

Carcass Yield and Haematological Response of Broiler Chickens to Spotted Pumpkin (*Lagenaria Breviflora* Robert) Fruit Extract

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Abstract

BACKGROUND

Over the years, therapeutic application of plants has been used in folkloric medicine to explore natural alternatives to usage of antibiotic growth promoters due to the development of resistant bacteria strains and presence of drug residues in poultry meat. This study investigated the effects of concentrations and frequencies of administration of *Lagenaria breviflora* fruit extract (LBFE) on the haematological indices and carcass yield of broiler chickens. 390 birds were randomly distributed into 8 treatments; T1: control, T2: 75 g of LBFE/litre as synthetic antibiotic replacer (SAR) when necessary, T3: 75 g of LBFE/litre for 3days/week, T4: 75 g of LBFE/litre for 5days/week, T5: control, T6: 125 g of LBFE/litre as SAR, T7: 125 g of LBFE/litre for 3days/week and T8: 125 g of LBFE/litre for 5days/week. The experiment was laid out in a 2×4 factorial and data were subjected to Completely Randomized Design. Packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and haemoglobin (HB) were determined using improved Neubaur Haemocytometer after dilution and Cyanomethamoglobin methods. Dressing percentage for the carcass yield were also obtained.

RESULTS

Eosinophil counts and Uric acid were significantly ($p < 0.05$) highest in birds administered 75 g of LBFE/ litre of water 5days/week. At the finisher phase, Packed cell volume was significantly ($P < 0.05$) highest (32.25) in birds administered 75 g of LBFE/ litre of water 5days/week, while birds administered 125 g of LBFE/ litre of water as antibiotic replacement had significantly ($P < 0.05$) highest (5.00) red blood cell count. The thigh and liver of birds administered LBFE 3 days/week had the highest values ($P < 0.5$) of 10.79% and 2.84% respectively.

CONCLUSION

The study infers that administration of LBFE to broilers enhances the haematological indices as well as carcass yield which ultimately promotes healthy living of the birds.

BACKGROUND

The poultry industry has become an important economic activity in many countries however, immunosuppression has posed a major challenge because affected flocks are susceptible to secondary infections, respond poorly to vaccines and do not perform as well as non-affected birds^[1]. The fruit of *Lagenaria breviflora* is widely used in folklore medicine in West Africa. It is used in animal production due to its antimicrobial property livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals, especially poultry^[2]. Researchers have validated the use of the plant as an anti-implantation agent, abortifacient^[3], miracidicide and cercaricide^[4], as well as an antibacterial agent with broad spectrum activity^[5]. Kalimuthu (2010)^[6] reported that extract of plant which form the basis for all traditional system of medicine have been used for the treatment of various diseases however, the lethal effects of these extracts maybe preceded by clinical signs of toxicity such as salivation, changes in animal eye colour, decreased respiratory rate and motor activity^[7].

Adeyemi *et al.* (2017)^[8] observed that the presence of active ingredients and their biological activities in the fruit of *Lagenaria breviflora* makes it a prospective alternative to synthetic drugs in preventing and treating diseases in both man and animals. The active constituents in the leaves, stem, seed, root and fruit of these medicinal plants are highly effective in combatting different diseases and also aiding digestion which would ultimately improve the performance of recipients^[9]. Compared to synthetic antibiotics or inorganic chemicals, products that are plant derivatives have been found to be less toxic, residue free, ideal feed additives for animal nutrition and most importantly they are natural^[10]. Herbal plants are safe and economical. A properly prescribed amount of plant extract intake has numerous health benefits. This can be particularly seen in its

application in animal feeding such as botanical additives in poultry feeds where results such as digestive secretions, immune stimulation, antibacterial coccidiostatical, antiviral or anti-inflammatory activity in birds have been recorded ^[11].

Haematological indices are useful parameters that can be employed to assess the toxic potentials of plant extracts in living systems^[12]. Balogun *et al.*, (2014)^[13] opined that haematological parameters provide information regarding the status of bone marrow activity and hemolysis. Changes in haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal^[14] because haematological indices are used as a base line in determination of damage to blood cells and in evaluation of responses of animal to therapy ^[15].

Overall, it has been observed that serum biochemical and haematological parameters are improved by inclusion of phytobiotics in broiler diets ^[16]. Serum biochemical and haematological parameters are reliable indicators of health status of animals, and may have important roles in diagnosis, prognosis, and treatments of poultry diseases. For example, alanine transaminase (ALT) and aspartate aminotransferase (AST) are considered diagnostic enzymes for liver diseases ^[17].

The study on administration of dosage gives an insight to safe use of *Lagenaria breviflora* fruit extract in poultry. Previous study had been carried out using laying birds focused on the effect on laying performance ^[18], toxicology and biochemical ^[7] as well as phytochemical^[8]. Based on the findings above, this research was aimed at determining the effect of different concentration and frequency of administration, of *Lagenaria breviflora* fruit on the Haematological, serological and carcass yield of broiler chickens.

