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Broiler Chickens' Growth, Haematological Indices, Guts Microbiota, Carcass and Meat Analysis in Response to Dietary Supplementation with *Anacardium occidentale* Leaf Powder and A Mix of Prebiotic, Probiotic and Acidifier

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Abstract

This 42-day study evaluates the broiler chickens' response to dietary supplementation with *Anacardium occidentale* leaf powder (ALP) and a mix of prebiotic, probiotic and acidifier (PPA). Two baseline diets were compounded for the starter phase (age 1-21 days) and finisher phase (age 22-42 days) and divided into four parts: Diet 1(control), Diet 2 (250mg/kg PPA), Diet 3 (2,500mg/kg ALP), and Diet 4 (250mg/kg PPA+2,500mg/kg ALP). 240 Cobb 500 broiler chicks were distributed randomly to the experimental diets (6 replicates/diet; 10 birds/replication). The relative growth rate of the birds fed diets 3, and 4 were similar (P>0.05) to those fed diet 2; but higher (P<0.05) than those fed diet 1. The packed cell volume, haemoglobin concentration and red blood cells were improved (*P<0.05*) by the dietary supplements. Meat catalase and glutathione peroxidase activities dressed weight and gut lactic acid-producing bacteria improved (P<0.05) by the treatments. Meat cholesterol level was reduced (P<0.05) by diet. Conclusively, 250mg/kg PPA and 2,500 mg/kg ALP improved the growth rate, dressed weight, erythrogram values, and gut's lactic acid-producing bacteria population, meat catalase, meat glutathione peroxidase of the broiler chickens.

Introduction

Broiler farming has been highlighted as a feasible solution to Africa's acute animal protein shortfall (Hatab et al., 2019). However, due to the negative impact of climate change on livestock output in the tropics, this may not be possible. In the tropics, for example, a 2°C–6°C increase in average ambient temperature by 2100 will increase heat stress impact and pose a considerable challenge to economical and sustainable broiler production (Sylla et al., 2016).

Heat stress caused by a climatic challenge has been demonstrated to harm growth, meat quality, immunity (Kpomasse et al., 2021), blood parameters (Altan et al., 2000), gut health (Rostagno, 2020) and carcass (Zeferino et al., 2016) of broiler chickens and other domestic animals. Antioxidant supplementation in the diet has been found to help birds cope with heat stress. As a result, dietary adjustments or alterations are made in reaction to climatic variations in the tropics (Attia and Hassan, 2017). Prebiotics, probiotics,

phytobiotics, and acidifiers have all been investigated as nutritional supplements to assist offset the detrimental effects of elevated environmental temperature and heat stress (Awaad et al, 2018; Awad et al., 2020). When used as dietary supplements, a variety of tropical botanicals or phytogens show antioxidant action (Manuelian et al., 2021; Oloruntola et a., 2022). Prebiotics has been associated with an increase in the beneficial microbial population, whereas probiotics have been connected to a reduction in antioxidative stress in animals (Zhang et al., 2011; Awad et al., 2020). Acidifiers work as antioxidants by preventing the generation of oxygen radicals. (Awaad et al, 2018).

Anacardium occidentale L, a tropical tree with components that could be used in animal production, is a prospective plant. According to a preliminary investigation by Oloruntola (2021), *Anacardium occidentale* leaf powder has nutritional profile, bioactive components and antioxidant properties, which qualifies it as a phytogenic supplement in an animal feeding system. As a result, the purpose of this study is to determine how supplementing *Anacardium occidentale* leaf powder and a mixture of prebiotics, probiotics, and acidifiers to broiler chickens' feed affects their growth, blood indices, gut microorganisms, carcass and meat.

Materials and Methods

Approval of Animal Protocols, Experimental Site and Dietary Supplements

The Research and Ethics Committee at Adekunle Ajasin University's Department of Animal Science in Akungba Akoko, Nigeria, approved the animal protocols. Between February and March 2021, the feeding trial was conducted at Adekunle Ajasin University's Teaching and Research Farm in Akungba Akoko, Nigeria.

The site is located between 7°28' and 7°0' north latitude and 5°44' and 5°0' east longitude of the Greenwich meridian. The average ambient temperature was 30.13℃ and the relative humidity was 68.27 percent.

