Effect of Fermented Lagenaria (Adenopus breviflorus) Fruit Extract on the Hematological and Serum Biochemical Indices of Broiler Chickens

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Abstract
The experiment involved 126 day old broiler chicks (Arbor acre plus) which lasted for six weeks after two weeks of acclimatization. The project was carried out in a completely randomized block design to evaluate the haematological and serum biochemical parameters of broilers served fermented lagenaria fruit extract (FLFE) at three days interval. The birds were weighed and randomly distributed into six dietary treatment group. Birds in treatment A (control) were given vaccine and drugs only, birds in treatment B were given vaccine only, birds in treatment C were given drugs only, but birds in treatments D, E and F were served (100, 200 and 300)ml of FLFE in 250ml of water, respectively. Each treatment was replicated three times with seven birds per replicate. The birds were maintained on starter and finisher marsh for starter and finisher phase, respectively. Feeds and water were served ad libitum. Data collected were subjected to Analysis of Variance (ANOVA) and comparisons were made using Duncan’s Multiple Range Test and significance was accepted at (P<0.05). The parameters tested were packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet (P), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration(MCHC), lymphocyte (LYM), heterocytes (HET), monocytes (Mn), eosinophils for haematological indices and total serum protein (TSP), albumin(Al), globumin (Gb), creatinine (Cr), alanine amino transminase (ALT), aspartate amino transminase (AST), alkaline phosphate (ALP), blood urea nitrogen (BUN) and cholesterol (CH) for serum biochemistry. The results showed significant (P<0.05) effects of the parameters studied across the treatment groups. The PCV was highest (40.00%) on the birds placed in control, and least on the birds served 100-300ml FLFE (29.50-33.00%). Similar scenario was observed for the Hb (13.20%) compared to those served 100-300ml FLFE (9.40-10.70%). The birds in treatments C (drugs only), D (100ml FLFE) and those in F (300ml FLFE) had the highest concentration of white blood cells which were 1.92, 2.12 and 1.87x10³/ml, respectively. The birds served 100-300ml FLFE had reduced concentration of Hb of 21.25% compared to control, and they had elevated concentration of WBC of 25.82% compared to control. The birds placed on vaccines only and drugs only had elevated WBC of 26.00 and 34.50%, respectively compared to control. The FLFE of 100-200ml had elevated platelet (353.00-314.50x10³/ml) with reference to control. The FLFE of 100-200ml had elevated platelet (353.00-314.50x10³/ml) with reference to control. The FLFE had no significant (P<0.05) effect on TSP, GB, AL, ALP and BUN. However, concentration of CH increased with increased concentration of FLFE as the bird offered 100ml had CH of 42.50mg/dl, compared to those on 200ml and 300ml whose value were 68.50 and 89.00mg/dl, respectively. Broiler chicken can tolerate 100-300ml FLFE for improved blood formation.

Keywords: Lagenaria, Hematology, Serum biochemistry, Broiler, Vaccine

Introduction
The fruit of lagernaria (Adenopus breviflorus) is widely used in folklore medicine in west Africa as herbal for the treatment of measles, digestive disorder and as wound antiseptic (e.g umbilical incision wound) in man, while the livestock farmers used it for the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry (Sonaiya, 1995). It is a perennial climber ascending to the forest canopy, occurring from Senegal to the West Cameroons, and generally wide spread in tropical Africa.

The family is a diverse family of plant in the temperate zones but also thrives in hot arid region of the world (Weihrauch and Feter, 1994). In Nigeria different tribal group have their indigenous name as: Ogbenwa in igbo, Tagiri in Yoruba, Luddal in Hausa (Burkill, 1995). In addition to its medicinal application, so much has been reported on the taxonomy (Morimote et al., 2005). Lagenaria breviflora (wild colocynth) is one of those plants with characteristics antibacterial and antiviral herbal remedies in local communities such as Nigeria (El-mahmood et al., 2008). Extract of plant which form the basis for all traditional system of medicine have been used for the treatment of various disease as reported by (Kalimuthu, 2010) and the medicinal values of these plant lie in their component photochemical mostly alkaloids, tannins, falconoid and phenotic compound. The leaves are extremely scabrid and sandpapery while the fruit are dark green with creamy breaches (Tomori et al., 2007). In some African countries including Nigeria, a good percentage of the populace relies exclusively on plants as a source of medicine to complement and supplement the increasingly expensive orthodox medicine service (Fashola, 2005 and Oridup, 2011). The stem when crushed has an unpleasant smell and a decoction
from it is said to be used in Africa for headache and a vermifuge (Ajayi et al., 2002). In both first and third world countries, the search alternative has also triggered a re-evaluation and appreciation of ethno-science: local or indigenous and so forth (Brokensha et al., 1993). In the bid to adequately prevent and control various disease, proper management, feeding and the use of different types of drugs are recommended and have found effective. The drugs used are mainly orthodox or conventional. However, because of foreign exchange and non-availability of the drugs, farmers are gradually embracing ethno-veterinary practice in the treatment of animal disease. The rational, being that the active ingredient in most drugs are from plant origin.

