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RESEARCH PAPER

Carcass Characteristics, Meat Antioxidative State, and Gut Microbiota of Broilers Fed With a Mixture of Bitter Melon and Basil Leaves Powder

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Abstract

The impact of a mixture of bitter melon leaf and basil leaf powder (BBLPB) on the carcass characteristics, meat antioxidant state, and gut microbiota of broilers was investigated. Three hundred one-day-old Cobb 500 broiler chicks were allocated to five diets: diet 1 (negative control), diet 2 (positive control; 0.1% blend of probiotic, prebiotic, and acidifier (BPPA) supplementation), diets 3 (0.25% BBLPB), 4 (0.5% BBLPB), and 5 (0.75% BBLPB) randomly. The 0.25% BBLPB supplementation improved ($P < 0.05$) slaughtered weight, dressed weight, dressing %, and growth rate of broilers, while organ weights remained unchanged ($P > 0.05$). Slaughtered weight recorded in diets 2 and 3 was higher ($P<0.05$) than those in the rest diets. The dressing percentages in diets 2 (76.45%), 3 (75.6%), and 1 (73.96%) were statistically equivalent but higher ($P<0.05$) than diets 4 (72.12%) and 5 (70.34%). Dietary supplementation with BBLPB significantly increased ($P < 0.05$) muscle glutathione peroxidase concentration while reducing meat lipid oxidation and cholesterol content in broilers compared to the control. Broilers fed BBLPB-supplemented diets exhibited higher ($P < 0.05$) levels of lactic acid-producing bacteria in the gut compared to the control group. The 0.25% BBLPB supplementation enhanced broilers carcass characteristics, meat quality, and gut health.

Introduction

In recent decades, the broiler industry has experienced remarkable progress compared to other livestock species, owing to extensive genetic selection and breeding programs aimed at achieving rapid growth rates, increased muscle yield, and enhanced nutritional content (Taverez and Solis, 2016). This surge in broiler production has been driven by consumer demand for leaner meat products with reduced fat and cholesterol levels, as well as the preference for products free from antibiotic residues (Mehdi *et al.*, 2018). Consequently, there has been a heightened focus on improving the quality of chicken meat (Mir *et al.*, 2017) and current global ban on the use of antibiotics in animal nutrition (Ma *et al* 2021).

To meet these demands, there is a growing trend towards the use of natural alternatives such as medicinal plants and spices in poultry diets, as substitutes for conventional antibiotic feed supplements. Research by Adu *et al.* (2020) and Oloruntola *et al.* (2021) has demonstrated that supplementation of medicinal plants as feed additives in broiler diets could positively impact of the broiler performance and meat quality. These studies have highlighted the beneficial effects of phytochemicals and other bioactive compounds present in medicinal plants, including improved growth performance, enhanced nutrient digestion, promotion of muscle development, elevation of endogenous antioxidant levels, and reduction of cholesterol levels in broiler meat (Adu *et al.*, 2020; Oloruntola *et al*., 2021).

Bitter melon (Momordica charantia) and basil (Ocimum gratissimum) plants are noteworthy among medicinal plants used as feed supplements in poultry nutrition. Bitter melon and basil, which belong to the Cucurbitaceae and Lamiaceae (Labiatae) families respectively, are widely cultivated in tropical and subtropical regions of the world, including Africa, Asia and Australia. Rich in essential nutrients such as crude protein, crude fiber, ash, crude fat, and nitrogen-free extract, bitter melon and basil possess significant nutritional value. Furthermore, their phytochemical composition analysis reveals high levels of bioactive substances like tannins, flavonoids, phenols, saponins, alkaloids, and phytate, underscoring their potential health benefits (Oloruntola *et al*., 2021).

Studies have also demonstrated the antioxidant properties of bitter melon and basil, showcasing their ability to scavenge free radicals and inhibit lipid peroxidation in biological systems (Oloruntola *et al*., 2021). Despite these attributes, scant information exists regarding the use of bitter melon and basil plants as feed supplements to enhance broiler carcass yield and meat quality. While various studies have investigated the effects of other medicinal plants such as oregano, rosemary, sage, thyme, moringa, and basil leaf meal on carcass and meat quality (Manessis *et al*., 2020), there is a dearth of research on composite blends of different medicinal plants (Puvača *et al*., 2020). In addition, recent study reveals that the multifaceted action of phytogenic supplements encompasses probiotic-like promotion of beneficial bacteria, prebiotic-like support for their growth, and acidifying effects that create a favorable gut environment, collectively contributing to improved gut health, meat quality and oxidative status in animals (Murugesan *et al*., 2015; Pandey *et al*., 2023).

