HAEMATO-BIOCHEMICAL INDICES, ANTIOXIDANT AND GUT HEALTH STATUS OF BROILER CHICKEN FED COMPOSITE LEAF MIX

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ABSTRACT

This study investigated the effects of supplementary composite leaf meal (CLM) on the haemato-biochemical, antioxidants and gut health indices of 150 broiler chickens (Ross 308). During the 42-days feeding trial, the birds were subjected to five dietary treatments (with CLM added at 0, 5, 10, 15 and 20 g/kg, respectively), replicated three times with 10 birds per replicate in a completely randomized design. The CLM was formulated from Annona murican, Ficus exasperate, Persea americana, Glyricidia sepium leaf meals in addition to Allium sativum meal, constituted in the ratio 2:2:2:1:1, respectively. From the study, there were significant differences (p<0.05) in the packed cell volume (PCV), haemoglobin and monocyte across all diets. Highest PCV (34.2 %) was recorded in birds fed Diet 4. All blood serum characteristics studied were not significantly influenced by the supplementation of CLM with the exception of creatinine and glucose. The microbes identified in the small intestine includes: Staphylococcus aureus, Lactobacillus spp. and Streptococcus spp. The microbial count recorded ranged from 7.10 to 40 ×10^6 cfu/ml. Salmonella spp. and Escherichia coli were identified in the jejunum of chicken fed Diets 1, 2 and 3 respectively. From the study, Diet 4 (containing 15 % CLM) showed a great prospect for use as a Prebiotics; given it ability to enhance lactobacillus colonization of the gut. CLM at 15 % supplementation level could be recommended as a sustainable substitute for synthetic antibiotics growth promoters in chicken performance and gut health improvement programmes.

Keywords: Haematological parameters, Serum biochemical, Antioxidant, Composite leaf meal, Gut microbes

INTRODUCTION

The rising need and demand for white meats across Nigeria and the world at large puts the poultry industry in a profitable business good light. A factor that accounts for this is the fact that, Nigerians are becoming more health conscious in their food and food products choices (Windhorst, 2006). Nutritionists in Nigeria have generally agreed that poultry production is the fastest means of bridging the protein deficiency gap prevalent in the country (Maidala and Istifanus, 2012). Hence, the choice of chicken meat which is considered one of the healthiest meats.

The increased interest of folks in poultry meat production is however not without a cost; a more intense competition has ensued between man and animals (poultry) for the scarcely available protein resource (Kuhad *et al*., 1997) and the costs of poultry feeds are roof high. According to Esonu *et al.* (2006), 30 % of poultry farms in Nigeria have been forced to reduce their production capacity. While more than 50 % have closed down due to shortage and high cost of commercial feeds. Some others, who had struggled to keep profitable production floating: trying to cut cost, have resolved into high use of synthetic antibiotic growth promoters (AGPs). Several studies have revealed that these antimicrobials have residual effect on the health of both the consumer and the animal itself (Yadav and Jha, 2019). Thus, with this action by farmers, the country is being overtly set back in its fight against the public health hazard of antibiotics resistance amidst a growing population. Today, research efforts are on-going for a prospective solution to the challenge, while simultaneously, efforts are on-going to evaluate the effects of phytogenic additive as a dietary supplement for broiler chickens. Tests are being carried out to check for the influence of the additive on haemato-biochemical indices and the gut microbial population of birds fed diets containing the additive. Haematological parameters, both in human and animal, are great indices for determination of the physiological and pathological state of an individual (Etim *et al*., 2014). Blood in body of an animal serves as a medium of nutrients transport. The blood picture varies with level of advancement of animal in age and also with level of nutrition amidst other factors. The haematological parameters which are of significant diagnostic values include the packed cell volume (PCV), haemoglobin (Hb), total protein (TP) and serum globulin (SG) are known to affect health, production and adaptability to environmental conditions in livestock (Adenkola *et al*., 2009: Medugu *et al*., 2010: Adenkola *et al*., 2011).

Naturally derived additives have been reported to possess inherent properties capable of positively modifying the haemato-biochemical, antioxidant and gut health indices of an animal. The beneficial properties of phytobiotic additives could be traced to the inherent active compounds such as phenol, tannins, alkaloids and terpenoïds (Alloui *et al*., 2011). Thus these reports formed the major aim of the present study.

MATERIALS AND METHODS

Description of the Study Area: The experimental study was carried out at the Poultry Unit of the Department of Animal Production and Health's Teaching and Research Farm, Federal University of Technology, Akure, Nigeria. Akure falls within 7°15' latitude north of the equator and of Greenwich's longitude 5°12', while also being at 350.52 m above the sea level. This describes the region to be located at humid tropical forest region. It has rainfall of about 1524 mm per year, temperature ranging from 28°C to 30°C and a relative humidity of about 80 % (Wikipedia, 2021).