MATERIALS AND METHODS

Experimental Site

The field experiment was carried out at the Poultry Unit, Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The area is situated in the South Western part of Nigeria. It is a region of 70m above the sea level of latitude 7° 5' to 7° 8'N and longitude 3°11'E.

Sourcing and preparation of *Lagenaria breviflora* fruit extract

Lagenaria breviflora fruits were purchased from a local market in Abeokuta, Ogun State, Nigeria. The fruit sample was identified and authenticated as *Lagenaria breviflora* at the Department of Botany, Federal University of Agriculture Abeokuta. The whole fruit of *Lagenaria breviflora* was weighed, rinsed with distilled water and chopped into smaller sizes. The fruits were properly washed, weighed (75 g and 125 g) into two separate containers of water at room temperature respectively and soaked for 72 hours, while being stirred vigorously at 12-hours intervals. After soaking for 72 hours, it was filtered using sieve with muslin cloth to obtain a clear solution.

Experimental Design

A total of three hundred and eighty – four birds were used for this experiment. Birds were fed with commercial broiler starter feed with crude protein of 23% and metabolizable energy of 2800Kcal/kg at the brooding phase while the broiler finisher feed had crude protein of 18% CP and metabolizable energy of 3000Kcal/kg, fed at the finishing stage. Feed and water/extract were provided *ad libitum* throughout the experiment. All vaccination and medication were administered and supplementation was carried out when and where necessary. After 2 weeks of brooding, the birds were balanced for weight and randomly distributed into experimental treatments. The birds were randomly distributed into 8 treatments; each treatment was assigned 48 birds. The treatments were further divided into 4 replicates with each replicate containing 12 birds. The experimental design is as shown in Table 1.

Table 1
Experimental design

Treatment	No of Birds/treatment	No of Replicates	No of Birds per replicate
T1 (control)	48	4	12
T2 (75 g/L as SAR)	48	4	12
T3 (75 g/L/3days/week)	48	4	12
T4(75 g/L/5days/ week)	48	4	12
T5 (control)	48	4	12
T6 (125 g/L as SAR)	48	4	12
T7 (125 g/L/3days/week)	48	4	12
T8 (125 g/L/5days/week)	48	4	12
Where:			

T1: Water and synthetic antibiotic as expected for routine medication (control), T2: 75 g of *Lagenaria breviflora* fruit extract as synthetic antibiotic replacer (SAR) in 1litre of drinking water, T3: 75 g of *Lagenaria breviflora* fruit extract in 1litre of drinking water for three consecutive days/week, T4: 75 g of *Lagenaria breviflora* fruit extract in 1litre of drinking water for five consecutive days/week, T5: Water and synthetic antibiotic as expected for routine medication (control), T6: 125 g of *Lagenaria breviflora* fruit extract as synthetic antibiotic replacer (SAR) in 1litre of drinking water, T7: 125 g of *Lagenaria breviflora* fruit extract in 1litre of drinking water for three consecutive days/week, T8: 125 g of *Lagenaria breviflora* fruit extract in 1litre of drinking water for five consecutive days /week.

Phytochemical screening of the *Lagenaria breviflora* fruit extract

Phytochemical screening of the *Lagenaria breviflora* fruit extract in cold water was carried out using standardized procedures to determine the presence of tannin, reducing sugar, cardiac glycoside, alkaloid, saponin, flavonoid and phenol.

Haematological Analysis of Broilers

Blood samples were collected at the 2nd and 6th week of the experiment from two broiler chickens per replicate using syringe and needle through the wing vein. Samples were collected into a set of sterilized tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) labeled bottles, for the analysis of haematological parameters. Packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and haemoglobin (HB) were determined using improved Neubaur Haemocytometer after dilution and Cyanomethamoglobin methods respectively as described by Bain *et al* (2016)^[41]. Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined by the method of Hyduke (1995) ^[42].

Serum Biochemical Analysis of broilers

Blood samples were collected at the 2nd and 6th week of the experiment from two broiler chickens per replicate using syringe and needle through the wing vein and kept inside a plan sample bottles. Serum urea and creatinine level were determined using spectrophotometric methods described by Coles (1986)^[43]. The activities of hepatic marker enzymes were determined in the serum. Alkaline phosphatase. Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined using the method described by Reitman and Frankel, (1957)^[44].

Antibody Titre

Haemagglutination inhibition tests for Newcastle disease (ND) antibodies: Blood samples were collected at the 1st, 3rd, 5th, 6th and 8 week of age for determination of antibody titre against Newcastle Disease and Blood samples were left without anticoagulant to clot. The serum was separated by centrifugation at 3000 rpm for 10 minutes. Microtechnique of haemagglutination inhibition test was done according to Takatasy (1955)^[45]. Geometric mean titre (GMT) was calculated according to Brugh (1978)^[46].