Studies by Oloruntola *et al*. (2018, 2020) have highlighted the potential synergistic and additive effects of composite meal mixtures containing various medicinal plants, suggesting that such blends may confer greater benefits on animal performance compared to individual plant additives. Using a blend of phytogenic supplements offers enhanced benefits for broiler chickens due to synergistic effects that amplify antioxidant, antimicrobial, and anti-inflammatory properties (Oso *et al*., 2019; Oloruntola *et al*., 2020). This combination provides a comprehensive nutritional profile, leading to improved nutrient absorption and balanced gut microbiota, which enhances digestion and reduces infection risks. The mix of phytochemicals more effectively boosts the immune system, offering better disease protection and improved meat quality and oxidative stutus through more efficient metabolic processes (Santhiravel *et al*., 2022). Overall, the blend maximizes positive effects on health and product quality by leveraging the complementary benefits of each supplement (Oso *et al*., 2019; Oloruntola *et al*., 2020; Santhiravel *et al*., 2022). The hypothesis of the study is that dietary supplementation of broilers with a mixture of bitter melon leaf powder and basil leaf powder will improve their carcass characteristics, internal organs, meat composition, and gut microbiota, thereby enhancing poultry nutrition and meat quality. Thus, this study aims to explore how blends of bitter melon leaf powder and basil leaf powder impact broiler chicken carcass characteristics, internal organs, meat quality and oxidative status, and gut microbiota, contributing valuable insights to poultry nutrition and meat quality enhancement.

Materials and Method

Ethical clearance, collection, processing, and chemical analysis of bitter melon and basil leaves

The Research and Ethics Committee of the Agricultural Technology Department at The Federal Polytechnic, Ado Ekiti, Nigeria, approved the experimental protocols and animal handling procedures (Ethics Reference No: AAUA/FA/ANS/1/4766/2023). Freshly harvested bitter melon (*Momordica charcantia*) and basil (*Ocimum gratissimum*) leaves were thoroughly washed with clean water, drained, and air-dried on polyethylene sheets in the shade for fourteen days. Subsequently, bitter melon leaf powder (BILP) and basil leaf powder (BALP) were obtained from the dried leaves. The proximate composition, phytochemical profile, antioxidant capacity, and mineral content of BILP and BALP were analyzed in a preliminary investigation, with the findings documented (Oloruntola *et al*., 2021). The bitter melon leaf and basil leaf powder blend (BBLPB) were prepared by mixing equal proportions (1:1) of BILP and BALP.

Blend of Probiotics, Prebiotics, and Acidifiers

The commercial blend of probiotic, prebiotic, and acidifier (BPPA) was obtained from a reputable feed mill in Akure. It was sourced from Xvet, GMBH, located at 22529 Hamburg, Germany. The composition of the BPPA includes: *Lactobacillus acidophilus* (5x4x109 CFU); *Bacillus licheniformis* + *Bacillus subtillis* (4x109 CFU); *Enterococcus foecium* (1x4x109 CFU); *Saccharomyces cerevisiae* (40.00%); Citric acid (2,000 mg); Formic acid (9,000 mg); Orthophosphoric acid (3,000 mg); Magnesium (5.00%); and Lactic acid (3,000 mg).

The experimental site, diets and experimental layout

The feeding trial took place at the Teaching and Research Farm of the Federal Polytechnic in Ado Ekiti, Nigeria. A basal diet was formulated for both the starter phase (0 to 21 days) and the finisher phase (21 to 42 days) to meet the nutritional requirements of broilers as recommended by the National Research Council (NRC, 1994). The basal diet was processed for proximate analysis following the guidelines of the Association of Official Analytical Chemists (AOAC, 1995).