The *Anacardium occidentale* leaves were collected, processed to *Anacardium occidentale leaf* powder (ALP) and analyzed as reported by Oloruntola (2021). The mix of prebiotic, probiotic, and acidifier (PPA) was prepared Xvet, GMBH, 22529, Hamburg, Germany. The PPA is composed of *Bacillus licheniformis*+*Bacillus subtills* ($4x10^9$ CFU); *Lactobacillus acidophilus* ($5x4x10^9$ CFU); *Saccharamyces cerevisae* (40.00%); *Enterococcus foecium* ($1×4×10^9$ CFU); Magnesium (5.00%); Citric acid (2,000 mg); Formic acid (9,000mg); Ortho-phosphoric acid (3,000 mg); and Lactic acid (3,000 mg).

The Experimental Diets and Design

Two separate basal diets (Table 1) were devised and compounded for the starter phase (age 1-21 days) and finisher phase (age 22-42 days), taking into account the nutritional needs of the birds at the two phases of broiler chicken production. At each stage of production, the baseline diet was divided into four equal parts and called diets 1 to 4.

Diet 1: No supplement
Diet 2: 250mg/kg PPA
Diet 3: 2,500mg/kg ALP
Diet 4: 250mg/kg PPA+2,500mg/kg ALP

In a completely randomised design, 240 Cobb 500 broiler chicks weighing 34.98±1.18 g were randomly distributed to the experimental diets (6 replicates per diet; 10 birds per replication). The floor of the experimental pen, which measured 2m x 1m and was covered in dry wood shavings to a depth of 3.5cm, was kept at 31 ±3 degrees Celsius for seven days, and then decreased by 2 degrees Celsius each week until it reached 26 ±3 degrees Celsius. The lights were left on for 24 hours on the first day, and then for 23 hours on successive days.

Collection of Data, Blood Samples and Data Analysis

The body weights of broiler chickens were measured every seven days. The relative growth rate (RGR) was calculated using the following formula:

$$RGR= [(w2-w1)/ ((w1+w2))/2] *100$$

w1= Bodyweight when the trial began; w2= Bodyweight at the conclusion of the study.

At the end of the sixth week of the feeding research, four birds were randomly selected, tagged, weighed, and slaughtered by cutting their jugular vein using a clean, sharp knife. The blood of the birds was allowed to flow into a blood sample bottle containing Ethylenediaminetetraacetic acid (EDTA) for haematological analysis. The haematological tests were completed within 120 minutes of the blood collection as outlined by Shastry (1983).

Following that, the sacrificed birds' breast meat was collected and packed aerobically in an oxygen-permeable bag. The samples were kept in the freezer for 20 days at 18 degrees Celsius. The disappearance of H_2O_2, as measured by a decrease in absorbance at 240 nm, was used to determine the beef catalase activity (Muhlisin et al., 2016). The glutathione peroxidase activity (Cichoski et al. (2012) while the thiobarbituric acid (TBA) assay method was used to determine the amount of lipid oxidation in the meat (Tokur et al. 2006). Using commercial kits (Asan Pharm. Co., Ltd. Seoul, Korea), the cholesterol concentrations were measured

Table 1. Experimental diets' make-up

Components (g/kg)	Starter feed	Finisher diet
Fish meal	30.00	30.00
Soybean meal	300.00	240.00
Maize bran	70.20	0.00
Rice bran	0.00	60.30
Maize	523.30	593.20
Soy oil	30.00	30.00
Limestone	5.00	5.00
Bone meal	30.00	30.00
Salt	3.00	3.00
Premix*	3.00	3.000
Lysine	2.50	2.50
Methionine	3.00	3.00
Analyzed composition (g/kg)		
Crude protein	221.30	200.60
Crude fat	44.40	39.80
Crude fibre	35.20	36.10
Calculated composition (g/kg)		
Metabolizable energy (KJ/kg)	12631.12	13004.46
Lysine	13.80	12.60
Methionine	6.90	6.60
Available phosphorus	4.50	4.00
Calcium	10.10	9.90