Carcass yield

At the 42nd day of the experiment, two birds of average weight per replicate were selected. The birds were fasted for 12 hours to empty their stomach to prevent carcass contamination by gut content but they had access to water to avoid dehydration. Birds were slaughtered and dressed following conventional procedure by severing the carotid artery and jugular vein. The birds were bled completely followed by scalding in water at 60°C. The live weight, plucked weight and dressed weight were recorded.

$$\text{Dressingpercentage (\%)} = \frac{\text{DressingWeight (g)} \times 100}{\text{LiveWeight (g)}}$$

Organs which include gizzard, liver, heart and the intestines were weighed. Cut parts of the birds such as the breast, back, thigh, drum stick, head, wings and shanks were weighed and expressed as percentages of the live weight.

$$\text{Cut – partpercentage (\%)} = \frac{\text{Cutpartweight (g)} \times 100}{\text{LiveWeight (g)}}$$

Statistical analysis

Data obtained were subjected to 2X4 factorial Analysis of Variance in a Completely Randomized Design. Significant differences among treatment means were separated using Duncan’s Multiple Range Test with the aid of SPSS package at 5% probability Frequency of administration.

RESULTS

Phytochemical Screening of the *Lagenaria breviflora* fruit extract

The results of the phytochemical screening show the presence of metabolites both at the 75g/litre and 125g/litre preparation as shown in Table 2.

Table 2
Phytochemical screening of *Lagenaria breviflora* fruit extract

Parameters	75 g of LBFE/ litre of water	125 g of LBFE/ litre of water
Tannin (mg/100g)	20.95	22.01
Reducing sugar (mg/100g)	30.26	27.31
Cardiac glycoside (mg/100g)	36.73	35.05
Alkaloid (mg/100g)	44.12	46.02
Saponin (mg/100g)	40.48	42.32
Flavonoid (mg/100g)	33.94	34.91
Phenol (mg/100g)	28.81	30.25

Main effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at starter phase

The main effects of concentration and frequency of administration of LBFE on haematological parameters of broiler chickens at starter phase are showed in Table 3. Lymphocyte and eosinophil counts differed significantly ($p < 0.05$) as influenced by concentration of LBFE. Lymphocyte count was higher ($p < 0.05$) in birds administered 125 g/ litres of the extract. However, eosinophil count was significantly ($p < 0.05$) higher (2.81%) in birds administered 75 g of LBFE / litre of water. The effect of frequency of administration of LBFE influenced only white blood cell count with significantly ($p < 0.05$) highest count (109125 g/l) observed in birds in the control and lowest (36375 g/l) in birds given antibiotic replacement.

Table 3
Main Effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at starter phase

Parameters	Concentration				Frequency of administration					
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/wk	5days/wk	SEM	P value
Packed Cell Volume (%)	35.03	34.25	1.23	0.655	34.38	33.75	35.88	34.56	1.74	0.851
White Blood Cell count (g/L)	73125	66125	10194	0.629	109125 ^a	36375 ^b	75250 ^{ab}	57750 ^{ab}	14416	0.006
Red Blood Cell Count ($10^{12}/l$)	4.47	4.91	0.29	0.290	4.50	4.44	4.88	4.94	0.41	0.762
Heterophil (%)	58.91	56.44	2.46	0.482	58.25	59.25	57.00	56.19	3.48	0.929
Lymphocyte (%)	30.09 ^b	36.97 ^a	2.11	0.025	35.63	31.75	30.69	36.06	2.98	0.484
Monocyte (%)	5.03	5.44	0.59	0.628	4.75	7.19	4.44	4.56	0.83	0.072
Eosinophil (%)	2.81 ^a	1.50 ^b	0.37	0.014	1.63	2.81	1.25	2.94	0.52	0.057
Basophil (%)	0.31	0.28	0.15	0.887	0.00	0.56	0.38	0.25	0.22	0.329
Heterophil : Lymphocyte	1.96	1.53			1.63	1.87	1.86	1.56		
^{ab} Means on the same row having different superscripts are significantly ($p < 0.05$) different										
wk week										

Effects interaction between of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at starter phase

Table 4 shows the interaction between concentration and frequency of administration of LBFE on haematological parameters of broiler chickens at starter phase. All parameters measured except eosinophil count were not significantly ($p > 0.05$) different across treatments. Eosinophil counts were highest (4.50%) in birds administered 75 g of LBFE / litre of water 5days/week and lowest (1.00%) in birds administered 125 g of LBFE / litre of water 3days/week.

Table 4
Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at starter phase

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Packed Cell Vol. (%)	34.38	35.25	36.38	34.13	34.38	32.25	35.38	35.00	2.46	0.875
WB C (g/L)	109125	30625	73625	79125	109125	42125	76875	36375	20387	0.549
R B C $\times 10^{12}/L$	4.50	4.13	4.50	4.75	4.50	4.75	5.25	5.13	0.58	0.921
Heterophil (%)	58.25	61.88	55.88	59.63	58.25	56.63	58.13	52.75	4.93	0.767
Lymphocytes (%)	35.63	28.63	24.88	31.25	35.63	34.88	36.50	40.88	4.22	0.539
Monocytes (%)	4.75	6.13	4.63	4.62	4.75	8.25	4.25	4.50	1.18	0.697
Eosinophils (%)	1.63 ^{ab}	3.63 ^{ab}	1.50 ^{ab}	4.50 ^a	1.63 ^{ab}	2.00 ^{ab}	1.00 ^b	1.38 ^{ab}	0.73	0.161
Basophil (%)	0.00	0.63	0.63	0.00	0.00	0.50	0.13	0.50	0.31	0.449
Heterophil:lymphocyte	1.63	2.16	2.25	1.91	1.63	1.62	1.59	1.29		
^{ab} Means on the same row having different superscripts are significantly ($p < 0.05$) different										