The experimental diets (Diets 1 to 5) were prepared by dividing the basal diet into equal parts for each phase and then supplemented as follows:

Diet 1: No supplementation (negative control)
Diet 2: 1% supplementation with BPPA
Diet 3: 0.25% supplementation with BBLPB
Diet 4: 0.50% supplementation with BBLPB
Diet 5: 0.75% supplementation with BBLPB

Using a completely randomized design, 300 one-day-old Cobb 500 broiler chicks (male and female) with an average weight of 42.06 ± 0.44 g were randomly allocated to the five experimental diets. Each diet was replicated six times, with 10 birds per replication. The broiler chicks were housed in experimental pens measuring 2m x 1m, with a 3cm thick layer of dried wood shavings serving as bedding material.

The temperature within the experimental facility was maintained at 31 ± 2 degrees Celsius during the first week and gradually decreased by 2 degrees Celsius each subsequent week until it reached 26 ± 2 degrees Celsius. Lighting conditions followed a schedule of 24 hours of light on the first day, followed by 23 hours of light on subsequent days. The feed and water were provided *ad libitum*.

Viability and relative growth rate

Viability and relative growth rate were assessed following the methods described by Adebayo *et al.* (2020). Viability percentage (V%) was determined using the formula:

$$V\,\% = \left[\frac{\text{Total number of live animals at the end}}{\text{Total number of animals at the start}}\right] * 100$$

The body weights of the broiler chicks were measured at the beginning and end of the feeding trial. Thereafter, the following formula was used to calculate the relative growth rate (RGR):

Table 1. The experimental basal diets' composition.

Ingredients	Starter feed	Finisher diet
Maize	52.35	59.35
Rice bran	0.00	6.00
Maize bran	7.00	0.00
Soybean meal	30.00	24.00
Fish meal	3.00	3.00
Soy oil	3.00	3.00
Salt	0.30	0.30
Bone meal	3.00	3.00
Limestone	0.50	0.50
Lysine	0.25	0.25
Methionine	0.30	0.30
*Premix	0.30	0.30
Analyzed composition (%)		
Crude fibre	3.52	3.61
Fat	4.21	2.32
Crude protein	22.18	20.03
Calculated composition (%)		
Metabolizable energy (Kcal/kg)	3018.89	3108.10
Available phosphorus	0.46	0.41
Calcium	1.01	0.99
Methionine	0.68	0.66
Lysine	1.36	1.24

*Premix: Vitamin A (10,000 iu) D (2,000,000 iu), E (35, 000 iu); K (1,900mg); B12 (19mg); Riboflavin (7,000mg). Nicotinic acid (45,000mg)' Folic acid (1,400mg); Pyridoxine (3800mg); Thiamine (2,200mg); Pantothenic acid (11,000mg); Biotin (113mg) and trace element such as Cu (8,00omg), Mn (64,000mg); Zn(40,000mg), Fe(32,000mg), Se(160mg), I(800mg); and other items as Ca (400mg); Chlorine (475,000mg) Methionine (50, 000mg); BHT (5,000mg) and Spiramycin (5,000mg) in 2.5kg of premix.

$$\text{RGR} = [(w2 - w1)/\frac{(w1+w2)}{2}] * 100.$$

W_1= Body weight at the start of the experiment;
W_2= Bodyweight at the conclusion of the trial.

Slaughtering techniques, blood sample collection, carcass analysis

On day 42 of the experiment, three birds per replication were randomly selected, weighed, and euthanized by severing the two jugular veins (Oloruntola *et al*., 2018). Prior to euthanasia, the birds were deprived of feed overnight. Breast meat samples were aseptically collected from the slaughtered chickens, packed aerobically in oxygen-permeable bags, and stored frozen at -18°C for 20 days. Simultaneously, the caecal contents of the slaughtered birds were collected for microbiological examination. Dressing procedures were performed following slaughtering, and the dressed weight and dressing percentage were calculated relative to the slaughter weight. Evaluation of internal organs including liver, heart, lungs, spleen, gall bladder, proventriculus, gizzard, and pancreas followed the method described by Oloruntola *et al*., (2018). Breast meat samples were then analyzed for lipid oxidation, glutathione peroxidase, and cholesterol levels. The TBA reactive species technique was employed to assess meat lipid oxidation (Tokur *et al*., 2006), while the method described by Cichoski *et al*., (2012) and de Almeida (2006) was used to determine meat glutathione peroxidase and cholesterol concentrations respectively. Enumeration of aerobic bacteria, lactic acid-producing bacteria, and intestinal negative bacteria in the caecal contents was conducted. Serial dilution examination of bacterial populations followed protocols outlined by Dibaji and Simoes (2014) and Seidavi and Simoes (2015).