Leaf Collection, Preservation and Milling: Soursop (*Annona murican* Linnaeus, Magnoliales: Annonaceae), Sandpaper plant (*Ficus exasperata* Vahl, Rosales: Moraceae), Avocado (*Persea Americana* Miller, Laurales: Lauraceae), Glyricidia (*Glyricidia sepium* Jacquin**,** Fabales: Fabaceae) were collected in and around the Federal University of Technology, Akure vicinity. Garlic (*Allium sativum* Linnaeus, Asparagales: Amaryllidaceae) was purchased from Oja Oba, a popular market in Akure, Ondo State. All plant materials were identified (Llamas, 2003) and authenticated by a plant taxonomist with the Department of Biology, Federal University of Technology, Akure, where voucher specimens were deposited for referral purposes (Table 1).

Table 1: Voucher specimen number of plant materials of used in the formulation of the composite leaf mix fed to broiler chicken

Plant materials	FUTA herbarium number
Soursop (*Annona murican*) leaf	0326
Sandpaper (*Ficus exasperata*) leaf	0329
Avocado (*Persea Americana*) leaf	0325
Glyricidia (*Glyricidia sepium*) leaf	0327
Garlic (*Allium sativum*) powder	0330

All other feed ingredients were purchased from a reputable feed mill in Akure, Ondo State, Nigeria. The plant materials were cut into pieces, spread and air-dried under shade to a constant weight. Thereafter, they were milled and stored in plastic containers under room temperature. Before the supplementation of the leaf mix, the four leaves *A. murican*, *F. exasperata, P. americana, G. sepium* together with *A. sativum* were weighed respectively and mixed in a ratio 2:2:2:1:1 (2, 2, 2, 1 and 1 g/kg) to produce the supplementary composite leaf meal (CLM). A sample from the composite mix was sent to laboratory for toxicity, phytochemical and proximate composition analyses prior to use.

Experimental Animals and Experimental Design: One hundred and fifty (150) broiler chicks were procured from a reputable Hatchery Limited in Ibadan, Nigeria. On arrival, the broiler chicks were randomly selected and allotted to five treatments replicated three times with 10 birds per replicate in a completely randomized design. The birds were fed their respective test diets (Table 2) for a period of six weeks (42 days).

Table 2: Gross composition of the experimental basal diet of broiler chickens fed with composite leaf mix based diets

Ingredients	Quantity (%)
Maize	60.00
Soybean Meal	15.00
Groundnut cake	18.25
Fishmeal	3.00
DCP	2.00
Limestone	1.00
Premix	0.25
Methionine	0.10
Lysine	0.10
Salt	0.30
Total	100.00
Calculated Analysis	
Crude protein (%)	21.96
Metabolizable energy (Kcal/kg)	2982.60
Calcium (%)	1.05
Available phosphorus (%)	0.64
Lysine (%)	1.09
Methionine (%)	0.44

Feed and water were provided for the birds *ad libitum* through the experimental period. Medications and vaccinations were given according to the hatchery recommendation.

Experimental Diets: A basal diet containing 21.96 % crude protein and 2982.60 kcal/kg metabolizable energy was formulated and used as straight diet for the birds from day 1 to day 42. The basal diet was then divided into five portions. The composite leaf mix (CLM) was added to the five portions at 0, 5, 10, 15 and 20 g/kg, respectively and designated as diets 1, 2, 3, 4 and 5 respectively. The gross composition of the basal diet is shown in Table 2.

Data Collection

Haematological and serum biochemical studies: Three birds were randomly selected from each replicate, bled through jugular vein and 10 ml of blood collected. 3 ml blood sample was collected into an EDTA plastic tube for haematological parameter studies. The parameters assayed were PVC, Hb count, red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), lymphocyte and monocyte as described by Dacie and Lewis (1991). The serum indices measured were creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, albumin, globulin, glucose using BIOBASE automatic biochemistry analyser.

Antioxidants assay: The serum glutathione peroxidase (GSH-Px) activity was determined according to the method described by Paglia and Valentine (1967) using a commercially available enzyme kit (Ransel, RANDOX/RS-504 by Randox Laboratories, Crumlin, United Kingdom). Superoxide dismutase (SOD) activity will be determined according to Nishikimi *et al.* (1972) using the commercially available enzyme kit (Ransod, RANDOX/SD-125 supplied by Randox Laboratories, Crumlin, United Kingdom). Malondialdehyde (MDA) level will be determined as described by Ohkawa *et al.* (1979) using MDA colorimetric assay kit (TBA method) (Elabscience, USA) and T-AOC according to

Koracevic *et al.* (2001) using auto analyzer kits (Bio-Med Diagnostics, Egypt).

