Canadian Journal of Animal Science

Effects of red-osier dogwood extracts on growth performance, intestinal digestive, and absorptive functions and meat quality of broiler chickens

Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2020-0191.R1
Manuscript Type:	Article
Date Submitted by the Author:	04-Feb-2021
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Keywords:	red osier dogwood, broiler chicken, growth performance, digestibility, meat quality

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RUNNING HEAD: RED-OSIER DOGWOOD EXTRACTS ON BROILERS**Effects of red-osier dogwood extracts on growth performance, intestinal digestive, and absorptive functions and meat quality of broiler chickens**

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ABSTRACT

A total of 320 1-day-old Cobb 500 chicks with an initial weight of 48.3 ± 3.3 g per pen were assigned to 4 dietary treatments with 8 replicates provided in 3 phases for 46 days. The treatments were fed as mash diets and included :1) negative control (**NC**) corn-soybean basal diet, 2) positive control (**PC**) basal diet with 30 ppm avilamycin, 3) basal diet supplemented with 1000 ppm RDE (**RDE1**), and 4) basal diet with 3000 ppm RDE (**RDE2**). Results showed reduced jejunal crypt depth in RDE1 and increased villus: crypt ratio in groups (either RDE1 or RDE2) ($P < 0.05$). Cationic amino acid transporter mRNA abundance was decreased ($P < 0.05$) in RDE1, RDE2, and PC treatments, but peptide and neutral amino acid transporter mRNA abundance were higher ($P < 0.05$) in RDE2 compared to NC. Apparent ileal digestibility of crude fat was increased in RDE2 and PC compared to NC, whereas amino acid digestibility was greater in RDE1, RDE2, and PC ($P < 0.05$). In conclusion, red osier dogwood had no effect on growth performance, improved the intestinal health and function of broiler chickens, and had no detrimental effects on meat quality.

Key words: red osier dogwood, broiler chicken, growth performance, digestibility, nutrient transporters, meat quality

INTRODUCTION

There is an increasing demand in the world for protein sources, and poultry meat products have become more popular recently. The Organisation for Economic Co-operation and Development statistics estimated that consumption of poultry meat will increase from the current 30.66 kg/capita in 2020 to 31.88 kg/capita by 2028 (OECD library, 2019). As the demand for poultry meat products increased, intensive farming practices such as the use of AGP have been employed to increase productivity and efficacy in commercial livestock agriculture (Castanon, 2007). The exact mechanism by which antibiotic growth promoter (AGP) increases livability and efficiency is not clearly understood, but has been explained to be partly as a result of interactions with gut microbiota (Dibner and Richards, 2005; Mehdi et al., 2018). Despite the beneficial effects of AGP, subtherapeutic dosages of antibiotics in broiler feed may lead to the development of antibiotic resistance in bacteria (Antunes et al., 2016; DiMarzio et al., 2013; Threlfall, 2002), which has shifted consumer preferences to chicken products labeled as 'raised without antibiotics. Those issues have contributed to restrictions on the preventative use of antibiotics (Kuttappan et al., 2012; Petracci et al., 2014; Wideman et al., 2016), and they have been banned in the European Union since 2006 and in Canada as of December 2018 (Maron et al., 2013; Mehdi et al., 2018). Therefore, it has become imperative to search for antimicrobial alternatives that can improve the performance and health of broiler chickens. Recently, there has been a lot of interest in the use of plants and their secondary metabolites as feed additives to improve animal health and productivity (Wallace et al., 2010). The use of aqueous and organic solvent extracts from plants that may include terpenoids, saponins, polyphenols, alkaloids, and organic acids have been employed as alternatives for AGP due to their antimicrobial, antioxidative, and anti-

inflammatory properties (Barbieri et al., 2017; Tholl, 2015; Zdunczyk et al., 2010). Red osier dogwood's leaves, stems, berries, and flowers are rich in polyphenolic compounds, including quercetin, rutin, kaempferol, gallic and ellagic acid, and various cyanidin - glycosides (Balasundram et al., 2006; Isaak et al., 2013). The polyphenolic compounds present in red osier dogwood extracts (RDE) have been demonstrated to have antimicrobial (Hirai et al., 2010; Nohynek et al., 2006; Song et al., 2011), antioxidative and anti-inflammatory potentials (Isaak et al., 2013; Seeram and Nair, 2002; Yang et al., 2019). Furthermore, RDE has been demonstrated to improve growth performance and gut health in weaned piglets potentially due to antioxidant capacity (Jayaraman et al., 2018; Koo et al., 2018). To our knowledge, there have been no studies conducted using RDE in broiler chickens. These findings can offer RDE an opportunity to be used as a potential antibiotic alternative in broiler chickens.

Due to the proven beneficial effects of polyphenolic compounds present in RDE, we hypothesized that RDE can improve growth performance, digestive and absorptive functions, and meat quality of broiler chickens. Therefore, the objective of this study was to evaluate the effects of 0.1% RDE and 0.3% RED on growth performance, organ weights, intestinal morphology, nutrient digestibility, nutrient transporters, and meat quality in broiler chickens.

MATERIALS AND METHODS

The broiler chickens were raised according to animal use protocol (F18-024), which was approved by the University of Manitoba Animal Care Protocol Management and Review Committee. The birds were cared for and handled in

accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Red-osier Dogwood

Red-osier dogwood plant was cultivated and obtained from Swan River Manitoba and extracted and distilled by Red Dogwood Enterprises (Swan River, Manitoba, Canada) to obtain the red-osier dogwood extract powder. The total phenolics content in the extract powder was 6277 mg/kg and the concentrations of glucogallic acid, ellagic acid, rutin, quercetin malonyl glucoside, and quercetin were 1682 mg/kg, 671 mg/kg, 2712 mg/kg, 313 mg/kg, and 10 mg/kg, respectively (Apea-Bah et al., 2020).