Blood profiles are important indices of the physiological state of animals (Khan and Zafar, 2005). Blood constituent provide valuable media for clinical investigation and nutrition evaluation of an animal, (Aderemi, 2004), the ingestion of numerous dietary materials has been reported by (Church et al., 1984) to have measurable effect on blood constituent. Thus, blood provides proximate measure for long term nutritional state of animal (Kerr et al., 1982). However, blood varies with certain condition such as stress, infection and toxicity (Khan and Zafar, 2005).

Lagenaria (Adenopus breviflorus) is used in animal production due to its anti-microbial property, however it is used in the treatment of measles in human being. It is also assumed that use of lagenaria improve the immunity of broiler birds against infection. It is available throughout the whole country, although seasonal; however, it is available in the market throughout the year.

**Importance of haematology and serum biochemistry in poultry production.**

For any successful livestock production enterprise, the analysis of blood is important clinically, because it provides a means of assessing the health status of animals. 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). Normal haematology parameters for a given species of animals are affected by sex, nutrition, strain, age, climate (Onyeli et al., 1991). Ross et al., (1978) concluded that blood parameters are important in assessing the suitability and quality of feed ingredients and in farm animals. Coles (1986) hinted that the excitement, exercise, state of bone marrow, blood loss, excessive destruction of the cells, infection and pregnancy dramatically affect the haemathology of most species of animal. Awotuyi (1999) revealed that pack cell volume (PVC) of local and commercial chickens in Ghana varied from 32.88 to 33.20 to 35.60% respectively. PVC of 23.33 to 30.50% when broiler were fed soldier fly larvae meal, while Nworgwu et al., (2003) reported PVC of 28 to 30.0% for cockerel chicks fed cocoa pod husk meal serum is the supranatant fluid which forms when blood clots and the measurement of its chemical constitutes is useful for the evaluation of physiologic and pathologic alterations in animals (Cole, 1986). Sturkle et al., (2000) noted that an increase in cholesterol in birds varies from 100-150mg/dl; higher values of cholesterol depict hyperlipemia indicating that the patient is likely to have heart disease. Serum creatinine value of 1.40-1.42mg/dl when broiler chickens were fed fermented copra meal-based diets are revealed that the serum creatinine which is an indirect measure of protein utilization was not significant.

**MATERIALS AND METHODS**

The experiment was carried out at the student project site Bora of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The experiment lasted for eight (8) weeks. A total number of 126 day-old broiler chicks of arbor acre plus were used for this experiment and randomly distributed to dietary treatment A, B, C, D, E and F respectively.

The experimental design was complete randomized design. There were six (6) treatments with three (3) replicates each. The dietary treatments were:

- **Treatment A:** Vaccination and conventional drugs (control).
- **Treatment B:** Vaccines only.
- **Treatment C:** Conventional drugs only.
- **Treatment D:** 100ml of fermented Lagenaria fruit extract in 250ml of water.
- **Treatment E:** 200ml of fermented Lagenaria fruit extract in 250ml of water.
- **Treatment F:** 300ml of fermented Lagenaria fruit extract in 250ml of water.

Fermented lagenaria fruit extract was prepared for four (4) days before administration. 2kg of freshly harvested lagenaria were detached from the stalk, washed, sliced and later immersed in one liter (1L) of water in a bucket and allow to ferment for 4 days. The lagenaria was drained and the extract was collected by squeezing the lagenaria and filtered with sieve to obtain a homogenous extract of the lagenaria 100ml, 200ml, 300ml of the extract were collected using measuring bottles into 250ml of water in a drinker for drinking.

The pens were thoroughly washed, cleaned and disinfected before the arrival of the birds. Eighteen pens were used for the experiment. The pens were allows to dry and wood shaving were spread on the floor of the pen before one week the birds were supplied to the pen. The birds.

