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Influence of Dietary Supplementation of Guava Leaf, Oxytetracycline, and Tert-Butylhydroxytoluene on Growth Performance, Gut Microbial Population, Immune Status, Carcass, and Meat Quality in Broiler Chickens

Research Article

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ABSTRACT

This study investigated the effects of dietary supplementation of guava leaf (GL), oxytetracycline, and tert-butylhydroxytoluene on growth, immune status, gut microbial population, and meat quality of broiler chickens. A total of 280 Ross 308 one-day-old chicks were randomly allotted to either G-0; basal diet (BD) without additive; G-1; BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2; BD + 2.5 g/kg GL; or G-3; BD + 5 g/kg GL for six weeks. At 1-21 d, G-1 and G-2 birds had higher (P<0.05) body weight gain (BWG) and feed efficiency compared with G-0 and G-3 birds. At 22-42 d, the supplemented birds consumed more feed than the G-0 birds. At 1-42 d, BWG and feed intake were higher (P<0.05) in the supplemented birds compared with the G-0 birds. Hematological indices were not affected by the diets. GL-supplemented birds had lower (P<0.05) serum and meat cholesterol than the G-0 and G-1 birds. The G-0 birds had higher tumor necrosis factor-α (83.69 pg/mL) and lower interleukin-10 (5.84 pg/mL) than birds fed other diets. The G-3 birds had lower (P<0.05) interleukin-1β and immunoglobulin M than other birds. Dietary supplements lowered (P<0.05) clostridium, coliforms, and salmonella counts in caecum and ileum. GL-supplemented birds had a higher ileal *Lactobacillus* count than G-0 and G-1 birds. Carbonyl and malondialdehyde contents were lower (P<0.05) in the supplemented meat on day 4 postmortem. Antioxidant enzymes and total antioxidant capacity were higher in the G-3 meat compared with other meats. Breast meat quality was not affected by diet. GL could be a potent antioxidant and antimicrobial in broiler diets.

KEY WORDS catalase, immunoglobulin, interleukin, salmonella.

INTRODUCTION

Antibiotics are often used in animal husbandry for the improvement of feed efficiency and growth, treatment of diseases, and prophylaxis (Pliego *et al.* 2020; Farinacci *et al.* 2021). Nonetheless, the indiscriminate and extensive use of antibiotics has led to the resistance of various pathogenic bacteria (Diarra *et al.* 2007; Ayeni *et al.* 2016; Oliveira *et al.* 2020; Selaledi *et al.* 2020) and the presence of antibiotic residues in poultry products (Adewuyi *et al.* 2011; Olatoye and Kayode, 2012; Granados-Chinchilla and Rodríguez, 2017; Bartkiene *et al.* 2020). These scenarios have led to a ban on the non-therapeutic use of antibiotics in animal production in the European Union in 2006 (Regulation 1831/2003/EC), with possible restrictions in other parts of the world (Nowakiewicz *et al.* 2020; Stoica and Cox, 2021). Practising animal husbandry systems devoid of antibiotics would be a logical step in reducing the deleterious

effects of antibiotics. However, the sustainability of antibiotic-free animal production is not feasible because the system is often characterized by low productivity, high morbidity, and mortality (Woodward, 2005; Leinonen *et al.* 2012). These developments have stimulated research interests in identifying potential alternatives to antibiotics in livestock production. Medicinal plants, in their various forms, offer numerous benefits for poultry production and health (Vase-Khavari *et al*. 2019; Khoobani *et al*. 2020; Farinacci *et al*. 2020; Pliego *et al*. 2020).

The concerns about the potential toxicity of synthetic antioxidants in humans (Ramadan and Suzuki, 2012) have stimulated consumers' abhorrence to their usage for preventing oxidative deteriorations in foods (Carocho *et al.* 2014). Emerging empirical evidence infer that medicinal plants could exert antimicrobial (Kim *et al.* 2013), immunomodulatory (Kim *et al.* 2013; Paraskeuas *et al.* 2017; Ahmadian *et al*. 2020), antioxidant (Shirzadegan and Falahpour, 2014; Kostadinović *et al.* 2015), and growth-promoting (Hassan and Awwad, 2017; Paraskeuas *et al.* 2017; Khoobani *et al*. 2020) effects in broiler chickens. However, results are at best inconsistent. Moreover, some level of consistency is needed to entrench an in-depth knowledge, and guarantee the efficacy and safety of phytogenic additives. This development has created an incentive for additional studies in diverse production systems to allow bespoke decisions and informed choices in the usage of medicinal plants in broiler nutrition.

