Haemato-biochemical indices and intestinal microbial population of broiler chickens fed diet supplemented with *Prosopis africana* (African mesquite) essential oil

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Abstract

The objective of this present study was to investigate the haemato-biochemical indices and intestinal microbial population of broiler chickens fed diets supplemented with *Prosopis africana* (African mesquite) essential oil (PAEO). A total of 540 one-day old broiler chicks of Ross 302 strain were allotted to six treatments with 6 replicates consisting of 15 birds each in a completely randomized design. Feed and water were given *ad libitum*, and all necessary management practices were strictly observed throughout the experiment, which lasted for 8 weeks. The birds in treatment 1 (T1) was fed a basal diet with no PAEO, T2 was fed basal diet plus 1.2 g kg^{-1} Oxytetracycline, T3, T4, T5 and T6 were fed basal diet plus PAEO at 200 mg, 400 mg, 600 mg and 800 mg kg^{-1} respectively. All haematological parameters and intestinal microbial population were influenced by the dietary treatments ($p < 0.05$). Total protein, triglycerides, cholesterol and urea were significantly different ($p < 0.05$) across the dietary treatments and are within the physiological ranges reported for healthy chicken. However, serum tryglycerides, low density lipoprotein, high density lipoprotein, magnesium and chloride ion values were not affected by PAEO ($p > 0.05$). It was concluded PAEO possess several properties – antioxidant, antimicrobial, hepatoprotective, immune-stimulatory, anti-inflammatory and physiological amongst others all of which are vital for the health enhancing effects in birds. *Prosopis africana* essential oil can be supplemented up to 800 mg kg^{-1} in the diets of broiler chickens without causing any deleterious effect on their blood profile.

Keywords: *Prosopis africana* oil, broiler chicken, *Prosopis* genus, phytochemicals, haematology.

Índices hemato-bioquímicos e população microbiana intestinal de frangos de corte alimentados com dieta suplementada com óleo essencial de *Prosopis africana* (mesquita Africana)

Resumo

O objetivo do presente estudo foi investigar os índices hemato-bioquímicos e a população microbiana intestinal de frangos de corte alimentados com dietas suplementadas com óleo essencial de *Prosopis africana* (*Mesquita Africana*) (PAEO). Um total de 540 pintos de corte de um dia da linhagem Ross 302 foram distribuídos em seis tratamentos com 6 repetições, consistindo 15 aves cada, em um delineamento inteiramente casualizado. Ração e água foram fornecidas *ad libitum*, e todas as práticas de manejo necessárias foram rigorosamente observadas durante todo o experimento, que durou 8 semanas. As aves do tratamento 1 (T1) receberam dieta basal sem PAEO, T2 receberam dieta basal mais 1,2 g kg^{-1} de Oxitetraciclina, T3, T4, T5 e T6 receberam dieta basal mais PAEO a 200 mg, 400 mg, 600 mg e 800 mg kg^{-1}, respectivamente. Todos os parâmetros hematológicos e população microbiana intestinal foram influenciados pelos tratamentos dietéticos (p < 0,05). Proteína total, triglicerídeos, colesterol e uréia foram significativamente diferentes (p < 0,05) entre os tratamentos dietéticos e estão dentro das faixas fisiológicas relatadas para frangos saudáveis. Entretanto, os valores séricos de triglicerídeos, lipoproteína de baixa densidade, lipoproteína de alta densidade, magnésio e íons cloreto não foram afetados pela PAEO (p > 0,05). Concluiu-se que o PAEO possui várias propriedades – antioxidante, antimicrobiana, hepatoprotetora, imunoestimulante, anti-inflamatória e fisiológica, entre outras, todas vitais para os efeitos benéficos à saúde das aves. O óleo essencial de *Prosopis africana* pode ser suplementado até 800 mg kg^{-1} na dieta de frangos de corte sem causar qualquer efeito deletério em seu perfil sanguíneo.

Palavras-chave: óleo de *Prosopis africana*, frango de corte, gênero *Prosopis*, fitoquímicos, hematologia.

1. Introduction

There is a growing legislation pressure to phase out the routine feeding of animals with low levels of antibiotics as feed additives due to possible presence of drug residues in edible animal products and the environment as well as transfer of antibiotic resistance to human pathogens has directed research towards alternative solutions such as use of the phytogenics or essential oils (Randolf, 2018; Singh et al., 2020; Singh et al., 2021). Essential oils (EOs) are natural products which do not show any nutrient, mineral or vitamin character but influence the performance and health of animals in a positive manner due to the presence of bioactive compounds or phytochemicals (Ines, 2010). They have been predicted to have a promising future in animal nutrition due to their broad range of efficacies and to their effects on sustainability and safety (Louis, 2012; Jan et al., 2013).