Blood collection was carried out at the 8th week of the experiment. Three birds per treatment were randomly selected and bled via wing vein. About 5ml of the blood was collected into two set of sterilized glass bottle for
haematology, the blood sample were collected into sterilized bottle containing ethylene diaminotetra acetic acid (EDTA). Blood sample for serum biochemical analysis were collected into plain sample bottles (i.e. without anti-coagulant) for serum separation. Serum was obtained by centrifugation and was delivered the same day to the laboratory for further analysis.

Results

Table 1: Haematological parameters of broilers administered fermented Lagenaria fruit extract

<table>
<thead>
<tr>
<th>Fruit extract Parameters</th>
<th>A (100ml) FLFE</th>
<th>B (200ml) FLFE</th>
<th>C (300ml) FLFE</th>
<th>D (100ml) FLFE</th>
<th>E (200ml) FLFE</th>
<th>F (300ml) FLFE</th>
<th>± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (%)</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>1.18</td>
</tr>
<tr>
<td>Haemoglobin (%)</td>
<td>13.20</td>
<td>13.20</td>
<td>13.20</td>
<td>13.20</td>
<td>13.20</td>
<td>13.20</td>
<td>0.43</td>
</tr>
<tr>
<td>Red blood cell (x10^6)</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>0.10</td>
</tr>
<tr>
<td>White blood cell (x10^6)</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
<td>0.10</td>
</tr>
<tr>
<td>Platelet (x10^3)</td>
<td>271.5</td>
<td>271.5</td>
<td>271.5</td>
<td>271.5</td>
<td>271.5</td>
<td>271.5</td>
<td>15.60</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>104.4</td>
<td>104.4</td>
<td>104.4</td>
<td>104.4</td>
<td>104.4</td>
<td>104.4</td>
<td>1.84</td>
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<tr>
<td>Mean haemoglobin(pg/cell)</td>
<td>33.47</td>
<td>33.47</td>
<td>33.47</td>
<td>33.47</td>
<td>33.47</td>
<td>33.47</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean cell haemoglobin concentration (%)</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>0.14</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>63.5</td>
<td>63.5</td>
<td>63.5</td>
<td>63.5</td>
<td>63.5</td>
<td>63.5</td>
<td>1.46</td>
</tr>
<tr>
<td>Heterocyte (%)</td>
<td>29.5</td>
<td>29.5</td>
<td>29.5</td>
<td>29.5</td>
<td>29.5</td>
<td>29.5</td>
<td>1.34</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>0.32</td>
</tr>
</tbody>
</table>

a, b, c, d : means with different superscript on the same horizontal row differ significantly (p<0.05)

VF - Vaccines and drugs  V - Vaccines  D - Drugs

FLFE = Fermented Lagenaria Fruit Extract

The results of the serum biochemical indices of broilers served FLFE revealed that total serum protein (TSP), albumin (Al), globulin (Gb), alkaline phosphates (ALP), blood Urea nitrogen (BUN) were not significant (p>0.05) unlike creatinine (Cr), glucose (Gl) aspartate amino transaminase (AST), alanine amino transaminase (ALT) and cholesterol(CH)which were significant (P<0.05)among the treatments. The concentration of TSP, Al and ASP were highest on the birds served 100ml of FLFE in 250ml of water and the concentration of Gb were highest on the birds served 100ml of FLFE in 250ml of water and on the birds given drugs only. The concentrations of Cr, Gl and BUN were highest on the birds served 200ml of FLFE in 250ml of water. The concentration of ALT and ALP were highest on the birds given drugs only and the concentration of CH were highest on the birds served 300ml of FLFE in 250ml of water (Table 1).

Table 2: Serum biochemistry of broilers administered fermented Lagenaria fruit extract