Animal Trial and Growth Performance

A total of 320 1-d-old Cobb500 male broiler chicks were distributed in a single room containing 32 floor pens with dimensions of 81.5 inches $\times$ 59 inches = 4,808.5 square inches and a depth of 4 inches of straw as bedding (8 pens per treatment, 10 birds per pen) based on their body weight (**BW**, 48.3 ± 3.3 g) and raised for 46 days. Broilers had free access to feed and water; and feed was provided as mash diets in trough feeders (d 1 - 5) and in hanging metal poultry feeders (d 6 - 45). Water pots (double-wall metal poultry fount 5 gallon) were used during the starter phase and water cups were used afterwards (6.5 inches in diameter and 2.25 inches deep). The broilers were assigned into 4 treatments during the entire experimental period, additives were added at the expense of energy ingredient corn, and all diets were prepared and mixed at the University of Manitoba feed mill (Glenlea Research Station, MB, Canada). Treatments included a negative control (corn-soybean based diet; **NC**), a positive control with basal diet + 30 ppm avilamycin (**PC**; Surmax 100

Premix, avilamycin premix 100 g/kg Elanco Canada Co. Ltd. Guelph, Ontario, Canada), **RDE1** (basal diet + 0.1% RDE) and **RDE2** (basal diet + 0.3% RDE). The diets were formulated in phases according to the genetic breeding company's guideline (Cobb - Vantress); starter (d 1 - 14), grower (d 15 - 28), and finisher (d 29 - 46), as shown in Table 1. The temperature was maintained at 31°C for day 1 then decreased by 1 °C every 3 d up to day 7. From d 7 - 14, temperature was maintained at 28 °C, reduced to 25 °C from day 21 - 28, reduced to 24 °C from day 28 - 35, then decreased further to 22 °C from d 35 to d 41 and maintained at 21 °C up to the end of the trial (42 - 46 d). The lighting program during the trial was as follows: 24L:0D from 0 to 3 d, 22L:2D from 4 to 7 d and 18L:6D in the period from 8 to 39 d and 23L:1D in the period of 40 to 46 days respectively to simulate commercial broiler rearing.

Feed intake and BW were recorded and calculated at the end of each phase (15d, 29d, 41d). The BW and feed intake were used to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**). Mortality was monitored daily. During the entire experimental periods, 5, 11, 10 and 4 broilers in the NC, PC, RDE1 and RDE2 treatment groups respectively were culled due to leg problems and recorded for calculating mortality.

Sampling and Sample processing

Tissue and digesta

At d 45, one bird per replicate pen was randomly selected (8 birds per treatment in total), euthanized performed using a CO₂ chamber and confirmed with cervical dislocation. and the BW of the bird was measured. The heart, bursa, spleen and liver were obtained from broilers and weighed for evaluation of relative organ weight. Mid-jejunal segments were obtained, rinsed with cold phosphate-buffered

saline (PBS), snap-frozen in liquid nitrogen, and then stored at - 80 °C for gene expression analysis. Duodenal (from the pylorus to the distal portion of the duodenum loop), jejunal (from the distal part of the duodenum loop to Meckel's diverticulum) and ileal segments (from Meckel's diverticulum to cecum openings) were obtained and flushed with cold PBS and then fixed in 10% neutral buffered formalin for intestinal morphological analysis. Ileal digesta was collected and stored at - 20 °C for digestibility studies. Ileum and cecum digesta were aseptically collected

Slaughtering and Breast Sample Collection

Upon completion of the trial, broiler chickens at 46 d of age were placed in plastic crates for transportation to a commercial slaughterhouse for processing after a 6 h fasting period. The carcasses were cleaned and chilled in a still ice water bath for 2 - 4 h, skinned and deboned to obtain the breast muscle (*pectoralis major*) for white striping (**WS**) and woody breast (**WB**) scoring and meat quality analysis.

After WS and WB scoring, samples were placed in labelled plastic bags, stored in ice, and transported to the lab. In the lab at 2 °C and 24 h postmortem, the samples were further subdivided to obtain five sub-samples that were used for meat quality analyses (Lu et al., 2020). Starting from the right breast, the first subsample was cut from the cranial section ($8 \times 4 \times 2 \text{ cm}^3$) and used to assess ultimate pH and myofibrillar fragmentation index (MFI). A rectangular cut ($8 \times 4 \times 3 \text{ cm}^3$, mid-region) was excised from the cranial and caudal parts for cooking traits and Warner-Bratzler shear force. In addition, a raw cylindrical meat cut (caudal region) 2.5 cm diameter $\times$ 6 cm length was obtained for gravimetric water holding capacity analysis (drip loss). From the left breast, the caudal portion ($8 \times 4 \times 2 \text{ cm}^3$) was excised for initial lipid oxidation analysis, and the rest of the breast was used for retail color and purge loss

evaluations. Except for retail color and purge loss evaluation samples, the samples were stored at - 40 °C.

Laboratory Analysis

Intestinal Morphology

The tissues were dehydrated with alcohol, fixed with paraffin, and embedded in wax. 6 µm sections were mounted onto slides deparaffinized in xylene, rehydrated, then stained with the Hemoxilin and eosin dyes for histological analysis. Carl Zeiss MicroImaging microscope equipped with a morphometric software system (Carl Zeiss Ltd., Göttingen, Germany) was used to capture the images of the intestine. Around 8 - 12 well-developed villus were selected from all parts of the lumen were used to measure villus height (**VH**) - from the tip of the villus to the villus - crypt junction and crypt depth (**CD**) - from the villus base to the bottom of the crypt and calculate VH to CD ratio (**VCR**).

Gene Expression

The samples were suspended in lysis solution RNA was extracted from tissues pulverized in liquid nitrogen and suspended in lysis buffer using the RNAqueous® Kit (AM1912) per the manufacturer's instructions. Extracted RNA was stored at -80 °C and used for cDNA synthesis using the iscript cDNA Synthesis Kit (Bio-Rad, Mississauga, ON, Canada) with the master mix containing 4 µl 5× iScript mix, 1 µl iScript R- transcriptase, and 5 µl water. cDNA was stored at -20 °C and used for Real Time-PCR. RT- PCR was performed using the IQ™ SYBR® Green Supermix (Bio-Rad Mississauga, ON, Canada) using 6 µL of cDNA samples, 10 µL supermix, 2 µL water and 1 µL forward and reverse primers. The thermal cycle was set as; 95° C for 3 min, 40 cycles at 95° C for 20 s, 60° C for 30 s, and 72° C for 30 s and the data was analyzed using the 2 delta delta method after normalization against the reference gene

β -actin (Livak and Schmittgen, 2001). The sequences of primers for the genes tested were designed according to Lu et al. (2020), and the sequences are located in GenBank.

Apparent Ileal Nutrient Digestibility

Broiler finisher diets were mixed with 0.3% chromic oxide as an indigestible marker from d 42 - 45. Frozen-dried ileal digesta was used for the analysis of crude fat, crude protein, dry matter, and amino acid digestibility. The chromium extraction method was used for the analysis of dry matter according to the AOAC (2000) method (procedure # 934.01). Approximately 0.9 - 1.1 g of diet and in-house standards were weighed with 0.25 - 0.35 g used for the analysis of digesta samples. Samples were dried for 12 h set at 600 °C and digested with acid and chromium content determined using an inductively coupled plasma spectrometer (Varian Inc., Palo Alto, CA, USA).