Guava (*Psidium guajava*) is a popular fruit in the tropics and subtropics, and belongs to the family *Myrtaceae* (Naseer *et al.* 2018).Guava leaf exhibits medicinal, immunomodulatory, antioxidant, and antimicrobial properties (Nwinyi *et al.* 2008; Metwally *et al.* 2010; Biswas *et al.* 2013; Jang *et al*. 2014; Naseer *et al.* 2018). Some works have explored the potential of guava leaf as a dietary protein source in broiler chickens (Rahman *et al.* 2013; Daing *et al.* 2020).

Nonetheless, there is a paucity of information on the potential of guava leaf as an antimicrobial and antioxidant in the broiler diet. The objective of this study was to determine the influence of guava leaf, oxytetracycline, and *tert*-butylhydroxytoluene on production traits, immune status, blood chemistry, gut microbial population, carcass, and meat quality in broiler chickens.

MATERIALS AND METHODS

Animal ethics

The experimental protocol was approved (FERC/ASN/2019/086) by the Animal Care and Use Committee, University of Ilorin.

Collection and processing of guava leaf

Fresh guava leaves were collected within Ilorin metropolis. The leaves were air-dried, ground to pass 1 mm sieve, and stored in Ziploc bags before use. The phytochemicals in guava leaf were determined according to the methods described by Trease and Evans (2002) and Sofowora (2008). Total flavonoid was determined by aluminum chloride method with quercetin as standard. Results were expressed as mg quercetin equivalent (QE)/g dry weight (DW). Total polyphenol was determined by the Folin-Cicocalteau assay using gallic acid as the standard. Results were expressed as mg gallic acid equivalent (GAE)/g DW.

Experimental diets and birds

One day old Ross 308 chicks (n=280) were obtained from a commercial hatchery in Ibadan, Nigeria. The chicks were weighed, and distributed into 24-floor pens (1.30 m^2 each). Wood shavings were spread to the depth of 5 cm on the floor of each pen. Birds were administered Gumboro vaccine on d 7 and 21, and Lasota vaccine on d 14 and 28. Birds were allowed free access to feed and water during the experiment. The birds were kept at 34 ˚C for the first 7 days. Afterward, the temperature was reduced by 3 ˚C per week until it reached 26 ˚C. During the first week, 22 h of light was provided. Thereafter, the light hour was reduced to 18L:6D and maintained till the end of the trial.

The feeding program consisted of starter (1-21 d) and finisher (22-42 d) basal diets that were formulated according to the Ross Aviagen guidelines. The pens were randomly allotted to either G-0; a basal diet (BD) only; G-1; BD + 0.5 g/kg oxytetracycline + 0.15 g/kg *tert*-butylhydroxytoluene (BHT); G-2; BD + 2.5 g/kg GL; or G-3; BD + 5 g/kg GL. The chemical composition of the basal diets was determined in accordance to AOAC (2000) methods and presented in Table 1. Feed was offered as mash (milled to pass through 2 mm-screen for starter diet and 4 mm-screen for finisher diet), and prepared weekly. Each supplement was added to its respective basal diet by mixing with a small quantity of the basal diet. This was added to the main portion of the diet and mixed thoroughly.

Growth indices

A weekly record of feed intake (FI) and body weight (BW) per pen was taken. Bodyweight gain (BWG) and feed conversion ratio (FCR) were calculated.

Blood sampling and analysis

Blood was collected from birds via brachial venipuncture into plain and EDTA bottles on d 40. Hematological parameters were determined with Sysmex-K 1000 (Sysmex Corporation, Kobe, Japan).

Serum lipids were determined using ELISA kit (ab65390, ABCAM, UK). Serum aspartate transaminase (AST) and alanine transaminase (ALT) were determined using Randox test kits (Randox Laboratories, WV, USA).

Slaughter and carcass analysis

On d 42, birds were deprived of feed overnight but had *ad libitum* access to water. Five birds per pen were randomly selected and euthanized. Carcasses were manually defeathered and gutted. The weight of abdominal fat, carcass, and different carcass cuts was measured. Carcass yield and relative weights of prime cuts and internal organs were calculated.

Immune status

Spleen samples were excised from three birds per pen. Spleen sample (100 mg) was rinsed with phosphate buffer saline (PBS), homogenized in 1 mL of PBS, and stored at -20 ˚C overnight. Thereafter, two freeze-thaw cycles were carried out to break the cell membranes. The homogenate was centrifuged for 3 min at 7500 × g, at 5 ˚C. The supernatant was removed and analyzed immediately. Splenic cytokines (interleukin IL-1β (IL-1β), interleukin IL-10 (IL-10) and Tumor necrosis factor (TNF-α)) and immunoglobulins (Ig) (IgA and IgM) were assayed with ELISA kits (Cusabio Technology, Houston, USA) following the manufacturer's procedure.

