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# Haematological Response and Carcass Yield of Broiler Chickens Administered Spotted Pumpkin (*Lagenaria Breviflora* Robert) Fruit Extracts

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**Abstract**

*Over the years, therapeutic application of plants has been used in folkloric medicine to explore natural alternatives to 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. A total of 384 1-day-old male Arbor acres chicks were randomly distributed into six treatments; T1: control, T2: 75 g of LBFE/litre for 3days/week, T3: 75 g of LBFE/litre for 5days/week, T4: control, T5: 125 g of LBFE/litre for 3days/week and T6: 125 g of LBFE/litre for 5days/week. The experiment was laid out in a 2×3 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 Haemycytometer after dilution and Cyanomethamoglobin methods. Dressing percentage for the carcass yield were also obtained. Eosinophil counts and Uric acid were significantly ($p<0.05$) higher in birds administered 75 g of LBFE/ litre of water 5days/week. At the finisher phase, Packed cell volume was significantly ($P<0.05$) higher (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 ($P<0.05$) higher (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. The study concluded that administration of Lagenaria breviflora fruit extract to broilers enhanced the haematological indices as well as carcass yield of the birds.*

**Keywords:** Haematological response; *Lagenaria breviflora*; carcass yield; Broilers

**Running title**: Effect of *Lagenaria breviflora* fruit extract on Haematology and Carcass yield of Broilers

## Réponse hématologique et rendement de carcasse des poulets de chair administrés avec des extraits de fruits de courge tachetée (*Lagenaria Breviflora* Robert)

**Résumé**

*Au fil des années, l'application thérapeutique des plantes a été utilisée dans la médecine folklorique pour explorer des alternatives naturelles aux promoteurs de croissance antibiotiques, en raison du développement de souches bactériennes résistantes et de la présence de résidus médicamenteux dans la viande de volaille. Cette étude a examiné les effets des concentrations et des fréquences d'administration de l'extrait de fruit de Lagenaria breviflora (EFLB) sur les indices hématologiques et le rendement en carcasse des poulets de chair. 384 oiseaux ont été répartis aléatoirement en six traitements : T1 : contrôle, T2 : 75 g de LBFE/litre pendant 3 jours/semaine, T3 : 75 g de LBFE/litre pendant 5 jours/semaine, T4 : contrôle, T5 : 125 g de LBFE/litre pendant 3 jours/semaine et T6 : 125 g de LBFE/litre pendant 5 jours/semaine. L'expérience a été conçue selon un plan factoriel 2×3 et les données ont été soumises à un plan complètement randomisé. Le volume des cellules packées (VCP), le nombre de globules rouges (GR), le nombre de globules blancs (GB) et l'hémoglobine (HB) ont été déterminés à l'aide d'un hémocytomètre Neubauer amélioré après dilution et des méthodes Cyanométhémoglobine. Le pourcentage de panage pour le rendement en carcasse a également été obtenu. Les comptages des éosinophiles et de l'acide urique étaient significativement ($p<0.05$) plus*

*élevés chez les oiseaux administrés avec 75 g de EFLB/litre d'eau pendant 5 jours/semaine. À la phase de finition, le volume des cellules packées était significativement (P<0.05) plus élevé (32.25) chez les oiseaux administrés avec 75 g de EFLB/litre d'eau pendant 5 jours/semaine, tandis que les oiseaux administrés avec 125 g de EFLB/litre d'eau en remplacement des antibiotiques avaient un nombre significativement (P<0.05) plus élevé (5.00) de globules rouges. Les cuisses et le foie des oiseaux administrés avec EFLB 3 jours/semaine avaient les valeurs les plus élevées (P<0.5) de 10,79% et 2,84%, respectivement. L'étude a conclu que l'administration d'extrait de fruit de Lagenaria breviflora aux poulets de chair améliorait les indices hématologiques ainsi que le rendement en carcasse des oiseaux.*