Essential oils are derived from group of spices and herbs and their combine the effects of antibiotics (antimicrobial activities of certain plant ingredient like *Prosopis africana*) with the effects of probiotics (well balanced gut flora and its stabilization) (Wouter, 2011; Maria, 2016; Alagbe, 2022). They are also well known for their beneficial effects on animals from flavoring and sensorial stimulation, antioxidant, hepato-protective, immune-stimulatory, anti-viral, antifungal and anti-inflammatory properties (Shittu; Alagbe, 2021; Oloruntola et al., 2019).

The chemical composition of essential oils (EOs) underlines a certain variation due to their ingredients and other influencing factors like climate, harvest, location, stage and storage conditions, explaining the differences in their efficacies (Oluwafemi et al., 2020; Axel, 2018). EOs tends to accumulate in specialized parts of the plants. For instance, leaves (cinnamon leaf oil, eucalyptus, lemon grass oil), bulbs (garlic), roots (ginger, vetiver), bark (cinnamon, *Polyalthia longifolia*), seeds (caraway, anis, pepper) or in fruit peels (lemon, orange) (Tobias, 2010).

Several studies indicate positive effects of EOs from lavender, turmeric, ginger, garlic, clove and other natural extracts on the growth performance, intestinal morphology, suppression of pathogenic organisms, increased enzymatic activity in the intestinal tract and improved nutrient utilization of broiler chickens when fed at different concentrations (Sandra, 2018). The presence of phyto-constituents like phenols, flavonoids, terpenoids, tannins and alkaloids can positively influence the gastrointestinal tract morphology and physiology and most likely stimulate or inhibit certain metabolic pathways (Suman et al., 2014). Phenolic compounds have free radicals scavenging properties, but also non-phenolic compounds may show considerable antioxidant activity by enhancing gene expression of antioxidant enzymes (Marc, 2010). These antioxidant effects are protecting the organism at cell and tissue level especially during stressful conditions (Axel, 2018).

Considering all the essential oils' diverse potentials. In this work, the intestinal microbial population and haemato-biochemical parameters of broiler chickens fed diets enhanced with *Prosopis africana* oil was examined.

2. Materials and Methods

2.1 Site of the experiment

This study was carried out in the Department of Animal Science, Faculty of Agriculture, University of Abuja Teaching and Research Farm, Main Campus, situated near Airport Road in Gwagwalada, Abuja, Nigeria. The administrative headquarters of the Gwagwalada Area Council are located in Gwagwalada, which is positioned between latitudes 8º571 and 8º551N and longitudes 7º051 and 7º061E. Gwagwalada (FCT) is one of the Federal Capital Territory's six area councils. Gwagwalada is the largest community in the study area and the third-largest urban center in the FCT, with a population of roughly 157,770. Additionally, it is one of the FCT's metropolitan areas that is expanding fast (NPC, 2006).

2.2 Taking a sample of seeds and identifying them

The *P. africana* seeds used in this investigation were bought from a Gwagwalada neighborhood market. The seeds were identified and authenticated at the Department of Crop Protection Herbarium, University of Abuja, Gwagwalada, Nigeria, with a voucher number of ULH 203RT/ANS.

2.3 Extraction of Prosopis africana oil

The steam-distillation process, which also requires the use of a digital scale, a round-bottom flask, distilled water, a heating mantle made of glass fibre, a measuring cylinder, and a separatory funnel, was used to extract the *P. africana* oil. A round-bottom flask was filled with 250 mL of distilled water and 150 g of ground *Prosopis* seed. The condenser was positioned atop the flask with a circular bottom after the mixture was added to a glass yarn heating mantle and warmed to a temperature of 80 ºC. The mixture is forced to boil for 15 min, and the distillate is then gathered in a beaker until no more oil drips are visible. To obtain *P. africana* essential oil, the distillate was poured into the separatory funnel.

2.4 Activities before experiment

The research was carried out using a battery cage that had normal dimensions (500×250×150 cm^3). Batteries were kept in a pen with good ventilation, hanging 100 cm above the ground. Each treatment group's cages were labeled for easy identification, thoroughly cleaned with Aquaclean 100 (20 mL of Aquaclean to 30 liters of water) and placed in an area where the birds could access the feed and water troughs. For the first week of the chicks' lives, old papers were scattered out at the bottom of the cage as litter.

2.5 The management of experimental animals

A private farm in Ibadan, Oyo State, Nigeria, 540 one-day-old broiler chicks (Ross 302), which were then shipped to the University of Abuja Teaching and Research Farm in Gwagwalada, Abuja. The birds were measured when they arrived and sequentially grouped into six groups, each of which contained six replicates and a total of fifteen birds in each replicate. Upon arrival and following each vaccination, vitamins with glucose were given at a rate of 1 g to 5 liters of water. Heat for the birds was provided by 200-watt electric lights .