Enumeration of gut microbiota

Caecal and ileal digesta were collected from three birds per pen. The culturing and enumeration of total aerobic counts, *Lactobacilli* spp., *Clostridium* spp., *Salmonella* spp., and coliforms were conducted according to standard techniques (Miller and Wolin, 1974; Mookiah *et al.* 2014). Digesta (1 g) was introduced into a test tube that contains 9 mL of sterile PBS. The mixture was vortexed and dilution was made up to 10^{10}. One mL of the mixture was put into petri dish and a sterile molten agar was added. Plates were incubated for 48 h at 37 ˚C and bacterial units were enumerated.

Total aerobic bacteria were cultured on nutrient agar, *Lactobacilli* spp. was cultured on Man Rogosa Sharpe agar, Coliform was cultured on coliform agar, *Salmonella* spp. was cultured on S*almonella shigella* agar, and *Clostridium* spp. was cultured on reinforced clostridial agar. The agars were prepared in accordance with the guidelines of the manufacturer.

Meat quality and antioxidant status

Meat quality attributes were assessed on breast meat samples that were excised from five birds per replicate. Before analysis, breast muscles were deskinned and trimmed free of epimysium connective tissue and external fat. Meat pH, cooking loss,drip loss, and color coordinateswere assessed as described by Adeyemi (2021).

Table 1 Ingredients (%) and chemical composition (% DM) of dietary treatments

Ingredient (%)	Diets[1]							
	Starter				Finisher			
	G-0	G-1	G-2	G-3	G-0	G-1	G-2	G-3
Maize	55.00	55.00	55.00	55.00	61.00	61.00	61.00	61.00
Soybean meal (44% CP)	35.80	35.80	35.80	35.80	31.25	31.25	31.25	31.25
Fish meal (72% CP)	4.00	4.00	4.00	4.00	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.50	1.50	1.50	1.50
Dicalcium phosphate	2.45	2.45	2.45	2.45	1.50	1.50	1.50	1.50
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Analyzed composition (% DM)								
Dry matter (%)	92.14	92.00	92.10	92.00	92.45	91.98	92.00	92.16
Crude protein (%)	22.68	22.70	22.68	22.69	20.50	20.54	20.60	20.50
Ether extract (%)	5.04	5.00	5.00	5.03	4.25	4.27	4.24	4.25
Crude fibre (%)	3.22	3.27	3.22	3.25	3.78	3.76	3.77	3.78
Ash (%)	3.52	3.50	3.50	3.50	3.86	3.84	3.85	3.86
Calculated analysis								
Metabolizable energy (kcal/kg)	2930	2930	2930	2930	3100	3100	3100	3100
Calcium (%)	1.40	1.40	1.40	1.40	1.20	1.20	1.20	1.20
Phosphorus (%)	0.78	0.78	0.78	0.78	0.68	0.68	0.68	0.68
Methionine (%)	0.88	0.88	0.88	0.88	0.63	0.63	0.63	0.63
Lysine (%)	1.52	1.52	1.52	1.52	1.20	1.20	1.20	1.20

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.

[2] Supplied per kg diet: α-tocopherol: 26.8 mg; Thiamine: 1.43 mg; Riboflavin: 3.44 mg; Retinol: 3.45 mg; Biotin: 0.05 mg; Niacin: 40.17 mg; Pantothenic acid: 6.46 mg; Cholicalciferol: 43.8 µg; Folic acid: 0.56 mg; Pyridoxine: 2.29 mg; Menadione: 2.29 mg; Cyanocobalamin: 0.05 mg; Zinc: 120 mg; Iron: 120 mg; Copper: 15 mg; Selenium: 0.3 mg; Cobalt: 0.4 mg; Iodine: 1.5 mg and Manganese: 150 mg.

Total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) protein carbonyls, and malondialdehyde (MDA) were assayed with ELISA kits (MyBiosource, San Diego, CA, USA) according to the manufacturer's instructions. Muscle cholesterol was determined by the method of Rudel and Morris (1973).

Statistical analysis

The experiment followed a completely randomized design with seven replicates per treatment. Data were checked for normality and homogeneity of variance. The growth performance, blood and immune indices, carcass traits, gut microbial population, and antioxidant enzymes data were subjected to a one-way analysis of variance (ANOVA) model using SAS (2004). Level of significance was $P < 0.05$. After a significant F test, differences between means were separated using Duncan multiple range test (Cilek and Tekin, 2005). Data on time-dependent meat oxidative stability and physicochemical traits were analyzed using the repeated statement of the MIXED procedure of SAS in which diet, chill storage, and interaction between diet and chill storage were fitted as fixed effects in a repeated measure analysis. Significance was declared at $P < 0.05$. Least-square means were separated using the PDIFF option of SAS.

