differential Count (Walker HK, Hall WD, and Hurst JW, Eds.). 3rd ed. Butterworths, Boston.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857 Available at https://www.nature.com/articles/s41587-019-0209-9 (verified 12 May 2022).

Borsoi, A., L. R. Santos, G. S. Diniz, C. T. P. Salle, H. L. S. Moraes, and V. P. Nascimento. 2011. *Salmonella* fecal excretion control in broiler chickens by organic acids and essential oils blend feed added. Rev. Bras. Cienc. Avic. 13:65–69 Available at http://www.scielo.br/j/rbca/a/456DjmrmNvVNzwsvXKGxLMn/?lang=en (verified 27 April 2022).

Boubendir, S., J. Arsenault, S. Quessy, A. Thibodeau, P. Fravalo, W. P. Thériault, S. Fournaise, and M. Lou Gaucher. 2021. *Salmonella* contamination of broiler chicken carcasses at critical steps of the slaughter process and in the environment of two slaughter plants: Prevalence, genetic profiles, and association with the final carcass status. J. Food Prot. 84:321–332.

Bradley, P. H., and K. S. Pollard. 2017. Proteobacteria explain significant significant functional variability in the human gut microbiome. Microbiome 5:1–23.

Brenes, A., A. Viveros, I. Goñi, C. Centeno, S.G. Sáyago-Ayerdy, I. Arija, F. Saura-Calixto. 2008. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult. Sci. 87:307–316. https://doi.org/10.3382/ps.2007-00297

Brenes, A., A. Viveros, S. Chamorro, and I. Arija. 2016. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. Anim. Feed Sci. Technol. 211:1–17.

Bruce, W.R., T.M.S. Wolever, and A. Giacca. 2000. Mechanisms linking diet and colorectal cancer: The possible role of insulin resistance. Nutr. Cancer, 37(1):19-26. doi:10.1207/S15327914NC3701_2.

Bukin, Y. S., Y. P. Galachyants, I. V. Morozov, S. V. Bukin, A. S. Zakharenko, and T. I. Zemskaya. 2019. The effect of 16S rRNA region choice on bacterial community metabarcoding results. Sci. Data 2019 61 6:1–14.

Bukina, Y. V., N. N. Polishchuk, H. V. Bachurin, O. S. Cherkovskaya, O. L. Zinych, O. L. Lazaryk, and M. B. Bezugly. 2021. *Salmonella*-induced changes of the rat intestinal microbiota. Russ. J. Infect. Immun. 11:865–874 Available at https://iimmun.ru/iimm/article/view/1507 (verified 8 May 2022).

Burkholder, K. M., K. L. Thompson, M. E. Einstein, T. J. Applegate, and J. A. Patterson. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella* Enteritidis colonization in broilers. Poult. Sci. 87:1734–1741.

Buttery, B.R. and R.I. Buzzell. 1977. The relationship between chlorophyll content and rate of photosynthesis in soybeans. Can. J. Plant Sci. 57: 1–5. NRC Research Press. doi:https://doi.org/10.4141/cjps77-001.

Canadian Council on Animal Care. 2009. The care and use of farm animals in research, teaching and testing. Ottawa, CCAC:12–15.

Cao, G. 2014. *Salmonella* Newport: Genetic diversity and phylogenetic analysis. Available at http://hdl.handle.net/1903/15186.

Carpenter, R., M.N. O'Grady, Y.C. O'Callaghan, N.M. O'Brien, J.P. Kerry. 2007. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. Meat Sci. 76:604–610. https://doi.org/10.1016/J.MEATSCI.2007.01.021

Carvalho, B. M., D. Guadagnini, D. M. L. Tsukumo, A. A. Schenka, P. Latuf-Filho, J. Vassallo, J. C. Dias, L. T. Kubota, J. B. C. Carvalheira, and M. J. A. Saad. 2012. Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. Diabetologia 55:2823–2834.

Carvalho, F. A., O. Koren, J. K. Goodrich, M. E. V. Johansson, I. Nalbantoglu, J. D. Aitken, Y. Su, B. Chassaing, W. A. Walters, A. Gonz´alez, J. C. Clemente, T. C. Cullender, N. Barnich, Darfeuille-Michaud, M. Vijay-Kumar, R. Knight, R. E. Ley, and A. T. Gewirtz. 2012. Transient inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice. Cell Host Microbe. 12:139–152.

Casewell, M., C. Friis, E. Marco, P. McMullin, and I. Phillips. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J. Antimicrob. Chemother. 52: 159–161. Oxford University Press. doi:https://doi.org/10.1093/jac/dkg313.

Castanon, J.I.R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86: 2466–2471. Elsevier.

Castro-Vargas, R. E., M. P. Herrera-Sánchez, R. Rodríguez-Hernández, and I. S. Rondón-Barragán. 2020. Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. Vet. World 13:2070–2084 Available at /pmc/articles/PMC7704309/ (verified 2 May 2022).

Catalkaya, G., K. Venema, L. Lucini, G. Rocchetti, D. Delmas, M. Daglia, A. De Filippis, H. Xiao, J. L. Quiles, J. Xiao, and E. Capanoglu. 2020. Interaction of dietary polyphenols and gut microbiota: Microbial metabolism of polyphenols, influence on the gut microbiota, and implications on host health. Food Front. 1:109–133 Available at https://onlinelibrary.wiley.com/doi/full/10.1002/fft2.25 (verified 18 May 2022).

CCAC-Canadian Council on Animal Care. 2009. The care and use of farm animals in research, teaching and testing. CCAC, Ottawa, 12–15.

Centers for Disease Control and Prevention (CDC). 2018. Reports of Selected *Salmonella* Outbreak Investigations. Available at https://www.cdc.gov/*Salmonella*/outbreaks.html (verified 11 August 2021).

Centers for Disease Control and Prevention. 2020. Serotypes and the Importance of Serotyping *Salmonella* | *Salmonella* Atlas | Reports and Publications | *Salmonella* | CDC. Available at https://www.cdc.gov/*Salmonella*/reportspubs/*Salmonella*-atlas/serotyping-importance.html (verified 11 September 2021).

Chacar, S., T. Itani, J. Hajal, Y. Saliba, N. Louka, J.F. Faivre, R. Maroun and N. Fares. 2018. The Impact of Long-Term Intake of Phenolic Compounds-Rich Grape Pomace on Rat Gut Microbiota. J. Food Sci. 83:246–251. https://doi.org/10.1111/1750-3841.14006

Chamorro, S., A. Viveros, A. Rebolé, B.D. Rica, I. Arija, and A. Brenes. 2015. Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. Food Res. Int. 73:197–203.

Chamorro, S., A. Viveros, A. Rebolé, I. Arija, C. Romero, I. Alvarez, A. Rey, and A. Brenes. 2017. Addition of exogenous enzymes to diets containing grape pomace: effects on intestinal utilization of catechins and antioxidant status of chickens. Food Res. Int. 96:226–234.

Chamorro, S., C. Romero, A. Brenes, F. Sánchez-Patán, B. Bartolomé, A. Viveros, I. Arija. 2019. Impact of a sustained consumption of grape extract on digestion, gut microbial metabolism and intestinal barrier in broiler chickens Food Funct. 10:1444-1454. https://doi.org/10.1039/c8fo02465k

Chang, C. H., P. Y. Teng, T. T. Lee, and B. Yu. 2020. Effects of multi-strain probiotic supplementation on intestinal microbiota, tight junctions, and inflammation in young broiler chickens challenged with *Salmonella* enterica subsp. enterica. Asian-Australasian J. Anim. Sci. 33:1797–1808 Available at /pmc/articles/PMC7649073/ (verified 16 May 2022).

Chapman, H. D., and T. K. Jeffers. 2014. Vaccination of chickens against coccidiosis ameliorates drug resistance in commercial poultry production. Int. J. Parasitol. Drugs Drug Resist. 4:214–217.

Cheikhyoussef, A., N. Cheikhyoussef, H. Chen, J. Zhao, J. Tang, H. Zhang, and W. Chen. 2010. Bifidin I - A new bacteriocin produced by Bifidobacterium infantis BCRC 14602: Purification and partial amino acid sequence. Food Control 21:746–753.

Chen, H. M., Y. Wang, L. H. Su, and C. H. Chiu. 2013. Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. Pediatr. Neonatol. 54:147–152.

Chen, S., X. Shen, S. Cheng, P. Li, J. Du, Y. Chang and H. Meng. 2013. Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. PLoS One 8: e79730. Public Library of Science. doi:https://doi.org/10.1371/journal.pone.0079730.

Chen, Y., Y. Cheng, W. Wang, A. Wang, and Y. Zhou. 2020. Protective effects of dietary Supplementation with a silicate clay mineral (palygorskite) in lipopolysaccharide-challenged broiler chickens at an early age. Anim. Feed Sci. Technol. 263:114459.

Chhabra, P. C., and M. C. Goel. 1980. Normal profile of immunoglobulins in sera and tracheal washings of chickens. Res. Vet. Sci. 29:148–152.

Chicken Farmers of Canada 2020. Antibiotics. [Online] Available: https://www.chickenfarmers.ca/antibiotics/ [2020 Oct. 23].

Colombino, E., I. Ferrocino, I. Biasato, L.S. Cocolin, D. Prieto-Botella, Z. Zduńczyk, J. Jankowski, J. Milala, M. Kosmala, B. Fotschki, M.T. Capucchio, and J. Juśkiewicz. 2020. Dried fruit pomace inclusion in poultry diet: growth performance, intestinal morphology and physiology. J. Anim. Sci. Biotechnol. 11:1–18. https://doi.org/10.1186/s40104-020-00464-z

Comeau, A. M., G. M. Douglas, and M. G. I. Langille. 2017. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. mSystems 2:e00127-16 Available at https://journals.asm.org/doi/full/10.1128/mSystems.0012 7-16 (verified 12 May 2022).

Conrad, K., and W. Stocker. 2013. Anti-Intestinal Goblet Cell Antibodies. in Autoantibodies: Third Edition. Third Edit. Elsevier B.V. Pages 425–431

Cooke, D., W.P. Steward, A.J. Gescher and T. Marczylo. 2005. Anthocyans from fruits and vegetables - Does bright colour signal cancer chemopreventive activity? Eur. J. Cancer 41: 1931–1940. Pergamon. doi:10.1016/j.ejca.2005.06.009.

Costa, M. C., J. A. Bessegatto, A. A. Alfieri, J. S. Weese, J. A. B. Filho, and A. Oba. 2017. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS One 12:e0171642.

Crisol-Martínez, E., D. Stanley, M. S. Geier, R. J. Hughes, and R. J. Moore. 2017. Understanding the mechanisms of zinc bacitracin and avilamycin on animal production: linking gut microbiota and growth performance in chickens. Appl. Genet. Mol. Biotechnol. 101:4547–4559 Available at http://www.primer-e.com/] (verified 31 October 2021).

da Silva, M.A., B.M. de Sousa Pessotti, S.F. Zanini, G.L. Colnago, M.R.A. Rodrigues, L. de C. Nunes, M.S. Zanini and I.V.F. Martins. 2009. Intestinal mucosa structure of broiler chickens infected experimentally with Eimeria tenella and treated with essential oil of oregano. scielo. doi:https://doi.org/10.1590/S0103-84782009005000135.

Dai, X. and A.P. Faciola. 2019. Evaluating Strategies to Reduce Ruminal Protozoa and Their Impacts on Nutrient Utilization and Animal Performance in Ruminants – A Meta-Analysis. Front. Microbiol. 10: 2648. Frontiers Media S.A. doi:10.3389/FMICB.2019.02648/BIBTEX.

Dailey, M. O. 2002. The Immune Functions of the Spleen - The Complete Spleen: Structure, Function, and Clinical Disorders.in Bowdler, A.J., ed. Humana Press, Totowa, NJ. 51-69.

Daneshmand, A., H. Kermanshahi, M. H. Sekhavati, A. Javadmanesh and M. Ahmadian. 2019. Antimicrobial peptide, cLF36, affects performance and intestinal morphology, microflora, junctional proteins, and immune cells in broilers challenged with E. coli. Sci. Rep, 9: 1-9.

David, L., V. Danciu, B. Moldovan and A. Filip. 2019. Effects of In Vitro Gastrointestinal Digestion on the Antioxidant Capacity and Anthocyanin Content of Cornelian Cherry Fruit Extract. Antioxidants 2019, Vol. 8, Page 114 8: 114. Multidisciplinary Digital Publishing Institute. doi:10.3390/ANTIOX8050114.

Davis, C. Y. 1985. Immunoglobulin concentrations in serum and secretions of vitamin A-deficient broiler chicks following Newcastle disease virus vaccination. Available at https://lib.dr.iastate.edu/rtd/7837 (verified 4 May 2022).

De Filippo, C., D. Cavalieri, M. Di Paola, M. Ramazzotti, J. B. Poullet, S. Massart, S. Collini, G. Pieraccini, and P. Lionetti. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. U. S. A. 107:14691–14696.

den Hartog, L.A., C.H.M. Smits and W.H. Hendriks. 2016. Feed additive strategies for replacement of antimicrobial growth promoters and a responsible use of antibiotics. Broadening Horizons. INRA.

