



Influence of dietary supplementation of *Crassocephalum crepidioides* leaf on growth, immune status, caecal microbiota, and meat quality in broiler chickens

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Abstract

The effect of dietary supplementation of *Crassocephalum crepidioides* leaf powder (CCLP) in comparison with oxytetracycline and butylated hydroxyanisole (BHA) on growth, caecal microbiota, immune status, blood chemistry, carcass traits, meat quality, and oxidative stability in broiler chickens was evaluated. Two hundred and eighty 1-day-old Arbor acre chicks were randomly assigned to a basal diet containing either no additive (control, CON), 400 ppm oxytetracycline + 150 ppm BHA (ANTIBIOX), 1000 ppm CCLP (CCLP-1), or 2000 ppm CCLP (CCLP-2) for 42 days. Each dietary group had seven replicates with ten birds per replicate. Supplemented birds had higher ($P < 0.05$) feed efficiency, hemoglobin, and hematocrit compared with the CON birds. Diet did not affect feed intake, body weight gain, splenic interleukin-1 β , interleukin-6, interleukin-10, and serum IgM. The CCLP-2 birds had lower ($P < 0.05$) serum total and LDL cholesterol than did birds fed other treatments. *Salmonella* spp. and *Escherichia coli* counts and serum IgG were higher in the CON birds than in the supplemented birds. The ANTIBIOX birds had lower ($P < 0.05$) *Lactobacillus* spp. count, and higher ($P < 0.05$) *E. coli* count compared with the CCLP-supplemented birds. Carcass, muscle pH, and cook loss were not affected by diet. The CON breast meat had higher drip loss and lower redness than did the breast meat of the supplemented birds. Carbonyl content and TBARS value in the thigh and breast meat of the supplemented birds were lower ($P < 0.05$) than those of the CON birds. These results infer that CCLP exhibited antioxidant and antimicrobial properties that were comparable to those of BHA and oxytetracycline in the diet of broiler chickens.

Keywords Carbonyl · *Escherichia coli* · Interleukin · Immunoglobulin · Serum cholesterol

Introduction

In recent times, there is a paradigm shift in the utilization of antibiotics in animal husbandry. The shift stems from the need to limit the occurrence and risks of propagation of multi-resistant bacteria currently linked to the usage of antibiotics (Furtula et al. 2010; Forgetta et al. 2012). However, the elimination of antibiotics could increase the incidence of pathogenic infections, which could consequently have detrimental effect on the performance and health of livestock (da costa

et al. 2011). This situation has sparked intense research on the potentials of alternatives to antibiotics (Yang et al. 2009). Medicinal plants contain myriad phytochemicals, which exhibit antimicrobial and immune modulation properties, and thus could be potential alternatives to antibiotics in promoting the production performance and health of livestock (Ramiah et al. 2014; Chang et al. 2016; Aditya et al. 2017).

The chicken gut is a host to a myriad of microbes, which confer numerous benefits and deleterious effects on its host (Yegani and Korver 2008; Sugiharto 2016). These microbes are extremely pliable and almost every aspect of production factors can alter their profile (Kabir 2009; Sugiharto 2016). Of particular interest in this modification are dietary supplements, which can assert changes to the gut microbiota (Sugiharto 2016). The changes in gut microbiota could elicit innate and adaptive immune responses, which could affect the health and production performance in chickens (Haghighi et al. 2008;

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Brisbin et al. 2010; Kogut 2013). Thus, assessing the alterations in gut microbiota and immune response elicited by the supplementation of antibiotics and their potential alternatives could provide data that will prove insightful and will allow for the comparative analysis of the feed additives, and provide science-based information to support livestock farmer-led decisions.

Broiler meat, because of its high polyunsaturated fatty acid contents, is readily susceptible to lipid oxidation (Adeyemi and Olorunsanya 2012; Adeyemi et al. 2020). Lipid oxidation could instigate protein oxidation, and both could limit the shelf life, nutritional value, and safety of meat (Nakyinsige et al. 2016; Sabow et al. 2016). Antioxidants can prevent or limit oxidative spoilage in muscle foods (Olorunsanya et al. 2012; Odhaib et al. 2018b). In this regard, the supplementation of antioxidants in diet seems to be an effective strategy for preventing oxidative spoilage in an intact muscle (Aditya et al. 2017; Yusuf et al. 2018). The growing demand for animal-derived foods that are devoid of synthetic additives has stimulated the use of natural antioxidants in foods.

A plethora of medicinal plants has been studied for their antimicrobial and antioxidant properties in poultry and their products (Ramiah et al. 2014; Chang et al. 2016; Aditya et al. 2017). Nonetheless, the antimicrobial and antioxidant activities of medicinal plants vary probably due to variations in the amounts and forms of phytochemical contents, part of medicinal plants, duration of supplementation, and the intricate interactions between dietary supplement and animal production factors (Jaberian et al. 2013; Sugiharto 2016). As such, further studies in different production systems are required to authenticate the use of specific medicinal plants in poultry nutrition.