RESULTS AND DISCUSSION

The guava leaf utilized in this study contained total polyphenol (98.80 mg GAE/ g DW), total flavonoids (76.42 mg QE/g DW), and tannin (0.72 mg/g DW). These values are somewhat comparable to those obtained in previous studies (Nantitanon *et al.* 2010; Venkatachalam *et al.* 2012). The dwindling consumers' interests in synthetic food additives (Carocho *et al.* 2014; Ronquillo and Hernandez, 2017) have propelled research interests in potential alternatives. In this study, the potential of guava leaf as an antimicrobial and antioxidant in broiler diets was assessed.

At 1-21 d, the G-1 and G-2 birds had higher BWG ($P<0.001$) and lower FCR ($P=0.012$) compared with the G-0 and G-3 birds (Table 2). Diets did not affect FI at 1-21 d. At 22-42 d, neither BWG nor FCR was affected by dietary treatments. The G-0 birds consumed less feed ($P=0.027$) compared with birds fed other dietary treatments. Feed intake in the G-1 and G-2 birds was not different but were higher than those of birds fed the G-3 diet. At 1-42 d, BWG and FI were higher ($P<0.05$) in the supplemented birds compared with the G-0 birds. The BWG and FI in the G-3 birds were lower than that of G-1 and G-2 birds.

The improved FI in the GL-supplemented birds may be due to the ability of phytochemicals in guava leaf to stimulate the activities of digestive enzymes and synthesis of bile acids, which enhance nutrient digestibility and ultimately improved body weight gain (Hashemi and Davoodi, 2011; Pliego *et al*. 2020). Further, the supplements can stabilize gut microbiota, thereby reducing microbial toxins (Windisch *et al.* 2008). This, in turn, lowers inflammation and; thus, essential nutrients are diverted for growth (Dibner and Richards, 2005; Windisch *et al.* 2008). Our observation is parallel to that of Kalavathy *et al*. (2008) who reported that oxytetracycline supplementation improved BWG in broiler chickens. Likewise, the supplementation of 5 g/kg thyme powder (Hassan and Awwad, 2017), and 0.10-0.20% *Chicorium intybus* powder improved BWG and feed efficiency in broiler chickens (Khoobani *et al*. 2020). Contrarily, the supplementation of lemongrass leaf and *Citrus sinensis* peel (Alzawqari *et al*. 2016) thyme and Sumac berries (Ahmadian *et al*. 2020), and *Crassocephalum crepidiodes* leaf (Adeyemi *et al.* 2021) did not alter BWG and FCR in broiler chickens. The percentage mortality was not related to diets and was within the recommended values for Ross 308 chickens.

The heavier ($P=0.039$) carcass weight in the supplemented birds (Table 3) reflected their improved body weights. However, dressing percentage, abdominal fat, and relative percentage of prime cuts and internal organs were unaffectedby dietary supplements (Table 3). This observation aligns with that of Alzawqari *et al*. (2016), who observed that carcass traits and relative weights were unaffected by lemon grass leaf and *Citrus sinensis* peel supplementation.

Dietary supplements did not affect hematological indices in broiler chickens (Table 4). Nonetheless, the hematological indices were within the normal range for healthy broiler chickens (Mitruka and Rausley, 1977). Guava leaf supplementation lowered ($P=0.043$) total serum cholesterol in a dose-dependent manner (Table 4). This observation could be attributed to the ability of phytochemical contents of guava leaf to suppress the activity of 3-hydroxy-3-methyl glutaryl-CoA reductase, which is essential for cholesterol synthesis (Crowell, 1999). In addition, plant polyphenols can suppress the synthesis of micelles in the small intestine thereby lowering intestinal cholesterol absorption (Vermeer *et al.* 2008). Likewise, the supplementation of thyme powder (Hassan and Awwad, 2017), lemongrass leaf and *Citrus sinensis* peel (Alzawaqari *et al.* 2016), 3% thyme and 1-3% Sumac berries (Ahmadian *et al*. 2020) lowered blood cholesterol in broiler chickens. The concentration of alanine transferase and aspartate aminotransferase did not differ among the diets.