Crude protein (CP) was estimated using total nitrogen content measured with a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA) and CP calculated as Nitrogen $\times$ 6.25. Amino acid digestibility was measured using acid hydrolysis method AOAC (2006; procedure # 994.12) followed by oxidation of methionine and cysteine with performic acid (AOAC, 2006; procedure # 985.18) and alkaline hydrolysis of tryptophan with barium hydroxide octahydrate Commission Directive (2000). Amino acid content was analyzed using the amino acid analyzer (Sykam, Germany). Nutrient digestibility was calculated as the following formula:

$$\text{Nutrient Digestibility (\%)} = \{1 - (\text{NF} \times \text{CD}) / (\text{ND} \times \text{CF})\} \times 100$$

Where; NF = nutrient concentration in digesta,

CD = chromium concentration in diet,

ND = nutrient concentration in diet,

CF = chromium concentration in digesta.

White Striping and Woody Breast Incidences

White striping (**WS**) and woody breast (**WB**) incidences were evaluated visually and scored by one person at the processing plant to minimize variation. The visual myopathies were recorded as the presence of either WS or WB, and severity scored. Breast fillets were manually palpated on both the right and left breast to evaluate WB incidence, and scoring was on a 4 - point scale as follows: normal (fillets are flexible throughout; 0), mild (hard in the cranial region but flexible; 1), moderate (hard throughout but flexible in mid to caudal region; 2) and severe (the whole fillet is extremely hard and rigid; 3) (Tijare et al., 2016). WS was visually scored on a 3 -point scale as: normal (no distinct white lines; 0), moderate (visible white lines < 1 mm thick; 1) and severe (large white lines 1 - 2 mm thick; 2) (Kuttappan et al., 2012).

Meat pH, Color and Drip loss Evaluations

The dorsal part of the muscle, pH was measured using a portable waterproof meat pH meter with a penetrating metal rode (HI 99163 Hanna instrument TM Carrollton, Texas, USA). The pH meter was calibrated using pH 4.01 and pH 7.0 solutions. The breast was split into a butterfly while avoiding contact on the exposed surface, plated on a Styrofoam tray (foam meat tray, 8.25 × 5.75 × 1 cm, Pack All Manufacturing Inc, Rockland, ON, Canada) with a soaking pad, and covered with oxygen-permeable polyvinyl chloride film (PVC; 037242 PUR Value Polyvinylchloride Standard Meat Films, AGL, Richmond Hill, Ontario, Canada) for retail display analysis. The trays were placed on a retail display cabinet (Model MI, Hussmann Corporation, Canada) at 3 °C under LED lighting (light-emitting diodes; Acuity Brands Dimmable Rigid 30-LED Light Strip Board HTG S7 - 94v-0 – 4000k) with intensity 1240 lx. The trays' positions were rotated randomly every 24 h to

account for temperature and lighting differences on the machine. The pH and objective colour were recorded at the beginning and at the end of the retail display period (96 h). The objective colour was measured at 3 locations on the meat surface using the Konica Minolta (Chroma Meter CR-410 Minolta Canada Inc., Mississauga, Ontario, Canada) using illuminant D65 and 10° standard observer angles and 2.54 cm aperture (Commission Internationale de l'éclairage, 1978) that was blanked against a white tile. CIE measurements L* (lightness), a* (redness) and b* (yellowness) were recorded. At the end of the display period and after colour evaluation, the breast fillets were removed from the retail cabinet, and weight and pH were measured again, vacuum packaged (6" × 10" FlairPak Vacuum Pouch, Flair Flexible Packaging Corporation, Canada/USA) and frozen at - 40°C for subsequent lipid oxidation analysis.

Drip loss was evaluated according to the method by Remignon et al. (1996) with some modifications. Briefly, approximately 2.5 cm diameter × 6 cm length of breast muscle was obtained from the caudal part and suspended perpendicularly in a flat-bottomed volumetric flask, ensuring the tissue did not touch the flask surfaces and sealed airtight. The flasks were placed at 2 °C for 48 h, and the initial and final weight were used to calculate drip loss percentage using the following equation;

$$\text{Drip loss \%} = [(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100.$$

Lipid Peroxidation

The breast muscle malondialdehyde (**MDA**) levels were evaluated using the thiobarbituric acid reactive substances (**TBARS**) method to evaluate lipid peroxidation from two time points according to Buege and Aust (1978). Breast meat sample weighing 10 g was suspended in 30 mL of cold deionized distilled water and homogenized for 60 s (Osterizer 10 speed blender). The homogenate was transferred

to 50 mL conical centrifuge tubes and centrifuged for 10 min at 1850 $\times g$ at 4 °C (Thermo Scientific™ Sorvall™ RC6 plus). Afterward, 2 mL of the supernatant was carefully transferred into 15 mL conical centrifuge tubes in duplicates and mixed with 4 mL TCA/TBA solution (15% TCA and 20Mm TBA- MW 144.15) and 100 μ L of 10% butylated hydroxyanisole (**BHA**) by a vortex. The tubes were placed in a boiling hot water bath (100 °C) for 15 min and afterwards transferred to an ice water bath for 10 min. The mixture was centrifuged at 1850 $\times g$, for 10 min at 4° C and supernatant carefully dispensed into 1.8 mL cuvettes for absorbance measurements read at 531 nm using a spectrophotometer machine (GENESYS 30 visible spectrophotometer, Thermo Scientific™).

Cooking Traits and Shear Force

Samples were thawed overnight at 2 °C, weighed then placed in sandwich bags and cooked in a water bath set at 85 °C to an internal temperature of between 75 °C – 78 °C. A thermometer was inserted in the thickest part of the breast meat used as temperature monitors. Samples were drained and left to cool down at room temperature for 2 h, lightly dried with paper towels, and the weight was recorded to calculate cook loss. Finally, cooking time and cook loss were indicated as cooking traits.

Cooked samples were kept in the cold room (2 °C) overnight and tempered to room temperature (20 °C) for 30 min before Warner Bratzler Shear Force (**WBSF**) measurement. Five rectangular strips of dimensions 1 cm wide, 1 cm thick and 2 - 4 cm long were obtained along the fibre axis. The analyzer was loaded with a 10 kg loading cell, and the weight was calibrated with a 2 kg weight and the return speed set at 10 mm/s. The strips were placed with fibres perpendicular to the blades of WBSF

analyzer (TA - XT Plus, Texture Technologies) for shredding, and two shear values were recorded from each strip.