Statistical analysis

The model: $Pxy = \mu + \alpha z + \beta zy$, was employed in this study, where Pzy = any of the response factors; x = the average on the whole; αz = the zth treatment's effect (*P*= diets 1, 2, 3, 4 and 5); and βzy = random error as a result of the study. Using SPSS version 20, One-way ANOVA was used to analyse all of the data. The SPSS Duncan multiple range tests were used to find discrepancies between the treatment means (P<0.05).

Results

Figure 1 illustrates the viability percentage of broiler chickens fed BBLPB-supplemented diets. The dietary inclusion of BBLPB did not result in significant changes (P > 0.05) in the viability percentage of broiler chickens across the experimental treatments. Furthermore, Figure 2 presents the relative growth rate of broiler chickens fed diets supplemented with BBLPB. The growth rates of broiler chickens fed diets supplemented with 0.25% BBLPB, 0.25% Bacflora, control, and 0.50% BBLPB were statistically similar (P > 0.05), while the growth rate of those fed 0.75% BBLPB was notably lower (P > 0.05) than the control group.

The impact of BBLPB on broiler chicken carcass and relative internal organ weights is presented in Table 2. Statistical analysis revealed a significant difference (P<0.05) in slaughtered weight, dressed weight and dressing percentage among treatments, while no significant (P>0.05) differences were observed in the

Table 2. Effects of BBLPB supplementation on broiler chicken carcass and relative internal organ weights (% slaughtered weight).

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Slaughter weigh (g/b)	2847.75^{b}	3168.06^{a}	2994.90^{a}	2659.52^{bc}	2523.07^{c}	115.04	0.02
Dressed weight (g/bird)	2106.21^{b}	2421.98^{a}	2256.95^{a}	1918.05^{bc}	1774.73^{c}	66.33	0.01
Dressing percentage (%)	73.96^{b}	76.45^{a}	75.36^{ab}	72.12^{bc}	70.34^{c}	0.66	0.01
Liver	2.15	1.84	2.19	2.02	2.03	0.05	0.25
Heart	0.45	0.47	0.46	0.45	0.49	0.01	0.89
Lung	0.71	0.12	0.17	0.14	0.11	0.03	0.49
Spleen	0.11	0.12	0.17	0.14	0.11	0.01	0.21
Gall bladder	0.23	0.12	0.16	0.08	0.15	0.01	0.20
Proventriculus	0.40	0.47	0.50	0.49	0.50	0.02	0.32
Gizzard	2.33	1.90	2.16	2.02	2.25	0.07	0.41

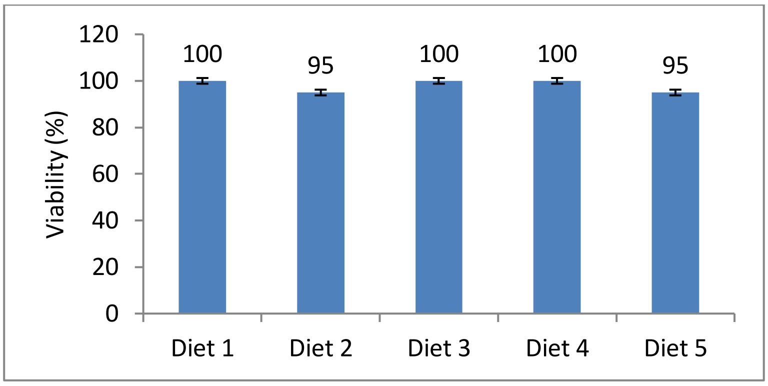

Figure 1. The effect of BBLPB supplementation on the viability percentage of broiler chicken. BBLPB: Bitter lemon and basil leaves blend; Diet1: control; Diet 2: 0.1% BPPA supplementation; Diet 3: 0.25% BBLPB supplementation; Diet 4: 0.50% BBLPB supplementation; Diet 5: 0.75% BBLPB supplementation; P>0.05.