2.6 Novel dietary practices

Birds received fed a base diet developed in accordance with NRC (1994). Two diets were given: a starter diet (0–4 weeks) with a crude protein (CP) content of 23.30% and metabolizable energy (ME) content of 2900.3 kcal kg^{-1}, and a finisher diet (5-8 weeks) with a CP content of 21.40% and ME content of 3200.8 kcal/kg^{-1}. The computed and ingredient makeup of the trial basal diet are both displayed in (Table 1). Birds in treatment 1 (T1) received a basal diet containing no *P. africana* oil (PAEO), treatment 2 (Oxytetracycline) was administered at 1.2 g kg^{-1} of feed, and treatments 3, 4, 5, and 6 (PAEO) were administered at 200 mg, 400 mg, 600 mg, and 800 mg kg^{-1} of feed, correspondingly.

Table 1. Ingredient composition of the experimental diets.

Components	Starters mash (0-4 weeks)	Finishers mash (5-8 weeks)
Maize	52.00	60.00
Wheat offal	2.50	5.00
Soya bean meal	30.00	25.00
Groundnut cake	8.00	4.00
Fish meal (72%)	2.00	2.00
Oyster shell	1.50	1.50
Bone meal	3.00	3.00

Lysine	0.20	0.20
Methionine	0.20	0.20
*Premix	0.25	0.25
Salt	0.30	0.30
Toxin binder	0.10	0.10
Total	100.0	100.0
Calculated analysis (% DM**)**		
Crude protein	23.30	21.40
Crude fibre	4.18	5.01
Ether extract	4.03	4.47
Calcium	1.50	1.60
Phosphorus	0.58	0.66
Energy (Kcal kg^{-1})	2900.3	3200.8

Note: *Premix supplied per kg^{-1} diet: - vit A, 13,000 I.U; vit E, 5 mg; vit D3, 3000 I.U, vit K, 3 mg; vit B2, 5.5 mg; Niacin, 25 mg; vit B12, 16 mg; choline chloride, 120 mg; Mn, 5.2 mg; Zn, 25 mg; Cu, 2.6 g; folic acid, 2 mg; Fe, 5 g; pantothenic acid, 10 mg; biotin, 30.5 g; antioxidant, 56 mg (starter's mash). **Premix supplied per kg^{-1} diet: - vit A, 9,000 I.U; vit E, 10 mg; vit D3, 1500 I.U, vit K, 3.8 mg; vit B2, 10 mg; Niacin, 15 mg; vit B12, 10 mg; choline chloride, 250 mg; Mn, 5.0 mg; Zn, 56 mg; Cu, 1.6 g; folic acid, 2.8 mg; Fe, 5.1 g; pantothenic acid, 10 mg; biotin, 30.5 g; antioxidant, 56 mg (finisher's mash). Source: Authors, 2023.

2.7 Data gathering

2.7.1 Blood sample estimation

On day 56, twelve birds selected per treatment for hemato-biochemical estimation. Selected animals were kept in a stress free environment to prevent oxygenated blood becoming deoxygenated during blood collection. The sampled birds were bled from punctured wing vein to draw 4 mL of blood from each birds out of which 2 mL was collected into bijou bottle treated with ethylenediaminetetraacetic acid (EDTA) for hematological assay while the remaining 2 mL was used for serum analysis.

Hamatological analysis of red blood cell, haemoglobin, pack cell volume, white blood cell and its differentials were carried out using Sysmex XN-3100™ automated analyzer system. The system provides reflex testing, reflexive slide preparation and optional digital cell imaging. The machine was caliberated before use according to the manufacturer's recommendation. Samples are arranged in the column chamber which makes use of infra radiation, thereafter individual results are displayed on the monitor.

Blood samples (2 mL) for serum biochemical indices collected into EDTA were analyzed for total protein, creatinine, uric acid, bilirubin, tryglycerides, lipoproteins, cholesterol, glucose, Calcium (Ca), Phosphorus (P), Potassium (K), Sodium (Na), bicarbonate and enzymes using Analytica 705™ clinical diagnostics with the following technical data; optical flow (30 µL quartz), reaction volume (350 – 1000 µL), photometric range (- 0.1 to 3.0 absorbance), filter (7 interference filters: 340, 405, 505, 546, 578, 620 and 670 nm).

2.*8 Intestinal microbiology estimation*

On the 8th week of the experiment, intestinal samples were collected from 12 chicken per treatments for microbiological analysis. Analysis was carried out using Microplate reader (DR – 200 BC – LED lamp) to identify the intestinal microbes. Samples were mixed with saline water and incubated at 35 ºC for 8 h. The equipment has the following specifications; ambient temperature (15 - 40 ºC), input (100 – 200 VAC, 50 – 60 Hz), output (24 VAC, direct current), optical chamber (OG550 glass filter), LED protector (transparent polycarbonate, body and lever (1.4462 stainless steel).