Deriu, E., J. Z. Liu, M. Pezeshki, R. A. Edwards, R. J. Ochoa, H. Contreras, S. J. Libby, F. C. Fang, and M. Raffatellu. 2013. Probiotic bacteria reduce *Salmonella* typhimurium intestinal colonization by competing for iron. Cell Host Microbe 14:26–37.

Dev, K., N. Akbar Mir, A. Biswas, J. Kannoujia, J. Begum, and R. Kant. 2020. Dietary Mannan-oligosaccharides potentiate the beneficial effects of Bifidobacterium bifidum in broiler chicken. Lett. Appl. Microbiol. 71:520–530 Available at https://onlinelibrary.wiley.com/doi/full/10.1111/lam.13360 (verified 18 May 2022).

Díaz Carrasco, J. M., E. A. Redondo, N. D. Pin Viso, L. M. Redondo, M. D. Farber, and M. E. Fernández Miyakawa. 2018. Tannins and Bacitracin Differentially Modulate Gut Microbiota of Broiler Chickens. Biomed Res. Int. 2018:1–11.

Dibner, J.J. and J.D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84,634–643.

Dubourg, G., J. C. Lagier, F. Armougom, C. Robert, I. Hamad, P. Brouqui, and D. Raoult. 2013. The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. Eur. J. Clin. Microbiol. Infect. Dis. 32:637–645.

Duda-Chodak, A., T. Tarko, P. Satora, and P. Sroka. 2015. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur. J. Nutr. 54:325–341 Available at https://link.springer.com/article/10.1007/s00394-015-0852-y (verified 17 May 2022).

Dudek-Wicher, R. K., A. Junka, and M. Bartoszewicz. 2018. The influence of antibiotics and dietary components on gut microbiota. Przegląd Gastroenterol. 13:85.

Dupak, R., J. Kovac, A. Kalafova, A. Kovacik, K. Tokarova, P. Hascik, et al. 2020. Supplementation of grape pomace in broiler chickens diets and its effect on body weight, lipid profile, antioxidant status and serum biochemistry. Biologia (Bratisl) 76:2511–8. https://doi.org/10.1007/S11756-021-00737-6/TABLES/3.

Dwyer, K., F. Hosseinian, and M. R. Rod. 2014. The market potential of grape waste alternatives. J. Food Res. 3:91.

Džinić, N., N. Puvača, T. Tasić, P. Ikonić and Đ. Okanović. 2015. How meat quality and sensory perception is influenced by feeding poultry plant extracts. Worlds. Poult. Sci. J. 71: 673–682. Cambridge University Press on behalf of World's Poultry Science Association. doi:https://doi.org/10.1017/S0043933915002378.

Ebrahimzadeh, S. K., B. Navidshad, P. Farhoomand, and F. Mirzaei Aghjehgheshlagh. 2018. Effects of grape pomace and vitamin E on performance, antioxidant status, immune response, gut morphology and histopathological responses in broiler chick- ens. S. Afr. J. Anim. Sci. 48:324. https://doi.org/10.4314/sajas.v48i2.13

Economic Research Service/USDA. U. S. Broiler Production . URL https://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-production/ (accessed 4.14.21).

El Gharras, H. 2009. Polyphenols: food sources, properties and applications−a review. Int. J. food Sci. Technol. 44:2512–2518.

Elbere, I., I. Kalnina, I. Silamikelis, I. Konrade, L. Zaharenko, K. Sekace, I. Radovica-Spalvina, D. Fridmanis, D. Gudra, V. Pirags, and J. Klovins. 2018. Association of metformin administration with gut microbiome dysbiosis in healthy volunteers. PLoS One 13:1–17.

El-Shall, N. A., A. M. Awad, M. E. A. El-Hack, M. A. E. Naiel, S. I. Othman, A. A. Allam, and M. E. Sedeik. 2020. The simultaneous administration of a probiotic or prebiotic with live *Salmonella* vaccine improves growth performance and reduces fecal shedding of the bacterium in *Salmonella*-challenged broilers. Animals 10:70 Available at https://www.mdpi.com/2076-2615/10/1/70/htm (verified 29 April 2022).

Embuscado, M.E. 2015. Spices and herbs: Natural sources of antioxidants–a mini review. J. Funct. Foods 18: 811–819. Elsevier. doi:https://doi.org/10.1016/j.jff.2015.03.005.

Erinle, T.J., Oladokun, S, MacIsaac J, Rathgeber B, Adewole D. 2022a. Dietary grape pomace – Effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens. Poult. Sci.; 101519. https://doi.org/10.1016/j.psj.2021.101519.

Erinle, T., J. MacIsaac, C. Yang, and D. Adewole. 2022b. Effect of red osier dogwood extract on growth performance, blood biochemical parameters, and gut functionality of broiler chickens challenged or unchallenged intraperitoneally with *Salmonella* Enteritidis lipopolysaccharide. Poult. Sci.:101861.

Erinle, T. J., M. Boulianne, Y. Miar, and R. Scales. 2022c. Red oseir dogwood and its use in animal nutrition - A Review. Anim. Nutr.:Submitted.

European Commission of the European Parliament. 2006. COMMISSION REGULATION (EC) No 1177/2006 of 1 August 2006 Implementing Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the trace. Off. J. Eur. Union Available at http://data.europa.eu/eli/reg/2006/1177/oj.

European Food Safety Authority. 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15:e05077 Available at /pmc/articles/PMC7009962/ (verified 9 May 2022).

European Parliament and the Council of the European Union. 2003. Regulation (EC) no 1831/2003. Off. J. Eur. Union 4:29–43.

Fadeev, E., M. G. Cardozo-Mino, J. Z. Rapp, C. Bienhold, I. Salter, V. Salman-Carvalho, M. Molari, H. E. Tegetmeyer, P. L. Buttigieg, and A. Boetius. 2021. Comparison of Two 16S rRNA Primers (V3–V4 and V4–V5) for Studies of Arctic Microbial Communities. Front. Microbiol. 12.

Farber, S. 1966. Chemotherapy in the Treatment of Leukemia and Wilms' Tumor. J. Am. Med. Assoc. 198:826–836 Available at https://jamanetwork.com/journals/jama/fullarticle/662326 (verified 19 May 2022).

Farhana, A., and Y. S. Khan. 2021. Biochemistry, Lipopolysaccharide. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK554414/ (Accessed 23-Sept-2021).

Fashingbauer, B. A., and J. B. Moyle. 1963. Nutritive Value of Red-Osier Dogwood and Mountain Maple as Deer Browse. J. Minnesota Acad. Sci. 31:73–81 Available at https://digitalcommons.morris.umn.edu/jmas/vol31/iss1/18 (verified 15 January 2022).

Fasina, Y. O., F. J. Hoerr, S. R. McKee, and D. E. Conner. 2010. Influence of *Salmonella* enterica serovar typhimurium infection on intestinal goblet cells and villous morphology in broiler chicks. Avian Dis. 54:841–847 Available at https://meridian.allenpress.com/avian-diseases/article/54/2/841/133749/Influence-of-*Salmonella*-enterica-Serovar (verified 29 April 2022).

Feduraev, P., G. Chupakhina, P. Maslennikov, N. Tacenko, and L. Skrypnik. 2019. Variation in phenolic compounds content and antioxidant activity of different plant organs from Rumex crispus l. and Rumex obtusifolius l. at different growth stages. Antioxidants 8. Multidisciplinary Digital Publishing Institute (MDPI). doi:10.3390/antiox8070237.

Ferenczi, S., K. Szegi, Z. Winkler, T. Barna, and K. J. Kovács. 2016. Oligomannan Prebiotic Attenuates Immunological, Clinical and Behavioral Symptoms in Mouse Model of Inflammatory Bowel Disease. Sci. Rep. 6:1–10 Available at https://www.nature.com/articles/srep34132 (verified 1 May 2022).

Ferrari, R. G., D. K. A. Rosario, A. Cunha-Neto, S. B. Mano, E. E. S. Figueiredo, and C. A. Conte-Juniora. 2019. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: A meta-analysis. Appl. Environ. Microbiol. 85:e00591-19 Available at /pmc/articles/PMC6606869/ (verified 8 May 2022).

Ferrer, M. D., A. López-López, T. Nicolescu, S. Perez-Vilaplana, A. Boix-Amorós, M. Dzidic, S. Garcia, A. Artacho, C. Llena, and A. Mira. 2020. Topic application of the probiotic *Streptococcus dentisani* improves clinical and microbiological parameters associated with oral health. Front. Cell. Infect. Microbiol. 10:465.

Field, T.S., D.W. Lee and N.M. Holbrook. 2001. Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol. 127: 566–574. Am Soc Plant Biol. doi:https://doi.org/10.1104/pp.010063.

Firrman, J., L. S. Liu, L. Zhang, G. Arango Argoty, M. Wang, P. Tomasula, M. Kobori, S. Pontious, and W. Xiao. 2016. The effect of quercetin on genetic expression of the commensal gut microbes Bifidobacterium catenulatum, Enterococcus caccae and Ruminococcus gauvreauii. Anaerobe 42:130–141.

Food and Agricultural Organization of United Nations (FAO) 2021. Crop. [Online] Available: http://www.fao.org/faostat/en/?#data/QC [2021 Apr. 4].

Forster, S. C., N. Kumar, B. O. Anonye, A. Almeida, E. Viciani, M. D. Stares, M. Dunn, T. T. Mkandawire, A. Zhu, Y. Shao, L. J. Pike, T. Louie, H. P. Browne, A. L. Mitchell, B. A. Neville, R. D. Finn, and T. D. Lawley. 2019. A human gut bacterial genome and culture collection for improved metagenomic analyses. Nat. Biotechnol. 37:186–192. https://doi.org/10.1038/s41587-018-0009-7

Gadde, U., W. H. Kim, S. T. Oh, and H. S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Heal. Res. Rev. 18:26–45.

Galanis, E., D. M. A. Lo Fo Wong, M. E. Patrick, N. Binsztein, A. Cieslik, T. Chalermchaikit, A. Aidara-Kane, A. Ellis, F. J. Angulo, and H. C. Wegener. 2006. Web-based surveillance and global *Salmonella* distribution, 2000-2002. Emerg. Infect. Dis. 12:381–388 Available at https://wwwnc.cdc.gov/eid/article/12/3/05-0854_article (verified 11 August 2021).

García-Lomillo, J., and M. L. Gonz´alez-SanJos´e. 2017. Applications of wine pomace in the food industry: approaches and functions. Compr. Rev. food Sci. food Saf. 16:3–22.

García-López, R., F. Cornejo-Granados, A. A. Lopez-Zavala, F. Sánchez-López, A. Cota-Huízar, R. R. Sotelo-Mundo, A. Guerrero, A. Mendoza-Vargas, B. Gómez-Gil, and A. Ochoa-Leyva. 2020. Doing more with less: a comparison of 16S hypervariable regions in search of defining the shrimp microbiota. Microorg. 8:134 8:134.

Garg, C.K., N.P. Sahu, N. Shamna, A.D. Deo, F.J. Fawole, S. Kumar, and M.K. Maiti. 2019. Effect of dietary Houttuynia cordata leaf meal and leaf extract on the growth performance, nutrient utilization and expression of IGF-I gene in Labeo rohita. Aquac. Nutr. 25: 702–711. John Wiley & Sons, Ltd. doi:10.1111/anu.12891.

Gaucher, M. L., S. Quessy, A. Letellier, J. Arsenault, and M. Boulianne. 2015. Impact of a drug-free program on broiler chicken growth performances, gut health, Clostridium perfringens and Campylobacter jejuni occurrences at the farm level. Poult. Sci. 94:1791–1801.

Ghareeb, K., W. A. Awad, S. Nitsch, S. Abdel-Raheem, and J. Böhm. 2008. Effects of transportation on stress and fear responses of growing broilers supplemented with prebiotic or probiotic. Int. J. Poult. Sci. 7:678–685.

Giannenas, I., E. Bonos, E. Christaki and P. Florou-Paneri. 2013. Essential oils and their applications in animal nutrition. Med. Aromat. Plants 2: 412–2167. doi:http://dx.doi.org/10.4172/2167-0412.1000140.

Giannenas, I., E. Bonos, I. Skoufos, A. Tzora, I. Stylianaki, D. Lazari, A. Tsinas, E. Christaki, and P. Florou-Paneri. 2018. Effect of herbal feed additives on performance parameters, intestinal microbiota, intestinal morphology and meat lipid oxidation of broiler chickens. Br. Poult. Sci. 59:545–553.

Gomaa, W. M. S., L. Y. Wei, G. M. Mosaad, H. Aamer, T. W. Alexander, and W. Z. Yang. 2018. In situ ruminal digestibility of red osier dogwood in finishing beef heifers. Can. J. Anim. Sci. 98:888–892. Available at https://cdnsciencepub.com/doi/abs/10.1139/cjas-2017-0092 (verified 18 January 2022).