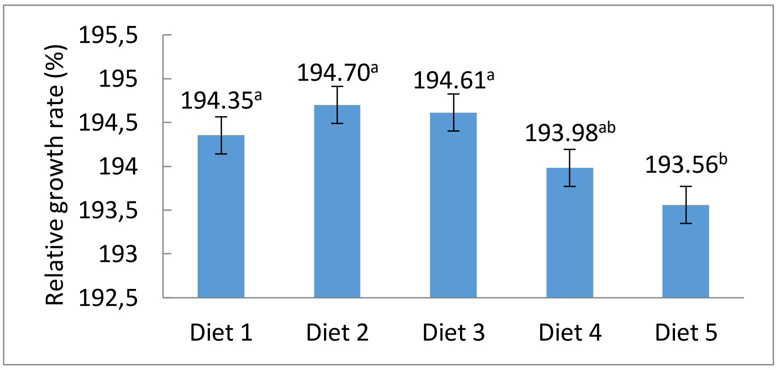

Figure 2. The effect of BBLPB supplementation on the relative growth rate of broiler chicken. BBLPB: Bitter lemon and basil leaves blend; Diet1: control; Diet 2: 0.1% BPPA supplementation; Diet 3: 0.25% BBLPB supplementation; Diet 4: 0.50% BBLPB supplementation; Diet 5: 0.75% BBLPB supplementation; P<0.05.

weights of the liver, heart, lung, spleen, gall bladder, proventriculus, gizzard, and pancreas. The slaughtered weight recorded for birds fed 0.1% BPPA and 0.25% BBLPB (diets 2 and 3) were higher than those in the diets 1, 4 and 5. Broiler chickens fed a diet containing 0.25% BBLPB exhibited the highest dressed weight (2256.95g), which were statistically similar (P>0.05) to birds on diet 2 (2421.98g), while those on diets 5 (0.75% BBLPB) and 4 (0.50% BBLPB) had the lowest dressed weight. The dressing percentages of birds fed diets 2 (76.45%), 3 (75.6%), and 1 (73.96%) were statistically equivalent but higher (P<0.05) than diets 4 (72.12%) and 5 (70.34%).

The effects of BBLPB on lipid oxidation, glutathione peroxidase, and cholesterol content of broiler meat are presented in Table 3. Broilers fed a control diet (1.55 mgMDA/g) exhibited significantly higher meat lipid oxidation than those fed diets supplemented with 0.25% BBLPB (0.75 mgMDA/g), BPPA (0.83 mgMDA/100g), 0.50% BBLPB (0.91 mgMDA/g), and 0.75% BBLPB (0.90 mgMDA/g). Similarly, the meat cholesterol content of broilers given a control diet (123.71 mg/dl) was considerably (P<0.05) greater than that of broilers fed diets supplemented with 0.25% BBLPB (30.35 mg/dl), BPPA (60.10 mg/dl), 0.50% BBLPB (17.25 mg/dl), and 0.75% BBLPB (22.47 mg/dl). Conversely, broiler chickens fed the control diet had the lowest glutathione peroxidase content compared to those fed diets supplemented with 0.25% BBLPB (57.38 mg/ml), 0.1% BPPA (60.12 mg/ml), 0.50% BBLPB (59.74 mg/ml), and 0.75% BBLPB (60.27 mg/ml).

The results of the broiler chicken's intestinal microbiology are detailed in Table 4. The microbial analysis indicated that the counts of aerobic bacteria and intestinal negative bacteria did not exhibit significant differences (P > 0.05) across the treatments. However, supplementation with BBLPB significantly (P < 0.05) increased the population of lactic acid-producing bacteria in broiler chickens compared to the control group. The highest count of lactic acid-producing bacteria (log10 CFU/g) was observed in broiler chickens fed a diet containing 0.75% BBLPB, followed by those on diets 2 (7.67), 4 (7.32), 3 (7.13), and the lowest count was recorded in the control group (6.71) (P=0.02).

Discussion

Antibiotic feed supplements and additives have exerted adverse effects on broiler productivity, prompting the quest for viable alternatives (Mehdi *et al*., 2018). Medicinal herbs have emerged as promising candidates in numerous studies (Adu *et al*., 2020; Oloruntola *et al*., 2021), owing to their potential to enhance animal growth, bolster health, and improve

Table 3. Effects of BBLPB supplementation on the broiler chickens meat analysis.