Myofibrillar Fragmentation Index

For myofibrillar fragmentation index analysis (**MFI**), 4 g of breast meat was minced using a knife in a cold room and suspended in 40 mL of cold MFI buffer (100 mM KCl, 20mM KPO₄, 2mM MgCl₂, 2mM EGTA, 1mM NaN₃, pH 7.0) and homogenized for 30s (Osterizer 10 speed blender). The homogenate was centrifuged at 1000 ×g for 15 min at 2° C (Sorvall™ RC6 plus Thermo Scientific™). The supernatant was discarded, and the pellet and fat cap resuspended in 40 mL cold MFI buffer using a stirring glass rod then centrifuged again. The supernatant and fat cap were discarded, and the pellet resuspended in 10 mL cold MFI buffer and mixed by vortexing. The mixture was poured through a strainer, and the suspension was obtained for protein estimation using bovine serum albumin (BSA), and 4 mL of biuret reagent absorbance was read at 540 nm using a spectrophotometer (GENESYS 30 visible spectrophotometer Thermo Scientific™). The suspension was then diluted to a final volume and concentration of 8 mL and 0.5 mg protein/mL respectively, vortexed and the absorbance read at 540 nm using a spectrophotometer (Thermo Scientific™ GENESYS™ 30 visible spectrophotometer). The MFI value was obtained using the equation $MFI = 200 * \text{Absorbance}$ (Culler et al., 1978).

Statistical Analysis

This experiment was designed as a completely randomized design and each pen was considered an experimental unit. Therefore, the variation associated with dietary treatment was considered a fixed effect, whereas replicates as a random effect. Statistical analysis of all data was performed using SAS 9.4 (SAS Institute, Cary NC, 2012). The data values were subjected to a normality test by using PROC

UNIVARIATE procedure. Using normal plot options and Shapiro–Wilk statistics, outliers were detected and discarded to satisfy the normal distribution assumption. Performance, intestinal parameters, and meat quality data were analyzed using the MIXED model procedures of SAS version 9.4 (SAS, 2012). Least squares means were separated (F test, $P < 0.05$) using the adjusted Tukey–Kramer's multiple comparison test. The degrees of freedom in the denominator was adjusted using the Kenward-Roger procedure. Chi-square analysis (Fisher's exact test) was analyzed by PROC FREQ to analyze the difference in the distribution of severity scores in WS and WB and mortality. Differences were considered significant at $P < 0.05$, and trends ($0.05 \leq P \leq 0.10$) were also presented.

RESULTS

Growth Performance

The ADG, ADFI, and FCR were similar between treatments in all phases and during the whole trial ($P > 0.05$). However, PC tended to have a decrease FCR in the grower phase ($P = 0.07$) as shown in Table 2. RDE1 and RDE2 treatments had the lowest mortality rate while PC showed the highest mortality rate.

Relative Organ Weight and Intestinal Morphology

The feeding treatments did not affect the relative weight of the heart, liver, bursa, and spleen ($P > 0.05$) as observed in Table 3.

The CD, VH and VCR of the duodenum and ileum were similar across treatments ($P > 0.05$). However, in the jejunum, RDE1 had lower CD compared to NC ($P < 0.05$). In addition, both RDE1 and RDE2 treatments presented highly

significant greater jejunal VCR compared to PC and NC ($P < 0.01$) as shown in Fig. 1.

Apparent Ileal Digestibility

Regarding apparent ileal digestibility (**AID**), there were no significant differences in AID of dry matter, and crude protein digestibility tended to differ ($P = 0.07$) among treatments. Conversely, RDE2 and PC had increased crude fat digestibility compared to NC and RDE1 ($P = 0.001$, Fig. 2). The AID of all amino acids was increased in RDE1, RDE2, and PC treatments compared to NC ($P < 0.01$), however, Pro and Ser showed decreased ($P < 0.01$) digestibility in RDE1 treatment compared to RDE2 as observed in Table 4.

Gene Expression

RDE2 presented an increased abundance in the mRNA expression of nutrient transporters PepT1 compared to RDE1 and PC ($P = 0.04$). RDE2 had a highly significant increased mRNA abundance of sodium-dependent neutral amino acid transporter (B⁰AT1) compared to NC and RDE1 ($P = 0.002$). On the other hand, RDE1, RDE2, and PC treatments showed reduced expression levels in cationic amino acid transporter (CAT1) than NC did ($P < 0.01$). No significant differences were observed in mRNA abundance of EAAC1, xCT1, and SGLT1 among treatments ($P > 0.05$, Fig. 3).

White Striping and Woody Breast Incidences, and Meat Quality

There was no significant difference among treatment means of WS and WB scores and their incidence on chicken breast ($P > 0.05$, Table 5).

Meat quality attributes such as lightness, yellowness, pH, drip loss, cook loss, WBSF, TBARS, and MFI were not affected by the feeding treatments ($P > 0.05$). RDE2 presented reduced redness values compared to NC ($P = 0.04$, Table 6); and NC and RDE1 treatments presented intermediate values ($P > 0.05$).

DISCUSSION

Red dogwood extracts are a combination of polyphenolic compounds extracted from the red osier dogwood plant. The chemical contents of red-osier dogwood extract used in this study was analyzed in a previous study (Apea-Bah et al., 2020). Interestingly, according to previous studies, the chemical contents of RDE are quite consistent which are rich in rutin, quercetin, kaempferol, gallic and ellagic acids, with the highest concentrations reported at 220 mg/g (Bate-Smith et al., 1975; Isaak et al., 2013; Obomsawin, 2001). Among these compounds, gallic acid, ellagic acid and quercetin were found to have antioxidant activity and red-osier dogwood have been shown to improve piglet performance and gut health in *E. coli* challenged piglets (Koo et al., 2018).

Similar performance results on BW and feed intake have been reported in previous studies using other kinds of plant extracts such as grape seed extracts, a strong antioxidant due to the high concentration of polyphenolic compounds, at concentrations between 125 – 2,000 ppm and dietary rosemary leaves, an aromatic plant with high antioxidant activity due to the presence of phenolic compounds, at 8,600 ppm and 11,500 ppm respectively (Farahat et al., 2017; Yesilbag et al., 2011). In contrast, other studies have reported that improvements in growth performance parameters such as BW and feed intake in 21 d old broilers that had supplementation

with 5,000 ppm *Labiatae* extracts and a 100 ppm mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin, which have antimicrobial effects antimicrobial activity (Bravo et al., 2014; Hernandez et al., 2004; Pirgozliev et al., 2014). In addition, Goliomytis et al. (2014) observed a reduction in the FCR of broiler chickens fed a quercetin supplemented diet. Differences in growth performance results may be attributed to a combination of factors such as nutrition, management and hygiene. In the current study, this is highlighted by similar performance between antibiotic-free (NC) and antibiotic-supplemented (PC) groups. It is important to note that PC had decreased FCR and increased ADG when compared to NC although the differences were not statistically significant. The experimental facility and environment may not have presented a challenging condition to the broilers, which making it difficult to observe statistical differences in growth performance. Research has shown that immunological and environmental stressors increases the likelihood of showing beneficial effects of plant extracts. In this study, all the broilers were in good health (Allen et al., 1997).