Crassocephalum crepidioides, also called fireweed, thickhead, and Okinawa spinach, is an annual herb that belongs to the family of *Asteraceae*, and it is widespread in numerous subtropical and tropical regions (Grubben and Denton 2004). *C. crepidioides* leaf and stem are commonly consumed as vegetables and various parts of the plant possess medicinal properties (Grubben and Denton 2004; Bahar et al. 2016). *C. crepidioides* has been used to treat stomachache, swollen lips, indigestion, sleeping sickness, and epilepsy, and it possesses anti-inflammatory properties (Karmakar 2018) and antitumor activity associated with nitric oxide production (Tomimori et al. 2012). It is a potent antioxidant and protects against hepatotoxicity (Aniya et al. 2005). In spite of the phytochemical contents and medicinal values of *C. crepidioides*, its antimicrobial and antioxidant potentials in poultry diet remain elusive. The objectives of this study were to determine the influence of dietary supplementation of *C. crepidioides* leaf powder on growth performance, caecal microbiota, immune status, blood chemistry, carcass traits, meat quality, and oxidative stability in broiler chickens.

Materials and methods

Animal welfare and ethics

The study was conducted in line with the protocol approved by the Animal Ethics Committee, University of Ilorin (Approval no: FERC/ASN/2018/050).

Source, preparation, and phytochemical analysis of *Crassocephalum crepidioides* leaf

Fresh leaves of *Crassocephalum crepidioides* were harvested around Ilorin Metropolis, Kwara State, Nigeria. The leaves were identified at the Department of Plant biology, University of Ilorin. The leaves were air dried, ground into powder, and stored in an airtight container until analysis and usage. *Crassocephalum crepidioides* leaf powder (CCLP) was subjected to qualitative phytochemical screening following the method of Odebiyi and Sofowora (1978). The phytochemicals were quantified following the protocol described by Obadoni and Ochuko (2001). The CCLP contained tannin (23.99 mg/100 g), flavonoids (25.6 mg/100 g), phenolics (53.09 mg/100 g), steroids (6.39 mg/100 g), and oxalate (7.8 mg/100 g).

Experimental birds, diet, and management

Two hundred and eighty, 1-day-old mixed-sex Arbor acre chicks were used for this experiment. On arrival, the chicks were individually wing banded, weighed, and randomly distributed into twenty-eight floor pens (1.5 m wide × 1.3 m long). Each pen had ten chicks. The pens were randomly assigned to four isocaloric and isonitrogenous dietary treatments, namely control, CON, a basal diet without antibiotic and antioxidant; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyanisole; CCLP-1, basal diet + 1000 ppm CCLP; and CCLP-2, basal diet + 2000 ppm CCLP. The feeding trial lasted 6 weeks. A two-phase feeding program was used, consisting of a starter diet from days 1 to 21 of age and finisher diet from days 22 to 42 of age. The basal diet was formulated to meet the nutrient requirements of broiler chickens following the recommendations of Arbor acre, Aviagen. The ingredients and the chemical composition of the basal diets are presented in Table 1. The birds were raised in a deep litter pens throughout the trial. The birds were vaccinated against infectious bursa disease on days 7 and 21, and Newcastle disease on days 14 and 28. The birds had ad libitum access to water and feed. The rearing conditions followed commercial recommendations.

Table 1 Ingredients and chemical composition of basal diets

	Starter	Finisher
Ingredient (g/kg as fed)		
Maize	500.00	550.00
Soybean meal	300.00	190.00
Groundnut cake	60.00	120.00
Maize offal	50.00	70.00
Fish meal	30.00	10.00
Bone meal	20.00	20.00
Oyster shell	16.50	10.50
Dicalcium phosphate	14.00	20.00
Methionine	1.50	1.50
Lysine	1.00	1.00
Salt	2.50	2.50
Vitamin-mineral premix ¹	2.50	2.50
Enzyme	1.00	1.00
Toxin binder	1.00	1.00
Analyzed composition		
Dry matter (%)	93.20	93.70
Crude protein (%)	23.65	21.24
Ether extract (%)	5.12	4.67
Crude fiber (%)	3.68	3.94
Ash (%)	3.56	3.93
Calculated analysis		
Metabolizable energy (kcal/kg)	3010	3195
Calcium (%)	1.02	1.08
Phosphorus (%)	0.64	0.56
Methionine (%)	0.70	0.70
Lysine (%)	1.17	1.20

¹ One kilogram premix contains folic acid 1000 mg, choline chloride 500 mg, biotin 80 mg, cobalt 240 mg, copper 6 mg, selenium 240 mg, iodine 1.4 mg, vitamin A 15,000 IU, vitamin B1 200 mg, vitamin B2–600 mg, vitamin B6 400 mg, vitamin D3 3000 IU, vitamin E 3000 IU, vitamin K 250 mg, zinc 60 mg, vitamin B12 20 mg

Growth indices and nutrient retention

On a weekly basis, feed intake (FI) and body weight gain were measured and recorded. The data obtained were used to estimate average daily gain (ADG), average daily feed intake, and the feed conversion ratio (FCR). Nutrient retention trial was conducted between days 33 and 37 of the feeding trial using the total collection method. Birds were placed in metabolism cages, weighed quantity of feed samples was supplied, and excreta samples were collected and weighed over 72 h. Feed and excreta samples were subjected to proximate analysis according to the AOAC (2000) procedure.

Blood sampling and analysis

On day 40 of the feeding trial, blood samples were collected via brachial venipuncture into EDTA and plain bottles. Hematological analysis was performed as described earlier (Adeyemi et al. 2020). Blood samples in the plain bottles were centrifuged (2000 ×g) for 15 min at 20 °C. Serum samples were removed, aliquot, and assayed for biochemical indices. Total serum protein was determined according to the method of Tietz (1995). Serum lipids were determined in line with the method of Qureshi et al. (1983). Uric acid and creatinine were assayed according to the protocol of Fossati et al. (1980) and Henry (1974) respectively. Serum aspartate transaminase (AST) and alanine transaminase (ALT) were determined as described by Huang et al. (2006).