Gut microbial population identification and counting: Digesta was collected from the duodenum and jejunum region of the gut. Bacteria colonies from the digesta grown on the plates were counted and calculation for the colony forming units was recorded as log cfu/ml using formula as described by Brugger *et al.* (2012).

Pure culture of bacteria species was obtained from the primary culture. Resulting pure culture of the bacteria species were then transferred into slants of nutrient agar for storage and subsequent identification.

Bacteria colonies were then identified using their staining, morphological and biochemical characteristics. Thereafter biochemical reactions, including catalase (CAT), coagulate and motility test was carried out as described by (Cheesbrough, 2005).

Statistical Analysis: Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS version 22 package and significant means were separated using Duncan Multiple Range Test of the same package. The microbial population in intestinal region (jejunum and duodenum) was tested using t-test of the same statistical package.

RESULTS AND DISCUSSION

The haematological parameters of broiler chickens fed basal diets supplemented with composite leaf mix are presented in Table 3. Among all the parameters measured, the PCV, Hb and monocyte had significant differences ($p<0.05$) across all diets. The highest PCV (34.20 ± 0.68 %) was recorded in birds fed Diet 4 and lowest in birds fed Diet 5 (29.40 ± 0.68 %). The highest Hb was in birds fed Diet 4 (11.40 ± 0.23 g/dl) and lowest in birds fed Diet 2 (9.80 ± 0.23 g/dl). The highest monocyte (2.00 ± 0.63 x109/L) was recorded in birds fed Diet 5 and lowest in birds fed Diets 2 and 3 (0.01 ± 0.01 x10^{9}/L). The highest RBC value was recorded in birds fed Diet 2 (2.64 ± 0.42 x10^{6}/L) and lowest value in birds fed Diet 4 (2.62 ± 0.42 x10^{6}/L). The highest MCHC was recorded in birds fed Diet 1 (32.53 ± 0.22 g/dl) and lowest was recorded in birds fed Diet 5 (31.21 ± 0.22 g/dl), while the highest MCV value was recorded in birds fed Diet 4 (132.36 ± 4.20 fL) and the lowest value was recorded in birds fed Diet 5 (112.47 ± 4.20 fL). The highest WBC count was recorded in birds fed Diet 1 (3.88 ± 0.22 x10^{9}/L) and lowest was recorded in birds fed Diet 2 (2.40 ± 0.22 x10^{9}/L), for the granulocytes, the highest was recorded in birds fed Diet 1 (1.54 ± 1.53 x10^{9}/L) and lowest was recorded in birds fed Diet 2 (0.82 ± 1.53 x10^{9}/L). Furthermore, the highest MCH value was recorded in birds fed Diet 4 (44.12 ± 0.22 pg) and lowest birds fed Diet 5 (37.49 ± 0.22 pg), while highest granulocytes was recorded in birds fed Diet 1 (39.20 ± 1.53 %) and lowest was recorded in birds fed Diet 5 (30.00 ± 1.53 %). The highest lymphocyte was recorded in birds fed Diet 5 (69.00 ± 2.28 %) and lowest in birds fed Diet 1 (59.60 ± 3.97 %).

Haematological indices had been recognized as one of the indicators for assessing the health status of animals. Therefore a haematological result that falls within normal range indicates a stable condition of an animal wellbeing. The haematological parameters of broiler chickens fed with the supplemented diets in this study indicated that the supplementary leaf mix meal had effects on the blood parameters. Given that the PCV values of the bird fed the diets ranged from 29.4 – 34.2 %**,** was within the normal range of 22.0 – 35.0 % reported by Odunitan-Wayas *et al.* (2018). This thus implies that birds fed with composite leaf mix are not prone to erythrocytosis or anaemia. According to Shohe *et al.* (2019), an increase in red blood cell mass of birds indicated that the birds were suffering from erythrocytosis and a decrease indicates anaemic condition.

The haemoglobin count in the birds fed CLM ranged from 9.84 – 11.40 g/dl, which falls within the normal range of 7 – 13 g/dl reported by Bounous and Stedman (2000). High haemoglobin levels could be indicative of a rare blood disease; polycythemia. Birds suffering from polycythemia are usually fatigued and also have troubles breathing. This suggests that birds fed are not under fatigue.