Gomaa, W.M.S., A.M. Saleem, T. Ran, L. Jin, M. Samir, E.J. McGeough, K. Ominski, L. Chen and W. Yang. 2021. Effect of Dried Distillers Grains With Solubles and Red Osier Dogwood Extract on Fermentation Pattern and Microbial Profiles of a High-Grain Diet in an Artificial Rumen System. Front. Vet. Sci. 8: 644738. Frontiers Media SA. doi:10.3389/FVETS.2021.644738.

Gong, J. and C. Yang. 2012. Advances in the methods for studying gut microbiota and their relevance to the research of dietary fiber functions. Food Res. Int. 48:916–929.

Goni, I., A. Brenes, C. Centeno, A. Viveros, F. Saura-Calixto, A. Rebol´e, I. Arija, and R. Estevez. 2007. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. Poult. Sci. 86:508–516. https://doi.org/10.1093/ps/86.3.508

Gorzynik-Debicka, M., P. Przychodzen, F. Cappello, A. Kuban-Jankowska, A. M. Gammazza, N. Knap, M. Wozniak, and M. Gorska-Ponikowska. 2018. Potential health benefits of olive oil and plant polyphenols. Int. J. Mol. Sci. 19:547 Available at /pmc/articles/PMC5877547/ (verified 3 May 2022).

Government of Canada. 2007. Laboratory surveillance data for enteric pathogens in Canada: annual summary 2006. National Microbiological Laboratory.

Government of Canada. 2016. Surveillance of salmonellosis (Salmonella). https://www.canada.ca/en/public-health/services/diseases/salmonellosis-salmonella/surveillance.html

Gowd, V., N. Karim, M. R. I. Shishir, L. Xie, and W. Chen. 2019. Dietary polyphenols to combat the metabolic diseases via altering gut microbiota. Trends Food Sci. Technol. 93:81–93.

Gowman, A.C., M.C. Picard, A. Rodriguez-Uribe, M. Misra, H. Khalil, M. Thimmanagari, and A.K. Mohanty. 2019. Physicochemical analysis of apple and grape pomaces. BioResources. 14:3210–3230.

Graham, J.P., J.J. Boland and E. Silbergeld. 2007. Growth promoting antibiotics in food animal production: an economic analysis. Public Health Rep. 122: 79–87. SAGE Publications Sage CA: Los Angeles, CA. doi:https://doi.org/10.1177/003335490712200111.

Granato, D., M. Magalhães Carrapeiro, V. de, Fogliano and S. M. van Ruth. 2016. Effects of geographical origin, varietal and farming system on the chemical composition and functional properties of purple grape juices: A review. Trends in Food Science & Technology, 52, 31–48. doi:10.1016/j.tifs.2016.03.013

Gressner, O. A., M. Fang, H. Li, L. G. Lu, A. M. Gressner, and C. F. Gao. 2013. Connective tissue growth factor (CTGF/CCN2) in serum is an indicator of fibrogenic progression and malignant transformation in patients with chronic hepatitis B infection. Clin. Chim. Acta 421:126–131.

Guaiume, E. A. 2005. Effects of continuous administration of low-dose of Escherichia coli lipopolysaccharide in chicks and poults fed non toxic doses of aflatoxin B1 and T-2 toxin. University of Missouri-Columbia.

Gucker, C. 2012. Cornus sericea. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). [Online] Available: https://www.fs.fed.us/database/feis/plants/shrub/corser/all.html.

Gungor, E., A. Altop and G. Erener 2021. Effect of raw and fermented grape pomace on the growth performance, antioxidant status, intestinal morphology, and selected bacterial species in broiler chicks. Animals. 11:1–14. https://doi.org/10.3390/ani11020364

Hafsa, S.H.A. and S.A. Ibrahim. 2018. Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. J. Anim. Physiol. Anim. Nutr. (Berl). 102:268–275. https://doi.org/10.1111/JPN.12688

Hagiwara, Y., T. Kasukabe, Y. Kaneko, N. Niitsu and J. Okabe-Kado. 2010. Ellagic acid, a natural polyphenolic compound, induces apoptosis and potentiates retinoic acid-induced differentiation of human leukemia HL-60 cells. Int. J. Hematol. 92: 136–143. Springer. doi:https://doi.org/10.1007/s12185-010-0627-4.

Hanušovský, O., D. Biro, M. Šimko, D. Bíro, B. Gálik, M. Juráček, et al. 2019. The Effect of Locality on the Grape Pomace Nutritional Value from the different Locations of Slovakia. J. Int. Sci. Publ. 7:282–8.

Harbowy, M.E., D.A. Balentine, A.P. Davies, and Y. Cai. 1997. Tea chemistry. CRC. Crit. Rev. Plant Sci. 16: 415–480. Taylor & Francis. doi:https://doi.org/10.1080/07352689709701956.

Hardy B.B.T. 1989. Manual of plant species suitability for reclamation in Alberta. Rep. no. RRTAC: 84–89.

Hashemi, S.R. and H. Davoodi. 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet. Res. Commun. 35: 169–180. Springer. doi:10.1007/s11259-010-9458-2.

Hassan, Y.I., V. Kosir, X. Yin, K. Ross and M.S. Diarra. 2019. Grape Pomace as a Promising Antimicrobial Alternative in Feed: A Critical Review. J. Agric. Food Chem. 67: 9705–9718. ACS Publications. doi:https://doi.org/10.1021/acs.jafc.9b02861.

Hatier, J.H. and K.S. Gould. 2008. Foliar anthocyanins as modulators of stress signals. J. Theor. Biol. 253: 625–627. doi:10.1016/j.jtbi.2008.04.018.

He, J., T.C. Wallace, K.E. Keatley, M.L. Failla, and M.M. Giusti. 2009. Stability of Black Raspberry Anthocyanins in the Digestive Tract Lumen and Transport Efficiency into Gastric and Small Intestinal Tissues in the Rat. J. Agric. Food Chem. 57: 3141–3148. American Chemical Society. doi:10.1021/JF900567T.

Hemetsberger, F., B. Zwirzitz, N. Yacoubi, W. Kneifel, K. Schedle, and K. J. Domig. 2022. Effect of Two Soybean Varieties Treated with Different Heat Intensities on Ileal and Caecal Microbiota in Broiler Chickens. Animals 12:1109 Available at https://www.mdpi.com/2076-2615/12/9/1109/htm (verified 20 May 2022).

Hernández, F., J. Madrid, V. García, J. Orengo, and M.D. Megías. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci. 83: 169–174. Elsevier. doi:10.1093/PS/83.2.169.

Heuze, V. and G. Tran. 2020. Grape pomace. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. Feedipedia. Accessed Feb. 2021. https://www.feedipedia.org/node/691.

Heyman, B., and M. J. Shulman. 2016. Structure, Function, and Production of Immunoglobulin M (IgM), In Encyclopedia of Immunobiology. Elsevier Inc. Pages 1–14.

Hickman, S., and S. Kornfeld. 1978. Effect of tunicamycin on IgM, IgA, and IgG secretion by mouse plasmacytoma cells. J. Immunol. 121:990–6 Available at http://www.jimmunol.org/content/121/3/990 (verified 3 May 2022).

Higgins, J. P., S. E. Higgins, A. D. Wolfenden, S. N. Henderson, A. Torres-Rodriguez, J. L. Vicente, B. M. Hargis, and G. Tellez. 2010. Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella* Enteritidis in neonatal broilers. Poult. Sci. 89:243–247.

Higgins, S. E., J. P. Higgins, A. D. Wolfenden, S. N. Henderson, A. Torres-Rodriguez, G. Tellez, and B. Hargis. 2008. Evaluation of a Lactobacillus-based probiotic culture for the reduction of *Salmonella* enteritidis in neonatal broiler chicks. Poult. Sci. 87:27–31.

Hogervorst, J. C., U. Milji´c, and V. Puˇskaˇs. 2017. Extraction of bioactive compounds from grape processing by-products. Pages 105−135 in Handbook of Grape Processing By-Products. Elsevier, Novi Sad, Serbia.

Holodick, N. E., T. Vizconde, T. J. Hopkins, and T. L. Rothstein. 2016. Age-Related Decline in Natural IgM Function: Diversification and Selection of the B-1a Cell Pool with Age. J. Immunol. 196:4348–4357 Available at /pmc/articles/PMC4874569/ (verified 4 May 2022).

Honda, K., and D. R. Littman. 2016. The microbiota in adaptive immune homeostasis and disease. Nat. 2016 5357610 535:75–84.

Hooper, L.V., T. Midwedt, and J.I. Gordon. 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu. Rev. Nutr. 22:283-307. https://doi.org/10.1146/annurev.nutr.22.011602.092259.

Hosseini-Vashan, S.J., M. Safdari-Rostamabad, A.H. Piray, H. Sarir. 2020. The growth performance, plasma biochemistry indices, immune system, antioxidant status, and intestinal morphology of heat-stressed broiler chickens fed grape (Vitis vinifera) pomace. Anim Feed Sci Technol. 259:114343. https://doi.org/10.1016/J.ANIFEEDSCI.2019.114343.

Hu, X. F., Y. M. Guo, J. H. Li, G. L. Yan, S. Bun, and B. Y. Huang. 2011. Effects of an early lipopolysaccharide challenge on growth and small intestinal structure and function of broiler chickens. Can. J. Anim. Sci. 91:379–384.

Huang, X., S. R. Palmer, S.-J. Ahn, V. P. Richards, M. L. Williams, M. M. Nascimento, and R. A. Burne. 2016. A highly arginolytic Streptococcus species that potently antagonizes Streptococcus mutans. Appl. Environ. Microbiol. 82:2187–2201.

Huycke, M.M., V. Abrams and D.R. Moore. 2002. Enterococcus faecalis produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. Carcinogenesis. 23:529–536. https://doi.org/10.1093/CARCIN/23.3.529

Huyghebaert, G., R. Ducatelle, and F. Van Immerseel. 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187:182–188.

Ijaz, A., E. J. A. Veldhuizen, F. Broere, V. P. M. G. Rutten, and C. A. Jansen. 2021. The interplay between *Salmonella* and intestinal innate immune cells in chickens. Pathogens 10:1512 Available at /pmc/articles/PMC8618210/ (verified 8 May 2022).

Ikusika, O. O., A. B. Falowo, C. T. Mpendulo, T. J. Zindove, and A. I. Okoh. 2020. Effect of strain, sex and slaughter weight on growth performance, carcass yield and quality of broiler meat. Open Agric. 5:607–616.

Isaak, C.K., J.C. Petkau, O. Karmin, K. Ominski, J.C. Rodriguez-Lecompte and Y.L. Siow. 2013. Seasonal variations in phenolic compounds and antioxidant capacity of Cornus stolonifera plant material: Applications in agriculture. Can. J. Plant Sci. 93: 725–734. NRC Research Press. doi:https://doi.org/10.4141/cjps2012-310.

Islam, M.R., D. Lepp, D.V. Godfrey, S. Orban, K. Ross, P. Delaquis and M.S. Diarra. 2019. Effects of wild blueberry (Vaccinium angustifolium) pomace feeding on gut microbiota and blood metabolites in free-range pastured broiler chickens. Poult. Sci. 98:3739–3755. https://doi.org/10.3382/PS/PEZ062

Islam, M.R., D.B. Oomah and M.S. Diarra. 2017. Potential immunomodulatory effects of non-dialyzable materials of cranberry extract in poultry production. Poult Sci 96:341–50. https://doi.org/10.3382/PS/PEW302.

Islam, M.R., Y.I. Hassan, Q. Das, D. Lepp, M. Hernandez, D.V. Godfrey, S. Orban, K Ross, Delaquis P, Diarra MS. 2020. Dietary organic cranberry pomace influences multiple blood biochemical parameters and cecal microbiota in pasture-raised broiler chickens. J. Funct. Foods. 72:104053. https://doi.org/10.1016/j.jff.2020.104053

Ismail, I. E., M. Alagawany, A. E. Taha, N. Puvača, V. Laudadio, and V. Tufarelli. 2020. Effect of dietary Supplementation of garlic powder and phenyl acetic acid on productive performance, blood haematology, immunity and antioxidant status of broiler chickens. Anim. Biosci. 34:363–370.

Jami, E., B. A. White, and I. Mizrahi. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. PLoS One 9:e85423.

Jang, I. S., Y. H. Ko, Y. S. Moon, and S. H. Sohn. 2014. Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. Asian-Australasian J. Anim. Sci. 27:749. https://doi.org/10.5713/ajas.2013.13852

Jayaraman, B., S. Amarakoon, B. Koo, K. O, and Nyachoti, C. 2018. PSIV-30 Effects of dietary supplementation with ground red-osier dogwood (Cornus stolonifera) on growth performance, blood profile, and ileal histomorphology in weaned pigs challenged with Escherichia coli K88+. J. Anim. Sci. 96: 322. Oxford University Press. doi:10.1093/jas/sky404.708.