*Premix composition: 2.5 kg of premix contains: Vitamin K3 (2000 mg), Vitamin E (12000 iu), Vitamin D3 (2500000 iu), Vitamin B1(2000 mg), Vitamin A (10000000 iu), Vitamin B6 (1500 mg), Niacin (15000 mg), Vitamin B12 (10 mg), Biotin (20 mg), Folic Acid (600 mg), Panthothenic Acid (7000 mg), Iron (40000 mg), Chlorine Chloride (150000 mg), Manganese (80000 mg), Copper (10 mg), Magnesium (100 mg), Selenium (150 mg), Iodine (1000 mg), Zinc (60000 mg), Ethoxyquine (500 g), BHT (700 g).

spectrophotometrically as described by de Almeida et al. (2006). The slaughtered birds were de-feathered, eviscerated and dressed. Following that, the carcass yield and carcass percentage were calculated. The heart, liver, gizzard, lung, and spleen were removed, weighed, and stated as a percentage of the slaughtered weight.

The caecal content of the sacrificed birds was collected for further serial dilution investigation of bacterial populations. Before collecting samples, the culture mediums were prepared and put into petri dishes 24 hours in advance. To cultivate the total aerobic bacterial counts, nutrient agar was utilized. Coliforms and intestinal lactose-negative bacteria were cultured on MacConkey agar. The lactic acid bacteria (LAB) were grown on de Man, Rogosa and shape agar (Seidavi and Simoes 2015; Oloruntola et al., 2020).

The model: $Dey = \mu + ae + bey$, was used in this experiment, where *Dey* is the response variable; e = the overall average; ae = the eth dietary effect (*D*= diets 1, 2, 3, and 4); and *bexy* = random error due to the investigation. In SPSS, all of the data were subjected to one-way ANOVA. To discover differences between the treatment averages, the SPSS Duncan multiple range tests were employed (P<0.05).

Results

Relative Growth

The effects of *Anacardium occidentale* leaf powder (ALP) and a commercial mix of prebiotic, probiotic, and acidifier (PPA) on the relative growth rate of broiler chicken are shown in Figure 1.

The relative growth rate of the birds fed diets supplemented with 2,500mg/kg ALP (Diet 3); and 2,500mg/kg ALP and 250mg/kg PPA (Diet 4) were similar (P>0.05) to those fed 250mg/kg PPA supplemented diet (Diet 2); but significantly (P<0.05) higher than those fed the control (Diet 1).

Blood Indices

The blood indices were significantly (P<0.05) affected by the dietary supplements in this study, except for the mean cell haemoglobin (Table 2). The packed cell volumes (PCV), red blood cell count (RBC) and haemoglobin concentration (HbC) of the experimental birds were enhanced by the ALP and PPA dietary supplementation. The PCV and HbC of the birds fed diets 2 and 4 were similar (P>0.05) but significantly higher than diet 1.