Table 3: Haematological characteristics of the broiler chickens fed with composite leaf mix based diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Packed cell volume (%)	34.00 ± 0.84[b]	33.20 ± 1.39[ab]	32.00 ± 0.70[ab]	34.20 ± 0.49[b]	29.40 ± 2.60[a]
Red blood cell (x10^6/L)	2.64 ± 0.12	2.92 ± 0.23	2.74 ± 0.24	2.62 ± 0.15	2.64 ± 0.22
Mean corpuscular haemoglobin concentration (g/dl)	32.53 ± 0.47	32.39 ± 0.64	32.50 ± 0.39	32.18 ± 0.47	31.21 ± 0.34
Mean corpuscular volume (fL)	130.01 ± 7.39	115.30 ± 6.77	121.61 ± 13.99	132.36 ± 8.36	112.47 ± 9.24
Haemoglobin (g/dl)	11.33 ± 0.28[b]	11.07 ± 0.46[ab]	10.67 ± 0.24[ab]	11.40 ± 0.16[b]	9.80 ± 0.87[a]
White blood cell (x 10^ 9/L)	3.88 ± 0.51	2.40 ± 0.26	3.48 ± 0.71	3.34 ± 0.34	3.66 ± 0.44
Granulocytes (x10^ 9/L)	1.54 ± 0.29	0.82 ± 0.06	1.30 ± 0.37	1.15 ± 0.13	1.10 ± 0.15
Mean corpuscular Haemoglobin (pg)	43.34 ± 2.46	38.43 ± 2.25	40.54 ± 4.66	44.12 ± 2.78	37.49 ± 3.08
Granulocytes %	39.20 ± 4.41	35.20 ± 3.07	35.60 ± 4.26	34.80 ± 2.87	30.00 ± 2.10
Lymphocyte (%)	59.60 ± 3.97	64.80 ± 3.07	60.40 ± 3.31	64.80 ± 2.94	69.00 ± 2.28
Monocytes (%)	1.20 ± 0.80[c]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.40 ± 0.40[b]	2.00 ± 0.63[d]

[a,b,c,d], Mean values with different superscripts on the same row differ significantly ($p<0.05$)

Monocyte counts of chickens fed the test diets was within the range of 0.01 – 0.07 %. This also falls within normal range of 0 – 3 % reported by Kermanshahi *et al.* (2008). The main causes of high monocyte levels (monocytosis) are chronic inflammation and infections. When the monocytes are too low also the birds have a higher risk of having infection. Monocytes are a type of white blood cell that are important to the immune system's ability to destroy invaders. The monocytes aids to protect the body from pathogen and carotenoids build up (Maggini *et al.*, 2018). The present results thus suggest that the chickens fed the CLM have enough immunity to destroy invaders.

MCH recorded in the treatments in this study was lower than the control with the exception of Diet 4. MCH indicates the blood carrying ability of RBC. The study therefore reveals that the treatments reduced the blood carrying ability of the RBC.

The value for MCV is within the normal range values of 113 – 144 fL as reported by Talebi *et al.* (2005) which is an indication that the experimental diets met the birds' nutritional requirements except Diet 5 where the MCV observed (112.47 ± 4.20 fL) was slightly lower than normal. A low MCV may indicate iron deficiency, a haemoglobin disorder such as anaemia due to blood cell destruction or bone marrow disorder (Akinsanmi *et al.*, 2020). This was confirmed as birds placed on Diets 2, 3 and 5 had lower PCV and Hb values than the control birds.

The present study results were not significant ($p>0.05$) for MCV, MCH, or MCHC in experimental broiler chickens. These results affirmed the findings of Oghenebrorhie and Oghenesuvwe (2016) and Abdul Basit *et al.* (2020) who reported no significant results for MCV, MCH and MCHC in broilers supplemented with *Moringa oleifera* leaf meal and *Persicaria odorata* leaf meal respectively.

The serum characteristics of the broiler chickens fed with the diet supplemented with composite leaf mix are presented in Table 4. Among all the parameters, only creatinine and glucose had significant differences ($p<0.05$) across all diets. The highest creatinine level was recorded in birds fed Diet 3 (50.67 ± 3.64 µmol/L) and lowest was recorded in birds fed

Table 4: Blood serum characteristics of broiler chicken fed diets containing composite leaf mix