Jazi, V., H. Mohebodini, A. Ashayerizadeh, A. Shabani, and R. Barekatain. 2019. Fermented soybean meal ameliorates *Salmonella* Typhimurium infection in young broiler chickens. Poult. Sci. 98:5648–5660.

Jiang, Q., H. Zhang, R. Yang, Q. Hui, Y. Chen, L. Mats, R. Tsao, and C. Yang. 2019. Red-Osier Dogwood Extracts Prevent Inflammatory Responses in Caco-2 Cells and a Caco-2 BBe1/EA. hy926 Cell Co-Culture Model. Antioxidants 8: 428. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/antiox8100428.

Jiang, X.R., H.J. Zhang, G. Mantovani, G.L. Alborali, J.M. Caputo, G. Savoini, V. Dell'Orto, and V. Bontempo. 2014. The effect of plant polyphenols on the antioxidant defence system of weaned piglets subjected to an Escherichia coli challenge. J. Anim. Feed Sci. 23(4):324-330. doi:https://doi.org/10.22358/jafs/65668/2014

Jonathan, O., C. Mguvane, M. Id, C. Kumanda and V. Mlambo. Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels. PLoS One 2021;16:e0259630. https://doi.org/10.1371/JOURNAL.PONE.0259630.

Jones, B.V., M. Begley, C. Hill, C.G.M. Gahan, J.R. Marchesi. 2008. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. Proc. Natl. Acad. Sci. 105:13580–13585. https://doi.org/10.1073/PNAS.0804437105

Jung, T.H., J.H. Park, W.M. Jeon and K.S. Han. 2015. Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway. Nutr. Res. Pract. 9:343.

Juśkiewicz, J, J. Jankowski, Z. Zduńczyk, K. Kołodziejczyk, D. Mikulski and P. Zduńczyk. 2015. The chemical composition of selected dried fruit pomaces and their effects on the growth performance and post-slaughter parameters of young turkeys. J. Anim. Feed Sci. 24:53–60.

Kabtni, S., D. Sdouga, I. Bettaib Rebey, M. Save, N. Trifi-Farah, M.L. Fauconnier, and S. Marghali. 2020. Influence of climate variation on phenolic composition and antioxidant capacity of Medicago minima populations. Sci. Reports 2020 101 10: 1–15. Nature Publishing Group. doi:10.1038/s41598-020-65160-4.

Kang, S.Y., N.P. Seeram, M.G. Nair, and L.D. Bourquin. 2003. Tart cherry anthocyanins inhibit tumor development in ApcMin mice and reduce proliferation of human colon cancer cells. Cancer Lett. 194: 13–19. Elsevier. doi:10.1016/S0304-3940(02)00583-9.

Kasapidou, E., E. N. Sossidou, N. Sossidou Evangelia, A. Zdragas, C. Papadaki, Vafeas G, et al. 2016. Effect of grape pomace supplementation on broiler meat quality characteristics. EuropPoultSci 80:135-42. https://doi.org/10.1399/eps.2016.135.

Kelly, D. and S. Conway. 2005. Bacterial modulation of mucosal innate immunity. Mol. Immunol. 42: 895–901. Elsevier. doi:https://doi.org/10.1016/j.molimm.2004.12.003.

Keyt, B. A., R. Baliga, A. M. Sinclair, S. F. Carroll, and M. S. Peterson. 2020. Structure, Function, and Therapeutic Use of IgM Antibodies. Antibodies 9:53 Available at /pmc/articles/PMC7709107/ (verified 1 May 2022).

Khandaker, L., M. B. Ali, and S. Oba. 2008. Total polyphenol and antioxidant activity of red amaranth (Amaranthus tricolor L.) as affected by different sunlight level. J. Japanese Soc. Hortic. Sci. 77:395–401.

Khare, M. L., S. Kumar, and J. Grun. 1976. Immunoglobulins of the chicken antibody to Newcastle disease virus (Mukteswar and F strain). Poult. Sci. 55:152–159.

Kholif, A.E., U.Y. Anele, A.K. Patra and Z. Varadyova. 2021.May 4. Editorial: The Use of Phytogenic Feed Additives to Enhance Productivity and Health in Ruminants. Frontiers Media SA. doi:10.3389/fvets.2021.685262.

Kim, S.W., J.F. Less, L. Wang, T. Yan, V. Kiron, S.J. Kaushik and X.G. Lei. 2019. Meeting global feed protein demand: challenge, opportunity, and strategy. Annu. Rev. Anim. Biosci. 7:221–243.

Kithama, M, Y.I. Hassan, K. Guo, E. Kiarie and M.S. Diarra. The Enzymatic Digestion of Pomaces From Some Fruits for Value-Added Feed Applications in Animal Production. Front. Sustain. Food Syst. 2021; https://doi.org/10.3389/fsufs.2021.611259

Klein, G., and S. Raina. 2019. Regulated assembly of LPS, its structural alterations and cellular response to LPS defects. Int. J. Mol. Sci. 20:356.

Kollarcikova, M., T. Kubasova, D. Karasova, M. Crhanova, D. Cejkova, F. Sisak, and I. Rychlik. 2019. Use of 16S rRNA gene sequencing for prediction of new opportunistic pathogens in chicken ileal and cecal microbiota. Poult. Sci. 98:2347–2353.

Konstantinidis, T., C. Tsigalou, A. Karvelas, E. Stavropoulou, C. Voidarou, and E. Bezirtzoglou. 2020. Effects of antibiotics upon the gut microbiome: a review of the literature. Biomedicines 8(11):502.

Koo, B., S. Amarakoon, B. Jayaraman, Y. Siow, S. Prashar and Y. Shang. 2018. Effects of dietary supplementation with ground red-osier dogwood (Cornus stolonifera) on oxidative status in weaned pigs challenged with Escherichia coli K88+. J. Anim. Sci. 96: 308. Oxford University Press. doi:10.1093/jas/sky404.677.

Koo, B., S. B. Amarakoon, B. Jayaraman, Y. L. Siow, S. Prashar, Y. Shang, K. O, and C. M. Nyachoti. 2020. Effects of dietary red-osier dogwood (Cornus stolonifera) on growth performance, blood profile, ileal morphology, and oxidative status in weaned pigs challenged with Escherichia coli K88+. Can. J. Anim. Sci. 101(1):96-105. https://doi.org/10.1139/cjas-2019-0188

Koo, B., S. B. Amarakoon, B. Jayaraman, Y. L. Siow, S. Prashar, Y. Shang, O. Karmin, and C. M. Nyachoti. 2021. Effects of dietary red-osier dogwood (Cornus stolonifera) on growth performance, blood profile, ileal morphology, and oxidative status in weaned pigs challenged with escherichia coli k88+. Can. J. Anim. Sci. 101:96–105.

Kopjar, M., A. Lončarić, M. Mikulinjak, Ž. Šrajbek, M. Šrajbek and A. Pichler. 2016. Evaluation of antioxidant interactions of combined model systems of phenolics in the presence of sugars. Nat. Prod. Commun. 11: 1934578X1601101008. SAGE Publications Sage CA: Los Angeles, CA. doi:https://doi.org/10.1177/1934578X1601101008.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Micro- biol. 79:5112–5120.

Kruczek, M., B. Drygaś and C. Habryka. 2016. Pomace in fruit industry and their contemporary potential application. World Sci. News. 48:259–265.

Kumanda, C., V. Mlambo and C. Mnisi. 2019. From Landfills to the Dinner Table: Red Grape Pomace Waste as a Nutraceutical for Broiler Chickens. Sustainability. 11:1931. https://doi.org/10.3390/su11071931

Kumar, S., C. Chen, N. Indugu, G.O. Werlang, M. Singh, W.K. Kim and H. Thippareddi. 2018. Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics, immunity, growth performance and prevalence of foodborne pathogens. PLoS One 13: e0192450. Public Library of Science San Francisco, CA USA. doi:https://doi.org/10.1371/journal.pone.0192450.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J. F. C. Meullenet, S. R. Mckee, and C. M. Owens. 2012. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240–1247.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.

L´opez, K. P., M. W. Schilling, and A. Corzo. 2011. Broiler genetic strain and sex effects on meat characteristics. Poult. Sci. 90:1105–1111.

Lala, V., A. Goyal, P. Bansal, and D. A. Minter. 2020. Liver function tests. StatPearls [Internet].

Larsson, A., R. M. Bålöw, T. L. Lindahl, and P. O. Forsberg. 1993. Chicken antibodies: taking advantage of evolution--a review. Poult. Sci. 72:1807–1812.

Lau, D.W. and A.J. King. 2003. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. J. Agric. Food Chem. 2003; 51:1602–1607. https://doi.org/10.1021/jf020740m

Laudadio, V., L. Passantino, A. Perillo, G. Lopresti, A. Passantino, R. U. Khan, and V. Tufarelli. 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult. Sci. 91:265–270.

Lazar, V., L.-M. Ditu, G.G. Pircalabioru, I. Gheorghe, C. Curutiu, A.M. Holban, A. Picu, L. Petcu and M.C. Chifiriuc. 2018. Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. Front. Immunol. 9: 1830. Frontiers. doi:https://doi.org/10.3389/fimmu.2018.01830.

Lebacq-Verheyden, A. M., J. P. Vaerman, and J. F. Heremans. 1974. Quantification and distribution of chicken immunoglobulins IgA, IgM and IgG in serum and secretions. Immunology 27:683–92 Available at /pmc/articles/PMC1445738/?report=abstract (verified 4 May 2022).

Lee, H. C., A. M. Jenner, C. S. Low, and Y. K. Lee. 2006. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res. Microbiol. 157:876–884.

Lee, J., J. Kim, C. Yang, K. O, and C. Nyachoti. 2018. PSI-31 Effects of red-osier dogwood (Cornus stolonifera) extract supplementation on nutrient digestibility and growth performance of weaned piglets. J. Anim. Sci. 96: 188. Oxford University Press US. doi:https://doi.org/10.1093/jas/sky404.409.

Lee, S. M., N. Kim, J. H. Park, R. H. Nam, K. Yoon, and D. H. Lee. 2018a. Comparative Analysis of Ileal and Cecal Microbiota in Aged Rats. J. Cancer Prev. 23:70–76 Available at /pmc/articles/PMC6037205/ (verified 20 May 2022).

Lewis Jr, J. L. 1973. Chemotherapy of gestational choriocarcinoma. Obstet. Gynecol. Surv. 28:478–480.

Ley, R. E., F. B€ackhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. 102:11070–11075.

Ley, R. E., P. J. Turnbaugh, S. Klein, and J. I. Gordon. 2006. Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023.

Li, K., W. Fu, Y. Bo, and Y. Zhu. 2018. Effect of albumin-globulin score and albumin to globulin ratio on survival in patients with heart failure: A retrospective cohort study in China. BMJ Open 8:e022960.

Li, Y., H. Zhang, Y. P. Chen, M. X. Yang, L. L. Zhang, Z. X. Lu, Y. M. Zhou, and T. Wang. 2015. Bacillus amyloliquefaciens Supplementation alleviates immunological stress and intestinal damage in lipopolysaccharide-challenged broilers. Anim. Feed Sci. Technol. 208:119–131.

Lim, T. H., M. S. Kim, D. H. Lee, Y. N. Lee, J. K. Park, H. N. Youn, H. J. Lee, S. Y. Yang, Y. W. Cho, J. B. Lee, S. Y. Park, I. S. Choi, and C. S. Song. 2012. Use of bacteriophage for biological control of *Salmonella* Enteritidis infection in chicken. Res. Vet. Sci. 93:1173–1178.

Lin, D, M. Xiao, J. Zhao, Z. Li, B. Xing, X. Li, M. Kong, L. Li, Q. Zhang, Y. Liu. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules. 21:1374.

Lin, D., L. Hu, H. You, D. Sarkar, B. Xing and K. Shetty. 2010. Initial screening studies on potential of high phenolic-linked plant clonal systems for nitrate removal in cold latitudes. J. Soils Sediments 10: 923–932. Springer. doi:DOI 10.1007/s11368-010-0214-6.

Litvak, Y., K. K. Z. Mon, H. Nguyen, G. Chanthavixay, M. Liou, E. M. Velazquez, L. Kutter, M. A. Alcantara, M. X. Byndloss, C. R. Tiffany, G. T. Walker, F. Faber, Y. Zhu, D. N. Bronner, A. J. Byndloss, R. M. Tsolis, H. Zhou, and A. J. Bäumler. 2019. Commensal Enterobacteriaceae Protect against *Salmonella* Colonization through Oxygen Competition. Cell Host Microbe 25:128-139.e5.

Liu, H.-Y., S. Roos, H. Jonsson, D. Ahl, J. Dicksved, J. E. Lindberg and T. Lundh. 2015. Effects of Lactobacillus johnsonii and Lactobacillus reuteri on gut barrier function and heat shock proteins in intestinal porcine epithelial cells. Physiol Rep. 3(4):e12355. doi:10.14814/phy2.12355.