Table 2 Growth performance in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatments[1]				SEM	P-value
	G-0	G-1	G-2	G-3		
1-21 d						
Body weight gain (g/bird)	741[b]	867[a]	857[a]	770[b]	15.0	< 0.0001
Feed intake (g/bird)	1134	1213	1191	1192	31.9	0.361
Feed conversion ratio	1.53[a]	1.40[b]	1.39[b]	1.54[a]	0.04	0.012
22-42 d						
Body weight gain (g/bird)	1365	1474	1513	1414	48.2	0.169
Feed intake (g/bird)	2541[c]	2760[a]	2725[a]	2636[b]	54.1	0.027
Feed conversion ratio	1.86	1.87	1.80	1.86	0.09	0.875
1-42 d						
Body weight gain (g/bird)	2106[c]	2341[a]	2362[a]	2184[b]	42.6	0.001
Feed intake (g/bird)	3675[c]	3973[a]	3916[a]	3828[b]	60.4	0.005
Feed conversion ratio	1.74	1.70	1.66	1.74	0.05	0.445
Mortality (%)	2.36	2.30	2.30	2.28	0.05	0.321

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Carcass attributes in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatments[1]				SEM	P-value
	G-0	G-1	G-2	G-3		
Carcass weight (g/bird)	1468.24[c]	1683.55[a]	1684.21[a]	1544.62[b]	58.78	0.039
Dressing percentage	68.29	70.61	69.76	69.31	5.30	0.272
Abdominal fat (% body weight)	0.70	0.75	0.67	0.65	0.04	0.109
Prime cut (% carcass weight)						
Drumstick	16.03	15.30	16.10	15.47	2.19	0.678
Thigh	15.22	16.07	15.99	16.30	2.10	0.770
Breast	31.61	31.91	31.37	32.25	2.49	0.831
Wing	12.05	11.52	11.56	11.56	1.50	0.697
Back	25.08	24.86	24.99	24.08	3.00	0.619
Organ weight (% body weight)						
Liver	1.96	1.89	1.84	1.84	0.09	0.913
Heart	0.50	0.48	0.48	0.48	0.03	0.696
Gizzard	2.17	2.18	2.37	2.22	0.16	0.910
Crop	0.38	0.41	0.40	0.40	0.02	0.108
Proventriculus	0.38	0.38	0.38	0.37	0.02	0.968
Caecum	0.62	0.50	0.53	0.51	0.03	0.394
Thymus	0.30	0.32	0.30	0.31	0.02	0.260
Spleen	0.18	0.18	0.19	0.20	0.02	0.310
Bursa of fabricius	0.15	0.16	0.17	0.16	0.04	0.123
Duodenum	0.81	0.70	0.81	0.62	0.06	0.186
Ileum	0.92	0.96	0.83	0.94	0.13	0.583
Jejunum	0.99	1.22	0.76	0.94	0.09	0.131
Pancreas	0.22	0.23	0.22	0.22	0.02	0.301
Colon	1.47	0.92	0.88	1.20	0.14	0.066

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Blood indices in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatment[1]				SEM	P-value
Hematological indices	G-0	G-1	G-2	G-3		
White blood cell (× 10^9/L)	220.00	230.44	258.20	224.23	14.35	0.656
Red blood cells (× 10^6/L)	2.01	2.23	2.28	2.02	0.23	0.864
Hemoglobin (g/L)	7.78	8.45	8.67	8.37	1.05	0.920
Hematocrit (g/L)	23.40	24.30	30.13	25.63	3.08	0.815
Platelets (× 10^4/L)	20.00	24.28	36.00	29.67	3.48	0.915
Serum indices						
Total cholesterol (mg/dL)	137.67[a]	139.60[a]	120.73[b]	101.33[c]	5.29	0.043
Triglycerides (mg/dL)	60.05	70.00	57.34	54.08	8.44	0.860
LDL-cholesterol (mg/dL)	71.40	72.00	69.98	63.34	6.32	0.852
HDL-cholesterol (mg/dL)	54.00	53.80	52.50	52.80	1.50	0.578
VLDL-cholesterol (mg/dL)	12.10	14.00	9.41	7.64	1.39	0.372
Alanine transaminase (IU/L)	35.55	36.80	37.68	35.06	1.49	0.673
Aspartate transaminase (IU/L)	82.68	72.10	78.84	68.60	5.94	0.696

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This observation suggests that the supplements did not exert deleterious effects on hepatic health and metabolism in the birds. A similar observation was reported following the supplementation of *C. crepidiodes* leaf in broiler chickens (Adeyemi *et al.* 2021).