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Creatinine (µmol/L)	28.50 ± 6.70^{a}	35.00 ± 2.29^{ab}	50.67 ± 6.12^{b}	19.20 ± 9.26^{a}	33.23 ± 5.33^{ab}
Aspartate aminotransferase (U/L)	115.17 ± 10.33	138.93 ± 4.87	134.03 ± 7.75	130.18 ± 3.04	129.48 ± 16.19
Alanine aminotransferase (U/L)	50.11 ± 1.02	53.25 ± 0.83	50.80 ± 0.82	51.81 ± 2.27	50.43 ± 0.21
Cholesterol (mmol\L)	3.20 ± 0.60	2.67 ± 0.40	2.60 ± 0.35	2.50 ± 0.23	3.07 ± 0.12
Albumin (g/L)	13.17 ± 4.68	7.60 ± 2.77	11.63 ± 0.37	12.80 ± 3.29	13.90 ± 1.61
Globulin (g/L)	37.77 ± 7.74	42.27 ± 0.23	40.37 ± 4.99	31.73 ± 5.87	40.77 ± 1.56
Glucose (mmol/L)	13.80 ± 1.12^{b}	11.00 ± 0.52^{a}	11.77 ± 1.59^{a}	11.10 ± 0.97^{a}	13.83 ± 0.56^{b}
Total Protein (g/L)	50.93 ± 3.08^{b}	49.87 ± 2.54^{a}	52.00 ± 5.21^{b}	44.53 ± 6.73^{a}	54.67 ± 0.27^{b}

a,b, Mean values with different superscripts on the same row differ significantly (p<0.05)

Diet 4 (19.2 ± 3.64 µmol/L). For glucose, the highest glucose level was recorded in birds fed Diet 5 (13.83 ± 0.39 mg/dl) and the lowest was recorded in birds fed Diet 2 (11.00 ± 0.39 mg/dl). Furthermore, the highest AST was recorded in birds fed Diet 2 (138.93 ± 4.21 U/L) and lowest was recorded in birds fed Diet 1 (115.17 ± 4.21 U/L), while the highest ALT was recorded in birds fed Diet 2 (53.25 ± 0.56 U/L) and lowest was recorded in the birds fed Diet 1 (50.11 ± 0.56 U/L). The highest cholesterol level was recorded in birds fed Diet 1 (3.20 ± 0.16 mmol/L) and lowest was recorded in birds fed Diet 4 (2.50 ± 0.16 mmol/L), while the highest albumin concentration was recorded in birds fed Diet 5 (13.90 ± 1.26 g/L) and lowest was recorded in birds fed Diet 2 (7.60 ± 1.26 g/L). The highest globulin was recorded in birds fed Diet 2 (42.27 ± 2.11 g/L) and lowest was recorded in birds fed Diet 4 (31.73 ± 2.11 g/L), highest total protein was recorded in birds fed Diet 5 (54.67 ± 1.82 g/L) and lowest was recorded in birds fed Diet 4 (44.53 ± 1.82 g/L). In this study, the serum total protein values were not significantly affected by the dietary treatments which suggest the adequacy and utilization of the dietary protein by the birds irrespective of the varying inclusion in the leaf meals.

Glucose is the most important source of energy in all organisms. The glucose level of the birds fed the supplementary composite leave mix ranges from 11.00 – 13.83 mmol/L which falls within the normal range of 9.31 – 12.13 mmol/L reported by Oloruntola *et al.* (2019). Hyperglycaemia (high blood glucose) is a condition where there is too much sugar in the blood because the body lacks enough insulin to metabolize them. A low blood sugar level, also called hypoglycaemia, occurs when the level of sugar (glucose) in the blood drops too low (Bernstein, 2021).

Creatinine level is applicable in determining the status of the kidney and its functions. Creatinine of the birds in this study has a range of 0.22 – 0.57 mg/dl which falls within the normal range (0.12 – 0.23 mg/dl) reported by Oloruntola *et al.* (2019). High creatinine level may signify early sign of kidney malfunctioning, while a low creatinine level can mean a lower muscle mass caused by a disease, such as muscular dystrophy, or by aging. Thus, birds fed the supplemented test diets had normal kidney function.

Albumin has been reported to aid blood clotting in farm animals, the higher the albumin content of the blood, the better the clotting ability as well as haemorrhage prevention (Vaughn and Beckel, 2012). However, the value of albumin observed in this study falls within the normal range of 2 – 3 g/dl as reported by Mitruka and Rawnsley (1977). Lee *et al.* (2021) reported that globulin aids infection prevention in birds. Adegbenro *et al.* (2016) also opined that decreased globulin levels could lead to increased mortality in pigs, this could also be true in their counterpart monogastric (broiler chicken) used in the present study. Agbede *et al*. (2011) reported that ALT and AST are important indicators used in assessing the liver condition of farm broiler chicken. ALT and AST activities were not influenced by leaf meal supplementation, which implies that the CLM might not compromise the health status of the birds, especially the liver, as increased activities of these enzymes are well known diagnostic indicator of liver injury (Agbede *et al.,* 2011).