Determination of serum immunoglobulins

Serum IgG and IgM were determined with chicken IgG ELISA kit (MyBioSource®, Cat # MBS260043, Mybiosource, Inc. San Diego, CA, USA) and chicken IgM ELISA kit (CUSABIO®, Cat # CSB-E16200C, Cusabio Technology, Houston, USA) respectively in line with the manufacturer's procedures.

Slaughter, carcass analysis, and sampling

On day 42, feed was withdrawn from the birds overnight but they had ad libitum access to water. Five birds per replicate were euthanized. Carcasses were manually defeathered and eviscerated. Weights of internal organs, abdominal fat, and prime cuts were measured. Dressing percentage and the relative weights of organ and carcass cuts were calculated.

Determination of splenic cytokine expression

Three birds per replicate were euthanized and spleen samples were aseptically excised and assayed for cytokine expression. The expression of interleukin 6 (IL-6) in the spleen was determined using chicken IL-6 ELISA kit (CUSABIO®, Cat # CSB-E08549Ch, CUSABIO Technology, Houston, USA). The expression of interleukin 1β (IL-1β) in the spleen was determined using chicken IL-1β ELISA kit (CUSABIO®, Cat # CSB-E11230Ch, CUSABIO Technology, Houston, USA). The expression of interleukin 10 (IL-10) in the spleen was determined using chicken IL-10 ELISA kit (CUSABIO®, Cat # CSB-E12835C, CUSABIO Technology, Houston, USA).

Determination of caecal microbial population

Fresh caecal digesta were collected from the birds used for the spleen sampling. The digesta were sampled from the right and

left caeca. One gram of digesta sample was weighed and introduced aseptically into a test tube containing 9 ml of sterile peptone water. The mixture was vortexed and dilution was made up to 10^{10} . One milliliter of the mixture was removed from the test tube and introduced into petri dishes and a sterile molten agar was introduced. Plates were incubated for 24 h at 37 °C and bacterial units were counted by a colony counter. *Salmonella* spp. was counted on *Salmonella shigella* agar, *Escherichia coli* was counted on eosin methylene blue agar, and *Lactobacilli* spp. was counted on Man Rogosa Sharpe agar. All agars were prepared according to the manufacturer's instruction.

Meat quality analyses

Meat quality analyses were assessed on the breast (*pectoralis*) and thigh (*sartorius*) muscles.

Determination of pH

The pH readings were taken from the sample using a handheld digital pH meter (MW102 pH meter, MILWAUKEE® instruments, Inc. NC, USA) fitted with pH (MA920B/1) and temperature (MA830R) probes. The pH meter was calibrated prior to taking readings by dipping the pH probe into buffer solution of pH 7.0 followed by pH 4.0. About 10 g of sample was homogenized with 50 ml of distil water using an electric blender. The homogenate was transferred into a beaker and the pH was read. Triplicate pH readings were taken from each sample. The pH probe was rinsed with distilled water after each measurement.

Measurement of color values

The meat samples were exposed to the air to bloom for 30 min prior to taking color readings. A hand-held colorimeter (WR-10, Shenzhen, China) was used to measure the meat color following the International Commission on Illumination (CIE) $L^* a^* b^*$ classification system with the D₆₅ illuminant. Three color readings were performed on each experimental unit, and the average was used for statistical analysis.

Determination of cook loss and drip loss

Cook loss and drip loss were measured as described by Adeyemi et al. (2020).

Determination of protein oxidation and lipid peroxidation

Protein carbonyl content was determined in line with the method of Levine et al. (1990). Lipid oxidation was determined by the protocol described by Buege and Aust (1978).

Statistical analysis

The trial was a completely randomized design with seven replicates. A pen of ten chicks was the experimental unit. The growth performance, blood chemistry, caecal microbial counts, and immune indices data were subjected to the generalized linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA) following the statistical model: $y_{ij} = \mu + \tau_i + \varepsilon_{ij}$ where y_{ij} is the j th observation in treatment i , μ is the overall mean, τ_i is the fixed effect of treatment i , and ε_{ij} is random error with mean 0 and variance σ^2 . Following a significant F test, means were separated using Tukey HSD test. The level of significance was set at $P < 0.05$.

The meat quality data were analyzed using the PROC MIXED procedure of SAS in which dietary treatment, chill storage, and interaction between dietary treatment and chill storage were fitted as fixed effects in a repeated measure analysis. The statistical model used is: $y_{ijk} = \mu + \tau_i + \delta_{ij} + t_k + (\tau \times t)_{ik} + \varepsilon_{ijk}$, where y_{ijk} is ijk^{th} observation, μ is the overall mean, τ_i is the fixed effect of treatment i , δ_{ij} is the random effect of birds nested within treatment i , t_k is the fixed effect of time k , $(\tau t)_{ik}$ is the interaction between treatment and time, and ε_{ijk} is random error with mean 0 and variance σ^2 . For each variable analyzed under this model, bird as a subject nested within treatment was subjected to five covariance structures. The covariance structure, which met the convergence criteria and yielded the smallest Akaike's information criterion was used. Least-square means were separated by using the PDIF option of SAS, and adjusted with Tukey HSD test.