The reduced mortality rate by RDE2 during the whole experimental period- is in agreement with previous studies using 5000 ppm *Labiatae* extracts, 8,000 ppm amaranth seeds and 3,000 ppm sea buckthorn and 3,000 ppm chokeberry pomace that improved livability to 100%. Amaranth seed and choke berry pomace are rich in antioxidant compounds, and amaranth contains flavonoids such as quercetin, tocopherols, and squalene, whereas choke berry pomace 10.58g/100g of phenolic compounds (Hernandez et al., 2004; Orczewska-Dudek et al., 2018). The bioactive compounds present in plants are thought to have an immunomodulatory effect by preventing inflammation, improving oxidative stability, and exerting antibacterial activity (Mishra et al., 2008; Orczewska-Dudek et al., 2018). The antioxidative effect

of polyphenolic compounds such as rutin and quercetin present in RDE may be the mechanism by which plant extracts reduced mortality in broiler chickens. However, this mechanism cannot be confirmed in the current study because no differences were observed in relative organ weights of immune-related organs such as liver, bursa, and spleen, and the oxidative status of the broilers was not assessed.

The VH, CD, and VCR are morphological parameters that indicate the digestive and absorptive capacity of the small intestines (Mekbungwan et al., 2002). An increase in the VH length enlarges the digestive surface, and a decrease in CD is an indicator of improved gut health due to less shedding at the villi tips, thus reducing enterocytes cell turnover and CD (Iji et al., 2001; Nabuurs et al., 1993). Our current results in improved morphology are consistent with previous studies utilizing 50 - 100 ppm of gallic acid and 500 ppm, and 1,000 ppm of elecampane rhizome extracts rich in rutin and gallic-acid equivalents in broiler chickens (Abolfathi et al., 2019; Samuel et al., 2017). Intestinal morphology is linked with intestinal health and has been associated with intestinal microbiota balance and gut oxidative status of broiler chickens (Abolfathi et al., 2019; Wu et al., 2016). An increase in VCR and decrease in crypt depth may be a result of the anti-inflammatory and antioxidative effect of polyphenolic compounds in RDE that exert a protective effect on intestinal enterocytes. Polyphenols from olive mill wastewater have been reported to have protective effects on intestinal enterocytes by exerting anti-inflammatory and antioxidant activities (Sabino et al., 2018). Additionally, polyphenolic compounds such as ellagitannins have been reported to increase cell proliferation by 20% via increasing the cell numbers at each stage of mitotic cycle (Losso et al., 2004). Pathogens and their toxic metabolites can cause damage to intestinal enterocytes resulting in cell death and increased villus shedding, thereby reducing VH and

increasing CD (Oviedo-Rondón, 2019). Plant extracts can reduce pathogenic bacteria in the gut such as *Escherichia coli* and *Clostridium perfringens* by increasing the population of beneficial bacteria (e.g. *Lactobacillus* spp.), resulting in competitive exclusion of bacterial pathogens and direct bactericidal or bacteriostatic effects on pathogens (Abolfathi et al., 2019; Barbieri et al., 2017; Giannenas et al., 2018). Therefore, the improvement of VCR and decreased CD in the current study may be due to a combination of factors such as the balance of intestinal microbiota, proliferative effects, and protective effects on the intestinal environment brought about by the antimicrobial, antioxidative and anti-inflammatory effects of polyphenolic compounds in RDE. Additionally, no effects on duodenal and ileal morphology were observed in the study. This may be because jejunum is the major site for nutrient absorptions including sugars, amino acids, and fatty acids; however, duodenum is the site mainly for the excretions and activations of digestive enzymes; and ileum is mainly for absorptions of remaining nutrients from the jejunum (Svihus, 2014). Another possible explanation is that microbiota such as *Lactobacillus* spp. and *Fructobacillus* spp. in the jejunum is highly related to feed efficiency compared to the duodenum, indicating jejunal microbiota is more sensitive to dietary changes (Shah et al., 2019). However, the mechanisms need to be further determined.

Apparent ileal CP and DM digestibility did not differ among treatment groups in the current study in agreement with previous researches on 1,500 – 2,500 ppm of natural chestnut rich in hydrolysable tannins and 15,000 – 60,000 ppm of polyphenol-rich grape pomace extracts (Brenes et al., 2008; Schiavone et al., 2008). Similarly, greater apparent ileal amino acid digestibility observed in supplemented groups was similar to amino acid digestibility of 21d, and 41d old broiler chickens in previous studies utilizing quercetin and kaempferol rich *Ginkgo biloba* leaves and leaves

extracts with significant differences observed at the lowest concentrations of 2,000 ppm and 4,000 ppm respectively (Ren et al., 2018). Digestive functions such as enzyme activities and digestive surface can affect the digestibility of nutrients. Jamroz et al. (2005) and Kamel (2001) described that the ability of phytogenic feed additives to increase enzyme production in the brush layers and increase liver lipase activity. Although the enzyme activity was not investigated in this study, we investigated and observed improved intestinal health and improved morphology observed by higher VCR that translated into greater digestibility of amino acids. The increased digestibility of crude fat in this study may be explained by the effects of plants and plant extracts on enzymes and microbiota. Hashemipour et al. (2013) and Platel and Srinivasan (2001) reported that herbs, spices, and essential oils can stimulate the secretion of bile acids and increase the activity of pancreatic and brush border enzymes. In addition, increased microbial populations are reported to reduce fat digestibility due to their hydrolytic action on bile acids (Smith et al., 1998). Therefore, the increased crude fat digestibility may also be attributed to the antimicrobial action of RDE on intestinal microbiota.