**Mots-clés** : Réponse hématologique ; *Lagenaria breviflora* ; Rendement en carcasse ; Poulets de chair

## Introduction

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 (Calcagni and Elenkov, 2006). 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. (Ekunseitan *et al.*, 2023). Farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals, especially poultry (Sonaiya, 2004). Researchers have validated the use of the plant as an anti-implantation agent (Elujoba *et al.,* 1985), abortificient (Elujoba and Hymete, 1986), miracicide and cercaricide (Ajayi *et al.,* 2002), as well as an antibacterial agent with broad spectrum activity (Tomori *et al.,* 2007). Adeyemi *et al*. (2017) 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 (Ashayerizadeh *et al.,* 2009). 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 (Wang and Bourne, 1998). 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 (Cross *et al.,* 2007). However, Kalimuthu (2010) reported that though extract of plant which form the basis for all traditional system of medicine have been used for the treatment of various diseases, 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 (Balogun *et al.,* 2014).

Haematological indices are useful parameters that can be employed to assess the toxic potentials of plant extracts in living systems (Sunmonu and Oloyede, 2010). Balogun *et al.,* (2014) 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 (Ashafa *et al.,* 2009) 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 (Awotuyi, 1999).

Overall, it has been observed that serum biochemical and haematological parameters are improved by inclusion of phytobiotics in broiler diets (Al-Kassie, 2010). 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 (Al-Jaff, 2011).

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 (Ekunseitan *et al.,*

2017), toxicology and biochemical (Balogun *et al.*, 2014) as well as phytochemical (Adeyemi *et al.*, 2017). 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. (Google Earth, 2021).

### *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-hour intervals. After soaking for 72 hours, it was sieved with muslin cloth to obtain a clear solution.

### *Experimental design*

A total of 384 male 1-day- old Arbor acres 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 containing the appropriate doses of *Lagenaria breviflora* fruit extracts were provided *ad libitum* throughout the experiment. All vaccinations and medications 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/3days/week) | 48 | 4 | 12 |
| T3 (75 g/L/5days/ week) | 48 | 4 | 12 |
| T4 (control) | 48 | 4 | 12 |
| T5 (125 g/L/3days/week) | 48 | 4 | 12 |
| T6 (125 g/L/5days/week) | 48 | 4 | 12 |

Where:

T1: Water and synthetic antibiotic as expected for routine medication (control for 75g/L), T2: 75 g of *Lagenaria breviflora* fruit extract in 1 litre of drinking water for three consecutive days/week, T3: 75 g of *Lagenaria breviflora* fruit extract in 1litre of drinking water for five consecutive days/week, T4: Water and synthetic antibiotic as expected for routine medication (control for 125g/L), T5: 125 g of *Lagenaria breviflora* fruit extracts in one litre of drinking water for three consecutive days/week, T6: 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 2$^{nd}$ and 6$^{th}$ 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 Haemycytometer after dilution and Cyanomethamoglobin methods respectively as described by Dacie and Lewis (1991). Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpusular Haemoglobin Concentration (MCHC) were determined by the method of Hyduke (1995).

***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 plain sample bottles. Serum urea and creatinine level were determined using spectrophotometric methods described by Coles (1986). 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).

***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 min. Microtechnique of haemagglutination inhibition test was done according to Takatasy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

***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.

$$\text{Dressing percentage (\%)} = \frac{\text{Dressing Weight (g)} \ \text{X } 100}{\text{Live Weight (g)}}$$

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.

$$\text{Cut} - \text{part percentage (\%)} = \frac{\text{Cut part weight (g)} \ \text{X } 100}{\text{Live Weight (g)}}$$

***Statistical analysis***

Data obtained were subjected to 2 x 3 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 (2021) 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 (mg/100g) | 75 g of LBFE/L of water |
|---|---|
| Tannins | 20.95 |
| Reducing sugars | 30.26 |
| Cardiac glycoside | 36.73 |
| Alkaloids | 44.12 |
| Saponins | 40.48 |
| Flavonoids | 33.94 |
| Phenol | 28.81 |