2.9 Statistical investigation

Using SPSS (25.0), one-way analysis of variance was performed on all data, and *Duncan* multiple range tests were used to identify significant means (Duncan, 1955). If $p < 0.05$, significance was deemed to exist.

3. Results and Discussion

3.1 Haematological characteristics of broiler chickens fed Prosopis africana essential oil

Hematological characteristics of broiler chickens given *P. africana* EO is captured in (Table 2). Pack cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), platelets, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), heterophils, lymphocytes, eosinophils, monocytes, and heterophils/lymphocytes (H/L) varied from 24.80 to 36.76%, 9.98 to 13.78 g dL^{-1}, 2.00 to 3.60 ($\times 10^{12}$ L^{-1}), 117.0 to 193.8 ($\times 10^{3}$ mL^{-1}), 30.46 to 43.79 pg, 29.83 to 60.02 fl, 20.18 to 33.97%, 16.06 to 26.52 ($\times 10^{9}$ L^{-1}), 24.80 to 27.60%, 58.22 to 73.87%, 0.15 to 0.71%, 1.00 to 2.11% and 0.34 to 0.47% respectively. Birds in T4, T5, and T6 had increased PCV, Hb, RBC, MCH, MCHC, and WBC values than other treatments ($p < 0.05$). Monocytes and lymphocytes were at their lowest rate ($p < 0.05$) for T1 compared to other treatments. Treatment had no effect on heterophils or eosinophils ($p > 0.05$). H/L ratio was higher in T1 compared to other groups ($p < 0.05$).

3.2 Prosopis africana essential oil-fed broiler chicken serum biochemical indexes

The serum biochemical parameters of broilers fed *P. africana* oil are shown in (Table 4).13. (5-8 weeks). Compared to other treatments, T1 had the smallest levels of total protein (3.57 - 6.95 g dL^{-1}), globulin (1.67 - 3.62 g dL^{-1}), albumin (1.90 - 3.28 g dL^{-1}), α1- globulin (0.92 - 1.50 g dL^{-1}), β-globulin (1.88 - 2.11 g dL^{-1}), and γ-globulin (0.54 - 1.49 g dL^{-1}) were lowest ($p < 0.05$) for T1, glucose (147.5 – 205.7 mg dL^{-1}), cholesterol (98.10 – 201.5 mg dL^{-1}), urea (2.67 – 5.61 mg dL^{-1}) and creatinine (0.40 – 0.95 mg dL^{-1}) were highest ($p < 0.05$) for T1 compared to other regimens. Conjugated bilirubin [0.42–0.52 (mol L^{-1}), total bilirubin [2.93–3.07 (mol L^{-1}), triglycerides [60.07–67.38 mg dL^{-1}], high density lipoprotein [HDL; 37.11–38.40 mg dL^{-1}], and low density lipoprotein [LDL; 16.51–19.08 mg dL^{-1}] have been unaffected by treatments ($p > 0.05$).

3.3 Broiler serum enzymes and electrolytes supplied oil from Prosopis africana

Electrolytes and serum enzymes in broiler chickens fed *P. africana* OE is displayed in (Table 4). The levels of alanine phosphatase (ALP; 219.5–310.2 (iu L^{-1}), alanine serum transaminase (AST; 38.09–64.23 (iu L^{-1}), and alanine amino transaminase (ALT; 15.00–24.09 (iu L^{-1})) were greatest in T1 compared with the other treatments ($p < 0.05$). The concentrations of Sodium (100.3 - 171.6 Mmol/L), Potassium (20.33 - 34.08 mg dL^{-1}), Calcium (11.40 - 33.74 mg dL^{-1}), Phosphorus (7.11 - 16.73 mg dL^{-1}), and bicarbonate (21.71 - 35.05 Mmol L^{-1}) were maximum in T4, T5, and T6, midrange in T3, and lowest in T1 and T2 ($p < 0.05$). Chloride (50.09 - 57.12 Mmol/L) and Magnesium (10.00 - 11.00 Mmol L^{-1}) also weren't altered by treatments ($p > 0.05$).

3.4 The impact of Prosopis africana oil on the broiler chickens intestine microbial population

The impact of *P. africana* OE on the intestinal microbial population of broiler chicken is shown in (Table 5). *Escherichia coli*, *Lactobacillus spp*, *Salmonella* spp, *Panicillum spp*, *Klebsiella* spp and *Streptococcus* spp logarithmic count varied from 9.05 to 20.08 (CFU/g^{-1}), 10.85 to 34.06 (CFU g^{-1}), 14.33 to 38.04 (CFU g^{-1}), 5.08 to 12.57 (CFU g^{-1}), 4.01 to 10.05 (CFU g^{-1}) and 10.02 to 27.29 (CFU g^{-1}) correspondingly. *P. africana* OE decreased ($p < 0.05$) the counts of *E. coli*, *Salmonella spp*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in the intestine when compared to the other treatments, with the exception of *Lactobacillus* spp, which was higher ($p < 0.05$) in the *P. africana oil* treatments.