Liu, J., Y.Y. Pu, Q. Xie, J.K. Wang and J.X. Liu. 2015b. Pectin Induces an In Vitro Rumen Microbial Population Shift Attributed to the Pectinolytic Treponema Group. Curr. Microbiol. 70: 67–74. Springer Science and Business Media, LLC. doi:10.1007/S00284-014-0672-Y/TABLES/4.

Llena, C., A. Almarche, A. Mira, and M. A. López. 2019. Antimicrobial efficacy of the supernatant of Streptococcus dentisani against microorganisms implicated in root canal infections. J. Oral Sci. 61:184–194.

Lou, S.N., Y.S. Lin, Y.S. Hsu, E.M. Chiu, C.T. Ho. 2014. Soluble and insoluble phenolic compounds and antioxidant activity of immature calamondin affected by solvents and heat treatment. Food Chem. 161:246–253. https://doi.org/10.1016/j.foodchem.2014.04.009

Louis, P., P. Young, G. Holtrop and H.J. 2010. Flint Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environ. Microbiol. 2010; 12:304–314. https://doi.org/10.1111/J.1462-2920.2009.02066.X

Lozupone, C. A., J. I. Stombaugh, J. I. Gordon, J. K. Jansson, and R. Knight. 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230.

Lu, X., C.F. Ross, J.R. Powers, D.E. Aston and B.A. Rasco. 2011. Determination of Total Phenolic Content and Antioxidant Activity of Garlic (Allium sativum) and Elephant Garlic (Allium ampeloprasum) by Attenuated Total Reflectance–Fourier Transformed Infrared Spectroscopy. J. Agric. Food Chem. 59: 5215–5221. American Chemical Society. doi:10.1021/JF201254F.

Magne, F., M. Gotteland, L. Gauthier, A. Zazueta, S. Pesoa, P. Navarrete, and R. Balamurugan. 2020. The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients 12:1474.

Maharjan, P., K. Hilton, J. Weil, N. Suesuttajit, A. Beitia, C. Owens and C. Coon. 2019. Characterizing Woody Breast Myopathy in a Meat Broiler Line by Heat Production, Microbiota, and Plasma Metabolites. Front. Vet. Sci. 6: 497. Frontiers.

Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50:882–889 Available at https://academic.oup.com/cid/article/50/6/882/419872 (verified 12 August 2021).

Makris, D. P., G. Boskou, and N. K. Andrikopoulos. 2007. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. J. Food Compos. Anal. 20:125–132.

Mandal, R. K., T. Jiang, R. F. Wideman, T. Lohrmann, and Y. M. Kwon. 2020. Microbiota Analysis of Chickens Raised Under Stressed Conditions. Front. Vet. Sci. 7:482637 Available at /pmc/articles/PMC7575692/ (verified 19 May 2022).

Mankola, E.S., M.H. Rabie, H.M.A. Abo El-Maaty and S.E. Asmaa. 2021. Utilization of Natural Antioxidants to Improve the Growth Performance of Broiler Chicks. J Anim Poult Prod 2021;12:281–6. https://doi.org/10.21608/JAPPMU.2021.197407.

Marcq, C., E. Cox, I. M. Szalo, A. Théwis, and Y. Beckers. 2011. *Salmonella* Typhimurium oral challenge model in mature broilers: Bacteriological, immunological, and growth performance aspects. Poult. Sci. 90:59–67.

Mariat, D., O. Firmesse, F. Levenez, V. D. Guimarˇaes, H. Sokol, J. Dor´e, G. Corthier, and J. P. Furet. 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. 9:1–6.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10 Available at https://journal.embnet.org/index.php/embnetjournal/article/view/200/479 (verified 12 May 2022).

Martinez, J. E., D. D. Kahana, S. Ghuman, H. P. Wilson, J. Wilson, S. C. J. Kim, V. Lagishetty, J. P. Jacobs, A. P. Sinha-Hikim, and T. C. Friedman. 2021. Unhealthy Lifestyle and Gut Dysbiosis: A Better Understanding of the Effects of Poor Diet and Nicotine on the Intestinal Microbiome. Front. Endocrinol. (Lausanne). 12:649.

Máthé, Á. and F. Hassan. 2015. Chemical Diversity of Medicinal Plants. In A. Máthé (Editor), Medicinal and Aromatic Plants of the World, Volume 1, pp. 35 - 60. doi: 10.1007/978-94-017-9810-5. New York, London: Springer Dordrecht Heidelberg.

Mazur-Kuśnirek, M., Z. Antoszkiewicz, K. Lipiński, M. Fijałkowska, C. Purwin, and S. Kotlarczyk. 2019. The effect of polyphenols and vitamin E on the antioxidant status and meat quality of broiler chickens fed diets naturally contaminated with ochratoxin A. Archive of Anim. Nutr. 73(6):431-444. https://doi.org/10.1080/1745039X.2019.1639445 73:431–444.

McGruder, E. D. 1995. Mechanisms of resistance to *Salmonella* enteritidis infections in Leghorn chicks conferred by *Salmonella* enteritidis-immune lymphokines.

Mehdi, Y., M.-P. Létourneau-Montminy, M.-L. Gaucher, Y. Chorfi, G. Suresh, T. Rouissi, S.K. Brar, C. Côté, A.A. Ramirez and S. Godbout. 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Anim. Nutr. 4: 170–178. Elsevier. doi:https://doi.org/10.1016/j.aninu.2018.03.002.

Mehrzadi, S., P. Hosseini, M. Mehrabani, A. Siahpoosh, M. Goudarzi, H. Khalili, and A. Malayeri. 2020. Attenuation of bleomycin-induced pulmonary fibrosis in wistar rats by combination treatment of two natural phenolic compounds: quercetin and gallic acid. Nutr. And Cancer. 73(10)2039-2049. https://doi.org/10.1080/01635581.2020.1820053 73:2039–2049.

Meyer-Sabellek, W., P. Sinha, and E. K€ottgen. 1988. Alkaline phosphatase. Laboratory and clinical implications. J. Chromatogr. B Biomed. Sci. Appl. 429:419–444.

Mielnik, M.B., E. Olsen, G. Vogt, D. Adeline and G. Skrede. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. LWT - Food Sci. Technol. 39:191–198. https://doi.org/10.1016/J.LWT.2005.02.003

Minitab 19 Statistical Software. 2019. [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com)

Mnisi, C.M., V. Mlambo, C. Kumanda and A. Crafford. 2021. Effect of graded levels of red grape pomace (Vitis vinifera L.) powder on physiological and meat quality responses of Japanese quail. Acta Agric. Scand. A. Anim. Sci. 70:100–6. https://doi.org/10.1080/09064702.2021.1923796.

Mogire, M. 2020. Red osier dogwood extracts as alternatives to in-feed antibiotics in broiler chickens. Master's Thesis, University of Manitoba. Available at tp://hdl.handle.net/1993/34883.

Mogire, M. K., J. Choi, P. Lu, C. Yang, S. Liu, D. Adewole, A. Rodas-Gonzalez, and C. Yang. 2021. Effects of red-osier dogwood extracts on growth performance, intestinal digestive and absorptive functions, and meat quality of broiler chickens. Can. J. Anim. Sci. 101:687–703 Available at https://cdnsciencepub.com/doi/abs/10.1139/cjas-2020-0191 (verified 24 November 2021).

Mountzouris, K.C., V. Paraskevas, P. Tsirtsikos, I. Palamidi, T. Steiner, G. Schatzmayr and Fegeros, K. 2011. Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol. 168: 223–231.doi:https://doi.org/10.1016/j.anifeedsci.2011.03.020

Muhlack, R. A., R. Potumarthi, and D. W. Jeffery. 2018. Sustainable wineries through waste valorisation: a review of grape marc utilisation for value-added products. Waste Manag. 72:99–118.

Mullen, W., S. Larcombe, K. Arnold, H. Welchman, and A. Crozier. 2010. Use of accurate mass full scan mass spectrometry for the analysis of anthocyanins in berries and berry-fed tissues. J. Agric. Food Chem. 58: 3910–3915. American Chemical Society. doi:10.1021/jf902267v.

Murry, A., A. Hinton and H.M.I.J.P. Sci. 2004. Inhibition of growth of Escherichia coli, *Salmonella* typhimurium, and Clostridia perfringens on chicken feed media by Lactobacillus salivarius and Lactobacillus. Int. J. Poult. Sci. 3:603–607.

Murugesan, G.R., B. Syed, S. Haldar and C. Pender. 2015. Phytogenic Feed Additives as an Alternative to Antibiotic Growth Promoters in Broiler Chickens. Front. Vet. Sci. 2:21. doi:https://doi.org/10.3389/fvets.2015.00021.

Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics 16:1–19.

Nagarajaiah, S.B. and J. Prakash. 2016. Chemical Composition and Bioactivity of Pomace from Selected Fruits. Int. J. Fruit Sci. 16:423–443. https://doi.org/10.1080/15538362.2016.1143433

Nair, G.G. and C.K.K. Nair. 2013. Radioprotective Effects of Gallic Acid in Mice. Biomed Res. Int. 2013: 953079. Hindawi Publishing Corporation. doi:10.1155/2013/953079.

Nardoia, M., C. Romero, A. Brenes, I. Arija, A. Viveros, C. Ruiz-Capillas, and S. Chamorro. 2020. Addition of fermented and unfermented grape skin in broilers' diets: effect on digestion, growth performance, intestinal microbiota and oxidative stability of meat. Animal 14:1371–1381.

National Research Council. 1994. Nutrient requirements of poultry: 1994. National Academies Press.

Neal-McKinney, J.M., X. Lu, T. Duong, C.L. Larson, D.R. Call, D.H. Shah, M.E. Konkel. 2012. Production of Organic Acids by Probiotic Lactobacilli Can Be Used to Reduce Pathogen Load in Poultry. PLoS One 7:e43928. https://doi.org/10.1371/JOURNAL.PONE.0043928

Neumann, A. P., and G. Suen. 2015. Differences in major bacterial populations in the intestines of mature broilers after feeding virginiamycin or bacitracin methylene disalicylate. J. Appl. Microbiol. 119:1515–1526.

Nguyen, D. H, K. Y. Lee, M. Mohammadigheisar and I. H. Kim. 2018. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers. Poult. Sci. 97: 4351-4358. Doi: 10.3382/ps/pey339.

Oakley, B. B., and M. H. Kogut. 2016. Spatial and temporal changes in the broiler chicken cecal and fecal microbiomes and correlations of bacterial taxa with cytokine gene expression. Front. Vet. Sci. 3:11.

Oakley, B. B., H. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedroso, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100–112.

Obomsawin, R. 2007. Traditional Medicines for Canada's First Peoples. Br. Columbia: 1–41.

OECD/FAO. 2020. "OECD-FAO Agricultural Outlook",OECD Agriculture statistics (database). https://doi.org/10.1787/e481f6bb-en

Oh, J. K., E. A. B. Pajarillo, J. P. Chae, I. H. Kim, and D. K. Kang. 2017. Protective effects of Bacillus subtilis against *Salmonella* infection in the microbiome of Hy-Line Brown layers. Asian-Australasian J. Anim. Sci. 30:1332–1339 Available at https://pubmed.ncbi.nlm.nih.gov/28423869/ (verified 16 May 2022).

Ojedapo, L., O. Akinokun, T. Adedeji, T. Olayeni, S. Ameen, and S. Amao. 2008. Effect of strain and sex on carcass characteristics of three commercial broilers reared in deep litter system in the derived savannah area of Nigeria. World J. Agric. Sci. 4:487–491.

Oladokun, S., A. Koehler, J. MacIsaac, E. M. Ibeagha-Awemu, and D. I. Adewole. 2021. Bacillus subtilis delivery route: effect on growth performance, intestinal morphology, caecal short-chain fatty acid concentration, and caecal microbiota in broiler chickens. Poult. Sci. 100: 100809.

Oliveira, M. C., E. A. Rodrigues, R. H. Marques, R. A. Gravena, G. C. Guandolini, and V. M. B. Moraes. 2008. Performance and morphology of intestinal mucosa of broilers fed mannan- oligosaccharides and enzymes [Desempenho e morfologia da mucosa intestinal de frangos de corte alimentados com mananoligossacarídeos e enzimas].

Orlewska, K., A. Markowicz, Z. Piotrowska-Seget, J. Smoleń-Dzirba, and M. Cycoń. 2018a. Functional diversity of soil microbial communities in response to the application of cefuroxime and/or antibiotic-resistant Pseudomonas putida Strain MC1. Sustain. 103549.

Orlewska, K., Z. Piotrowska-Seget, J. Bratosiewicz-Wąsik, and M. Cycoń. 2018b. Characterization of bacterial diversity in soil contaminated with the macrolide antibiotic erythromycin and/or inoculated with a multidrug-resistant Raoultella sp. Strain using the PCR-DGGE approach. Appl. Soil Ecol. 126:57–64.

OurWorldinData 2021. FAOSTAT - Data. [Online] Available: https://ourworldindata.org/grapher/animals-slaughtered-for-meat.

Özkan G., O. Sagdic¸ , N. G. Baydar, and Z. Kurumahmutoglu. 2004. Antibacterial activities and total phenolic contents of grape pom- ace extracts. J. Sci. Food Agric. 84:1807–1811.