***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/L of the extract. However, eosinophil count was significantly ($p<0.05$) higher (2.81%) in birds administered 75 g of LBFE /L 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**

| | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/L** | **125 g/L** | **SEM** | **P value** | **Control** | **3days/wk** | **5days/wk** | **SEM** | **P value** |
| Packed Cell Volume (%) | 35.03 | 34.25 | 1.23 | 0.655 | 34.38 | 35.88 | 34.56 | 1.74 | 0.851 |
| White Blood Cell count (g/L) | 73125 | 66125 | 10194 | 0.629 | 109125[a] | 75250[ab] | 57750[ab] | 14416 | 0.006 |
| Red Blood Cell Count ($10^{12}$/L) | 4.47 | 4.91 | 0.29 | 0.290 | 4.50 | 4.88 | 4.94 | 0.41 | 0.762 |
| Heterophil (%) | 58.91 | 56.44 | 2.46 | 0.482 | 58.25 | 57.00 | 56.19 | 3.48 | 0.929 |
| Lymphocyte (%) | 30.09[b] | 36.97[a] | 2.11 | 0.025 | 35.63 | 30.69 | 36.06 | 2.98 | 0.484 |
| Monocyte (%) | 5.03 | 5.44 | 0.59 | 0.628 | 4.75 | 4.44 | 4.56 | 0.83 | 0.072 |
| Eosinophil (%) | 2.81[a] | 1.50[b] | 0.37 | 0.014 | 1.63 | 1.25 | 2.94 | 0.52 | 0.057 |
| Basophil (%) | 0.31 | 0.28 | 0.15 | 0.887 | 0.00 | 0.38 | 0.25 | 0.22 | 0.329 |
| Heterophil : Lymphocyte | 1.96 | 1.53 | | | 1.63 | 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** | **SEM** | **P value** |
|---|---|---|---|---|---|---|---|---|
| Packed Cell Vol. (%) | 34.38 | 36.38 | 34.13 | 34.38 | 35.38 | 35.00 | 2.46 | 0.875 |
| WB C (g/L) | 109125 | 73625 | 79125 | 109125 | 76875 | 36375 | 20387 | 0.549 |
| R B C $\times 10^{12}$/L | 4.50 | 4.50 | 4.75 | 4.50 | 5.25 | 5.13 | 0.58 | 0.921 |
| Heterophil (%) | 58.25 | 55.88 | 59.63 | 58.25 | 58.13 | 52.75 | 4.93 | 0.767 |
| Lymphocytes (%) | 35.63 | 24.88 | 31.25 | 35.63 | 36.50 | 40.88 | 4.22 | 0.539 |
| Monocytes (%) | 4.75 | 4.63 | 4.62 | 4.75 | 4.25 | 4.50 | 1.18 | 0.697 |
| Eosinophils (%) | 1.63[ab] | 1.50[ab] | 4.50[a] | 1.63[ab] | 1.00[b] | 1.38[ab] | 0.73 | 0.161 |
| Basophil (%) | 0.00 | 0.63 | 0.00 | 0.00 | 0.13 | 0.50 | 0.31 | 0.449 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Heterophil:lymphocyte | 1.63 | 2.25 | 1.91 | 1.63 | 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**

| HN | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/L** | **125 g/L** | **SEM** | **P value** | **Control** | **3 days/week** | **5 days/week** | **SEM** | **P value** |
| Uric acid (mg/dL) | 22.06 | 23.67 | 3.46 | 0.744 | 14.38[b] | 20.78[ab] | 35.19[a] | 4.90 | 0.030 |
| Creatinine (mg/dL) | 1.919 | 1.413 | 0.502 | 0.479 | 0.863 | 1.388 | 2.025 | 0.710 | 0.444 |
| ALT (U/L) | 16.06 | 18.97 | 4.49 | 0.649 | 10.13 | 21.94 | 18.87 | 6.34 | 0.586 |
| AST (U/L) | 21.62 | 20.06 | 6.08 | 0.856 | 8.75 | 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 /L of water 5 days/week while lowest values (11.50mg/dL) was recorded in birds administered 75 g of LBFE /L 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** | **SEM** | **P value** |
|---|---|---|---|---|---|---|---|---|
| Uric acid (mg/dL) | 14.38[b] | 11.63[b] | 50.75[a] | 14.38[b] | 29.92[ab] | 19.62[b] | 6.93 | 0.002 |
| Creatinine (mg/dL) | 0.86 | 0.63 | 2.87 | 0.86 | 2.15 | 1.17 | 1.00 | 0.298 |
| ALT (U/L) | 10.13 | 8.63 | 30.12 | 10.13 | 35.25 | 7.62 | 8.97 | 0.064 |
| AST (U/L) | 8.8 | 26.5 | 38.6 | 8.8 | 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**

| | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/L** | **125 g/L** | **SEM** | **P value** | **Control** | **3days/ week** | **5days/ week** | **SEM** | **P value** |
| Packed Cell Volume (%) | 26.56 | 26.84 | 1.11 | 0.858 | $22.13^{b}$ | $27.62^{ab}$ | $29.87^{a}$ | 1.57 | 0.008 |
| White Blood Cell (g/L) | 18000 | 21937 | 4311 | 0.521 | 16875 | 27500 | 20562 | 6097 | 0.486 |
| Red Blood Cell ($\times10^{12}$/L) | 3.688 | 3.875 | 0.147 | 0.370 | 3.375 | 3.500 | 4.125 | 0.207 | 0.016 |
| Heterophil (%) | 62.16 | 63.81 | 2.18 | 0.593 | 63.88 | 58.25 | 63.31 | 3.08 | 0.300 |
| Lymphocytes (%) | 30.44 | 28.06 | 2.08 | 0.423 | 26.13 | 35.06 | 30.06 | 2.95 | 0.102 |
| Monocytes (%) | 3.531 | 4.656 | 0.404 | 0.054 | 5.000 | 3.563 | 3.938 | 0.571 | 0.318 |
| Eosinophils (%) | 3.531 | 3.281 | 0.424 | 0.678 | $4.875^{a}$ | $2.813^{ab}$ | $2.313^{b}$ | 0.599 | 0.022 |
| Basophil (%) | 0.187 | 0.188 | 0.116 | 1.000 | 0.125 | 0.125 | 0.250 | 0.164 | 0.900 |
| Heterophil :Lymphocyte | 2.04 | 2.27 | | | 2.44 | 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** | **SEM** | **P value** |
|---|---|---|---|---|---|---|---|---|
| Packed Cell Volume (%) | $22.13^{b}$ | $29.25^{ab}$ | $32.25^{a}$ | $22.13^{b}$ | $26.00^{ab}$ | $27.50^{ab}$ | 2.21 | 0.013 |
| White Blood Cell (g/L) | 16875 | 28250 | 7750 | 16875 | 26750 | 33375 | 8622 | 0.226 |
| Red Blood Cell($\times10^{12/}$L) | $3.375^{b}$ | $3.875^{ab}$ | $4.250^{ab}$ | $3.375^{b}$ | $3.125^{b}$ | $4.000^{ab}$ | 0.293 | 0.001 |
| Heterophil (%) | 63.88 | 54.88 | 65.25 | 63.88 | 61.63 | 61.38 | 4.36 | 0.645 |
| Lymphocytes (%) | 26.13 | 38.25 | 29.37 | 26.13 | 31.88 | 30.75 | 4.17 | 0.763 |
| Monocytes (%) | 5.000 | 3.000 | 2.375 | 5.000 | 4.125 | 5.500 | 0.808 | 0.214 |
| Eosinophils (%) | 4.875 | 3.500 | 2.250 | 4.875 | 2.125 | 2.375 | 0.847 | 0.754 |
| Basophil (%) | 0.125 | 0.000 | 0.500 | 0.125 | 0.250 | 0.000 | 0.231 | 0.331 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Heterophil : Lymphocyte | 2.44 | 1.43 | 2.22 | 2.44 | 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**

| | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/1l** | **125 g/1l** | **SEM** | **P value** | **Control** | **3days/ wk** | **5days/ wk** | **SEM** | **P value** |
| Uric acid (mg/dL) | 22.59 | 25.56 | 2.24 | 0.353 | 17.88[b] | 20.63[b] | 25.00[ab] | 3.17 | 0.009 |
| Creatinine (mg/dL) | 0.988 | 1.337 | 0.160 | 0.128 | 0.850 | 1.150 | 1.006 | 0.226 | 0.087 |
| ALT (U/L) | 15.47 | 12.41 | 2.38 | 0.368 | 23.25[a] | 10.88[ab] | 10.56[b] | 3.37 | 0.024 |
| AST (U/L) | 11.28 | 9.25 | 1.76 | 0.417 | 10.25 | 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** | **SEM** | **P value** |
|---|---|---|---|---|---|---|---|---|
| Uric acid (mg/dL) | 17.88 | 15.00 | 24.87 | 17.88 | 26.25 | 25.12 | 4.49 | 0.523 |
| Creatinine (mg/DL) | 0.850 | 1.013 | 0.925 | 0.850 | 1.287 | 1.087 | 0.320 | 0.460 |
| ALT (U/L) | 23.25 | 12.63 | 14.50 | 23.25 | 9.13 | 6.63 | 4.77 | 0.843 |
| AST (U/L) | 10.25 | 8.63 | 13.25 | 10.25 | 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**

| | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/L** | **125 g/L** | **SEM** | **P value** | **Control** | **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 | 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 | 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

T**able 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** | **SEM** | **P value** |
|---|---|---|---|---|---|---|---|---|
| Antibody titre at starter phase ($log_2$gmt) | 13.8 | 13.8 | 20.7 | 13.8 | 20.3 | 4.5 | 24.7 | 0.418 |
| Antibody titre at finisher phase ($log_2$gmt) | 12.00 | 14.75 | 11.50 | 12.00 | 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**

| | Concentration | | | | Frequency of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **75 g/L** | **125 g/L** | **SEM** | **P value** | **Control** | **3days/wk** | **5days/wk** | **SEM** | **P value** |
| Live weight (kg) | 1.75 | 1.70 | 0.04 | 0.377 | 1.76 | 1.71 | 1.67 | 0.05 | 0.629 |
| Defeathered weight(kg) | 1.60 | 1.56 | 0.03 | 0.416 | 1.63 | 1.58 | 1.51 | 0.05 | 0.391 |
| Dressed weight (kg) | 1.23 | 1.19 | 0.03 | 0.474 | 1.27 | 1.18 | 1.17 | 0.04 | 0.269 |
| Dressing % | 70.17 | 70.46 | 0.55 | 0.711 | 71.79 | 69.09 | 69.62 | 0.77 | 0.075 |
| Head (%) | 2.55[a] | 2.37[b] | 0.06 | 0.026 | 2.45 | 2.39 | 2.49 | 0.08 | 0.803 |
| Shank (%) | 4.12 | 3.91 | 0.10 | 0.137 | 4.08 | 4.10 | 3.86 | 0.14 | 0.632 |
| Neck (%) | 2.69 | 2.91 | 0.08 | 0.056 | 2.88 | 2.61 | 2.83 | 0.11 | 0.319 |
| Breast (%) | 19.74 | 19.74 | 0.34 | 0.992 | 20.17 | 20.14 | 18.67 | 0.48 | 0.094 |
| Back (%) | 14.95 | 14.78 | 0.24 | 0.613 | 15.41 | 14.16 | 15.17 | 0.34 | 0.057 |
| Thigh (%) | 9.89 | 9.86 | 0.17 | 0.902 | 9.52 | 9.51 | 10.24 | 0.24 | 0.041 |
| Drumsticks (%) | 9.49 | 9.58 | 0.15 | 0.682 | 10.03 | 9.25 | 9.30 | 0.21 | 0.045 |
| Wings (%) | 7.95 | 8.11 | 0.16 | 0.482 | 8.29 | 7.66 | 8.08 | 0.22 | 0.241 |
| Liver (%) | 2.60 | 2.59 | 0.07 | 0.980 | 2.64[ab] | 2.84[a] | 2.58[ab] | 0.10 | 0.013 |
| Heart (%) | 0.54 | 0.56 | 0.03 | 0.548 | 0.54 | 0.59 | 0.53 | 0.04 | 0.625 |
| Gizzard (%) | 1.88 | 1.85 | 0.05 | 0.749 | 1.85 | 1.84 | 1.79 | 0.07 | 0.356 |
| Proventriculus (%) | 0.45 | 0.47 | 0.02 | 0.421 | 0.46 | 0.50 | 0.44 | 0.03 | 0.330 |
| GIT (%) | 9.31 | 8.79 | 0.25 | 0.148 | 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 | SEM | P value |
|---|---|---|---|---|---|---|---|---|
| Live weight (kg) | 1.76 | 1.76 | 1.66 | 1.76 | 1.66 | 1.68 | 0.075 | 0.763 |
| Defeathered weight (kg) | 1.63 | 1.64 | 1.49 | 1.63 | 1.51 | 1.53 | 0.073 | 0.638 |
| Dressed weight (kg) | 1.27 | 1.22 | 1.13 | 1.27 | 1.14 | 1.20 | 0.057 | 0.401 |
| Dressing percent (%) | 71.80 | 69.35 | 67.83 | 71.80 | 68.84 | 71.41 | 1.09 | 0.088 |
| Head (%) | 2.45 | 2.58 | 2.66 | 2.45 | 2.23 | 2.33 | 0.12 | 0.314 |
| Shank (%) | 4.08 | 4.52 | 3.88 | 4.08 | 3.69 | 3.85 | 0.20 | 0.116 |
| Neck (%) | $2.88^{ab}$ | $2.79^{ab}$ | $2.26^{b}$ | $2.88^{ab}$ | $2.44^{b}$ | $3.40^{a}$ | 0.16 | <0.001 |
| Breast (%) | 20.17 | 19.66 | 18.62 | 20.17 | 20.61 | 18.72 | 0.68 | 0.544 |
| Back (%) | 15.41 | 14.37 | 14.77 | 15.41 | 13.94 | 15.56 | 0.48 | 0.287 |