Results

Growth indices and nutrient retention

Diet had no effect ($P > 0.05$) on ADG and FI in broiler chickens throughout the production cycle (Table 2). Dietary treatments did not affect ($P > 0.05$) the FCR at the starter and finisher phases. However, at the end of the production cycle, the CON birds had higher ($P < 0.05$) FCR compared with the supplemented birds. Diets did not affect ($P > 0.05$) the retention of protein, fat, ash, and fiber. Mortality was not influenced by dietary supplements.

Caecal microbial population and immune indices

The CON birds had higher ($P < 0.05$) *E. coli* and *Salmonella* spp. counts compared with the supplemented birds (Table 3). *E. coli* count in the CCLP-1 and CCLP-2 birds was lower ($P < 0.05$) than that of the ANTIBIOX birds. *Salmonella* spp. counts in the ANTIBIOX and CCLP-2 birds were lower ($P < 0.05$) than that of CCLP-1 birds. *Lactobacillus* spp. count

Table 2 Growth indices in broiler chickens fed diets supplemented with different additives for 42 days

Item	Dietary treatment ¹					<i>P</i> value
	CONT	ANTIBIOX	CCLP-1	CCLP-2	SEM	
Average body weight (g/bird)						
1 days	44.00	43.50	43.50	43.50	0.49	0.368
21 days	794.00	823.50	793.50	803.50	10.45	0.092
42 days	2090.00	2158.0	2147.00	2138.00	40.12	0.065
Average daily gain (g/bird/day)						
1–21 days	35.71	37.14	35.71	36.19	4.20	0.214
22–42 days	63.80	65.62	66.52	65.62	6.29	0.191
1–42 days	48.71	50.34	50.10	49.89	6.30	0.086
Average daily feed intake (g/bird/day)						
1–21 days	47.50	48.20	47.20	47.00	3.90	0.167
22–42 days	121.00	125.21	123.40	125.21	9.28	0.125
1–42 days	89.06	87.70	86.36	85.87	4.33	0.192
Feed conversion ratio						
1–21 days	1.33	1.29	1.32	1.30	0.21	0.190
22–42 days	1.89	1.90	1.85	1.91	0.14	0.215
1–42 days	1.83 ^a	1.74 ^b	1.72 ^b	1.72 ^b	0.06	0.028
Coefficient of nutrient retention						
Crude protein	0.60	0.60	0.61	0.60	0.15	0.935
Crude fat	0.55	0.54	0.54	0.52	0.10	0.768
Ash	0.46	0.46	0.48	0.48	0.07	0.957
Crude fiber	0.48	0.49	0.49	0.51	0.11	0.678
Mortality (%)	2.80	2.40	2.50	2.50	0.28	0.072

^{a,b,c} Means bearing different superscript along a row are significantly different ($p < 0.05$)

¹ CON, basal diet without additive; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyl anisole; CCLP-1, basal diet + 1000 ppm *Crassocephalum crepidioides* leaf powder; and CCLP-2, basal diet + 2000 ppm *Crassocephalum crepidioides* leaf powder. SEM standard error of mean

Table 3 Caecal microbial population and immune indices in broiler chickens fed diet supplemented with different additives for 42 days

Dietary treatment ¹						
Item	CONT	ANTIBIOX	CCLP-1	CCLP-2	SEM	<i>P</i> value
Caecal microflora (log ₁₀ cfu/g caecal content)						
<i>E. coli</i>	3.90 ^a	1.03 ^b	0.30 ^c	0.13 ^c	0.19	< .0001
<i>Salmonella</i> spp.	3.10 ^a	1.22 ^c	2.40 ^b	1.53 ^c	0.21	0.0010
<i>Lactobacillus</i> spp.	3.17 ^a	2.68 ^c	5.20 ^a	5.23 ^a	0.18	0.0004
Immune indices						
Immunoglobulin M (μmol/L)	256.33	241.11	247.80	232.72	20.19	0.473
Immunoglobulin G (μmol/L)	273.60 ^a	190.90 ^b	211.90 ^b	196.60 ^b	22.22	0.045
Interleukin-1β (pg/mL)	20.85	19.12	16.98	18.41	7.05	0.054
Interleukin-10 (pg/mL)	17.94	21.67	20.64	28.75	5.03	0.068
Interleukin-6 (pg/mL)	451.00	393.40	427.50	400.60	42.8	0.105

^{a,b,c} Means bearing different superscript along a row are significantly different ($p < 0.05$)

¹ CON, basal diet without additive; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyl anisole; CCLP-1, basal diet + 1000 ppm *Crassocephalum crepidioides* leaf powder; and CCLP-2, basal diet + 2000 ppm *Crassocephalum crepidioides* leaf powder. SEM standard error of mean

in the CON and ANTIBIOX birds was lower ($P < 0.05$) than that of the CCLP-supplemented birds.

Splenic IL-10, IL-6, and IL-1 β and serum IgM were not different ($P > 0.05$) among the dietary treatments (Table 3). The CON birds had higher ($P < 0.05$) serum IgG compared to the supplemented birds.

Hematology and serum biochemistry

Dietary treatments did not influence ($P > 0.05$) the concentration of leucocytes, erythrocytes, granulocytes, and lymphocytes in broiler chickens (Table 4). The concentration of hemoglobin and hematocrit was higher ($P < 0.05$) in the supplemented birds compared with the CON birds. The CCLP-2 birds had lower serum total cholesterol and low-density lipoprotein (LDL) cholesterol compared with birds fed other dietary treatments (Table 4). LDL cholesterol in the CON and ANTIBIOX birds was higher ($P < 0.05$) than that of birds fed diet supplemented with CCLP. Dietary treatment did not affect ($P > 0.05$) serum triglyceride, uric acid, high-density lipoprotein (HDL) cholesterol, aspartate transaminase, total protein, alanine transaminase, urea, and creatinine in broiler chickens.