Table 2. Haematological characteristics of broiler chickens fed *Prosopis africana* essential oil.

Parameters	T1	T2	T3	T4	T5	T6	SEM	*P*-value
PCV (%)	24.80^{c}	29.87^{bc}	34.56^{a}	35.06^{a}	36.10^{a}	36.76^{a}	0.32	0.81
Hb (g/dL)	9.98^{c}	10.09^{b}	13.32^{a}	13.00^{a}	13.40^{a}	13.78^{a}	0.22	0.001
RBC ($\times 10^{12}$/L)	2.00^{c}	2.91^{bc}	3.16^{a}	3.32^{a}	3.59^{a}	3.60^{a}	0.059	0.02
Platelets ($\times 10^{3/}$mL)	117.0	138.6	162.1	175.4	188.9	193.8	3.03	0.60

MCH (pg)	30.46^b	32.00^b	38.40^b	42.65^a	42.75^a	43.79^a	0.41	0.92
MCV (fl)	29.83^c	30.22^c	38.77^c	44.95^b	56.88^a	60.02^a	0.32	0.10
MCHC (%)	20.18^b	26.18^b	32.00^a	33.50^a	33.83^a	33.97^a	0.18	0.01
WBC ($\times 10^9$/L)	16.06^b	20.07^a	21.44^a	23.06^a	23.87^a	26.52^a	0.81	0.04
Heterophils (%)	27.60	24.90	25.85	24.91	24.88	24.80	0.95	0.06
Lymphocytes (%)	58.22^c	66.08^b	67.10^b	68.40^b	71.30^a	73.87^a	1.28	0.25
Eosinophils (%)	0.15	0.40	0.56	0.61	0.65	0.71	0.03	0.001
Monocytes (%)	1.00^b	1.24^b	1.63^b	2.00^a	2.09^a	2.11^a	0.05	0.15
H/L ratio	0.47^a	0.38^b	0.39^b	0.36^b	0.35^b	0.34^b	0.03	0.001

Note: Results in cells with various characters differ considerably ($p < 0.05$); LYM: lymphocytes; MON: monocytes; BAS: basophils; HET: heterophils; EOS: eosinophils; SEM: standard error of mean; Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; T1: control diet with no *Prosopis africana* oil; T2: control diet plus 1.2 g kg^{-1} oxytetracycline; T3: baseline diet plus 200 mg; T4: baseline diet plus 400 mg; T5: control diet plus 600 mg; T6: basal diet plus 800 mg PAEO. Source: Authors, 2023.

Table 3. *Prosopis africana* essential oil-fed broiler chicken serum biochemical indexes.

Parameters	T1	T2	T3	T4	T5	T6	SEM	*P*-value
Total protein (g/dL)	3.57^c	4.08^b	6.44^a	6.83^a	6.87^a	6.95^a	0.17	0.52
Albumin (g/dL)	1.90^b	2.04^b	3.03^a	3.21^a	3.30^a	3.28^a	0.55	0.001
α_1-globulin (g/dL)	0.92^b	1.04^a	1.20^a	1.47^a	1.50^a	1.55^a	0.30	0.00
β-globulin (g/dL)	1.88^b	1.95^b	2.00^a	2.08^a	2.11^a	2.17^a	0.40	0.001
γ-globulin (g/dL)	0.54^c	1.07^{ab}	1.00^{ab}	1.34^a	1.47^a	1.49^a	0.17	0.10
Globulin (g/dL)	1.67^c	2.04^b	3.41^a	3.62^a	3.57^a	3.67^a	0.47	0.001
Glucose (mg/dL)	205.7^a	200.8^a	171.5^b	156.2^b	150.1^b	147.5^b	8.71	0.42
Cholesterol (mg/dL)	201.5^a	150.8^{bc}	123.8^{bc}	110.2^{bc}	100.5^{bc}	98.10^d	5.33	0.31
Urea (mg/dL)	5.61^a	5.05^a	3.00^b	2.83^c	2.80^c	2.67^c	0.26	0.001
Creatinine (mg/dL)	0.95^a	0.75^b	0.70^b	0.51^c	0.45^c	0.40^c	0.25	0.00
C. bilirub (µmol/L)	0.52^a	0.50^a	0.41^b	0.44^b	0.42^b	0.40^b	0.20	0.00
T. bilrub (µmol/L)	2.73^b	3.00^a	3.03^a	3.01^a	3.04^a	3.07^a	0.60	0.01
HDL (mg/dL)	37.31	38.40	37.52	38.00	37.94	37.11	2.46	0.50
LDL (mg/dL)	19.08	19.33	17.57	17.00	16.84	16.51	1.33	0.20
TRY (mg/dL)	67.38	65.11	59.05	60.09	61.13	60.07	3.08	0.61