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Lipid oxidation (mgMDA/g)	1.55[a]	0.83[bc]	0.75[c]	0.91[b]	0.90[b]	0.08	0.00
Glutathione peroxidase (mg/ml)	31.07[b]	60.12[a]	57.38[a]	59.74[a]	60.27[a]	3.09	0.00
Cholesterol (mg/dl)	123.71[a]	60.10[b]	30.35[c]	17.25[c]	22.47[c]	10.81	0.00

*Means within a row with different letters are significantly different (P<0.05); BPPA: Blend of probiotic, prebiotic and acidifier; BBLPB: Bitter melon and basil leave blend; SEM Standard error of the mean.

Table 4. Effects of the BBLPB supplementation on intestinal microbiology (log10 CFU/g) of broiler chickens.

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Aerobic bacteria	6.46	5.70	6.31	6.62	5.84	0.15	0.23
Lactic acid-producing bacteria	6.71[c]	7.67[ab]	7.13[bc]	7.32[abc]	7.83[a]	0.13	0.02
Intestinal negative bacteria	6.32	6.39	6.47	6.56	6.44	0.06	0.87

*Means within a row with different letters are significantly different (P<0.05); BPPA: Blend of probiotic, prebiotic and acidifier; BBLPB: Bitter melon and basil leave blend; SEM Standard error of the mean.

meat quality, thus yielding nutritious muscle food. The observed increase in slaughtered weight and dressed weight of broiler chickens receiving feed supplemented with 0.25% BBLPB in this trial, relative to the control treatment, suggests that BBLPB at this concentration has the capacity to enhance muscle yield in broiler chickens. However, it is noteworthy that when BBLPB is administered at concentrations exceeding 0.25%, the slaughtered weight, dressed weight and dressing percentage of broiler chickens decline compared to the control treatment. The presence of significant phytochemical concentrations such as tannin, saponin, alkaloid, and phytate in BBLPB could be attributed to this decline (Oloruntola *et al*., 2021). Dietary intake of elevated levels of tannin, saponin, phytate, and alkaloid has been linked to the impairment of animal growth rate and nutrient absorption, thereby reducing carcass production (Sobayo *et al*., 2012; Sugiharto *et al*., 2019). In contrast, Adeyeye *et al*. (2020) observed that the nutritional inclusion of sunflower leaf meal and goat weed leaf meal blends had no significant impact on broiler chicken slaughter weight, dressed weight, or dressing percentage when compared to the control group. Similarly, Adegbenro *et al*. (2017) reported no significant difference in the dressed weight of broiler chickens fed diets supplemented with composite leaf meal.

The similarity in the relative internal organ values of broiler chickens across treatments suggests that the inclusion of BBLPB may promote the growth of internal organs without adversely affecting their integrity (Ayodele *et al*., 2016). Adeyeye *et al*. (2020) similarly demonstrated no significant difference in the relative organ weight of broiler chickens fed a diet supplemented with a blend of wild sunflower and goat weed leaf meal. However, this finding contrasts with the results of Adegbenro *et al*. (2017), who reported a significant difference in the relative organ weight of broiler chickens fed a diet containing composite leaf meal.

The ability of the inherent antioxidants, such as phenol, flavonoid, saponin, and phytate, present in the plants to scavenge free radicals and inhibit oxidation production in animals could account for the significant reduction in meat lipid oxidation observed in broiler chickens fed a BBLPB-inclusive diet. Previous studies have identified *Momordica charantia* and *Ocimum gratissimum* plant leaves as possessing high antioxidant scavenging abilities against free radicals and other reactive oxygen species that cause peroxidation in biological systems (Oloruntola et al., 2021). Lipid oxidation has been identified as the primary cause of meat quality deterioration during storage (Falowo *et al*., 2014), suggesting that BBLPB supplementation in the diet could serve as a preservative to extend the meat's shelf life. This finding is consistent with the results of Adu *et al*. (2020), who observed a reduction in lipid oxidation in the breast meat of broiler chickens fed a diet supplemented with *Syzygium aromaticum* leaf powder. Moreover, studies have demonstrated that adding phytogenics or phytochemicals to animal feed helps protect meat against lipid oxidation (Jiang *et al*., 2007; Simitzis *et al*., 2011; Valenzuela-Grijalva *et al*., 2017).