Cholesterol is a separate type of lipids which circulate throughout the body as part of lipoproteins. Abundance of cholesterol is related with coronary illness which is as a result of the accumulation of cholesterol on the walls of the artery consequently causing the narrowing of the arteries thereafter reducing the flow of blood into the heart (Oloruntola *et al.,* 2019). This suggests that the CLM supplementation in the diets of broiler chickens could help to reduce heart related problems.

The antioxidants characteristics of the broiler chickens fed with the composite leaf mix supplemented diets are presented in Table 5. Among all the parameters only CAT and superoxide dismutase (SOD) had significant difference ($p<0.05$) across all diets. The highest CAT level was recorded in birds fed Diet 5 Diet 5 (54.50 ± 2.22 Ku) and lowest was recorded in birds fed Diet 2 (34.72 ± 2.22 Ku). The highest SOD was recorded in birds fed Diet 2 (87.09 ± 0.01 %) and lowest was recorded in birds fed Diet 3 (63.74 ± 3.72 %). Although statistical significant difference was not observed in glutathione (GST), numerically higher GST was observed in birds fed Diet 2 (212.59 ± 3.00 %) than in birds fed the control diet (200.84 ± 3.00 %), while birds fed Diet 5 (195.09 ± 3.00 %) had lower GST than the control birds.

Catalase is one of the most important antioxidant enzymes responsible for the break-down of two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water in a two-step reaction (Dringen *et al*., 2005). CAT deficiency destroys the cell membranes. It causes pain, turns hair grey, and causes peroxidation in lipids which leads to bad cholesterol ratios, diabetes and heart attack. The birds fed the supplementary composite leaf mix did not suffer any condition related to high or low CAT concentrations.

Superoxide dismutase is the first detoxification enzyme and most powerful antioxidant in the cell. The normal range of SOD as reported by Oloruntola *et al.* (2019) falls within (57.33 – 68.99 %). The birds fed composite leaf mix had the SOD range of 67.74 – 87.10 %. Low SOD level can cause acute respiratory distress syndrome (ARDS) or chronic obstructive pulmonary disease (COPD).

Afolabi and Oloyede (2014) reported in their study on rats that antioxidant enzyme like glutathione peroxidase can forestall oxidation by balancing transition metal radicals such as Fe^{2+} or Cu^{+} or by gathering initiated free radicals such as superoxide and hydrogen peroxide, the most reactive free radical *in-vivo*. In the same vein, the low plasma values of glutathione, in broilers fed the dietary treatment as compared to birds placed on control diet also showed the antioxidant properties of the composite leaf meal in use in this study. This affirms the utilization of the composite leaf meals as non-toxic feed ingredients in the diets of broiler.

The effect of the diets on the gut microbial population counts in the gut duodenum and the jejunum region of broiler chickens fed with composite leaf mix supplemented diet are presented in Table 6. There were no significant differences ($p>0.05$) in the gut microbial populations. However, out of the two intestinal regions of the birds, the duodenum had the lowest microbial population mean (MPM) at 7.10 ± 7.08 $\times10^6$ cfu/ml, while the jejunum had the highest MPM at 27.00 ± 7.08 $\times10^6$ cfu/ml .

In the jejunum, there were no significant differences ($p>0.05$) for each of the birds fed the test diets. Moreover, the jejunum of birds fed Diet 1 had the highest population count value at 40.50 ± 6.17 $\times10^6$ cfu/ml, while the jejunum of birds fed with Diet 4 had the lowest count at 11.50 ± 6.17 $\times10^6$ cfu/ml.

In the duodenum, there were no significant ($p>0.05$) differences for each of the diets fed to the chickens. Duodenum of the birds fed Diet 3 had the highest count value at 18.00 ± 2.16 $\times10^6$ cfu/ml, while duodenum of

Table 5: The antioxidants status of the broiler chickens fed diets containing composite leaf mix

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Glutathione (%)	200.84 ± 12.12	212.59 ± 1.01	206.34 ± 4.04	204.84 ± 0.01	195.09 ± 7.65
Catalase (kU)	36.28 ± 0.41^{a}	34.72 ± 4.28^{a}	46.52 ± 1.02^{b}	50.18 ± 1.67^{bc}	54.50 ± 0.36^{c}
Superoxide dismutase (%)	80.65 ± 3.72^{bc}	87.10 ± 0.01^{c}	67.74 ± 3.72^{a}	73.39 ± 0.47^{b}	80.65 ± 3.72^{bc}

^{a,b,c}, Mean values with different superscripts on the same row differ significantly (p<0.05)

Table 6: Gut microbial population count of chickens fed diets containing composite leaf mix