Amino acid absorption in the small intestines can take place as either free form or as a di- or tri-peptide. PepT1 is a proton-dependent transporter and is the only known intestinal peptide transporter with broad specificity. B⁰AT1 is a neutral amino acid transporter, whereas CAT1 is cationic amino acid transporter. B⁰AT1 and CAT1 (basolateral amino acid transporter) are responsible for the transportation of methionine and threonine, lysine and arginine (Hatzoglou et al., 2004; Poncet and Taylor 2013). Nutrient transporter gene expression can be altered by phytogenic feed additives containing phenolic plant extracts, although the research is scarce (Santos et al., 2019). In this study, amino acid transporters PepT1 and B⁰AT1 were highly

expressed in RDE treated groups, but CAT1 was decreased in expression compared to the control group. To our knowledge, this study is the first published report of its kind evaluating plant extracts effects on nutrient transporters in broiler chickens under non-challenge conditions; therefore, the mechanism of action is not clear. Previous studies have reported that the up-regulation of nutrient transporters is correlated to increased levels of circulating amino acids and improved amino acid digestibility (Osmany et al., 2018; Yu et al., 2017). Therefore, the observed improvement in digestive surface and amino acid digestibility (see discussions above) may have contributed to the up-regulation of amino acid transporters in response to an increase in amino acid digestibility and concentration. It is well known that overabundance of cationic amino acids in enterocytes can cause a downregulation of CAT1 in order to maintain homeostatic levels of plasma amino acid concentrations (Hatzoglou et al., 2004). In this study, the observed decrease in mRNA levels of CAT1 in the jejunum may have been as a result of increased amino acid concentration in the enterocytes correlated with an upregulation of PepT1 transporter gene. Additionally, no differences in the expressions of SGLT-1, xCT-1, and EAAC-1 were observed among dietary treatments. SGLT-1 is sodium-dependent glucose cotransporters, which is a family of glucose transporter that could be found in enterocytes of small intestines (intestinal mucosa); xCT-1 is a member of anionic amino acid (cysteine, glutamate) transporter which could be detected in many tissues including intestinal segments; and EAAC-1 is excitatory amino acid transporter 1 plays important role in transporting glutamate across plasma membrane, which is expressed in tissues including small intestines. Since this is the first study investigating the effects of red on intestinal gene expressions of nutrient transporters in broiler chicken, the mechanism is not quite clear. Interestingly, a previous study reported that most phenolic compounds had no effects of nutrient

transporter expressions such as SGLT-1 (Hossain et al., 2002). Another possible explanation is that since RDE increased glutamate and cysteine digestibility (Table 4) which elevated AA levels, and high AA levels could increase expressions of AA transporters (Wu et al., 2015). However, high AA content may also lead to downregulation of AA transporters in order to maintain homeostatic levels of plasma amino acid concentrations (Hatzoglou et al., 2004). Consequently, no difference in the gene expressions of AA transporters was observed. However, the mechanism remains to be further determined.

The occurrence of white striping and woody breast affects nutrient composition of meat, consumer preferences, and further processing abilities of meat (Mudalal, et al., 2015; Petracci et al., 2014). The WS and WB are caused by different factors that trigger hypoxic conditions and affect muscle metabolic processes of the TCA cycle, fatty acid oxidation, arginine and taurine metabolism which is mainly due to oxidative stress and inflammation (Boerboom et al., 2018). Previous studies have demonstrated that the ability of rutin and RDE to decrease LPS-induced inflammation in vitro by preventing the production of IL-8 and the expression of pro-inflammatory cytokines in Caco- 2 and C2C12 cells that initiate inflammatory reactions (Jiang et al., 2019; Liu et al., 2019). In addition, RDE has been reported to have the antioxidative ability in vitro by upregulating expression of hemeoxygenase-1 (**HO-1**), superoxide dismutase (**SOD**), and glutathione peroxidase (**GSH-Px**) enzymes in Caco-2 cells involved in endogenous antioxidant activity effectively mitigating oxidative stress (Yang et al., 2019). These results suggest that the supplementation of the antioxidant compound rutin or rutin-containing plant extracts (e.g., RDE) may present a promising approach to control WS and WB in broiler chickens. However, in the current study, no statistically significant differences among treatment mean scores of

WS and WB and their incidence of chicken breast were observed. However, the supplementation of RDE tended to show numerical reductions in their incidence. These numerical reductions may be associated with the antioxidative and anti-inflammatory activity of RDE. Another potential cause of WS and WB is a reduction in protein synthesis rate, and an increase in protein breakdown rate and lipidosis are associated with oxidative stress and inflammation (Baldi et al., 2017; Vignale et al., 2016). Future studies are needed to investigate the effects of RDE on nutrient concentration, such as nitrogen retention, protein and lipid profiles, oxidative stress and inflammation in breast muscle of broiler chickens.

Several studies have indicated no significant effects of plant extracts treatments, namely cinnamon oil at 0.5 ppm to 1.0 ppm and commercial phytochemical compounds including a bacteriostatic herbal growth promotor at 5,500 ppm on meat quality traits of broiler breast meat such as pH and lipid oxidation (Orlowski et al., 2018; Symeon et al., 2014), thyme essential oils at 500 ppm on cook loss, and 3000 ppm of antioxidant-rich garlic powder on shear force values (Alfaig et al., 2013; Choi et al., 2010) similar to the results observed in the current study. Regarding objective color traits, Wang et al. (2016) and Young et al. (2003) reported an increase in redness and yellowness values with oregano at 3000 ppm and marigold extracts at a concentration of 750 ppm, respectively but Yesilbag et al. (2011) reported no differences in objective color values using rosemary leaves supplementation with increasing concentrations of up to 11,500 ppm. However, in the current study, the 0.3% RDE supplementation treatment reduced redness values, but there was no difference in other color traits. These differences in results may be due to different plant extracts used in the experiments; marigold and oregano contains lutein and other pigment-containing carotenoids, whereas RDE has flavonoids and other phenolic

compounds. Additionally, no differences were observed between redness values between the NC and 0.1 % RDE treatments. The results suggested that 0.1 % of RDE did not affect meat quality characteristics. It is important to mention that in vivo studies investigating plant extracts effects on meat quality largely concentrate on essential oil and known herbs and spices used to treat or marinate meat. For this reason, the studies discussed above may have similar antioxidative effects to RDE, although the bioactive compounds differ.

In conclusion, the supplementation of RDE improved the digestibility of crude fat and amino acids, intestinal morphology, and the mRNA levels of nutrient transporter in the present study. Although RDE did not significantly reduce the incidence of WS and WB, the supplementation of RDE tended to show a numerical reduction in their incidence. Moreover, the supplementation of RDE reduced the mortality rate and produced a limited effect on meat quality. Therefore, RDE may be used as a potential alternative to in-feed antibiotics in broiler chickens.