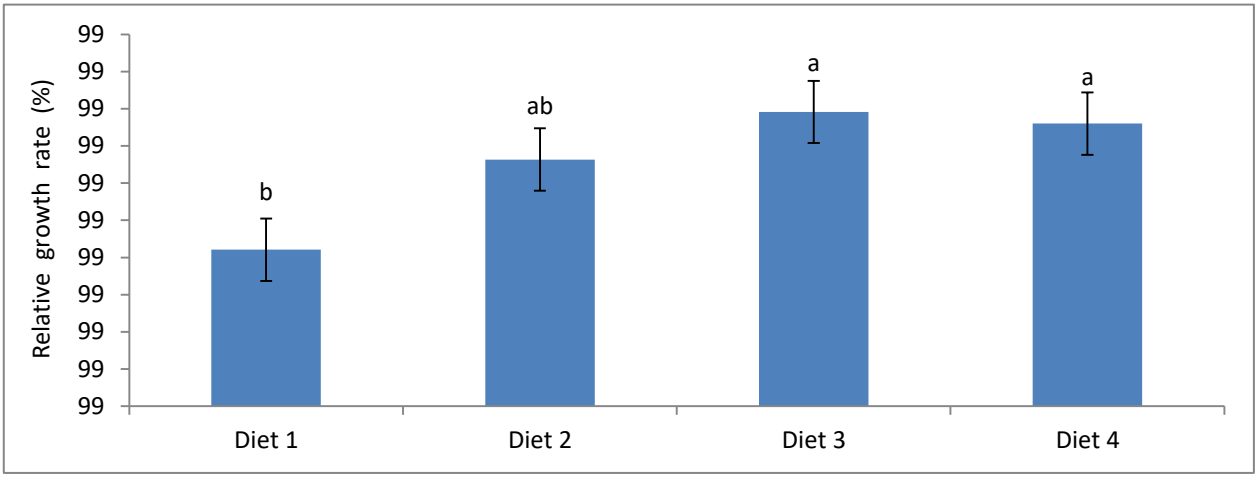

Figure 1. The effects of *Anacardium occidentale* leaf powder and PPA on the relative growth rate of broiler chickens; PPA: Commercial mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA.

Table 2 . The effects of *Anacardium occidentale* leaf powder and PPA on blood indices of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Packed cell volume (%)	30.50^{c}	37.00^{a}	33.50^{bc}	35.50^{ab}	1.46	0.01
Red blood cell ($x10^{6}$/l)	1.70^{b}	2.35^{a}	2.50^{a}	2.31^{a}	0.18	0.01
Haemoglobin conc. (g/dl)	10.20^{c}	12.30^{a}	11.20^{bc}	11.8^{ab}	0.48	0.02
White blood cells ($x10^{9}$/l)	2.96	2.79	2.85	2.51	0.13	0.72
Granulocytes ($x10^{9}$/l)	1.20	1.29	1.18	1.24	0.02	0.15
Lymphocytes ($x10^{9}$/l)	1.16	1.14	1.12	1.21	0.07	0.98
Monocytes ($x10^{9}$/l)	0.34	0.36	0.55	0.06	0.03	0.48

Means in a row without a common superscript letter differ (*P<0.05*); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

The RBC significantly (P<0.05) increased in birds fed diets 2, 3 and 4, compared to the control. The White blood cells (WBC) count and differentials (granulocyte count and monocyte counts) were stable (P>0.05) among the birds fed the various experimental diets.

Meat antioxidant activities, meat cholesterol, carcass traits and internal organ weight

When compared to the control diet, the catalase activity and glutathione peroxidase activity of broiler meat were considerably (P<0.05) greater in broiler birds fed diets 2, 3, and 4. When compared to the control, dietary supplements tend to lower the extent of lipid oxidation in broiler meat (P=0.06). However, as compared to the control diet, the meat cholesterol in the birds fed diets 2, 3, and 4 was considerably (P<0.05) lower (Table 3). Diets 2, 3, and 4 significantly (P<0.05) increased the dress weight of the birds compared to diet 1. Across the diet groups, the dressed percentage, and relative weight of the heart, liver, gizzard, lung and spleen were similar (P>0.05).

Gut Microflora

Table 5 shows the effects of ALP and PPA on gut microflora of broiler chickens. The aerobic bacteria, and coliform bacteria counts were similar (P>0.05) across the diets. However, the lactic acid bacteria count of birds fed diets 2 and 4 were similar (P>0.05) to those fed diet 3, but significantly (P<0.05) higher than those fed the control diet.

Discussion

The increased growth rate observed in broiler chickens fed diets supplemented with 250 mg/kg PPA and 250 mg/kg ALP in this study reflects the growth-promoting characteristics of the aforementioned dietary supplements. The antioxidant capabilities of ALP, probiotics, prebiotics and acidifiers (Awaad et al, 2018; Awad et al., 2020; Oloruntola, 2021), the activities of bioactive substances (tannin, flavonoids, alkaloids, phenols, saponins, etc.) could have improved the growth of experimental chickens (Oloruntola, 2021).