Carcass traits and relative organ weights

Diet had no effect ($P > 0.05$) on dressing percentage, carcass weight, abdominal fat percentage, percentage of carcass cuts, and relative organ weights in broiler chickens (Table 5).

Meat oxidative stability and physicochemical properties

The carbonyl content and the TBARS value of the *pectoralis* and *sartorius* muscles of the CON birds were higher ($P < 0.05$) than those of the supplemented birds (Table 6). In the *pectoralis* and *sartorius* muscles, carbonyl content and TBARS value increased ($P < 0.05$) over storage. There was a significant interaction between diet and chill storage days on the carbonyl content of *pectoralis* and *sartorius* muscles. However, interaction between diet and chill storage on TBARS values was significant in the *pectoralis* muscle only.

Diets did not affect ($P > 0.05$) the pH of *pectoralis* and *sartorius* muscles (Table 6). In both muscles, the pH on day 0 was higher ($P < 0.05$) than the pH on day 3, and 5 *postmortem*. Diet and chill storage had no effect on L^* and b^* of *pectoralis* and *sartorius* muscles. The *pectoralis* muscle of CON birds had lower ($P < 0.05$) a^* than that of the supplemented birds. Drip loss in the CON and CCLP-1 *pectoralis* meat was higher ($P < 0.05$) than those of CCLP-2 and ANTIBIOX. Dietary supplements had no effect on drip loss of the *sartorius* meat. In both muscles, drip loss increased ($P < 0.05$) over *postmortem* chill storage. Regardless of

muscle, cook loss was not affected by diet and chill storage. The interaction between diet and chill storage was not significant for color coordinates, pH, cook loss, and drip loss of *pectoralis* and *sartorius* muscles in broiler chickens.

Discussion

There was an improvement in feed efficiency following the supplementation CCLP, and oxytetracycline in broiler diets. This observation may reflect the antimicrobial properties of the additives as evidenced in the changes in caecal microbiota in the supplemented birds. Our finding corroborates that of Yazdi et al. (2014), who reported an improvement in feed efficiency following the supplementation of anise seed powder in broiler chickens. Further, the supplementation of oxytetracycline improved feed efficiency in broiler chickens (Khadem et al. 2014; Attia et al. 2017). Contrarily, the supplementation of garlic and pennywort powder did not affect feed efficiency in broiler chickens (Ramiah et al. 2014). Notwithstanding the improvement in feed efficiency, nutrient retention, feed intake, and ADG were not influenced by dietary supplements. The similarity in ADG may reflect the homogenous nutrient retention. Contrarily, the supplementation of garlic and pennywort powder improved FI and ADG in broilers (Ramiah et al. 2014).

The supplementation of CCLP induced significant changes in caecal microbial population as did the oxytetracycline-supplemented diet. The reduction in the counts of *E. coli* and *Salmonella* spp. in the caecum of birds maintained on CCLP-supplemented diet may reflect the antimicrobial potential of the phytochemicals present in *C. crepidioides*. Nonetheless, the CCLP-2 was more potent than the CCLP-1 in reducing the caecal *Salmonella* spp. counts. The antimicrobial potential of *C. crepidioides* leaf has been documented (Adjatin et al. 2013; Ayodele et al. 2020). Previous works have shown that the supplementation of garlic (Kumar et al. 2010; Ramiah et al. 2014) and pennywort powder (Ramiah et al. 2014) reduced caecal *E. coli* and *Salmonella* spp. counts in broiler chickens.

Contrary to the reduction in the population of *Salmonella* spp. and *E. coli*, the population of *Lactobacillus* spp. increased in response to dietary supplementation of CCLP. This observation suggests that *C. crepidioides* exhibited a selective antimicrobial activity. An improvement in *Lactobacillus* counts was observed when garlic and pennywort powder (Ramiah et al. 2014) and *Bidens pilosa* (Chang et al. 2016) were supplemented in chickens. Previous studies have reported that lactic acid bacteria were resistant to the antimicrobial effects of some spices and herbs (Rees et al. 1993; Patterson and Burkholder 2003). The change in caecal microbiota towards the *Lactobacillus* spp. may promote the synthesis of lactate and short-chain fatty acids that may create

Table 4 Hematology and serum indices in broiler chickens fed diet supplement with different additives for 42 days