ACKNOWLEDGMENT

The authors appreciate Jason Bourcier and Gemmar Maramot at the Glenlea Research Station, University of Manitoba for their help on the chicken trial.

This project was funded by the Natural Sciences and Engineering Research Council (NSERC) Engage grant (C. Yang, EGP 523472-2018) and the University of Manitoba Start-Up Grant (C. Yang, 46561) and the Graduate Enhancement of Tri-Council Stipends (GETS) program from the University of Manitoba.

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Fig. 1. The effect of non-supplemented and red-osier dogwood extracts supplemented diets on the villus height (A), crypt depth (B) and the villus height/crypt depth (C) of broiler chickens at day 45.

NC, negative control (basal diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, basal diet with 0.1% red-osier dogwood extracts; RDE2, basal diet with 0.3% red-osier dogwood extracts. All data reported are treatment means $\pm$ standard error. Different letters in the same figure indicate significant differences among treatments ($P < 0.05$).

Fig. 2. The effect of non-supplemented and red-osier dogwood extracts supplemented diets on the apparent ileal digestibility of crude fat (A), crude protein (B) and dry matter (C) of broiler chickens at day 45. NC, negative control (basal diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, basal diet with 0.1% red-osier dogwood extracts; RDE2, basal diet with 0.3% red-osier dogwood extracts. All data reported are treatment means $\pm$ standard error. Different letters in the same figure indicate significant differences among treatments ($P < 0.05$).

Fig. 3 .The effect of non-supplemented and red-osier dogwood extracts supplemented diets on the mRNA abundance of nutrient transporters B⁰AT1 (A), CAT1 (B); EAAC1 (C); PepT1 (D); SGLT1 (E) and XCT (F) of broiler chickens at day 45.

NC, negative control (basal diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, basal diet with 0.1% red-osier dogwood extracts; RDE2, basal diet with 0.3% red-osier dogwood extracts. All data reported are treatment means $\pm$ standard error. Different letters in the same figure indicate significant differences among treatments ($P < 0.05$).

Table 1. The diet formulation and nutrient levels of the basal diets (g/kg, as fed basis unless otherwise stated)

Ingredients	Starter				Grower				Finisher			
	NC	PC	RDE1	RDE2	NC	PC	RDE1	RDE2	NC	PC	RDE1	RDE2
Maize	522.29	522.28	521.76	520.72	529.38	529.37	528.85	527.79	566.00	565.99	565.43	564.30
Soybean meal	305.00	305.00	305.00	305.00	261.00	261.00	261.00	261.00	225.00	225.00	225.00	225.00
Corn gluten meal	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Wheat	25.00	25.00	25.00	25.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Canola meal	25.00	25.00	25.00	25.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Soybean oil	22.60	22.60	22.60	22.60	43.80	43.80	43.80	43.80	45.80	45.80	45.80	45.80
Corn DDGS	20.00	20.00	20.00	20.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Limestone	15.00	15.00	15.00	15.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Vitamin premix ¹	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
BIOFOS ²	9.00	9.00	9.00	9.00	7.00	7.00	7.00	7.00	5.00	5.00	5.00	5.00
Mineral premix ³	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
99% DL-methionine	2.65	2.65	2.65	2.65	2.37	2.37	2.37	2.37	2.05	2.05	2.05	2.05
Lysine-HCl	2.25	2.25	2.25	2.25	2.46	2.46	2.46	2.46	2.31	2.31	2.31	2.31
Threonine	0.71	0.71	0.71	0.71	0.49	0.49	0.49	0.49	0.34	0.34	0.34	0.34
Xylanase 8000G	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase 5000G	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
RDE	-	-	0.52	1.57	-	-	0.53	1.59	-	-	0.57	1.70
Avilamycin	-	0.01	-	-	-	0.01	-	-	-	0.01	-	-
Calculated composition												
ME, kcal/kg	3000.00				3150.00				3200.00			
CP, %	22.30				20.80				19.40			
Ca, %	0.86				0.74				0.70			
Total P, %	0.60				0.54				0.52			
SID Lys, %	1.18				1.10				1.00			
SID Met, %	0.60				0.55				0.50			

SID Thr, %	0.78	0.74	0.67
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¹Provided per kilogram of diet: vitamin A, 8,255 IU; vitamin D3, 3,000 IU; vitamin E, 30 IU; vitamin B12, 0.013 mg; vitamin K3, 2.0 mg; niacin, 41.2 mg; choline, 1300.5 mg; folic acid, 1.0 mg; biotin, 0.25 mg; pyridoxine, 4.0 mg; thiamine, 4.0 mg; calcium pantothenic acid, 11.0 mg; riboflavin, 6.0 mg.

² BIOFOS = 21% Monocalcium phosphate

³Provided per kilogram of diet: manganese, 70.0 mg; zinc, 80.0 mg; iron, 80.0 mg; iodine, 0.5 mg; copper, 10 mg; selenium, 0.3 mg.

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Table 2. Effect of non-supplemented and red-osier dogwood extracts supplemented diets on growth performance of broiler chickens

Items ¹	Treatments ²				SEM ³	<i>P</i> -value
	NC	PC	RDE1	RDE2		
Starter (d 1-14)						
ADG, g	33.32	32.34	34.14	35.18	0.45	0.13
ADFI, g	44.99	46.07	47.98	47.54	0.52	0.13
FCR, g/g	1.35	1.43	1.41	1.35	0.02	0.29
Grower (d 15-28)						
ADG, g	88.18	91.64	88.13	86.93	1.14	0.52
ADFI, g	118.64	119.03	120.21	118.79	1.31	0.98
FCR, g/g	1.35	1.3	1.37	1.37	0.01	0.07
Finisher (d 29-41)						
ADG, g	116.54	118.09	121.69	119.08	2.02	0.85
ADFI, g	185.56	182.63	185.81	192.69	2.43	0.53
FCR, g/g	1.6	1.55	1.54	1.62	0.01	0.12
Whole phase (d 1-41)						
ADG, g	76.57	77.88	78.43	77.46	0.90	0.87
ADFI, g	110.06	108.87	111.63	114.56	1.18	0.21
FCR, g/g	1.44	1.4	1.43	1.48	0.01	0.11
Mortality (%)	2.5 ^b	3.75 ^a	1.25 ^c	1.25 ^c	-	0.02

Data was the mean of 4 dietary treatments with 8 replicates.

¹ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

²NC, negative control (non-antibiotics diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, diets containing 0.1% red-osier dogwood extracts; RDE2, diets containing 0.3% red-osier dogwood extracts.