Palafox-Carlos, H., E.M. Yahia and G.A. González-Aguilar. 2012. Identification and quantification of major phenolic compounds from mango (Mangifera indica, cv. Ataulfo) fruit by HPLC–DAD–MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. Food Chem. 135: 105–111. doi:https://doi.org/10.1016/j.foodchem.2012.04.103.

Park, S.H., A. Perrotta, I. Hanning, S. Diaz-Sanchez, S. Pendleton, E. Alm and S.C. Ricke. 2017. Pasture flock chicken cecal microbiome responses to prebiotics and plum fiber feed amendments. Poult. Sci. 96: 1820–1830. doi:https://doi.org/10.3382/ps/pew441.

Patil, P. and Killedar, S. 2021. Formulation and characterization of gallic acid and quercetin chitosan nanoparticles for sustained release in treating colorectal cancer. J. Drug Deliv. Sci. Technol. 63: 102523. Elsevier. doi:10.1016/J.JDDST.2021.102523.

Pazos, M., J.M. Gallardo, J.L. Torres and I. Medina. 2005. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chem. 92:547–557. https://doi.org/10.1016/J.FOODCHEM.2004.07.036

Peng, L., Z.R. Li, R.S. Green, I.R. Holzman, J. Lin. 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J. Nutr. 139:1619–1625.

Petiwala, S. M., A. G. Puthenveetil, and J. J. Johnson. 2013. Polyphenols from the Mediterranean herb rosemary (Rosmarinus officinalis) for prostate cancer. Front. Pharmacol. 4:29 Available at /pmc/articles/PMC3607075/ (verified 3 May 2022).

Pié, S., A. Awati, S. Vida, I. Falluel, B.A. Williams and I.P. Oswald. 2007. Effects of added fermentable carbohydrates in the diet on intestinal proinflammatory cytokine-specific mRNA content in weaning piglets. J. Anim. Sci. 85: 673–683. Oxford Academic. doi:10.2527/JAS.2006-535.

Pijut, P.M. 2004. “Cornus sericea.” Pages 249–251 *in* E. Francis, John K., ed. Wildland shrubs of the United States and its territories. Thamnic. Vol. 1. Gen. Tech. Wildland shrubs of the United States and its territories, 26th edition. US Department of Agriculture, Forest Service, International Institute of Tropical Forestry, Rio Piedras, Puerto Rico.

Pop, I.M., S.M. Pascariu and D. Simeanu. 2015. The grape pomace influence on the broiler chickens growing rate. Lucr. Științifice-Universitatea Științe Agric. și Med. Vet. Ser. Zooteh. 2015; 64:34–39.

Popović, Z., R. Matić, J. Bajić-Ljubičić, V. Tešević and S. Bojović. 2018. Geographic variability of selected phenolic compounds in fresh berries of two Cornus species. Trees 32: 203–214. doi:10.1007/s00468-017-1624-5.

Prado-Rebolledo, O. F., J. de J. Delgado-Machuca, R. J. Macedo-Barragan, L. J. Garcia-Márquez, J. E. Morales-Barrera, J. D. Latorre, X. Hernandez-Velasco, and G. Tellez. 2017. Evaluation of a selected lactic acid bacteria-based probiotic on *Salmonella* enterica serovar Enteritidis colonization and intestinal permeability in broiler chickens. Avian Pathol. 46:90–94 Available at https://www.tandfonline.com/doi/abs/10.1080/03079457.2016.1222808 (verified 27 April 2022).

Proctor, A. and G.J. Phillips. 2019. Differential effects of bacitracin methylene disalicylate (BMD) on the distal colon and cecal microbiota of young broiler chickens. Front. Vet. Sci. 6: 114. Frontiers. doi:https://doi.org/10.3389/fvets.2019.00114.

Public Health Agency of Canada (PHAC) (2008): C-EnterNet Annual Report. (2008). Available at: http://www.phac-aspc.gc.ca/c-enternet/pubs/2008/index-eng.php.

Public Health Agency of Canada (PHAC) (2009): Annual Report (2009). Available at: http://www.nmllnm.gc.ca/NESPPNSME/assets/pdf/NESP_2009_Annual_Report_ENG pdf.

Putecova, K., K. Nedbalcova, I. Bartejsova, M. Zouharova, K. Matiaskova, E. Jeklova, M. Viskova, P. Zouzelkova, M. Jerabek, and K. Stastny. 2021. Experimental determination of the pharmacokinetic properties of trimethoprim and sulfamethoxazole combination in the blood serum of broiler chickens. Vet. Med. (Praha). 66:248–256 Available at https://doi.org/10.17221/190/2020-VETMED (verified 19 May 2022).

Puvača, N., V. Stanaćev, D. Glamočić, J. Lević, L. Perić, V. Stanaćev and D. Milić. 2013. Beneficial effects of phytoadditives in broiler nutrition. Worlds. Poult. Sci. J. 69: 27–34. Taylor & Francis. doi:10.1017/S0043933913000032.

Qaid, M. M., S. I. Al-Mufarrej, M. M. Azzam, M. A. Al-Garadi, H. H. Albaadani, I. A. Alhidary, and R. S. Aljumaah. 2021. Growth performance, serum biochemical indices, duodenal histomorphology, and cecal microbiota of broiler chickens fed on diets supplemented with cinnamon bark powder at prestarter and starter phases. Animals 11:94.

Qaisrani, S. N., P. C. A. Moquet, M. M. Van Krimpen, R. P. Kwakkel, M. W. A. Verstegen, and W. H. Hendriks. 2014. Protein source and dietary structure influence growth performance, gut morphology, and Hindgut fermentation characteristics in broilers. Poult. Sci. 93:3053–3064.

Qin, J., R. Li, J. Raes, M. Arumugam, K.S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez, T. Yamada, D.R. Mende, J. Li, J. Xu, S. Li, D. Li, J. Cao, B. Wang, H. Liang, H. Zheng, Y. Xie, J. Tap, P. Lepage, M. Bertalan, J.M. Batto, T. Hansen, D. Le Paslier, A. Linneberg, H.B. Nielsen, E. Pelletier, P. Renault, T. Sicheritz-Ponten, K. Turner, H. Zhu, C. Yu, S. Li, M. Jian, Y. Zhou, Y. Li, X. Zhang, S. Li, N. Qin, H. Yang, J. Wang, S. Brunak, J. Doré, F. Guarner, K. Kristiansen, O. Pedersen, J. Parkhill, J. Weissenbach, P. Bork, S.D. Ehrlich, J. Wang, M. Antolin, F. Artiguenave, H. Blottiere, N. Borruel, T. Bruls, F. Casellas, C. Chervaux, A. Cultrone, C. Delorme, G. Denariaz, R. Dervyn, M. Forte, C. Friss, M. Van De Guchte, E. Guedon, F. Haimet, A. Jamet, C. Juste, G. Kaci, M. Kleerebezem, J. Knol, M. Kristensen, S. Layec, K. Le Roux, M. Leclerc, E. Maguin, R. Melo Minardi, R. Oozeer, M. Rescigno, N. Sanchez, S. Tims, T. Torrejon, E. Varela, W. De Vos, Y. Winogradsky and E. Zoetendal. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 464:59–65. https://doi.org/10.1038/nature08821

R Development Core Team. 2013. R: A language and environment for statistical computing.

Rathnapraba, S., G. D. Raj, and V. Thiagarajan. 2007. Development of monoclonal antibodies against chicken IgM and its application in immunity studies. 6:187–193.

Rauber, R. H., V. J. Perlin, C. D. Fin, A. L. Mallmann, D. P. Miranda, L. Z. Giacomini, and V. P. do Nascimento. 2014. Interference of *Salmonella* typhimurium lipopolysaccharide on performance and biological parameters of broiler chickens. Brazilian J. Poult. Sci. 16:77–81.

Razavi, A. C., K. S. Potts, T. N. Kelly, and L. A. Bazzano. 2019. Sex, gut microbiome, and cardiovascular disease risk. Biol. Sex Differ. 10:1–14.

Reisinger, N., C. Emsenhuber, B. Doupovec, E. Mayer, G. Schatzmayr, V. Nagl, and B. Grenier. 2020. Endotoxin translocation and gut inflammation are increased in broiler chickens receiving an oral lipopolysaccharide (LPS) bolus during heat stress. Toxins 2020, Vol. 12, Page 622 12:622.

Reyes, P., I. Urquiaga, G. Echeverría, E. Durán, M.S. Morales and C. Valenzuela. 2020. Wine grape pomace flour in broiler diets effects growth and some meat characteristics. Anim. Prod. Sci. 60(9):1210-1216. https://doi.org/10.1071/AN19385.

Ribeiro, A. M. L., L. K. Vogt, C. W. Canal, M. R. de I. Cardoso, R. V. Labres, A. F. Streck, and M. C. Bessa. 2007. Effects of prebiotics and probiotics on the colonization and immune response of broiler chickens challenged with *Salmonella* Enteritidis. Brazilian J. Poult. Sci. 9:193–200.

Rodríguez Montealegre, R., R. Romero Peces, J. L. Chacon Vozmediano, J. Martínez Gascuena, and E. García Romero. 2006. Phenolic com- pounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J. Food Compos. Anal. 19:687–693.

Rodríguez-Daza, M. C., E. C. Pulido-Mateos, J. Lupien-Meilleur, D. Guyonnet, Y. Desjardins, and D. Roy. 2021. Polyphenol-Mediated Gut Microbiota Modulation: Toward Prebiotics and Further. Front. Nutr. 8:347.

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ 2016:e2584 Available at https://peerj.com/articles/2584 (verified 12 May 2022).

Rossi, R., G. Pastorelli, S. Cannata, S. Tavaniello, G. Maiorano and C. Corino. 2013. Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork. Meat Sci. 95: 542–548. doi:https://doi.org/10.1016/j.meatsci.2013.05.037.

Ruas-Madiedo, P, M. Gueimonde, M. Fernández-García, C.G. de los Reyes-Gavilán and A. Margolles. 2008. Mucin degradation by Bifidobacterium strains isolated from the human intestinal microbiota. Appl. Environ. Microbiol. 74:1936–1940. https://doi.org/10.1128/AEM.02509-07

Russell, J.B. and H.J. Strobel. 1989. Effect of lonophores on Ruminal Fermentation. Appl. Environ. Microbiol. 55: 1–6.

Russell, S.L., M.J. Gold, M. Hartmann, B.P. Willing, L. Thorson, M. Wlodarska, N. Gill, M.-R. Blanchet, W.W. Mohn, K.M. McNagny and B.B. Finlay. 2012. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. 13: 440–447. John Wiley & Sons, Ltd. doi:https://doi.org/10.1038/embor.2012.32.

Ryang, Y. S., J. A. Im, I. S. Kim, Y. K. Cho, H. J. Sung, J. Y. Park and J. Y. Ha. 2001. Antiparasitic effects of an herb extract from Gentiana scabra var buergeri on Trichomonas vaginalis. Biomed. Sci. Lett. 7(2), 53-58.

Sayago-Ayerdi, S. G., A. Brenes, A. Viveros, and I. Gon~i. 2009. Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored chicken patties. Meat Sci. 83:528–533.

Saleh A.A., T.A. Ebeid and A.M. Abudabos. 2018. Effect of dietary phytogenics (herbal mixture) supplementation on growth performance, nutrient utilization, antioxidative properties, and immune response in broilers. Environ. Sci. Pollut. Res. 25: 14606–14613. doi:10.1007/s11356-018-1685-z.

Saleh, A. A., K. Amber, and A. A. Mohammed. 2020. Dietary supplementation with avilamycin and Lactobacillus acidophilus effects growth performance and the expression of growth-related genes in broilers. Anim. Prod. Sci. 60:1704–1710 Available at https://www.publish.csiro.au/an/AN19030 (verified 28 April 2022).

Salobir J., T. Frankič, and V. Rezar. 2012. Animal nutrition for the health of animals, human and environment. Acta Agric. Slov.: 41–9.

Samuel, K. G., J. Wang, H. Y. Yue, S. G. Wu, H. J. Zhang, Z. Y. Duan, and G. H. Qi. 2017. Effects of dietary gallic acid supplementation on performance, antioxidant status, and jejunum intestinal morphology in broiler chicks. Poult. Sci. 96:2768–2775.

Sarmah, A. K., M. T. Meyer, and A. B. A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (Vas) in the environment. Chemosphere 65:725–759.

Sawanon, S., S. Koike and Y. Kobayashi 2011. Evidence for the possible involvement of Selenomonas ruminantium in rumen fiber digestion. FEMS Microbiol. Lett. 325: 170–179. Oxford Academic. doi:10.1111/J.1574-6968.2011.02427.X.

Sáyago-Ayerdi, S.G., A. Brenes, A. Viveros and I. Goñi. 2009. Antioxidative effect of dietary grape pomace concentrate on lipid oxidation of chilled and long-term frozen stored chicken patties. Meat Sci. 83:528–533. https://doi.org/10.1016/j.meatsci.2009.06.038

Scales, R. 2015. Anti-oxidant properties of Cornus sericea, US Patent 20150093460.