Dietary supplements influenced gut microbial counts, though the results varied slightly between ileum and caecum (Table 5). Ileal total aerobic count was not influenced by dietary treatments. Dietary supplements lowered ileal *Clostridium* spp. (P=0.031), *Coliforms* (P=0.029), and *Salmonella* spp. (P=0.044) in birds. The GL-supplemented birds had higher (P=0.042) ileal *Lactobacillus* spp. count compared with the G-0 and G-1 birds. Caecal total aerobic counts (P=0.034), *Clostridium* spp. (P=0.031), *Coliforms* (P<0.001), *Salmonella* spp. (P=0.024) counts were lower in the supplemented birds compared with the G-0 birds (Table 5). Caecal *Lactobacillus* spp. count was not affected (P=0.446) by diets.

Gut microbiota plays a pivotal role in the health, immune status, and productivity of poultry (Windisch *et al.* 2008). The lower counts of *Clostridium* spp., coliforms, and *Salmonella* spp. counts in the supplemented birds reflected the antimicrobial properties of the additives. Oxytetracycline exerts its antimicrobial effects by inhibiting cellular protein synthesis (Chopra and Roberts, 2001). Moreover, plant secondary metabolites exert their antimicrobial properties by disrupting the cellular membrane of pathogenic microbes, promoting the hydrophobicity of microbes, which may hamper the proliferation of pathogenic microbes, and stimulating the proliferation of beneficial microbes (Windisch and Kroismayr, 2006; Windisch *et al.* 2008). The higher ileal *Lactobacillus* spp. count in the GL-supplemented birds may partly account for the reduction in the populations of the pathogenic microbes.

Lactobacilli spp. can inhibit the proliferation of pathogenic bacterial through the competitive exclusion of pathogens (Fuller *et al.* 1977; Patterson *et al.* 2003), and the synthesis of bacteriocins (Fuller *et al.* 1977; Kawai *et al.* 2004), that could exert bacteriostatic (Patterson *et al.* 2003) and bacteriocidal effects (Fuller *et al.* 1977; Pascual *et al.* 1999).

Our findings align with those of Lin *et al.* (2002) who observed that guava leaf extract inhibited the growth of *E. coli* and *Salmonella in vitro*. Moreover, the supplementation of different herbs enhanced gut *Lactobacillus* spp. count and lowered the population of pathogenic bacteria in broiler chickens (Giannenas *et al.* 2018; Vase-Khavari *et al.* 2019; Khoobani *et al.* 2020; Adeyemi *et al.* 2021).

The G-3 diet down-regulated (P<0.0001) the expression of splenic IL-1β compared with other diets (Table 6). Dietary supplements down-regulated (P<0.0001) the expression of splenic TNF-α and up-regulated the expression of splenic IL-10.

The G-1 and G-3 diets repressed (P<0.0001) IgM and IgA compared with the G-0 and G-2 diets. Splenic IgM was lower in the G-3 birds compared with G-1 birds.

In chickens, innate immunity serves as the first line of defense against oxidative and microbial challenges (Alkie *et al.* 2019). In this context, cytokines play crucial roles as signaling molecules in cellular communication and as effector molecules of the acquired and innate immunity (Kaiser and Stäheli, 2014).

The immunoglobulins are synthesized by the B-cells in response to oxidative stress, infection, or other immune stressors (Ratcliffe, 2006). Plant polyphenols and antibiotics can stimulate the immune system by altering the gut microbiota, and by activating lymphocytes, macrophages, and natural killer cells (Windisch *et al.* 2008).

Table 5 Gut microbial counts (log_{10} CFU^2/g) in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatment[1]				SEM	P-value
	G-0	G-1	G-2	G-3		
Ileum						
Total aerobic bacteria	8.21	8.03	8.54	8.30	0.54	0.101
Clostridium spp.	4.56^a	3.28^c	3.72^b	3.20^c	0.33	0.031
Coliform	6.34^a	5.01^b	5.26^b	5.08^b	0.42	0.029
Salmonella spp.	5.01^a	3.78^b	4.03^b	3.93^b	0.37	0.044
Lactobacillus spp.	5.62^b	5.14^c	6.67^a	6.78^a	0.45	0.042
Caecum						
Total aerobic bacteria	9.56^a	8.22^b	8.55^b	8.34^b	0.41	0.034
Clostridium spp.	5.45^a	3.43^b	3.70^b	3.40^b	0.33	0.031
Coliform	8.10^a	6.28^b	6.42^b	6.11^b	0.03	< 0.0001
Salmonella spp.	6.45^a	4.83^b	4.12^c	4.02^c	0.21	0.024
Lactobacillus spp.	7.52	7.80	7.35	7.56	0.81	0.446