³SEM, Pooled standard error of mean.

Table 3. Effect of non-supplemented and red-osier dogwood extracts supplemented diets on relative organ weight of 45-days old broiler chickens

Items	Treatments ¹				SEM ²	<i>P</i> -value
	NC	PC	RDE1	RDE2		
Heart	0.42	0.42	0.42	0.45	0.01	0.19
Liver	2.25	2.16	2.21	2.12	0.06	0.81
Bursa	0.15	0.14	0.16	0.14	0.01	0.53
Spleen	0.08	0.08	0.09	0.09	0.01	0.78

Data was the mean of 4 dietary treatments with 8 replicates.

¹NC, negative control (non-antibiotics diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, diets containing 0.1% red-osier dogwood extracts; RDE2, diets containing 0.3% red-osier dogwood extracts.

²SEM, Pooled standard error of mean

Table 4. Effect of non-supplemented and red-osier dogwood extracts supplemented diets on apparent ileal AA digestibility of 45-days old broiler chickens

Item	Treatments ¹				SEM ²	P value
	NC	PC	RDE1	RDE2		
Ala	77.53 ^b	85.32 ^a	83.36 ^a	85.60 ^a	1.01	< 0.01
Arg	77.99 ^b	87.65 ^a	86.00 ^a	87.98 ^a	1.10	< 0.01
Asp	73.77 ^b	79.66 ^a	77.25 ^b	81.51 ^a	1.02	< 0.01
Cys	69.64 ^b	79.81 ^a	76.97 ^a	79.31 ^a	1.42	< 0.01
Glu	81.94 ^b	87.58 ^a	86.29 ^a	87.67 ^a	0.83	< 0.01
Gly	70.68 ^b	78.28 ^a	75.38 ^b	80.05 ^a	1.18	< 0.01
His	53.45 ^b	64.26 ^a	60.65 ^{ab}	63.05 ^a	1.34	< 0.01
Ile	61.11 ^b	81.33 ^a	79.52 ^a	83.87 ^a	1.96	< 0.01
Leu	77.61 ^b	85.96 ^a	85.06 ^a	86.43 ^a	1.05	< 0.01
Lys	81.24 ^b	88.35 ^a	84.22 ^b	86.55 ^a	0.94	< 0.01
Met	89.28 ^b	92.40 ^a	91.90 ^a	93.50 ^a	0.51	< 0.01
Phe	75.47 ^b	85.64 ^a	83.28 ^a	86.04 ^a	1.16	< 0.01
Pro	79.23 ^c	84.19 ^{ab}	82.83 ^{bc}	84.58 ^a	0.82	< 0.01
Ser	75.10 ^c	81.33 ^{ab}	78.22 ^{bc}	82.14 ^a	1.01	< 0.01
Thr	65.45 ^b	76.97 ^a	74.28 ^a	78.99 ^a	1.17	< 0.01
Trp	77.87 ^b	81.32 ^{ab}	83.25 ^a	80.77 ^{ab}	0.84	< 0.01
Tyr	72.33 ^b	85.18 ^a	83.11 ^a	87.00 ^a	1.40	< 0.01
Val	61.62 ^b	79.99 ^a	77.70 ^a	82.02 ^a	1.79	< 0.01

Data was the mean of 4 dietary treatments with 8 replicates.

a, b, c Different letters within the same row indicates significant difference

¹NC, negative control (non-antibiotics diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, diets containing 0.1% red-osier dogwood extracts; RDE2, diets containing 0.3% red-osier dogwood extracts.

²SEM, Pooled standard error of mean

Table 5. Effect of non-supplemented and red-osier dogwood extracts supplemented diets on WS and WB incidence and relative frequency in breast meat of 46-days old broiler chickens

Items ¹	Treatments ²				SEM ³	<i>P</i> -value
	NC	PC	RDE1	RDE2		
White stripping						
% (n)						
WS scores	0.19	0.32	0.24	0.21	0.05	0.42
Normal	95.24(20)	90.91(20)	96.55(28)	100(28)	-	0.43
Moderate	4.76(1)	9.09(2)	3.45(1)	0.00(0)	-	
Woody breast						
% (n)						
WB score	0.08	0.27	0.28	0.14	0.07	0.20
Normal	100(21)	86.30(19)	89.66(26)	96.43(27)	-	0.39
Mild	0.00(0)	13.64(3)	6.90(2)	3.57(1)	-	
Moderate	0.00(0)	0.00(0)	3.45(1)	0.00(0)	-	
Myopathy % n	4.76(1)	13.64(5)	10.34(4)	3.57(1)	-	0.57

¹WS score, white stripping score; WB score, woody breast score; n, counts; myopathy, either WS or WB present

²NC, negative control (non-antibiotics diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, diets containing 0.1% red-osier dogwood extracts; RDE2, diets containing 0.3% red-osier dogwood extracts.

³SEM, Pooled standard error of mean

Table 6. Effect of non-supplemented and red-osier dogwood extracts supplemented diets on breast meat quality of 46-days old broiler chickens.

Items ¹	Treatments ²				SEM ³	P-value
	NC	PC	RDE1	RDE2		
repH ₂₄	5.78	5.9	5.79	5.91	0.05	0.51
Purge loss (%)	1.34	1.26	1.09	0.80	0.35	0.52
L* ₂₄	58.68	59.86	59.30	60.00	0.57	0.33
a* ₂₄	11.52 ^a	10.91 ^{ab}	10.91 ^{ab}	10.50 ^b	0.24	0.04
b* ₂₄	18.70	19.07	17.46	18.57	0.46	0.11
Cooking loss (g)	31.46	34.82	31.39	33.07	2.27	0.53
Cooking loss (%)	23.11	23.63	22.81	22.68	0.66	0.75
Cooking time (min)	37.62	38.13	36.31	36.95	1.32	0.43
WBSF (kg)	1.59	1.34	1.89	1.72	0.16	0.10
TBARS	0.17	0.17	0.13	0.17	0.22	0.67

Data was the LS means of 4 dietary treatments.

^{a, b} Different letters within the same row indicates significant difference

¹ L*, lightness; a*, redness; b*, yellowness; WBSF, Warner-Bratzler Shear Force; TBARS, thiobarbituric acid reactive substances

²NC, negative control (non-antibiotics diet); PC, positive control (basal diet with 30 ppm avilamycin); RDE1, diets containing 0.1% red-osier dogwood extracts; RDE2, diets containing 0.3% red-osier dogwood extracts.

³SEM, Pooled standard error of mean