Schloss, P. D. 2009. A high-throughput DNA sequence aligner for microbial ecology studies. PloS One 4:e8230.

Schokker, D., A.J.M. Jansman, G. Veninga, N. de Bruin, S.A. Vastenhouw, F.M. de Bree, A. Bossers, J.M.J. Rebel, and M.A. Smits. 2017. Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. BMC Genomics 18: 241. doi:10.1186/s12864-017-3625-6.

Seal, C.J. and C.K. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6: 185–208. Nutr Res Rev. doi:10.1079/NRR19930012.

Sekirov, I., N. M. Tam, M. Jogova, M. L. Robertson, Y. Li, C. Lupp, and B. B. Finlay. 2008. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infect. Immun. 76:4726–4736 Available at https://journals.asm.org/doi/full/10.1128/IAI.00319-08 (verified 8 May 2022).

Serreli, G. and M. Deiana. 2018. Biological Relevance of Extra Virgin Olive Oil Polyphenols Metabolites. Antioxidants 2018, Vol. 7, Page 170 7: 170. Multidisciplinary Digital Publishing Institute. doi:10.3390/ANTIOX7120170.

Shah, N. P., and R. Dave. 2002. Antimicrobial substances including bacteriocins produced by lactic acid bacteria. Biosci. microflora 21:217–223.

Shang, Q. H., S. J. Liu, T. F. He, H. S. Liu, S. Mahfuz, X. K. Ma, and X. S. Piao. 2020. Effects of wheat bran in comparison to antibiotics on growth performance, intestinal immunity, barrier function, and microbial composition in broiler chickens. Poult. Sci. 99:4929–4938.

Shang, Y., A. Regassa, J. H. Kim, and W. K. Kim. 2015. The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology, and immune responses in broiler chickens challenged with *Salmonella* Enteritidis lipopolysaccharides. Poult. Sci. 94:2887–2897.

Shang, Y., S. Kumar, B. Oakley, and W. K. Kim. 2018. Chicken gut microbiota: importance and detection technology. Front. Vet. Sci. 5:254.

Sharma, V., K. K. Jakhar, V. Nehra, and S. Kumar. 2015. Biochemical studies in experimentally Escherichia coli infected broiler chicken supplemented with neem (Azadirachta indica) leaf extract. Vet. World 8:1340.

Shen, H., X. Hu, C. Liu, S. Wang, W. Zhang, H. Gao and J. Chen. 2010. Ethyl pyruvate protects against hypoxic-ischemic brain injury via anti-cell death and anti-inflammatory mechanisms. Neurobiol. Dis. 37(3)711-722.

Shi, J., J. Yu, J.E. Pohorly and Y. Kakuda. 2003. Polyphenolics in Grape Seeds - Biochemistry and Functionality. J. Med. Food. 6: 291-299. https://doi.org/10.1089/109662003772519831

Shin, N. R., T. W. Whon, and J. W. Bae. 2015. Proteobacteria: micro- bial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33:496–503.

Shuman, M., T. lee Demler, E. Trigoboff, and L. A. Opler. 2012. Hematologic impact of antibiotic administration on patients taking clozapine. Innov. Clin. Neurosci. 9:18–30 Available at /pmc/articles/PMC3552460/ (verified 1 May 2022).

Singh, K. M., T. Shah, S. Deshpande, S. J. Jakhesara, P. G. Koringa, D. N. Rank, and C. G. Joshi. 2012. High through put 16S rRNA gene-based pyrosequencing analysis of the fecal microbiota of high FCR and low FCR broiler growers. Mol. Biol. Rep. 39:10595–10602.

Singh, M., S.S. Chauhan and P. Kumar. 2008. Effect of supplementation of diets with BMD and virginiamycin on the growth performance, carcass characteristics and bacterial population in broiler chickens. Vet. World 1: 141. Citeseer.

Smeriglio, A., D. Barreca, E. Bellocco, and D. Trombetta. 2016. Chemistry, Pharmacology and Health Benefits of Anthocyanins. Phyther. Res. 30: 1265–1286. doi:10.1002/ptr.5642.

Soltan, M. A., R. S. Shewita, and M. I. El-Katcha. 2008. Effect of dietary anise seeds supplementation on growth performance, immune response, carcass traits, and some blood parameters of broiler chickens. Int. J. Poult. Sci. 7:1078–1088.

Song, J., Q. Li, N. Everaert, R. Liu, M. Zheng, G. Zhao, and J. Wen. 2020. Effects of inulin supplementation on intestinal barrier function and immunity in specific pathogen-free chickens with *Salmonella* infection. J. Anim. Sci. 98 Available at https://academic.oup.com/jas/article/98/1/skz396/5693378 (verified 1 May 2022).

Spernakova, D., D. Mate, R. Hanna, and G. Kovac. 2007. Effects of dietary rosemary extract and alpha-tocopherol on the performance of chickens, meat quality, and lipid oxidation in meat storaged under chilling conditions. Bull. Vet. Inst. Pulawy 51: 585–589.

Stafford, N., C. Wilson, D. Oceandy, L. Neyses, and E. J. Cartwright. 2017. The plasma membrane calcium ATPases and their role as major new players in human disease. Physiological reviews, 97(3):1089-1125.

Stanley, D., M. S. Geier, S. E. Denman, V. R. Haring, T. M. Crowley, R. J. Hughes, and R. J. Moore. 2013. Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. Vet. Microbiol. 164:85–92.

Stanley, D., R. J. Hughes, M. S. Geier, and R. J. Moore. 2016. Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: challenges presented for the identification of performance enhancing probiotic bacteria. Front. Microbiol. 7:187.

Stanley, D., S.E. Denman, R.J. Hughes, M.S. Geier, T.M. Crowley, H. Chen, V.R. Haring, and R.J. Moore. 2012. Intestinal microbiota associated with differential feed conversion efficiency in chickens. Appl. Microbiol. Biotechnol. 96: 1361–1369. doi:10.1007/s00253-011-3847-5.

Steiner, T., and B. Syed. 2015. Phytogenic Feed Additives in Animal Nutrition. In A. Máthé (Editor), Medicinal and Aromatic Plants of the World. 1: 403-423. New York, London: Springer Dordrecht Heidelberg.

Steyn, W.J., S.J.E. Wand, D.M. Holcroft, and G. Jacobs. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155: 349–361. John Wiley & Sons, Ltd. doi:https://doi.org/10.1046/j.1469-8137.2002.00482.x.

Strain, R., C. Stanton, and R. P. Ross. 2022. Effect of diet on pathogen performance in the microbiome. Microbiome Res. Reports 1:13 Available at https://oaepublish .com/mrr/article/view/4730 (verified 16 May 2022).

Sun Q., Y. Shi, F. Wang, D. Han, H. Lei, Y. Zhao, and Q. Sun. 2015. Study on the effects of microencapsulated Lactobacillus delbrueckii on the mouse intestinal flora. https://doi.org/10.3109/02652048.2015.1057249 32: 669–676. Informa Healthcare. doi:10.3109/02652048.2015.1057249.

Swanson, K.S., C.M. Grieshop, G.M. Clapper, R.G. Shields, T. Belay, N.R. Merchen, G.C. Fahey. 2001.Fruit and vegetable fiber fermentation by gut microflora from canines. J. Anim. Sci. 79:919–926. https://doi.org/10.2527/2001.794919x

Swanson, M.A. 2019. Cornus mas l. cultivar selection based on hardiness and propagation. PhD Dissertation, North Dakota State University.

Tagliazucchi, D., E. Verzelloni, and A. Conte. 2005. Effect of Some Phenolic Compounds and Beverages on Pepsin Activity during Simulated Gastric Digestion. J. Agric. Food Chem. 53: 8706–8713. American Chemical Society. doi:10.1021/jf058074n.

Talavéra, S., C. Felgines, O. Texier, C. Besson, J.L. Lamaison, and C. Rémésy. 2003. Anthocyanins Are Efficiently Absorbed from the Stomach in Anesthetized Rats. J. Nutr. 133: 4178–4182. Oxford Academic. doi:10.1093/JN/133.12.4178.

Tarko, T., A. Duda-Chodak, and N. Zając. 2013. Digestion and absorption of phenolic compounds assessed by in vitro simulation methods. A review. Rocz. Państwowego Zakładu Hig. 64. -.

Tavárez, M.A., D.D. Boler, K.N. Bess, J. Zhao, F. Yan, A.C. Dilger, F.K. McKeith, J. Killefer. 2011. Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poult. Sci. 90:922–930. https://doi.org/10.3382/PS.2010-01180

Team R. C. 2013. R: A Language and Environment for Statistical Computing.

Teixeira, A., N. Baenas, R. Dominguez-Perles, A. Barros, E. Rosa, D.A. Moreno, C. Garcia-Viguera. 2014. Natural bioactive compounds from winery by-products as health promoters: a review. Int. J. Mol. Sci. 15:15638–15678.

Thiam, M., Q. Wang, A. L. B. Sánchez, J. Zhang, M. Zheng, J. Wen, and G. Zhao. 2021. Association of heterophil/lymphocyte ratio with intestinal barrier function and immune response to *Salmonella* enteritidis infection in chicken. Animals 11 Available at https://pubmed.ncbi.nlm.nih.gov/34944274/ (verified 1 May 2022).

Thiam, M., Q. Wang, A. L. Barreto Sánchez, J. Zhang, J. Ding, H. Wang, Q. Zhang, N. Zhang, J. Wang, Q. Li, J. Wen, and G. Zhao. 2022. Heterophil/Lymphocyte Ratio Level Modulates *Salmonella* Resistance, Cecal Microbiota Composition and Functional Capacity in Infected Chicken. Front. Immunol. 13:1524 Available at https://www.frontiersin.org/articles/10.3389/fimmu.2022.816689/full (verified 17 May 2022).

Thomas, M., S. Wongkuna, S. Ghimire, R. Kumar, L. Antony, K.C. Doerner, A. Singery, E. Nelson, T. Woyengo, Chankhamhaengdecha, S., Janvilisri, T., and Scaria, J. 2019. Gut Microbial Dynamics during Conventionalization of Germfree Chicken. mSphere 4: e00035-19. doi:10.1128/mSphere.00035-19.

Thompson, L.U., J.H. Yoon, and D.J.A. Jenkins. 1984. Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. Am. J. Clin. Nutr. 39: 745–751. Oxford Academic. doi:10.1093/AJCN/39.5.745.

Thrall, M.A., 2007. Hematologia e bioquímica clínica veterinária. Editora Roca.

Toghyani, M., M. Toghyani, A. Gheisari, G. Ghalamkari, and S. Eghbalsaied. 2011. Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. Livest. Sci. 138:167–173.

Tokić, V., M. Lazarević, Z. Sinovec, and A. Tokić. 2007. The influence of different feed additives to performances and immune response in broiler chicken. Acta Vet. 57: 217–229. Univerzitet u Beogradu-Fakultet veterinarske medicine, Beograd.

Touré, R., E. Kheadr, C. Lacroix, O. Moroni, and I. Fliss. 2003. Production of antibacterial substances by bifidobacterial isolates from infant stool active against Listeria monocytogenes. J. Appl. Microbiol. 95:1058–1069 Available at https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2672.2003.02085.x (verified 18 May 2022).

Tournour, H.H., M.A. Segundo, L.M. Magalhães, L. Barreiros, J. Queiroz, LM. Cunha. 2015. Valorization of grape pomace: Extraction of bioactive phenolics with antioxidant properties. Ind. Crops Prod 74:397–406. https://doi.org/10.1016/j.indcrop.2015.05.055

Treena, H. 2018. A new era of antibiotics use. [Online] Available: https://www.canadianpoultrymag.com/a-new-era-of-antibiotics-use-30821/ [2020 Sep. 17].

Ursell, L. K., J. C. Clemente, J. R. Rideout, D. Gevers, J. G. Caporaso, and R. Knight. 2012. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. J. Allergy and Clinical Immunol. 129(5):1204-1208.

Vacca, M., G. Celano, F. M. Calabrese, P. Portincasa, M. Gobbetti, and M. De Angelis. 2020. The controversial role of human gut lach- nospiraceae. Microorganisms 8:1–25.

Valdez, J. M. 2021. Systemic Inflammation Affects the CNS in an Age, Duration, and Sex Dependent Manner (Doctoral dissertation, University of California, Riverside).

Van Immerseel, F., J. De Buck, F. Pasmans, L. Bohez, F. Boyen, F. Haesebrouck, and R. Ducatelle. 2004. Intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella* enteritidis. Poult. Sci. 83:1911–1916.

Vareed, S.K. 2005. Health-beneficial compounds in Cornus fruits. Michigan State University.