Table 3. The effects of *Anacardium occidentale* leaf powder and PPA on meat antioxidant enzymes and cholesterol of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Catalase (kU)	16.43[b]	36.69[s]	38.23[s]	39.80[s]	3.31	0.01
Glutathione peroxidase (mg/ml)	188.23[b]	267.48[s]	288.18[s]	284.81[s]	9.44	0.01
Lipid oxidation (mgMDA/g)	0.50	0.29	0.17	0.13	0.06	0.06
Cholesterol (mg/dl)	42.09[a]	19.65[b]	17.28[b]	21.96[b]	3.45	0.01

Means in a row without a common superscript letter differ (*P<0.05*); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Table 4. The effects of *Anacardium occidentale* leaf powder and PPA on carcass trait and internal organ relative weight (% SW) of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Carcass weight (g/bird)	1859.33[b]	2097.67[a]	2122.00[a]	2083.66[a]	37.07	0.01
Carcass percentage (%)	73.20	75.44	76.54	74.50	0.54	0.16
Heart	0.35	0.33	0.31	0.33	0.04	0.65
Liver	1.33	1.15	1.42	1.39	0.09	0.08
Gizzard	1.94	2.03	1.77	1.88	0.11	0.21
Lung	0.41	0.38	0.42	0.38	0.05	0.75
Spleen	0.08	0.10	0.08	0.07	0.01	0.37

Means in a row without a common superscript letter differ (P<0.05); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Table 5. The effects of *Anacardium occidentale* leaf powder and PPA on gut microflora (log 10 CFU/g) of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Aerobic bacteria	7.82	7.96	8.39	8.22	0.21	0.11
Coliform bacteria	8.25	8.32	8.79	8.68	0.64	0.80
Lactic acid bacteria	7.76[b]	9.92[a]	9.02[ab]	9.74[a]	0.33	0.04

Means in a row without a common superscript letter differ (P<0.05); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Flavonoids, for example, could boost chicken growth by modulating growth hormone (GH) and enhancing insulin-like growth factor (IGF-1) production. Furthermore, flavonoids stimulate growth by stimulating muscle protein synthesis and epiphyseal cartilage proliferation (Ouyang et al., 2016, Setiawan et al., 2018). Additionally, acidifiers, prebiotics, and probiotics have been shown to enhance growth by preventing micronutrient shortages, improving gut health, reducing infection, and improving zinc, vitamin B_{12}, and calcium absorption (Scholz-Ahrens et al. 2007; Pearlin et al., 2020).

Blood indices can be used to determine an animal's health state (Agbede et al., 2019), and nutrition has a major impact on haematological indices and performance (Oloruntola et al., 2018). The improved haematocrit value, red blood cell count and haemoglobin concentration of birds fed the supplemented diets (diets 2, 3 and 4) in this study suggest the supplements under study enhanced the adequate ingestion, absorption or utilization of essential nutrients that are needed in the processes involved in erythropoiesis (Beckman et al., 2010; Oloruntola et al., 2022). This is advantageous since the nutritional supplements help the birds maintain their regular overall health. The potential for phytogenic feed supplements to promote nutrient absorption from the intestine and, as a result, improve performance and blood indices has been documented (Alaeldein et al., 2018; Oloruntola et al., 2022). This result concurred with Sjofjan et al., (2022), who reported improved red blood cell counts in birds fed probiotics supplemented diets. Dietary acidifiers and probiotics, on the contrary, had no effect on haematocrit, red blood cell count, or haemoglobin concentration (Ogunwole et al., 2017; Aguihe et al., 2018). The fundamental role of white blood cells is to protect the bodily system from infections. In addition, the phytogens', probiotics',

prebiotics' and acidifiers' immunomodulatory activities were reported (Rajput et al., 2013; Oloruntola et al., 2016; Pearlin et al., 2020).

As a result, the similar white blood cell count and differentials across the dietary treatments in this study indicate that dietary supplements did not have an unfavourable effect on the birds' immunological condition. The principal peroxide-removing enzymes found in the cytosol are glutathione peroxidase and catalase (Utama et al., 2016). When superoxide dismutase scavenges superoxide anions by producing hydrogen peroxide, catalase safely decomposes hydrogen peroxide to water and oxygen, whereas glutathione peroxidase decomposes both hydrogen peroxide and lipoperoxides generated during lipid oxidation (Terevinto et al., 2010). This study's findings of improved meat catalase and glutathione peroxidase activities, as well as a tendency for decreased lipid oxidation in broiler birds that were fed diets fortified with ALP and PPA, are similar to those of Hosseindoust et al., (2020), who reported increased antioxidant status, as well as a decreased lipid peroxidation in the meat of broiler chickens that were fed diets supplemented with astaxanthin. Phytosupplements, for example, increase antioxidant activity in meat and reduce lipid oxidation, resulting in an extended meat storage period (Valenzuela-Grijalva et al., 2017). A previous study found that after prebiotic absorption and systemic circulation, poultry meat has substantial antioxidant activity.