Similarly, the significant decrease in cholesterol levels observed in broiler chickens supplemented with BBLPB compared to the control suggests that these plants contain hypocholesterolemic compounds. Various studies have reported the potency of medicinal plants as hypocholesterolemic agents, reducing cholesterol levels in muscle food due to their inherent phytochemicals and high fiber content (Oloruntola *et al.*, 2021). Phytochemicals found in medicinal plants have been shown to reduce cholesterol by inhibiting cholesterogenesis and fat storage in the carcass and other body areas, as well as decreasing fatty acid and triglyceride production (Santoso *et al.*, 2000). Alternatively, they may form insoluble complexes bonded with cholesterol from food in the gut, preventing cholesterol reabsorption by the intestine (Ueda, 2001; Dong *et al.*, 2007). This finding aligns with Adeyeye *et al.* (2020), who found that broiler chickens fed a diet inclusive of a blend of wild sunflower and goat weed leaf meal had significantly lower cholesterol levels than the control group. However, this result contrasts with the report of Adeyemi *et al.* (2021), who observed no discernible effect of mango leaf on broiler meat cholesterol levels.

Furthermore, the higher glutathione peroxidase recorded in broiler chickens fed a BBLPB-inclusive diet reveals the ability of BBLPB to enhance the animal's endogenous antioxidant capacity when used as an additive at this level. Endogenous antioxidant enzymes play a crucial role in protecting cells from the harmful effects of free radicals and reactive oxygen species. Specifically, glutathione peroxidase is involved in oxidizing glutathione and catalyzing the degradation of various peroxides to protect cells against oxidative damage (Jomova *et al.*, 2024). This could also explain why broiler chickens fed a BBLPB-enriched diet showed decreased lipid oxidation. Adu *et al.* (2020) previously reported reduced meat glutathione peroxidase levels in broiler chickens fed a diet enriched with Syzygium aromaticum leaf meal.

It was observed in this study that supplementation with BBLPB significantly increased the production of lactic acid-producing bacteria (LAB) in the gut of broiler chickens compared to the control group. This indicates the ability of BBLPB to enhance the growth of LAB in the gut. The combined phytochemicals from both plants may modify the broilers' gut environment in favor of LAB proliferation by strengthening the gut barrier, preventing harmful bacteria from colonizing, reducing inflammation, and promoting a healthier microbial balance, thereby creating a favorable environment for beneficial bacteria like LAB (Kikusato, 2021; Santhiravel *et al.*, 2022). LABs play a crucial role in broiler production due to their ability to inhibit the growth or adhesion of harmful microorganisms, improve nutrient acquisition, and stimulate the immune system to enhance feed intake and growth rate (Vieco-Saiz *et al.*, 2019). This result is consistent with the findings of Sjofjan *et al.* (2019), who reported higher levels of lactic acid-producing bacteria in broiler chickens fed diets containing bay leaf meal at various inclusion rates compared to the control.

The observed similarities in viability percentages between broiler chickens fed BBLPB and the control diet indicate that the medicinal plant is not detrimental to the health and livability of the broiler chickens. Similarly, the similar relative growth rates observed at 0.25% BBLPB and 0.50% BBLPB dietary inclusion rates in broiler chickens compared to the control suggest the potential of the plant to maintain or support normal growth performance when used as an additive at relatively high dietary levels in broiler production. The utilization of medicinal plants as feed additives at relatively low concentrations has been reported to enhance growth performance in broiler chickens (Adeyeye *et al.*, 2020).

Conclusion

In conclusion, supplementation of broiler chicken diets with 0.25% bitter melon and basil leaves powder blend (BBLPB) had notable effects on carcass characteristics, meat antioxidative state, and gut microbiota. BBLPB inclusion at 0.25% positively influenced dressed weight and antioxidative parameters in meat, while also enhancing the population of beneficial lactic acid-producing bacteria in the intestine. However, excessive supplementation, particularly at 0.75% BBLPB, may have adverse effects on growth performance.

Conflict of Interest

The authors declare there is no conflict of interest.

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