WBC - White Blood Cell, RBC - Red Blood Cell

Main effect of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at starter phase

The main effects of concentration and frequency of administration of LBFE on serum biochemical parameters of broiler chickens at starter phase are revealed in Table 5. All parameters measured were not significantly ($p > 0.05$) affected by concentration of LBFE. However, the effect of frequency of administration of LBFE significantly ($p < 0.05$) affected uric acid. Uric acid was significantly ($p < 0.05$) highest (35.19mg/DL) in birds fed LBFE 5days/week and lowest (14.38mg/DL) in birds in the control.

Table 5
Main Effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at starter phase

Parameters	Concentration				Frequency of administration					
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/week	5days/week	SEM	P value
Uric acid (mg/DL)	22.06	23.67	3.46	0.744	14.38 ^b	21.13 ^{ab}	20.78 ^{ab}	35.19 ^a	4.90	0.030
Creatinine (mg/DL)	1.919	1.413	0.502	0.479	0.863	2.388	1.388	2.025	0.710	0.444
ALT (U/L)	16.06	18.97	4.49	0.649	10.13	19.13	21.94	18.87	6.34	0.586
AST (U/L)	21.62	20.06	6.08	0.856	8.75	18.44	34.00	22.19	8.59	0.231
^{ab} Means on the same row having different superscripts by factor are significantly ($p < 0.05$) different										

Effect of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at starter phase

The effect of interaction between concentration and frequency of administration of LBFE on serum biochemical parameters of broiler chickens at starter phase is presented in Table 6. All parameters measured except Uric acid were not significantly ($p > 0.05$) different across treatments. Uric acid was significantly ($p < 0.05$) highest (50.75mg/DL) in birds administered 75 g of LBFE / litre of water 5 days/week while lowest values (11.50mg/DL) was recorded in birds administered 75 g of LBFE / litre of water as antibiotic replacement which was similar to the value obtained from the other groups.

Table 6
Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at starter phase

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Uric acid (mg/DL)	14.38 ^b	11.50 ^b	11.63 ^b	50.75 ^a	14.38 ^b	30.75 ^{ab}	29.92 ^{ab}	19.62 ^b	6.93	0.002
Creatinine (mg/DL)	0.86	3.31	0.63	2.87	0.86	1.46	2.15	1.17	1.00	0.298
ALT (U/L)	10.13	15.38	8.63	30.12	10.13	22.87	35.25	7.62	8.97	0.064
AST (U/L)	8.8	12.6	26.5	38.6	8.8	24.2	41.5	5.8	12.2	0.196
^{ab} Means on the same row having different superscripts by factor are significantly ($p < 0.05$) different										

Main effect of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at finisher phase

The main effects of concentration and frequency of administration of LBFE on haematological parameters of broiler chickens at finisher phase is shown in Table 7. Effect of concentration of the extract had no significant ($p > 0.05$) effect on all the measured parameters. However, effect of frequency of administration of LBFE influenced significantly ($p < 0.05$) Packed cell volume and Eosinophil count. Packed cell volume was significantly ($p < 0.05$) increased (29.87%) with the frequency of administration of the extract while eosinophil count was reversed other.

Table 7

Main Effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at finisher phase

Parameters	Concentration				Frequency of administration					
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/week	5days/week	SEM	P value
Packed Cell Volume (%)	26.56	26.84	1.11	0.858	22.13 ^b	27.19 ^{ab}	27.62 ^{ab}	29.87 ^a	1.57	0.008
White Blood Cell (g/L)	18000	21937	4311	0.521	16875	14937	27500	20562	6097	0.486
Red Blood Cell ($\times 10^{12}/L$)	3.688	3.875	0.147	0.370	3.375	4.125	3.500	4.125	0.207	0.016
Heterophil (%)	62.16	63.81	2.18	0.593	63.88	66.50	58.25	63.31	3.08	0.300
Lymphocytes (%)	30.44	28.06	2.08	0.423	26.13	25.75	35.06	30.06	2.95	0.102
Monocytes (%)	3.531	4.656	0.404	0.054	5.000	3.875	3.563	3.938	0.571	0.318
Eosinophils (%)	3.531	3.281	0.424	0.678	4.875 ^a	3.625 ^{ab}	2.813 ^{ab}	2.313 ^b	0.599	0.022
Basophil (%)	0.187	0.188	0.116	1.000	0.125	0.250	0.125	0.250	0.164	0.900
Heterophil :Lymphocyte	2.04	2.27			2.44	2.58	1.66	2.11		
^{ab} Means on the same row having different superscripts by factor are significantly ($p < 0.05$) different										

Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at finisher phase

Interaction between concentration and frequency of administration of LBFE on haematological parameters of broiler chickens at finisher phase is presented in Table 8. Packed cell volume and Red blood cell count were significantly ($p < 0.05$) different across treatments. Packed cell volume was significantly ($P < 0.05$) highest (32.25%) in birds administered 75 g of LBFE / litre of water 5days/week, while lowest value (22.13%) was obtained in birds in the control group administered 75 g of LBFE / litre of water, and birds in the control group. Birds administered 125 g of LBFE / litre of water as antibiotic replacement had significantly ($P < 0.05$) highest (5.00L) red blood cell count.