Additionally, the antioxidant component in the prebiotic prevents the oxidation of broiler breast meat (Biswas et al., 2021). In another study, Wang et al. (2020) credited dietary acidifiers with improving nutrient digestion, antioxidant capacity, and meat quality. As a result of the increased meat catalase and glutathione peroxidase activities seen in broiler chickens fed ALP and PPA supplemented diets in this study, these supplements may be used in an attempt to improve the meat quality of broiler chickens. Producers' interest in producing animal products or proteins with lower cholesterol content has grown as the demand for lowering human dietary cholesterol consumption has become more urgent (Ponte et al., 2004). This is because eating a lot of high-cholesterol meat raises blood cholesterol levels and increases the risk of coronary heart disease. (Oloruntola et al., 2018). As a result, this study's findings of lower meat cholesterol levels in chicken that were fed ALP and PPA enriched diets are intriguing. Earlier, phytosupplements (Valenzuela-Grijalva et al., 2017; Oloruntola et al., 2022) and prebiotics (Biswas et al., 2021) have been shown to have hypocholesterolemic properties. For instance, tannin, one of the bioactive compounds found in PPA, has been shown to have a retarding effect on lipid absorption in the intestine and hence regulate excess lipid accumulation in the blood and tissues (Oloruntola et al., 2022).

Some phytochemicals and feed supplements have recently been postulated to have comparable effects on animal metabolism as anabolic steroids, acting as growth enhancers by altering animal metabolism in favour of building muscle tissue (Valenzuela-Grijalva et al., 2017). The increased dressed weight observed in the birds fed the supplemented feed followed the same pattern as the broiler chickens' relative growth rate in this study. This is consistent with a prior study that identified live weight as one of the elements influencing animal dressing weight, and that heavier and larger animals have higher dressing weight (Boler, 2014; Oloruntola et al., 2021). Furthermore, dietary constituents can affect the weight of animals' internal organs (Ayodele et al., 2016). Therefore, the fact that relative heart, gizzard, liver, lung and spleen weights remained consistent throughout dietary regimens suggests that the supplements employed in this investigation are nutritionally safe for broiler chicken production. The chickens' capacity to realise their genetic potential is influenced by their microbiome makeup and health. Phytogenic, prebiotic, and probiotic supplements have been shown to impact the microbial profile and health condition of broiler chicken guts in recent investigations (Oloruntola et al., 2019; Emami et al., 2020). The stable broiler chickens' gut aerobic bacteria and Coliform bacteria population across the dietary treatments is of health benefit because the inhibition of pathogen proliferation and enhanced performance require the preservation of normal gut microbiome and/or increase of non-pathogenic gut flora (Oloruntola et al., 2019).

Furthermore, the increased gut population of Lactic acid-producing bacteria found in birds fed supplemented diets reveals further nutritional benefits of the supplements under study in broiler production by encouraging the multiplication of beneficial and non-pathogenic gut microbes. Some harmful bacteria have the potential to stick to the gut epithelium; however, lactic acid-forming bacteria prevent these bacteria from clinging to the gastric epithelium by competitive and direct inhibition and exclusion, eliminating harmful microbes (Servin and Coconnier, 2003; Emami et al., 2020).

Conclusion

Conclusively, the 250mg/kg PPA and 2,500 mg/kg ALP, either singly or in combination improved the growth rate, dressed weight, erythrogram values, and gut's lactic acid-producing bacteria population of the broiler chickens. In addition, the dietary supplements improved the antioxidant status of the broiler meat by increasing its catalase, and glutathione peroxidase concentration. The PPA and ALP have hypocholesterolemic properties and decreased the broiler meat cholesterol content.

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