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Immune status in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Parameters	Dietary treatment[1]				SEM	P-value
	G-0	G-1	G-2	G-3		
Interleukin-1β (pg/mL)	263.89^a	230.00^a	232.00^a	114.44^b	26.79	< 0.0001
Interleukin-10 (pg/mL)	5.84^c	15.11^a	9.47^b	12.73^{ab}	1.14	< 0.0001
Tumor necrosis factor-α (pg/mL)	83.69^a	37.33^c	44.30^b	42.48^b	5.08	< 0.0001
Immunoglobulin A (pg/L)	724.40^a	142.9^c	562.4^b	189.9^c	86.03	< 0.0001
Immunoglobulin M (ng/L)	15.90^a	8.11^b	14.83^a	2.07^c	1.77	< 0.0001

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

It appears that in the absence of infection, the additives up-regulated the expression of anti-inflammatory cytokine (IL-10), which, in turn, repressed the expression of pro-inflammatory cytokines (TNF-α and IL-1β). Likewise, the supplementation of botanicals down-regulated the expression of pro-inflammatory cytokines (Kim *et al.* 2013; Paraskeuas *et al.* 2017) and up-regulated the expression of IL-10 (Kim *et al.* 2013) in uninfected birds. Similarly, the supplementation of antibiotics and quercetin suppressed IgA and IgM in broiler chickens (Kim *et al.* 2015). Contrarily, splenic cytokines were not affected by the supplementation of *C. crepidiodes* leaf in broiler chickens (Adeyemi *et al.* 2021). It was surprising that despite the changes in the gut microbial population of the G-2 birds, their IgA and IgM were not different from that of the G-0 birds. From the foregoing, it appears that the immunomodulatory properties of guava leaf were dose-dependent.

The GL-meats had lower (P=0.027) cholesterol contents compared with other meats (Table 7). The lower meat cholesterol in the GL-supplemented birds mimicked the reduction in serum total cholesterol and the plausible reason could be that guava leaf repressed the activity of enzymes involved in cholesterol synthesis. Our result agrees with that of Stanacev *et al.* (2012) who reported that garlic supplementation lowered breast meat cholesterol.

Meat cholesterol has been in the spotlight in recent times due to its implication on human health. Lowering meat cholesterol via dietary strategy would thus align with contemporary consumers' demands.

The G-3 meat presented higher SOD (P=0.025), CAT (P<0.001), and TAC (P=0.039) than other meats (Table 7). Moreover, meat from the supplemented birds presented higher (P<0.05) TAC, GPx, SOD, and CAT than the G-0 meat. Antioxidant enzymes are the first line of defense against oxidative challenge in animals, where they play a preventive antioxidant role by inhibiting free radical formation via quenching singlet oxygen and superoxide, sequestering metal ions, and lowering hydroperoxides (Shi and Noguchi, 2001). Thus, assessing the levels of antioxidant enzymes in postmortem muscle may partly reflect the antioxidant status of muscle. Our observations infer that the additives stimulate or spare the antioxidant enzyme activities. Similarly, the supplementation of *Artemisia absinthium* enhanced GPx activity in broiler meat (Kostadinović *et al.* 2015).

Diet-chill storage interaction was insignificant for carbonyl (P=0.845) and malondialdehyde (P=0.647) contents and quality attributes of breast meat (Table 8). Dietary supplements reduced meat carbonyl (P=0.039) and malondialdehyde (P=0.031) contents on day 4 postmortem.

Table 7 Meat cholesterol and antioxidant enzymes in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatments[1]				SEM	P-value
	G-0	G-1	G-2	G-3		
Cholesterol (mg/100 g wet tissue)	71.81^{a}	90.94^{a}	39.56^{b}	43.64^{b}	14.04	0.027
Superoxide dismutase (U/mg protein)	1017.6^{b}	1039.8^{b}	1087.0^{b}	1327.0^{a}	72.38	0.025
Catalase (U/mg protein)	33308^{d}	62447^{c}	148082^{b}	263339^{a}	4323	< 0.0001
Glutathione peroxidase (U/mg protein)	100.39^{c}	156.83^{a}	131.96^{b}	133.14^{b}	16.12	0.043
Total antioxidant capacity (mg/dL)	46.11^{c}	58.00^{b}	54.64^{b}	65.08^{a}	4.33	0.039

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Meat oxidative stability and quality in broiler chickens supplemented with guava leaf, oxytetracycline, and tert-butylhydroxytoluene for 42 d