Table 8
Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on haematological parameters of broiler chickens at finisher phase

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Packed Cell Volume (%)	22.13 ^b	22.63 ^{ab}	29.25 ^{ab}	32.25 ^a	22.13 ^b	31.75 ^{ab}	26.00 ^{ab}	27.50 ^{ab}	2.21	0.013
White Blood Cell (g/L)	16875	19125	28250	7750	16875	10750	26750	33375	8622	0.226
Red Blood Cell($\times 10^{12}/L$)	3.375 ^b	3.250 ^b	3.875 ^{ab}	4.250 ^{ab}	3.375 ^b	5.000 ^a	3.125 ^b	4.000 ^{ab}	0.293	0.001
Heterophil (%)	63.88	64.63	54.88	65.25	63.88	68.38	61.63	61.38	4.36	0.645
Lymphocytes (%)	26.13	28.00	38.25	29.37	26.13	23.50	31.88	30.75	4.17	0.763
Monocytes (%)	5.000	3.750	3.000	2.375	5.000	4.000	4.125	5.500	0.808	0.214
Eosinophils (%)	4.875	3.500	3.500	2.250	4.875	3.750	2.125	2.375	0.847	0.754
Basophil (%)	0.125	0.125	0.000	0.500	0.125	0.375	0.250	0.000	0.231	0.331
Heterophil : Lymphocyte	2.44	2.31	1.43	2.22	2.44	2.91	1.93	2.00		
^{ab} Means on the same row having different superscripts are significantly (p < 0.05) different										

Main effect of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at finisher phase

The main effects of concentration and frequency of administration of LBFE on serum biochemical parameters of broiler chickens at finisher phase are shown in Table 9. All parameters measured were not significantly (p > 0.05) affected by concentration of LBFE. However, the effect of Frequency of administration of LBFE significantly (p < 0.05) affected uric acid and ALT. Uric acid was significantly (p < 0.05) highest (32.81mg/DL) in birds fed antibiotic replacement and lowest values (17.88 and 20.63mg/DL) were recorded in birds in the control and birds administered LBFE 3days/week, respectively. ALT was significantly (p < 0.05) highest (23.25U/L) in birds in the control and lowest (10.56U/L) in birds administered LBFE 5days/week.

Table 9
Main Effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at finisher phase

Parameters	Concentration				Frequency of administration					
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/wk	5days/wk	SEM	P value
Uric acid (mg/DL)	22.59	25.56	2.24	0.353	17.88 ^b	32.81 ^a	20.63 ^b	25.00 ^{ab}	3.17	0.009
Creatinine (mg/DL)	0.988	1.337	0.160	0.128	0.850	1.644	1.150	1.006	0.226	0.087
ALT (U/L)	15.47	12.41	2.38	0.368	23.25 ^a	11.06 ^{ab}	10.88 ^{ab}	10.56 ^b	3.37	0.024
AST (U/L)	11.28	9.25	1.76	0.417	10.25	9.31	8.13	13.38	2.49	0.489
^{ab} Means on the same row having different superscripts by factor are significantly (p < 0.05) different										

Effect of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at finisher phase

The interaction between concentration and frequency of administration of LBFE on serum biochemical parameters of broiler chickens at finisher phase shown on Table 10 revealed all the measured parameters were not significantly ($p > 0.05$) different across treatments.

Table 10

Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on serum biochemical parameters of broiler chickens at finisher phase

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Uric acid (mg/DL)	17.88	32.63	15.00	24.87	17.88	33.00	26.25	25.12	4.49	0.523
Creatinine (mg/DL)	0.850	1.163	1.013	0.925	0.850	2.125	1.287	1.087	0.320	0.460
ALT (U/L)	23.25	11.50	12.63	14.50	23.25	10.63	9.13	6.63	4.77	0.843
AST (U/L)	10.25	13.00	8.63	13.25	10.25	5.63	7.63	13.50	3.51	0.667

Main effect of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Antibody titre to Newcastle Disease vaccination of broiler chickens

The main effects of concentration and frequency of administration of LBFE on immuno-modulatory of broiler chickens are presented in Table 11 All measured parameters were not significantly ($p > 0.05$) affected both concentration and frequency of administration of LBFE. Although, there was no significant difference however the mean titer of serum antibodies of Newcastle disease was higher in the birds that received 75 g of LBFE / litre of water at the starter phase compared to the control ($p > 0.05$).