Parameters	Total Microbial Count (10^6 cfu/ml)
Microbial population in intestinal region	
Jejunum	27.00 ± 4.50*
Duodenum	7.10 ± 4.50
Microbial population in diets	
Diet 1	23.75 ± 27.20^{b}
Diet 2	21.25 ± 30.70^{b}
Diet 3	23.75 ± 17.30^{b}
Diet 4	7.25 ± 4.80^{a}
Diet 5	9.25 ± 6.10^{a}
Microbial population in jejunum as influence by diets	
Diet 1	40.50 ± 31.50^{c}
Diet 2	40.00 ± 36.00^{c}
Diet 3	29.50 ± 20.50^{b}
Diet 4	11.50 ± 1.50^{a}
Diet 5	13.50 ± 23.60^{a}
Microbial population in duodenum as influence by diets	
Diet 1	7.00 ± 4.00^{b}
Diet 2	2.50 ± 1.50^{a}
Diet 3	18.00 ± 15.00^{c}
Diet 4	3.00 ± 0.01^{a}
Diet 5	5.00 ± 3.00^{b}
Microbial population in jejunum and duodenum as influence by diets	
Diet 1	23.75 ± 27.20^{c}
Diet 2	21.25 ± 30.70^{b}
Diet 3	23.75 ± 17.30^{c}
Diet 4	7.25 ± 4.80^{a}
Diet 5	9.25 ± 6.10^{a}

*^{a,b,c}, Mean values with different superscripts on the same row differ significantly (p<0.05), *Mean value differ significantly using t-test pairwise comparison*

birds fed Diet 2 had the lowest count at 2.50 ± 2.16 ×10^6 cfu/ml. Between the jejunum and the duodenum, there was no significant (p>0.05) differences for each of the diets fed to the chickens. The highest microbial count in the jejunum was recorded in birds fed Diet 1 at 40.50 ± 6.17 ×10^6 cfu/ml, while birds fed with Diet 3 had the highest count in the duodenum, at 18.00 ± 2.16 ×10^6 cfu/ml. This study showed that diverse microbial population resides in the gut of broiler chickens fed the supplementary composite leaf mix; this was in agreement with the report of Shang *et al.* (2018). The gastrointestinal (GI) tracts of chickens harbours diverse and complex congregation of micro-biotas working together to play vital roles in the digestion and absorption of feed nutrients. However, not all microbes resident in the chickens' gut are beneficial. According to Diaz Carrasco *et al.* (2019) some microbes could be detrimental to the health and immunity of chicken; inhibiting the performance of the beneficial ones. Hence, the use of CLM as an additive because of its antimicrobial properties: could help reduce or eliminate the detrimental microbes' population in the gut of broiler chickens.

It was observed that the gut of bird fed with Diets 4 and 5 had a reduced number of *Salmonella* and *Escherichia coli* population as against the high range of these scavenging microbes found in the gut of the birds fed the control diet. This was in agreement with the study of Engberg *et al.* (2000) who suggested that the discovery of antimicrobial containing additives could lead to the control of infectious pathogens in the gut of poultry animals. And thus, CLM could serve as a substitute for synthetic antibiotics.

The effect of the diets on isolates identified in the gut regions of broiler chickens fed with the supplemented diets is presented in Table 7. For lactic acid bacteria (LAB), bird fed Diet 4 had the highest identified isolate counts (9.25 ± 0.43 log10/CFU). While birds fed with Diet 3 had the lowest gut isolates (7.30 ± 0.59 log10/CFU). For total aerobic bacteria (TAB), values were similar for all the diets.

Tables 7: Identified isolate count (log10 cfu/ml) of chickens fed diets containing composite leaf mix

Bacterial counts	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Lactic acid bacteria	9.09 ± 0.11^b	8.07 ± 0.42^{ab}	7.30 ± 0.59^a	9.25 ± 0.43^b	8.73 ± 0.08^b
Total aerobic bacteria	9.16 ± 0.35	8.64 ± 0.21	9.07 ± 0.31	8.70 ± 0.19	9.01 ± 0.35
Coliform bacteria	9.57 ± 0.01^b	8.00 ± 0.01^a	8.34 ± 0.01^a	8.14 ± 0.01^a	8.55 ± 0.01^a

a,b, Mean values with different superscripts on the same row differ significantly (p<0.05)

Coliform bacteria had the highest isolate count (9.57 ± 0.01 log10/CFU) in birds fed Diet 1, while birds fed Diets 3, 4, and 5 had similar value. Birds fed Diet 2 had the lowest bacterial count (8.00 ± 0.01 log10/CFU).