Vattem, DA, Y.T. Lin, R. Ghaedian, K. Shetty. 2005. Cranberry synergies for dietary management of Helicobacter pylori infections. Process Biochem. 40:1583–1592. https://doi.org/10.1016/J.PROCBIO.2004.06.024

Videnska, P., F. Sisak, H. Havlickova, M. Faldynova, and I. Rychlik. 2013. Influence of *Salmonella* enterica serovar Enteritidis infection on the composition of chicken cecal microbiota. BMC Vet. Res. 9:1–8 Available at https://bmcvetres.biomedcentral.com/articles/10.1186/1746-6148-9-140 (verified 19 May 2022).

Vinci, G. and M.L. Antonelli. 2002. Biogenic amines: quality index of freshness in red and white meat. Food Control 13: 519–524. doi:https://doi.org/10.1016/S0956-7135(02)00031-2.

Viveros, A., S. Chamorro, M, Pizarro. I. Arija, C Centeno and A. Brenes. 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult. Sci. 90:566–578. https://doi.org/10.3382/ps.2010-00889

Vlaicu, P.A., Dumitra Panaite T., Dragotoiu D., Ropota M., Bobe. E., M. Olteanu. et al. 2017. Feeding quality of the meat from broilers fed with dietary food industry by-products (flaxseed, rapeseeds and buckthorn meal, grape pomace). Sci Pap Ser D Anim Sci. 60:123–30.

Wang, R., H. Yu, H. Fang, Y. Jin, Y. Zhao, J. Shen, C. Zhou, R. Li, J. Wang, and Y. Fu. 2020. Effects of dietary grape pomace on the intestinal microbiota and growth performance of weaned piglets. Arch. Anim. Nutr. 74:296–308.

Wang, X., H. Tong, F. Chen, and J. D. Gangemi. 2010. Chemical characterization and antioxidant evaluation of muscadine grape pomace extract. Food Chem 123:1156–1162.

Wassenaar, T. M., and K. Zimmermann. 2018. Lipopolysaccharides in Food, Food Supplements, and Probiotics: Should We be Worried? Eur. J. Microbiol. Immunol. (Bp). 8:63.

Wei, L.Y., P.X. Jiao, T.W. Alexander, and W.Z. Yang. 2018. InclusIon of red osIer dogwood In hIgh-forage and hIgh-graIn dIets affected in vitro rumen fermentation. Anim. Sci 18: 453–467. doi:10.1515/aoas-2017-0042.

Wei, L.Y., W.M.S. Gomaa, B.N. Ametaj, T.W. Alexander, and W.Z. Yang. 2019. Feeding red osier dogwood (Cornus sericea) to beef heifers fed a high-grain diet affected feed intake and total tract digestibility. Anim. Feed Sci. Technol. 247: 83–91. doi:https://doi.org/10.1016/j.anifeedsci.2018.11.006.

Wenk, C. 2003. Herbs and botanicals as feed additives in monogastric animals. Asian-Australas. J. Anim. Sci. 16: 282-289.

White, D. G., S. Zhao, R. Sudler, S. Ayers, S. Friedman, S. Chen, P. F. McDermott, S. McDermott, D. D. Wagner, and J. Meng. 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. N. Engl. J. Med. 345:1147–1154.

WHO. 2018. *Salmonella* (non-typhoidal). Available at https://www.who.int/en/news-room/fact-sheets/detail/*Salmonella*-(non-typhoidal) (verified 25 April 2022).

Wierup, M. 2001. The Swedish Experience of the 1986 Year Ban of Antimicrobial Growth Promoters, with Special Reference to Animal Health, Disease Prevention, Productivity, and Usage of Antimicrobials. Microb. Drug Resist. 7: 183–190. Mary Ann Liebert, Inc., publishers. doi:10.1089/10766290152045066.

Williams, K., K. Ruotsalo, and M. S. Tant. 2021. Serum Biochemistry | VCA Animal Hospital. Available at https://vcacanada.com/know-your-pet/serum-biochemistry (verified 30 November 2021).

Williamson, G. 2013. Possible effects of dietary polyphenols on sugar absorption and digestion. Mol. Nutr. Food Res. 57: 48–57. John Wiley & Sons, Ltd. doi:10.1002/MNFR.201200511.

Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86: E140–E148. Oxford University Press. doi:10.2527/jas.2007-0459.

Wise, M. G., and G. R. Siragusa. 2007. Quantitative analysis of the intestinal bacterial community in one-to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. J. Appl. Microbiol. 102:1138–1149.

Witzig, M., A. Camarinha da Silva, R. Green-Engert, K. Hoelzle, E. Zeller, J. Seifert, L.E. Hoelzle, and M. Rodehutscord. 2015. Spatial Variation of the Gut Microbiota in Broiler Chickens as Affected by Dietary Available Phosphorus and Assessed by T-RFLP Analysis and 454 Pyrosequencing. PLoS One 10: e0143442. Public Library of Science. doi:https://doi.org/10.1371/journal.pone.0143442.

Wojcikowski, K., L. Stevenson, D. Leach, H. Wohlmuth, and G. Gobe. 2007. Antioxidant Capacity of 55 Medicinal Herbs Traditionally Used to Treat The Urinary System: A Comparison Using A Sequential Three-Solvent Extraction Process. J. Altern. Complement. Med. 13: 103–110. Mary Ann Liebert, Inc., publishers. doi:10.1089/acm.2006.6122.

Woof, J.M. and M.A. Ken. 2006. The function of immunoglobulin A in immunity. J. Pathol. 208: 270–282. J Pathol. doi:10.1002/PATH.1877.

World Health Organization 2019. New report calls for urgent action to avert antimicrobial resistance crisis. Joint News Release, New York. Available online: https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis

World Health Organization 2019. New report calls for urgent action to avert antimicrobial resistance crisis. Joint News Release, New York. Available online: https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis

World Health Organization. 2015. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization, Switzerland.

Wu, Q. J., Y. M. Zhou, Y. N. Wu, L. L. Zhang, and T. Wang. 2013. The effects of natural and modified clinoptilolite on intestinal barrier function and immune response to LPS in broiler chickens. Vet. Immunol. Immunopathol. 153:70–76.

Xie, H., N. C. Rath, G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Effects of *Salmonella* typhimurium lipopolysaccharide on broiler chickens. Poult. Sci. 79:33–40.

Xue, B., J. Xie, J. Huang, L. Chen, L. Gao, S. Ou, Y. Wang, and X. Peng. 2016. Plant polyphenols alter a pathway of energy metabolism by inhibiting fecal Bacteroidetes and Firmicutes in vitro. Food Funct. 7:1501–1507.

Yáñez, A., S. G. Coetzee, A. Olsson, D. E. Muench, B. P. Berman, D. J. Hazelett, N. Salomonis, H. L. Grimes, and H. S. Goodridge. 2017. Granulocyte-Monocyte Progenitors and Monocyte-Dendritic Cell Progenitors Independently Produce Functionally Distinct Monocytes. Immunity 47:890-902.e4.

Yang, R., Q. Hui, Q. Jiang, S. Liu, H. Zhang, J. Wu, F. Lin, K. O, and C. Yang. 2019. Effect of Manitoba-Grown Red-Osier Dogwood Extracts on Recovering Caco-2 Cells from H2O2-Induced Oxidative Damage. Antioxidants (Basel, Switzerland) 8. doi:10.3390/antiox8080250.

Yang, R., Q. Hui, Q. Jiang, S. Liu, H. Zhang, J. Wu, F. Lin, K. O, and C. Yang. 2019. Effect of Manitoba-grown red-osier dogwood extracts on recovering Caco-2 cells from H2O2-induced oxidative damage. Antioxidants. 8(8):250. Available at https://europepmc.org/articles/PMC6721146.

Yang, X., Y. Guo, X. He, J. Yuan, Y. Yang, and Z. Wang. 2008. Growth performance and immune responses in chickens after challenge with lipopolysaccharide and modulation by dietary different oils. Animal 2:216–223.

Yang, Y., G. Tellez, J. D. Latorre, P. M. Ray, X. Hernandez, B. M. Hargis, S. C. Ricke, and Y. M. Kwon. 2018. *Salmonella* excludes *Salmonella* in poultry: Confirming an old paradigm using conventional and barcode-tagging approaches. Front. Vet. Sci. 5:1–7.

Yason, C. V., B. A. Summers, and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am. J. Vet. Res. 48:927–938.

Yeon, M.J., M.H. Lee, D.H. Kim, J.Y. Yang, H.J. Woo, H.J. Kwon, C. Moon, S.H. Kim, and J.B. Kim. 2019. Anti-inflammatory effects of Kaempferol on Helicobacter pylori-induced inflammation. Biosci. Biotechnol. Biochem. 83: 166–173. Oxford Academic. doi:10.1080/09168451.2018.1528140.

Yu, Q., L. Zhu, Z. Wang, P. Li, and Q. Yang. 2012. Lactobacillus delbrueckii ssp. lactis R4 prevents *Salmonella* Typhimurium SL1344-induced damage to tight junctions and adherens junctions. J. Microbiol. 2012 504 50: 613–617. Springer. doi:10.1007/S12275-012-1596-5.

Zabek, K., D. Szkopek, M. Michalczuk, and P. Konieczka. 2020. Dietary phytogenic combination with hops and a mixture of a free butyrate acidifier and gluconic acid maintaining the health status of the gut and performance in chickens. Animals 10:1335.

Zeineldin, M., R. Barakat, A. Elolimy, A.Z.M. Salem, M.M.Y. Elghandour and Monroy, J.C. 2018. Synergetic action between the rumen microbiota and bovine health. Microb. Pathog. 124: 106–115. Academic Press. doi:10.1016/J.MICPATH.2018.08.038.

Zhang, H., Y. Chen, Y. Chen, Y. Li, P. Jia, S. Ji, Y. Zhou, and T. Wang. 2020. Dietary pterostilbene supplementation attenuates intestinal damage and immunological stress of broiler chickens challenged with lipopolysaccharide. J. Anim. Sci. 98.

Zhang, S., and I. H. Kim. 2020. Effect of quercetin (flavonoid) supplementation on growth performance, meat stability, and immunological response in broiler chickens. Livest. Sci. 242:104286.

Zhang, S., J. Huang, X. Xie, Y. He, F. Mo, and Z. Luo. 2017. Quercetin from Polygonum capitatum Protects against Gastric Inflammation and Apoptosis Associated with Helicobacter pylori Infection by Affecting the Levels of p38MAPK, BCL-2 and BAX. Mol. 2017, Vol. 22, Page 744 22: 744. Multidisciplinary Digital Publishing Institute. doi:10.3390/MOLECULES22050744.

Zhang, X. 2011. Application of total bile acid, ALT, and AST in serum. Jilin Med. J. 32:4840–4841.

Zhang, Y., P. B. Limaye, H. J. Renaud, and C. D. Klaassen. 2014. Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice. Toxicol. Appl. Pharmacol. 277:138.

Zhang, Z., X. Peng, S. Li, N. Zhang, Y. Wang, and H. Wei. 2014. Isolation and identification of quercetin degrading bacteria from human fecal microbes. PLoS One 9:e90531 Available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0090531 (verified 17 May 2022).

Zhen, W., Y. Shao, X. Gong, Y. Wu, Y. Geng, Z. Wang, and Y. Guo. 2018. Effect of dietary Bacillus coagulans supplementation on growth performance and immune responses of broiler chickens challenged by *Salmonella* enteritidis. Poult. Sci. 97:2654–2666.

Zheng, C. J., R. Liu, B. Xue, J. Luo, L. Gao, Y. Wang, S. Ou, S. Li, and X. Peng. 2017. Impact and consequences of polyphenols and fructooligosaccharide interplay on gut microbiota in rats. Food Funct. 8:1925–1932 Available at https://pubs.rsc.org/en/content/articlehtml/2017/fo/c6fo01783e (verified 19 May 2022).

Zheng, S., J. Song, X. Qin, K. Yang, M. Liu, C. Yang, and C. M. Nyachoti. 2021. Dietary supplementation of red-osier dogwood polyphenol extract changes the ileal microbiota structure and increases Lactobacillus in a pig model. AMB Express 11:1–12 Available at https://amb-express.springeropen.com/articles/10.1186/s13568-021-01303-8 (verified 23 January 2022).

Zheng, X. C., Q. J. Wu, Z. H. Song, H. Zhang, J. F. Zhang, L. L. Zhang, T. Y. Zhang, C. Wang, and T. Wang. 2016. Effects of Oridonin on growth performance and oxidative stress in broilers challenged with lipopolysaccharide. Poult. Sci. 95:2281–2289.

Zhenping, S., L. Wenting, Y. Ruikui, L. Jia, L. Honghong, S. Wei, W. Zhongmie, , L. Jingpan, S. Zhe, and Q Yuling. 2013. Effect of a straw-derived xylooligosaccharide on broiler growth performance, endocrine metabolism, and immune response. Can. J. Vet. Res. 77: 105–109.

Zhou, Y. and F. Zhi. 2016. Lower Level of Bacteroides in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis. Hindawi 2016:5828959 https://doi.org/10.1155/2016/5828959

Zulkifli, I., M. T. Che Norma, C. H. Chong, and T. C. Loh. 2000. Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. Poult. Sci. 79:402–406