Table 11

Main effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Antibody titre to Newcastle Disease vaccination of broiler chickens

Parameters	Concentration				Frequency of administration					
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/week	5days/week	SEM	P value
Antibody titre at starter phase ($\log_2$ GMt)	37.9	18.2	12.4	0.265	13.8	68.7	17.0	12.6	17.5	0.075
Antibody titre at finisher phase ($\log_2$ GMt)	12.50	14.00	3.70	0.775	12.00	14.63	18.88	7.50	5.23	0.483

Effect of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Antibody titre to Newcastle Disease vaccination of broiler chickens

The interaction between concentration and frequency of administration of LBFE on immuno-modulatory of broiler chickens is shown in Table 12. No significant ($p > 0.05$) differences were observed across treatment means

Table 12

Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Antibody titre to Newcastle Disease vaccination of broiler chickens

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Antibody titre at starter phase (log ₂ gmt)	13.8	10.3	13.8	20.7	13.8	34.3	20.3	4.5	24.7	0.418
Antibody titre at finisher phase (log ₂ gmt)	12.00	11.75	14.75	11.50	12.00	17.50	23.00	3.50	7.40	0.701

Main effect of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Carcass characteristics of broiler chickens

The main effects of concentration and frequency of administration of LBFE on carcass characteristics of broiler chickens were presented in Table 13. All parameters except head were not significantly ($p > 0.05$) affected by concentration of *Lagenaria breviflora* fruit extract with significantly ($p < 0.05$) higher (2.55%) head measured in birds administered 75 g of LBFE / litre of water. The effect of frequency of administration of LBFE influenced significantly ($p < 0.05$) the liver portion with highest value (2.84%) obtained in birds administered LBFE 3days/week and lowest (2.35%) in birds administered antibiotic replacement.

Table 13

Main Effects of concentration and frequency of administration of *Lagenaria breviflora* fruit extract on carcass characteristics of broiler chickens

Parameters	Concentration		Frequency of administration							
	75 g/l	125 g/l	SEM	P value	Control	AR	3days/wk	5days/wk	SEM	P value
Live weight (kg)	1.75	1.70	0.04	0.377	1.76	1.75	1.71	1.67	0.05	0.629
Defeathered weight(kg)	1.60	1.56	0.03	0.416	1.63	1.61	1.58	1.51	0.05	0.391
Dressed weight (kg)	1.23	1.19	0.03	0.474	1.27	1.24	1.18	1.17	0.04	0.269
Dressing percent (%)	70.17	70.46	0.55	0.711	71.79	70.75	69.09	69.62	0.77	0.075
Head (%)	2.55 ^a	2.37 ^b	0.06	0.026	2.45	2.50	2.39	2.49	0.08	0.803
Shank (%)	4.12	3.91	0.10	0.137	4.08	4.01	4.10	3.86	0.14	0.632
Neck (%)	2.69	2.91	0.08	0.056	2.88	2.87	2.61	2.83	0.11	0.319
Breast (%)	19.74	19.74	0.34	0.992	20.17	19.98	20.14	18.67	0.48	0.094
Back (%)	14.95	14.78	0.24	0.613	15.41	14.74	14.16	15.17	0.34	0.057
Thigh (%)	9.89	9.86	0.17	0.902	9.52	10.21	9.51	10.24	0.24	0.041
Drumsticks (%)	9.49	9.58	0.15	0.682	10.03	9.54	9.25	9.30	0.21	0.045
Wings (%)	7.95	8.11	0.16	0.482	8.29	8.11	7.66	8.08	0.22	0.241
Liver (%)	2.60	2.59	0.07	0.980	2.64 ^{ab}	2.35 ^b	2.84 ^a	2.58 ^{ab}	0.10	0.013
Heart (%)	0.54	0.56	0.03	0.548	0.54	0.55	0.59	0.53	0.04	0.625
Gizzard (%)	1.88	1.85	0.05	0.749	1.85	1.97	1.84	1.79	0.07	0.356
Proventriculus (%)	0.45	0.47	0.02	0.421	0.46	0.44	0.50	0.44	0.03	0.330
GIT (%)	9.31	8.79	0.25	0.148	8.88	8.88	9.85	8.59	0.35	0.072
^{ab} Means on the same row having different superscripts are significantly (p < 0.05) different										

Effect of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on Carcass characteristics of broiler chickens

Interaction between concentration and frequency of administration of LBFE on carcass characteristics of broiler chickens is depicted in Table 14. Neck, thigh, liver and GIT were significantly ($p < 0.05$) different across treatments. Neck was significantly ($P < 0.05$) highest (3.40%) in birds administered 125 g of LBFE / litre of water 5days/week, while lowest values (2.26 and 2.22%) were obtained in birds administered 75 g of LBFE / litre of water 5days/week and birds administered 125 g of LBFE / litre of water 3days/week, respectively. Thigh was significantly ($P < 0.05$) highest (10.79%) in birds administered 125 g of LBFE / litre of water 5days/week and lowest (9.22%) in birds administered 125 g of LBFE / litre of water 3days/week. Liver was significantly ($P < 0.05$) highest (2.95%) in birds administered 125 g of LBFE / litre of water 3days/week and lowest (2.23%) in birds administered 75 g of LBFE / litre of water as antibiotic replacement. The GIT was significantly ($P < 0.05$) highest (10.68%) in birds administered 75 g of LBFE / litre of water 3days/week and lowest (8.35%) in birds administered 125 g of LBFE / litre of water 5days/week.