Note: Results in cells with various characters differ considerably ($p < 0.05$); Total protein, albumin, globulin, glucose, cholesterol, creatinine, high density lipoprotein, low density lipoprotein, triglycerides, and standard error of the mean are all abbreviations for total protein. T1: Baseline diet plus 0% *Prosopis africana* oil; T2: Baseline diet plus 1.2 g kg^{-1} oxytetracycline; T3: Baseline diet plus 200 mg PAEO; T4: Baseline diet plus 400 mg PAEO; T5: Baseline diet plus 600 mg PSAEO; T6: Baseline diet plus 800 mg PAEO. Source: Authors, 2023.

Table 4. Broiler chicken serum enzymes and electrolytes fed *Prosopis africana* essential oil.

Parameters	T1	T2	T3	T4	T5	T6	SEM	*P*-value
ALP (U/L)	310.2^a	287.4^b	255.0^b	234.1^b	220.7^b	219.5^b	21.40	0.06
AST (U/L)	64.23^a	59.84^{ab}	50.87^{ab}	43.88^b	41.90^b	38.09^c	4.12	0.23
ALT (U/L)	24.09^a	20.80^a	18.40^b	18.03^b	16.54^b	15.00^c	1.21	0.01

B. C (Mmol/L)	21.71^{b}	30.88^{ab}	32.95^{a}	33.48^{a}	34.10^{a}	35.05^{a}	1.37	0.01
Ca (mg/dL)	11.40^{c}	17.49^{c}	23.80^{b}	30.67^{a}	30.88^{a}	33.74^{a}	1.55	0.72
P (mg/dL)	7.11^{c}	9.22^{c}	11.45^{b}	14.03^{a}	15.63^{a}	16.73^{a}	1.02	0.02
K (mg/dL)	20.33^{b}	27.18^{b}	30.04^{a}	30.80^{a}	32.33^{a}	34.08^{a}	2.71	0.01
Na (Mmol/L)	100.3^{b}	100.8^{b}	156.2^{a}	160.6^{a}	169.1^{a}	171.6^{a}	8.56	0.04
Cl (Mmol/L)	57.12	54.08	51.44	56.07	50.09	57.11	4.04	0.62
Mg (Mmol/L)	10.04	11.00	10.88	10.21	10.00	10.03	1.07	0.02

Note: Implies in cells with various characters differ considerably ($p < 0.05$); ALP stands for "alanine phosphatase," AST for "alanine serum transaminase," ALT for "alanine tranferase," B.C. for "bicarbonate," CAL for "Calcium," P for "Phosphorus," Na for "Sodium," Cl for "Chloride," Mg for "Magnesium," and SEM for "standard error of mean"; T1: control diet with no *Prosopis africana* oil; T2: control diet plus 1.2 g kg^{-1} oxytetracycline; T3: baseline diet plus 200 mg; T4: baseline diet plus 400 mg; T5: diet supplemented plus 600 mg; T6: basal diet plus 800 mg PAEO. Source: Authors, 2023.

Table 5. The impact of *Prosopis africana* oil on the broiler population's intestine microbial population.

Trt (CFU/g^{-1})	*E. coli*	*L. spp*	*Salmonella spp*	*P. spp*	*Klebsiella spp*	*Streptococcus spp*
T1	20.08^{a}	10.85^{c}	38.04^{a}	12.57^{a}	10.05^{a}	27.29^{a}
T2	16.22^{b}	14.08^{c}	21.67^{ab}	9.40^{ab}	7.40^{b}	18.05^{ab}
T3	15.83^{b}	20.07^{b}	17.54^{b}	6.71^{b}	4.94^{c}	12.75^{b}
T4	10.95^{ab}	25.64^{b}	15.60^{b}	5.58^{b}	4.21^{c}	11.62^{b}
T5	9.77^{c}	31.31^{a}	15.00^{b}	5.11^{b}	4.08^{c}	10.88^{b}
T6	9.05^{c}	34.06^{a}	14.33^{b}	5.08^{b}	4.01^{c}	10.02^{b}
SEM	1.52	2.55	2.08	1.33	1.02	1.48
P-value	0.62	0.84	0.71	0.05	0.03	0.02

Note: Implies in rows with various characters vary markedly ($p < 0.05$); Standard error of the mean (SEM) T1: control diet with no *Prosopis africana* oil; T2: control diet plus 1.2 g kg^{-1} oxytetracycline; T3: baseline diet plus 200 mg; T4: baseline diet plus 400 mg; T5: control diet plus 600 mg; T6: basal diet plus 800 mg PAEO. Source: Authors, 2023.