Table 8a showed the bacteria isolate species present in the duodenum of chicken fed across each of the test diets. The species identified and characterised in this intestinal region were *Staphylococcus aureus*, *Lactobacillus* spp. and *Streptococcus* spp. For the bacteria species; *S. aureus* was found in the duodenum region of all chickens fed Diet 1, 2 and 3, while absent in the duodenum of birds fed with test Diet 4 and 5. *Lactobacillus* species were identified in the duodenum of birds fed all the test diets. Also, *Streptococcus* spp. was identified in the duodenum of all birds fed test Diets 1, 3 and 5, while absent in birds fed Diets 2 and 4.

Table 8a: Isolate bacteria in duodenum of chickens fed diets containing composite leaf mix

Bacterial species	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Staphylococcus aureus	+	+	+	-	-
***Lactobacillus* spp.**	+	+	+	+	+
***Streptococcus* spp.**	+	-	+	-	+

Key: + = present, - = absent

Table 8b showed the microbial isolates population identified and characterized in the jejunum region of the bird's intestine. Microbes identified include; *S. aureus*, *Lactobacillus* spp. and *Streptococcus* spp. Microbes such as; *Salmonella* and *E. coli* were also identified in this intestinal region.

The bacteria species; *S. aureus* was identified in the jejunum of the birds fed test diets 2, 4 and 5. *Lactobacillus* spp. was identified in the jejunum of the birds fed the entire test Diets 1, 2, 3, 4 and 5.

Table 8b: Isolate bacteria in jejunum of chickens fed diets containing composite leaf mix

Bacterial species	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Staphylococcus aureus	-	+	-	+	+
***Lactobacillus* spp.**	+	+	+	+	+
***Streptococcus* spp.**	-	+	+	+	+
Salmonella	+	+	+	-	-
Escherichia coli	+	-	-	-	-

Key: + = present, - = absent

Streptococcus spp. was absent only in birds fed test Diet 1. *Salmonella* species were identified in the jejunum region of the entire groups: except in group of birds fed diets 4 and 5. *E. coli* species were also identified in the jejunum of the chickens fed Diet 1, while being absent in birds fed test Diets 2, 3, 4 and 5.

The aerobic microbial isolates identified from the digesta harvested from the slaughtered birds' duodenums and jejunums includes: *S. aureus*, *Lactobacillus* spp., *Streptococcus* spp., *Salmonella* and *E. coli*.

Lactobacillus spp. was adjudged to be abundant in both sections of the small intestine. This is in line with the report of Apajalahti and Vienola (2016) that the small intestinal section of the chicken gut is predominately colonized by lactic acid producing bacteria species. *Lactobacillus* also known as lactic acid bacteria (LAB) is adjudged one of the most valuable species colonizing the chickens GIT. *Lactobacilli* spp. has for years been used as a prebiotics: due to their inherent abilities to help in energy metabolism, enhanced growth and performance; and as well as help improve carcass quality (Valeriano *et al.*, 2016). And so, the increased population of *Lactobacillus* spp. observed in the intestinal regions of the birds fed diets containing CLM could be said to be of benefits to the chickens. This was in line with Li *et al.* (2018) findings which stated that the dietary supplementation of *Lactobacillus* spp. is capable of improving the gut health of broiler chickens.

Results of the study indicated that out of the two intestinal segments studied, the duodenum region of the intestine contained the least bacteria population density, at 7.10×10^6 cfu/ml, and the jejunum had the highest microbial population at 27.00×10^6 cfu/ml. This is in coherence with the findings of Shapiro and Sarles (1949) who identified that, of the three segments of the small intestine, the duodenums of chicken accommodates the lowest microbial density. This was suggested to be due to the dilution by bile; as well, the fast passing time of digesta in the segment. The data obtained also showed the presence of aerobic bacteria at 8.9×10^6 cfu/ml and coliforms at 8.52×10^6 cfu/ml.

Also, according to the reports of Shang *et al.* (2018), aerobic microbes in collaborative activity with other beneficial microbes, perform basic duties in immune system development and pathogen exclusion. Thus, it could be generally suggested that CLM supplemented diets could promote beneficial microbes resident in birds gut, thereby performing mutualistic roles during feed metabolism activities of the GIT.

Conclusion: It could be concluded with respect to the study that CLM in test diets positively influenced the haematological, serum, antioxidants properties, and the gut microbiome profile of the broiler chickens. The supplementation levels of CLM could help to improve gut beneficial microbial population: acting as a prebiotic, while reducing the population of pathogenic microbes. And given the stable influence of the test diets on the haematology, serum, antioxidants, and gut health indices of broiler chickens used in the experiment, supplementation of broiler diets with CLM at 15 g/kg could be recommended for safe use as a prebiotic, and as a reliable substitute for sub-therapeutic antibiotics or AGPs.

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