Table 14
Effects of interaction between concentration and frequency of administration of *Lagenaria breviflora* fruit extract on carcass characteristics of broiler chickens

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P value
Live weight (kg)	1.76	1.80	1.76	1.66	1.76	1.69	1.66	1.68	0.075	0.763
Defeathered weight (kg)	1.63	1.66	1.64	1.49	1.63	1.57	1.51	1.53	0.073	0.638
Dressed weight (kg)	1.27	1.29	1.22	1.13	1.27	1.18	1.14	1.20	0.057	0.401
Dressing percent (%)	71.80	71.71	69.35	67.83	71.80	69.79	68.84	71.41	1.09	0.088
Head (%)	2.45	2.53	2.58	2.66	2.45	2.47	2.23	2.33	0.12	0.314
Shank (%)	4.08	4.01	4.52	3.88	4.08	4.01	3.69	3.85	0.20	0.116
Neck (%)	2.88 ^{ab}	2.83 ^{ab}	2.79 ^{ab}	2.26 ^b	2.88 ^{ab}	2.92 ^{ab}	2.44 ^b	3.40 ^a	0.16	< 0.001
Breast (%)	20.17	20.49	19.66	18.62	20.17	19.46	20.61	18.72	0.68	0.544
Back (%)	15.41	15.26	14.37	14.77	15.41	14.21	13.94	15.56	0.48	0.287
Thigh (%)	9.52 ^{ab}	10.52 ^{ab}	9.81 ^{ab}	9.69 ^{ab}	9.52 ^{ab}	9.89 ^{ab}	9.22 ^b	10.79 ^a	0.34	0.046
Drumsticks (%)	10.03	9.34	9.43	9.15	10.03	9.74	9.07	9.46	0.29	0.575
Wings (%)	8.29	7.99	7.64	7.89	8.29	8.22	7.67	8.27	0.31	0.922
Liver (%)	2.64 ^{ab}	2.23 ^b	2.72 ^{ab}	2.82 ^{ab}	2.64 ^{ab}	2.46 ^{ab}	2.96 ^a	2.34 ^{ab}	0.15	0.054
Heart (%)	0.55	0.57	0.54	0.51	0.55	0.52	0.64	0.54	0.05	0.520
Gizzard (%)	1.85	2.02	1.91	1.72	1.85	1.92	1.77	1.88	0.09	0.443
Proventriculus (%)	0.46	0.46	0.47	0.41	0.46	0.43	0.53	0.47	0.04	0.510
GIT (%)	8.88 ^{ab}	8.84 ^{ab}	10.68 ^a	8.84 ^{ab}	8.88 ^{ab}	8.93 ^{ab}	9.03 ^{ab}	8.35 ^b	0.49	0.287
^{ab} Means on the same row having different superscript are significantly ($p < 0.05$) different										

DISCUSSION

A reliable and fast means of assessing clinical and nutritional health status of animals include the use of blood analysis, because ingestion of bioactive components have measurable effects on blood composition^{[19][20]} and may be considered as appropriate measure of long term nutritional status^[21]. Haematological studies are good indicators of the physiological status of animals^[22] and have been found useful for disease prognosis, therapeutic as well as feed stress monitoring^[23]. Haematological results in the starter phase revealed that lymphocyte and eosinophil counts differed significantly as influenced by concentration of *Lagenaria breviflora* fruit extract. This is further confirming report of Ekunseitan *et al.* (2016)^[24] that *Lagenaria breviflora* Robert (Spotted Pumpkin) possesses relevant ethno-veterinary properties in the prevention and treatment of rural poultry health management. The same trend was observed in the finisher phase where birds in the control had higher eosinophil count than birds administered *Lagenaria breviflora* fruit extract for 5days/week. Although birds were in a good state of health during this study, however, elevated WBC and eosinophil counts can be an indicator of disease or infection. It shows that the birds are producing more antibodies to fight off infection. A number of in vitro and animal studies have shown that bioactive compounds from plants increase immunologic activity by increasing phagocytosis^{[25][26]}. Phagocytosis by granulocytes is the first and major defense mechanism against invasion of bacteria, fungi, and parasites.

Nonetheless, the values obtained are within the normal reference range for domestic chickens [27]. The higher Packed cell volume in treatments administered *Lagenaria breviflora* fruit extract observed in finisher broilers in this study can be due to their inability to cause haemolysis inherent from the anti-inflammatory potential inherent in *Lagenaria breviflora*. Similar results were reported by Ekunseitan *et al.* (2017)^[18] where packed cell volume and red blood cell counts were highest in laying birds administered *Lagenaria breviflora* fruit extract.