4. Discussion

4.1 Hematological evaluation of broiler chickens fed various amounts of Prosopis africana oil

Blood examination may be a quick and easy way to determine the physiological and nutritional health condition of animals participating in feeding experiments since dietary components have quantifiable impacts on the blood's composition (Olajide et al., 2009). The incorporation of PAEO in the diet enhanced ($p < 0.05$) the blood concentration of red blood cells (RBC), hemoglobin (Hb), pack cell volume (PCV), platelets, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) especially in comparison to those given antibiotics. These results are analogous to those of Maziar & Moein (2017), who found that adding oregano essential oil at a concentration of 500 ppm significantly boosted the RBC, Hb, and PCV counts when compared to the control group. Similar findings were made by Kassie (2009), who discovered that broiler chickens given thyme oil (100–200 ppm) had statistically ($p < 0.05$) greater RBC, PCV, and Hb levels than the control group.

A high RBC level in birds fed T3 to T6 is a sign that the animals are getting enough oxygen in their blood stream. Phyto-constituents in PAEO (flavonoids, terpenoids, tannins, alkaloids, steroids and saponins) may also account for the major increase in Hb, PCV and platelet counts (Oluwafemi et al., 2020; Shittu; Alagbe, 2021; Agubosi et al., 2021). Normal RBC, Hb, PCV, and platlets readings dispelled any concerns about an impending danger that might be connected to using PAEO. They are also crucial indicators for assessing circulatory erythrocytes and also helpful indicators of an animal's ability to generate red blood cells in the bone marrow (Chineke et al., 2006). The functional significance of hemoglobin is to carry carbon dioxide from an animal's body and deliver oxygen to its tissues for the oxidation of food consumed to provide energy for other bodily activities (Soetan et al., 2013).

A minimum level of eosinphils found in birds fed PAEO is a definite sign that the immunological health of the animals was unaffected. The regulation of allergy and inflammatory processes, host defenses against parasitic illnesses like helminthiasis and ectoparasitic infestation, and blood detoxification are all key functions of eosinophils (Butterworth, 2009). According to Aster (2004), monocytes mature into macrophages, which are responsible for producing antigens and phagocytosing big particles (such as fungi and protozoa). Broiler chickens fed diets containing PAEO had lower heterophils: lymphocytes (H/L ratio) levels, which decreased oxidative responses (Abd El-Hady et al., 2020). H/L ratio is a reliable biological predictor of stress (Alagbe et al., 2023), the results of this current study, revealed that dietary supplementation of PAEO did not have any negative consequences on their overall health.

4.2 Serum biochemical measurements from chickens fed diets supplemented with Prosopis africana oil

Blood levels of dietary protein and organ illness in animals can be determined via serum biochemical indicators (Adeyemi et al., 2000). The concentration of serum proteins, which are predominantly produced in the liver, indicates the health of the hepatocytes. According to Tothova et al. (2016), any major drop in blood protein levels is a blatant symptom of malnutrition, poor feed quality, inflammation brought on by infections, and immunological deterioration. According to the findings of the current study, birds fed PAEO noticed a substantial ($p < 0.05$) increase in total protein (albumin, globulin, α-globulin, β-globulin, and γ-globulin). The investigation's albumin and protein levels fell within the typical values noted by Ibrahim (2012). Protein electrophoresis is a good screening method for assessing the relative or total levels of γ-globulin fractions (Grasman et al., 2000).

According to Alikwe et al. (2010), plasma cells, lymphocytes, or both can produce gamma globulin in the reticuloendothelial system outside of the liver. Though the albumin and globulin are all connected to bacterial susceptibility in some way, antigens are only present in the y-fraction, and the values discovered in this investigation were consistent with the normal range described by Nworgu et al. (2007). The current findings on serum protein in broiler chickens are consistent with those of Islam et al. (2004), who found that treatment with turmeric powder (100 g/kg^{-1} and 300 g/kg^{-1}) significantly increased total protein, albumin, and globulin levels. Extreme hunger, eating disorders, a weak habitation system, and digestive disturbances can all lead to higher blood glucose levels (Peters et al., 2002). Yang et al. (2019) reported that administration of cinnamon oil at 200 mg/kg^{-1} - 300 mg/kg^{-1} decreased the level of glucose in the blood of broiler chickens. Corresponding to this, low cholesterol levels in birds fed PAEO may indicate that the oil has the ability to prevent cardiovascular infection (Oloruntola et al., 2018).