Item	Dietary treatment ¹				SEM	<i>P</i> value
	CONT	ANTIBIOX	CCLP-1	CCLP-2		
Hematological indices						
White blood cell (× 10 ⁹ /L)	12.03	12.67	15.60	17.47	2.25	0.317
Lymphocytes (%)	80.24	85.13	84.3	81.7	7.44	0.940
Middle cell (%)	9.20	8.10	7.20	12.2	1.32	0.065
Granulocytes (%)	9.70	10.10	8.4	8.0	1.54	0.100
Red blood cells (× 10 ⁶ /L)	6.37	7.80	8.29	6.92	0.84	0.115
Hemoglobin (g/L)	7.56 ^b	13.40 ^a	15.67 ^a	12.48 ^a	0.91	0.000
Hematocrit (g/L)	35.70 ^b	49.40 ^a	50.90 ^a	47.60 ^a	3.66	0.016
Mean corpuscular volume (N ³)	60.34	62.00	61.30	63.00	2.15	0.723
Mean cell hemoglobin (pg)	18.13	18.44	18.83	18.12	12.30	0.146
MCHC ² (g/L)	260.46	290.00	307.23	298.00	23.15	0.101
RDWSD ³ (%)	21.13	22.23	22.60	23.30	4.44	0.100
RDWCV ⁴ (%)	12.37	13.50	15.93	15.23	2.85	0.093
Platelet (× 10 ³ /L)	354.07	360.10	365.07	373.60	28.71	0.071
Mean platelet volume (fL)	8.88	9.40	11.93	12.17	0.56	0.160
Platelet distribution width (%)	10.30	9.80	12.60	9.30	0.44	0.100
PLCR ⁵ (%)	48.37	49.00	45.93	48.23	0.85	0.093
Plateletcrit (%)	0.74	0.61	0.65	0.73	0.12	0.081
Serum biochemical indices						
Total cholesterol (mg/dL)	250.11 ^a	275.00 ^a	251.98 ^a	216.76 ^b	15.28	0.040
Triglycerides (mg/dL)	87.12	90.21	85.15	89.88	4.05	0.391
LDL cholesterol (mg/dL)	132.00 ^a	143.02 ^a	100.33 ^b	90.34 ^b	12.26	0.030
HDL cholesterol (mg/dL)	99.78	122.82	132.81	100.87	20.99	0.497
Uric acid (mg/dL)	5.040	4.900	4.440	4.707	0.17	0.149
Aspartate transaminase (IU/L)	138.50	116.00	118.24	120.02	5.98	0.447
Alanine transaminase (IU/L)	25.76	44.99	43.83	44.86	1.02	0.635
Creatinine (μmol/L)	0.62	0.65	0.60	0.68	0.16	0.113
Urea (mmol/L)	1.90	1.78	1.58	1.67	0.24	0.153
Total protein (g/L)	14.42	12.80	12.84	14.92	1.53	0.410

^{a,b,c} Means bearing different superscript along a row are significantly different ($p < 0.05$)

¹ CON, basal diet without additive; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyl anisole; CCLP-1, basal diet + 1000 ppm *Crassocephalum crepidioides* leaf powder; and CCLP-2, basal diet + 2000 ppm *Crassocephalum crepidioides* leaf powder. SEM standard error of mean

² MCHC mean cell hemoglobin concentration

³ Red blood cell distribution width standard deviation

⁴ Red blood cell distribution width coefficient of variation

⁵ Plateletcrit-large cell ratio; SEM standard error of mean

an acidic environment, which could hinder the proliferation of pathogenic microbes (Rees et al. 1993; Jazi et al. 2018).

Diet can affect blood components and may be a reliable indicator of short- and long-term nutritional status (Maxwell et al. 1990). Here, the supplementation of CCLP improved the hemoglobin and hematocrit concentration in broiler chickens, as did the diet supplemented with oxytetracycline and BHA. This observation may indicate the antioxidant properties of the

supplements, which prevents lipid oxidation of erythrocyte membranes, and stimulates hematopoiesis and erythropoietin production. Similarly, the supplementation of *Pimpinella anisum* powder improved hemoglobin and hematocrit in broiler chickens (Al-Shammari et al. 2017).

Diet supplemented with 2000 ppm CCLP induced a significant decrease in serum total cholesterol. Further, dietary CCLP-1 and CCLP-2 reduced LDL cholesterol in broiler

Table 5 Carcass traits and relative organ weight in broiler chickens fed diet supplemented with different additives for 42 days

Parameters	Dietary treatment ¹					P value
	CONT	ANTIBIOX	CCLP-1	CCLP-2	SEM	
Body weight, BW	2090.00	2158.00	2147.00	2138.00	40.12	0.065
Dressing %	67.98	68.21	67.56	67.97	4.57	0.973
Abdominal fat (% BW)	0.75	1.13	0.71	0.72	0.34	0.646
Carcass weight, CW	1420.78	1471.97	1459.32	1453.14	27.15	0.107
Carcass cuts (% CW)						
Breast	32.98	30.53	30.14	31.67	2.54	0.257
Thigh	15.55	16.14	16.31	16.05	1.24	0.814
Drumstick	14.45	16.15	15.61	16.37	1.59	0.122
Wing	12.49	12.74	11.91	11.89	1.81	0.278
Back	24.47	23.45	22.19	24.02	2.06	0.853
Organ weight (% BW)						
Heart	0.52	0.47	0.38	0.41	0.03	0.118
Liver	1.70	1.71	1.86	1.63	0.16	0.709
Gizzard	1.98	1.97	1.96	1.99	0.08	0.132
Crop	0.32	0.32	0.35	0.36	0.02	0.054
Proventriculus	0.52	0.50	0.52	0.49	0.05	0.855
Duodenum	1.38	1.34	1.31	1.39	0.19	0.760
Ileum	0.95	0.85	1.17	0.79	0.14	0.307
Jejunum	1.26	1.32	1.45	1.24	0.11	0.120
Caecum	0.50	0.48	0.48	0.48	0.08	0.431
Pancreas	0.25	0.22	0.24	0.21	0.02	0.142
Thymus	0.23	0.25	0.26	0.25	0.02	0.215
Spleen	0.13	0.12	0.11	0.12	0.01	0.585
Bursa of Fabricus	0.11	0.11	0.12	0.11	0.01	0.595