Biochemical components are sensitive to elements of toxicity in feeds and plant extract and are particularly valuable in monitoring toxicity especially with feed constituents that affect the formation of blood [28]. The results of serum biochemical indices measured in this study revealed higher uric acid in treatments at both starter and finisher phases and it agrees with the findings of Ekunseitan *et al.* (2017)^[18] who reported significantly higher serum uric acid in laying birds administered *Lagenaria breviflora* extract than birds in the control. The kidney has been recognized as the main regulator of serum uric acid and the excretion of renal uric acid is determined by the balance of urate re-absorption and re-secretion [29]. Therefore, serum uric acid frequency of administration could be regarded as a therapeutic target for the prevention of thromboembolic events and may be an indicator of antithrombotic therapy decision [30]. In addition, ALT was higher in birds in the control than in birds administered *Lagenaria breviflora* fruit extract thus, indicating the liver health was in the best condition in birds in this treatment. Plant extracts have been reported by previous reports by Muhammad *et al.* (2020)^[31] to contain certain compounds that reduce hepatic damage. These plants are rich source of alkaloids, flavonoids, diterpenoid, tannins, lipids, sterols etc. with anti-microbial and hepatic protective effects [32].

Furthermore, the antibody titre against Newcastle disease effects of *Lagenaria breviflora* fruit extract in broiler chickens at starter and finisher phases as investigated in this present study is negligible. Though reports on the immune response of broiler chickens to *Lagenaria breviflora* extract in cold water, similar reports were presented by Faluyi and Agbede (2018)^[33] who observed no significant differences when investigating the immuno-modulatory effects of aqueous leaf extracts of *Moringa oleifera* in broiler chickens in response to Newcastle disease vaccinations.

The antibody titre values reduced as the WBC reduces in both concentrations 75 g and 125 g of *Lagenaria breviflora* fruit extract per litre of water at the starter and finisher phases. Also the titre values reduce as the concentration increased. There is consistency in the trend of immune response as evident in lymphocyte observed at both 75 g and 125 g of the extract per litre of water in the starter phase. The increase in B-lymphocyte could be attributed to the improved immune response of the birds receiving *Lagenaria breviflora* extract. This study agrees with Ali *et al.* (2018)^[34] who observed Titer of serum antibodies of Newcastle disease was maximum in treatment supplemented with CLA + HMB compared to the control. The effect of *Lagenaria breviflora* extract observed in the current study was a significant increase in WBC and lymphocytes respectively which showed an improvement in immune system. The present study is in agreement with those obtained by Jamroz *et al.*, (2005)^[35] who concluded that enzyme had no effect of HI titer on broiler chicks when compared with the chicks fed on the same diet containing different levels of palm kernel cakes without enzyme supplementation.

The response of the birds to *Lagenaria breviflora* may pose some stress in the body system of the birds thereby increasing the production of white blood cell count and lymphocyte. Moreover, the effect of the extract may have modulated the immune response of the birds. The increased lymphocyte count may be reflection of higher activity of humeral immune responses in chicks. Ali *et al.* (2018)^[34] reported that increasing in lymphocyte populations may be indicative of higher activity of humeral immune responses in chicks fed HMB and CLA supplemented diets.

The significant differences across treatments observed in carcass traits such as neck, thigh, liver and GIT as influenced by the interaction between concentration and frequency of administration of LBFE agrees with previous studies such as Alçiçek *et al.* (2003)^[36] and Jamroz and Kamel (2002)^[37] who reported significant effect of herbal extract on carcass characteristics of broiler chickens. Al Kassie (2010)^[38] also recorded positive effect in liver yield of broiler given different frequency of administration of peppermint. In contrast, Hernandez *et al.* (2004)^[39] and Amouzmehr *et al.* (2012)^[40] reported that use

herbal extracts had no significant effect on carcass characteristics. These disparities could result from differences in plant extract used and frequency of administration.

CONCLUSION

The utilization of natural plant extracts, such as *Lagenaria breviflora* extract, in broiler production offers promising results in terms of improving haematological parameters and carcass yield. The bioactive compounds present in the extract exhibit various beneficial properties, including antioxidant, antimicrobial, and immunomodulatory effects. By enhancing the birds' immune system, oxygen-carrying capacity, and nutrient utilization, *Lagenaria breviflora* extract can contribute to the overall health, performance, and profitability of broiler chicken production.

It is important to note that further research and field trials are necessary to determine the optimal dosage, duration, and long-term effects of *Lagenaria breviflora* extract supplementation. Nonetheless, the potential benefits observed in existing studies highlight the promising nature of this natural alternative in the poultry industry.

Declarations

ETHICAL APPROVAL

This research has been carried out in accordance to the Animal Ethics and guidelines as approved by the Ethics committee of the Federal University of Agriculture,, Abeokuta Ogun state, Nigeria.

CONSENT FOR PUBLICATION

Not Applicable

DATA AVAILABILITY

The datasets generated during and/or analyzed during this study are included in this article

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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AUTHORS' CONTRIBUTION

Conceptualization and study design was performed by ADELEYE O.O., material preparation, data collection and analysis were performed by ABATAN, M.O., OLORUNSOGBON, B.F. and EGBEYALE, L. T. Project administration and supervision was done by ADELEYE O.O and SONIBARE A.O. The first draft of the manuscript was written by KOLAWOLE A. O. and OLORUNSOGBON B. F. and all authors read and approved the final manuscript.

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