Item	Dietary treatments[1]				SEM	P-value		
	G-0	G-1	G-2	G3		Diet (D)	Time (T)	D × T
Carbonyl (umol/mg protein)								
1 d	0.28^{x}	0.25^{x}	0.24^{x}	0.23^{x}	0.08	0.039	< 0.0001	0.845
4 d	2.40^{ay}	1.15^{by}	1.38^{by}	1.23^{by}				
Malondialdehyde (nmol/mg protein)								
1 d	0.13^{ax}	0.11^{ax}	0.10^{ax}	0.06^{bx}	0.03	0.031	< 0.0001	0.647
4 d	0.50^{ay}	0.32^{by}	0.30^{by}	0.27^{by}				
pH								
0 h (20 min)	6.25^{x}	6.20^{x}	6.20^{x}	6.23^{x}	0.09	0.731	< 0.0001	0.774
24 h	5.86^{y}	5.88^{y}	5.84^{y}	5.88^{y}				
Drip loss (%)								
1 d	3.92^{x}	3.78^{x}	3.54^{x}	3.86^{x}	0.69	0.642	< 0.0001	0.832
4 d	7.48^{y}	7.60^{y}	7.47^{y}	7.77^{y}				
Cook loss (%)								
1 d	10.20	10.71	10.04	9.70	0.86	0.109	0.101	0.167
4 d	10.30	9.22	9.24	10.33				
Lightness (L*)								
1 d	48.89	50.14	51.14	50.71	1.23	0.192	0.158	0.051
4 d	47.30	48.22	49.19	50.74				
Redness (a*)								
1 d	4.40	4.46	4.42	4.40	0.59	0.997	0.082	0.994
4 d	3.24	3.23	3.37	3.58				
Yellowness (b*)								
1 d	12.14	11.00	11.42	11.51	0.33	0.984	0.139	0.173
4 d	11.56	12.21	12.11	11.56				

[1] G-0: basal diet (BD) without additive; G-1: BD + 0.5 g/kg oxytetracycline + 0.15 g/kg tert-butylhydroxytoluene; G-2: BD + 2.5 g/kg guava leaf (GL) and G-3: BD + 5 g/kg GL.
$^{a, b}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{x, y}$: the means within the same column with different letter, are significantly different (P<0.05).
SEM: standard error of the means.

However, on 1 d postmortem, the G-3 meat presented lower malondialdehyde content than other meats. These observations could be ascribed to the improved antioxidant enzyme activities and TAC of the supplemented meats. Moreover, BHA and plant polyphenols function as the second line of antioxidant defense via scavenging of free radicals, inhibiting chain initiation, and breaking chain propagation (Shi and Noguchi, 2001). Similar to our findings, the supplementation of *Artemisia absinthium* (Kostadinović *et al*. 2015) reduced malondialdehyde content in broiler meat.

Both carbonyl and malondialdehyde contents increased (P<0.0001) over chill storage reflecting a breakdown of muscle antioxidant defense over aging.

The quality traits of breast meat were not affected by dietary treatments (Table 8). The similarity (P=0.731) in muscle pH could be due to the homogenous husbandry conditions and dietary energy utilized during the trial. The homogenous muscle pH could be responsible for the similar drip loss (P=0.642), and cook loss (P=0.109) of meat among the dietary treatments.

Moreover, the similar meat color may infer that the additives did not affect the concentration and oxidative status of meat pigments, in particular, myoglobin (Adeyemi *et al.* 2020). The reduction in muscle pH (P<0.001) over chill storage was due to postmortem glycolysis characterized by the conversion of glycogen to lactic acid (Salwani *et al.* 2016). Drip loss on day 1 was higher (P<0.001) than that of day 4. This finding was due to the rigor-induced decrease in the water holding capacity of myofibrillar proteins (Adeyemi *et al.* 2017). Meat cook loss (P=0.101) and color (P=0.05) were not affected by chill storage.

CONCLUSION

Dietary supplementation of oxytetracycline-BHT and guava leaf enhanced BWG and feed efficiency, reduced gut *Salmonella* and *Coliform* counts, and altered immune indices in broiler chickens. Dietary supplementation of guava leaf reduced cholesterol content in serum and meat and improved ileal *Lactobacillus* spp. count in broiler chickens. Dietary supplements enhanced the antioxidant status of breast meat in broiler chickens. Overall, the immunomodulatory and antioxidant potential of 5 mg/kg guava leaf was higher than that of 2.5 g/kg guava leaf. Conversely, the 2.5 g/kg guava leaf induced feed intake and BWG than the 5 g/kg guava leaf. These results suggest that guava leaf may be a potent antioxidant and antimicrobial in broiler diets. Nonetheless, we recommend further studies to assess the antimicrobial, immunomodulatory and antioxidant potential of guava leaf in diseased and/or oxidatively challenged birds.

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