| Thigh (%) | $9.52^{ab}$ | $9.81^{ab}$ | $9.69^{ab}$ | $9.52^{ab}$ | $9.22^{b}$ | $10.79^{a}$ | 0.34 | 0.046 |
| Drumsticks (%) | 10.03 | 9.43 | 9.15 | 10.03 | 9.07 | 9.46 | 0.29 | 0.575 |
| Wings (%) | 8.29 | 7.64 | 7.89 | 8.29 | 7.67 | 8.27 | 0.31 | 0.922 |
| Liver (%) | $2.64^{ab}$ | $2.72^{ab}$ | $2.82^{ab}$ | $2.64^{ab}$ | $2.96^{a}$ | $2.34^{ab}$ | 0.15 | 0.054 |
| Heart (%) | 0.55 | 0.54 | 0.51 | 0.55 | 0.64 | 0.54 | 0.05 | 0.520 |
| Gizzard (%) | 1.85 | 1.91 | 1.72 | 1.85 | 1.77 | 1.88 | 0.09 | 0.443 |
| Proventriculus (%) | 0.46 | 0.47 | 0.41 | 0.46 | 0.53 | 0.47 | 0.04 | 0.510 |
| GIT (%) | $8.88^{ab}$ | $10.68^{a}$ | $8.84^{ab}$ | $8.88^{ab}$ | $9.03^{ab}$ | $8.35^{b}$ | 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 (Church *et al.,* 1984; Maxwell *et al.,* 1990) and may be considered as appropriate measure of long term nutritional status (Togun and Oseni, 2005, Olabanji *et al.,* 2007). Haematological results in the starter phase of this study 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) 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 the 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 (Geetha *et al.,* 2012; Lillehoj *et al*., 2018). 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 (Jain, 1986). 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) 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 (Oyawoye and Ogunkunle, 1998). 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) on 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 (Bobulescu and Moe, 2012). 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 (Dogan *et al.,* 2016). 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) 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 (Mangwani *et al.,* 2019).

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. Similar reports were presented by Faluyi and Agbede (2018) 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 reduced 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 reduced 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) 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 Soltan (2009) 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) 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 by Jamroz and Kamel (2002) and Alçiçek *et al.* (2003) that significant effect of herbal extract was recorded on carcass characteristics of broiler chickens. Al Kassie (2010) also recorded positive effect in liver yield of broiler administered different frequency of administration of peppermint. In contrast, Hernandez *et al.* (2004) and Amouzmehr *et al*. (2012) reported that use of herbal extracts had no significant effect on carcass characteristics. These disparities could result from differences in plant extract used and frequency of administration.

**Conclusion**

This study has been able to show that the administration of *Lagenaria breviflora* fruit extract to broiler birds can potentiate their haematological indices as well as improve the carcass yield. This invariable implies higher yield and more availability of meat to consumers.

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