Creatinine and urea levels were significantly different amongst the treatments. Although the readings obtained by the birds fed PAEO were lower than those of the other groups, they were all inside the usual range described by (De Marinis; Martini, 2008). Serum bilirubin evaluation is employed to determine the detoxification functions of the liver (Alagbe and Akintayo, 2020; Subhadarsini; Silpa, 2020). According to He et al. (2015), triglycerides constitute significant markers of lipid metabolism. The outcome of the current investigation demonstrates that PAOE possesses considerable cholesterol-lowering properties to prevent the occurrence of cardiovascular illnesses as well as the ability to protect tissues from lipid peroxidation (Miguel, 2010).

4.3 Serum electrolytes and enzymes of broiler chickens fed various PAEO doses

Birds' normal biological status depends on electrolytes, and a shortage in the blood might cause animal mortality (Malahubban and Aziz, 2016). Varying PAOE treatments considerably ($p < 0.05$) enhanced the concentrations of Ca, bicarbonate, P, and K. For the normal production of bones and the conveyance of nerve impulses in birds, calcium and phosphorus are required (Vasudevan; Sreekumari, 2007). Osmotic pressure, intracellular enzyme activity, and acid-base balance are all regulated by potassium (Alagbe, 2020).

According to Gupta et al. (2005), bicarbonate is a crucial component of pH homeostasis and is used by a number of transporters to move other ions and organic substrates across cell membranes. Ca, P, K, and bicarbonate readings in the current investigation were all within the usual levels noted by (Subhadarsini; Silpa, 2020). Sodium aids in the regulation of P, osmotic pressure, water balance, transmission of nerve impulses, and active movement of glucose and amino acids while magnesium is essential for enzymatic processes that support healthy body metabolism (Edeoga et al., 2005).

The diagnostic enzymes such alkaline phosphatase, alanine amino transferase, and aspartate amino transferase are used in reliable markers of liver, kidney, or cardiac dysfunction brought on by toxins or infections (Vos et al., 1993; Alhidary et al., 2016). While the concentrations of ALT are predicted to rise during injury to the liver, AST activities are driven by necrosis and inflammation of the heart, liver, and muscular tissues (Ahmad and Wudil, 2013; Musa et al., 2020). The outcomes of the present investigation revealed that adding more PAEO to birds' diets resulted in lower ($p < 0.05$) blood activity of AST, ALT, and ALP.

4.4 Impact of Prosopis africana oil on the caeca microbial population of broiler chickens

Bird wellbeing and regular gastrointestinal functions are significantly influenced by intestinal microbiota (Adewale et al., 2020). It is noteworthy in this investigation that the numbers of *E. coli*, *Salmonella* spp, *Panicillum* spp, *Klebsiella* spp, and *Streptococcus* spp decreased as the level of PAEO increased across the treatments. This shows that PAEO can inhibit the activities of pathogenic bacteria and keep the intestinal microbiota of birds in balance. In vitro investigations have shown that essential oils have potent antibacterial, antiviral, and antifungal effects (Oluwafemi et al., 2020). A broad range of antibacterial activity against both Gram-positive and Gram-negative bacteria are displayed by several phyto-constituents in PAEO (Wong et al., 2008).

Broiler chicks fed 800 mg/kg^{-1} rosemary oil experienced a significant ($p < 0.05$) decrease in *E. coli* count, according to Yesilbag et al. (2011). The results of this experiment provide strong evidence that PAEO can inhibit the activities of microbial pathogens by competitive inhibition, surface charge, and blocking the emergence of virulent disease structures (Alagbe et al., 2021). This current research additionally demonstrated a rise in *Lactobacillus* counts among birds fed PAEO compared to the other treatments ($p < 0.05$). Useful bacteria like *Lactobacillus* spp help metabolize food, obtain nutrients, and fend off harmful organisms (Alagbe, 2020). These findings are in line with those of Khaksar et al. (2012) who fed broiler chicken food with 200 mg/kg^{-1} thyme oil and observed a substantial ($p < 0.05$) elevation in intestinal *Lactobacillus* spp count and a reduction in the population of *E. coli*, *Salmonella* spp, and *Streptococcus* spp.

5. Conclusions

It was concluded that *Prosopis africana* essential oil (PAEO) show a wider range of activities in broilers nutrition than conventional antibiotics due to the presence of phyto-constituents which are efficient, safe and environmental friendly. The research have indicated that PAEO can be supplemented in the diet of broiler chickens up to 800 mg/kg^{-1} without causing any deleterious effect on the blood profile and performance of animals.

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7. Auhors' Contributions

Alagbe, J. O.: Performed the experiment; analyzed and interpreted the data; wrote the paper. *Agubosi, O. C. P.* and *Oluwafemi, R. A*: Designed the experiments. *Akande, T. O*, *Adegbite, A. E.* and *Emiola, I. A.*: Contributed analysis tools or data.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Yes applicable, n°. ANSJ2009/004

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