CON, basal diet without additive; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyl anisole; CCLP-1, basal diet + 1000 ppm *Crassocephalum crepidioides* leaf powder; and CCLP-2, basal diet + 2000 ppm *Crassocephalum crepidioides* leaf powder

chickens. Nonetheless, serum triglycerides and HDL-cholesterol did not differ among the treatments. The reduction in serum cholesterol in the CCLP-supplemented birds could reflect the higher caecal *Lactobacillus* spp. counts. *Lactobacillus* spp. can bind cholesterol on cellular surfaces and convert intestinal cholesterol to coprostanol (Shokryazdan et al. 2017). Moreover, phytochemicals can impede the activity of 3-hydroxy-3-methyl glutaryl-CoA, which is an important enzyme in cholesterol synthesis thereby lowering tissue cholesterol (Crowell 1999). Similarly, Bahar et al. (2016) reported that methanolic extract of *C. crepidioides* reduced serum total cholesterol and LDL cholesterol in rats fed high fat diet. Daramola (2019) reported a reduction in serum cholesterol following the supplementation of 0.2% *Moringa oleifera* leaf and bitter leaf in broiler chickens. However, the supplementation of green tea powder (El-Deek and Al-Harthi 2004) and oxytetracycline (El-Deek et al. 2012; Attia et al. 2017) did not affect serum lipids in broiler chickens. The

evaluation of ALT and AST could serve as a reliable indicator for hepatic function and health (Odhaib et al. 2018a). The non-significant difference in the concentration of ALT and AST among the dietary treatments implies that dietary CCLP did not have deleterious effect on hepatic health. In contrast, Daramola (2019) reported a significant reduction in AST following the supplementation of 0.2% bitter leaf and *Moringa oleifera* leaf in broilers. In addition, Al-Shammari et al. (2017) reported a significant reduction in AST and ALT in birds supplemented with *Pimpinella anisum* powder.

Cytokines are crucial effector molecules of acquired and innate immune system and are important signaling molecules in cellular communication (Kaiser et al. 2000). In this study, we evaluated the expression of IL-1 β and IL-6 that play pro-inflammatory roles, and the expression of IL-10 that plays anti-inflammatory roles (Kaiser et al. 2000; Haghighi et al. 2008) in the spleen of broiler chickens. Our results revealed that dietary supplements did not alter the expression of splenic

Table 6 Oxidative stability and physicochemical properties of *pectoralis* and *sartorius* muscles in broiler chickens fed diet containing different additives for 42 days

Item									
Muscle	Factor		Carbonyl (mmol/kg)	TBARS (mg MDA/kg)	pH	Drip loss (%)	Cook loss (%)	<i>L</i> *	<i>a</i> * <i>b</i> *
Pectoralis	Diet ¹	CON	0.83 ^a	0.19 ^a	5.83	13.31 ^a	14.62	46.17	3.56 ^b 9.03
		ANTIBIOX	0.43 ^b	0.09 ^b	5.88	6.52 ^b	16.27	43.49	5.77 ^a 9.61
		CCLP-1	0.54 ^b	0.11 ^b	5.97	11.28 ^a	14.62	47.97	6.01 ^a 8.90
		CCLP-2	0.44 ^c	0.09 ^b	5.93	6.60 ^b	14.44	45.50	5.97 ^a 8.57
		SEM	0.03	0.01	0.04	1.29	1.74	1.80	0.61 0.55
		<i>P</i> value	<.0001	0.0002	0.128	<.0001	0.8665	0.389	0.009 0.616
	Storage days (SD)	0	0.34 ^c	0.03 ^b	6.05 ^a				
		1				6.65 ^b	13.12 ^b	45.62	4.12 8.74
		3	0.46 ^b	0.05 ^b	5.85 ^b	8.93 ^{ab}	19.69 ^a	44.97	4.01 9.64
		5	1.18 ^a	0.29 ^a	5.79 ^b	11.20 ^a	12.15 ^b	46.77	4.09 8.71
		SEM	0.03	0.01	0.54	1.12	1.51	1.56	0.53 0.48
		<i>P</i> value	<.0001	0.0002	<.0001	0.028	0.003	0.716	0.989 0.311
		<i>P</i> value	0.0054	0.0005	0.5910	0.987	0.230	0.856	0.702 0.799
Sartorius	Diet ¹	CON	1.27 ^a	0.25 ^a	5.90	7.44	13.30	46.02	7.38 9.49
		ANTIBIOX	0.91 ^b	0.14 ^b	5.93	6.56	12.11	46.88	5.58 7.32
		CCLP-1	0.85 ^b	0.11 ^b	5.80	5.23	12.62	43.87	7.33 8.29
		CCLP-2	0.50 ^c	0.09 ^b	5.81	7.99	10.39	48.95	6.75 8.91
		SEM	0.05	0.02	0.04	0.96	1.56	1.94	0.83 0.93
		<i>P</i> value	<.0001	0.0005	0.0447	0.221	0.602	0.339	0.405 0.407
	SD	0	0.47 ^c	0.08 ^b	6.14 ^a				
		1				4.59 ^b	12.88	45.69	8.15 9.05
		3	0.92 ^b	0.14 ^b	5.73 ^b	6.81 ^{ab}	13.12	47.88	6.29 7.56
		5	1.25 ^a	0.22 ^a	5.71 ^b	9.02 ^a	10.32	45.71	5.84 8.90
		SEM	0.04	0.02	0.03	0.83	1.35	1.68	0.72 0.80
		<i>P</i> value	<.0001	0.0006	<.0001	0.003	0.288	0.579	0.075 0.371
		<i>P</i> value	0.0024	0.8137	0.9707	0.099	0.094	0.917	0.976 0.586

a,b,c. Means bearing different superscript along a column for each factor are significantly different ($p < 0.05$)

¹ CON, basal diet without additive; ANTIBIOX, basal diet + 400 ppm oxytetracycline + 150 ppm butylated hydroxyl anisole; CCLP-1, basal diet + 1000 ppm *Crassocephalum crepidioides* leaf powder; and CCLP-2, basal diet + 2000 ppm *Crassocephalum crepidioides* leaf powder. SEM standard error of mean

IL-1 β , IL-10, and IL-6 in chickens. It seems likely that the trend of results suggests that in the absence of substantial oxidative and immune challenge, and/or disease conditions, the supplementation of CCLP may not induce splenic inflammatory responses in broiler chickens. Elevated expressions of proinflammatory cytokines have been reported following protozoal (Laurent et al. 2001), bacterial (Okamura et al. 2004), and viral (Khatri et al. 2005) infections in chickens.

Immunoglobulins are glycoproteins, which possess antibody activity and are present in the vascularized tissues, blood, and lymph of all the jawed vertebrates (Härtle et al. 2014). Our findings revealed that the supplementation of CCLP and ANTIBIOX lowered serum concentration of IgG in broiler chickens. The finding may be due to the lower

counts of *E. coli* and *Salmonella* spp. in the caecum of the supplemented birds. This finding contradicts that of Aditya et al. (2017), who reported that the supplementation of onion extract enhanced serum IgG in broiler chickens.

Dietary supplements did not influence carcass traits and relative organ weights in broilers. The homogenous dietary energy and protein may be responsible for this observation. Rafeeq et al. (2017) reported that dietary *Artemisia scoparia*, *Cassia angustifolia*, and *Allium sativum* did not affect the carcass traits and relative organ weights in broilers. Appearance is one of the key meat quality traits because it is a good indicator of meat freshness and it influences consumers' decision during purchase (Sabow et al. 2016). Our results indicate that dietary CCLP and ANTIBIOX improved

the redness of *Pectoralis* muscle but did not influence the color coordinates of *Sartorius* muscle in broiler chickens. The improvement in redness may be related to the antioxidant potential of *C. crepidioides* (Wijaya et al. 2011) and BHA, which enhanced the oxidative stability of myoglobin. In poultry, the *pectoralis* muscle is more susceptible to color changes than the *sartorius* muscle because the already light appearance of the *pectoralis* muscle makes slight changes more noticeable. Moreover, the *pectoralis* muscle is more sensitive to factors that contribute to color changes and it accounts for a large portion of live weight (Northcutt 2009).

Meat pH is the function of the amount of glycogen in the muscle before slaughter and the rate of postmortem glycolysis (Lawrie and Ledward 2006). Meat pH was not affected by dietary treatments. This observation could be attributed to the similar dietary energy, and the homogenous rearing and slaughtering conditions employed in this trial. The pH on day 0 was higher than that of days 3 and 5 in both *pectoralis major* and *sartorius* muscles. The lower pH on days 3 and 5 could be due to postmortem glycolysis, typified by the conversion of muscle glycogen to lactate (Salwani et al. 2016).

Dietary CCLP reduced the drip loss of *Pectoralis* muscle as did the BHA-supplemented diet. The observation could be due to the phytochemical contents in CCLP, which prevent/reduce the oxidative degradation of myofibrillar proteins. Oxidative deterioration of amino acids can generate carbonyl compounds that can affect the functionality of meat proteins (Serpen et al. 2012). Contrary to the observation for drip loss, diet had no effect on the cook loss in *pectoralis* and *sartorius* muscles. The oxidation of proteins and lipids in meat and meat products can have deleterious effects on the nutritional quality, physicochemical properties, and safety of foods, which can affect consumer acceptability and cause huge economic loss to the meat industry (Serpen et al. 2012; Adeyemi et al. 2016). Dietary CCLP reduced the MDA and carbonyl contents in the *pectoralis* and *sartorius* muscles of broiler chickens, as did the BHA-supplemented diet. This observation may reflect the antioxidant properties of the phytochemicals present in CCLP (Wijaya et al. 2011; Bahar et al. 2016; Bello et al. 2019). This finding is in accord with that of Aditya et al. (2017), who observed that the supplementation of onion extract reduced the MDA values in broiler meat. Regardless of dietary treatment, there was an increase in carbonyl content and TBARS values in the *pectoralis* and *sartorius* muscles over chill storage. This observation suggests a reduction in oxidative stability over postmortem chill storage.

Conclusion

The results of this study revealed that the dietary supplementation of *Crassocephalum crepidioides* leaf powder improved feed efficiency, hemoglobin and hematocrit concentration,

and muscle oxidative stability in broiler chickens, as did the diet supplemented with oxytetracycline and BHA. These results suggest that the supplementation of 2000 ppm *Crassocephalum crepidioides* leaf powder exerts antioxidant and antimicrobial properties that are comparable to that of oxytetracycline and BHA in the diet of broiler chickens under the current experimental conditions. Nonetheless, additional studies are required to ascertain the potential of CCLP as an alternative to oxytetracycline, and BHA in broiler diet under disease or compromised health conditions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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