<table>
<thead>
<tr>
<th>Fruit extract Parameters</th>
<th>A (100ml) FLFE</th>
<th>B (200ml) FLFE</th>
<th>C (300ml) FLFE</th>
<th>D (100ml) FLFE</th>
<th>E (200ml) FLFE</th>
<th>F (300ml) FLFE</th>
<th>± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein(g/dl)</td>
<td>5.15</td>
<td>5.25</td>
<td>5.30</td>
<td>5.55</td>
<td>4.95</td>
<td>5.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
<td>1.50</td>
<td>1.20</td>
<td>1.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Globulin (g/gl)</td>
<td>3.90</td>
<td>4.00</td>
<td>4.05</td>
<td>4.05</td>
<td>3.75</td>
<td>3.90</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>272.5</td>
<td>402.5</td>
<td>327.5</td>
<td>424.00</td>
<td>449.50</td>
<td>379.00</td>
<td>20.98</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>271.5</td>
<td>188.5</td>
<td>268.00</td>
<td>353.00</td>
<td>314.50</td>
<td>221.50</td>
<td>15.60</td>
</tr>
<tr>
<td>Aspartate amino</td>
<td>178.00</td>
<td>190.50</td>
<td>186.50</td>
<td>231.00</td>
<td>192.00</td>
<td>197.00</td>
<td>4.80</td>
</tr>
<tr>
<td>Alanine amino</td>
<td>29.50</td>
<td>32.00</td>
<td>24.00</td>
<td>35.00</td>
<td>40.00</td>
<td>29.50</td>
<td>1.79</td>
</tr>
<tr>
<td>Transaminase (µ/L)</td>
<td>237.5</td>
<td>370.5</td>
<td>268.50</td>
<td>222.50</td>
<td>241.50</td>
<td>245.00</td>
<td>19.86</td>
</tr>
<tr>
<td>Alkaline phosphates</td>
<td>14.50</td>
<td>14.60</td>
<td>15.30</td>
<td>14.70</td>
<td>15.70</td>
<td>13.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>78.50</td>
<td>42.00</td>
<td>46.00</td>
<td>42.50</td>
<td>68.50</td>
<td>89.00</td>
<td>5.09</td>
</tr>
</tbody>
</table>

abc: means with different superscript on the same horizontal row differ significantly (P<0.05); VF - Vaccines and drugs; V – Vaccines; D – Drugs FLFE - Fermented Lagenaria Fruit Extract
4.2 Discussion

The diets met the recommendation nutrient requirement for broiler starter and finisher and it is in line with (NRC, 1994) standard. The fermented lagenaria fruit extract (FLFE) is a valuable fed supplement for broiler, especially during the late dry season of the year and the methanol extracts of FLFE contain some secondary metabolites such as alkaloid, tannins, terpenoid, flavonoids and reducing sugar as established by (Cowan, 1999). The flavonoid and tannins which are present in the FLFE is indicative of its anti-inflammatory and analgesic effects, the flavonoids and tannins are phenolic compounds suggestive of its primary antioxidants as documented by (Ayoola et al., 2008) and (Adedapo, 2013).

*Lagenaria breviflora* has high water content which was evident in the moisture value reported in this study and also have a cooling and lubricating effect when used (Tomori et al., 2007). The phytochemicals present in Lagenaria fruit extract (FLFE) display various mechanisms of action such as inhibiting intestinal maturity, while some components have been shown to inhibit specific pathogen (Ahmad et al., 2006). Balogun et al. (2014) reported that the liver section from group of animals administered Lagenaria fruit extract orally at all doses of the extract revealed or showed no gross lesion when compared with the control.

The birds in control group that have the highest PCV, Hb and RBC among all the birds served FLFE. The FLFE significantly increase (P<0.05) the serum levels of creatinine in all treatment groups when compared to the control and it is similar to the submission of Balogun et al., (2014) unlike when the authors experimented with *Ocimum gratissimum* effect on blood parameters in growing pullets.

However, the TSP in this study was maintain by slightly increase of albumin fraction with corresponding decrease in globulin fraction. Creatinine and Total Serum Protein values in the present study are lower with reference to Nworgu et al., (2003). Balogun et al., (2014) reported that albino rats served Lagenaria ethanolic fruit extract had elevated urea, creatinine and total bilirubin in all treatment group when compared to the control in close-dependent manner. The authors further revealed that 100, 200, 300 and 400mg/kg/b.w.t/day of ethanolic extract of *Adenopus breviflorus* (EEAB) had no significant (p<0.05) effect on haematological indices of albino rats. Variation could be due to frequency and dosage of administration. Ekunseitan et al., (2017) concluded that *Adenopus breviflorus* fruits extract of up to 300g/4l of water had no deleterious effects on the health of laying birds, while Oyedele et al., (2015) concluded that *Adenopus breviflorus* had a little toxic effect and a lot of beneficial potentials on the hematological and blood chemistry parameters of male Wistar rats. Lagernaria has been employed ethnomedically as therapeutic cure for a variety of diseases (Oriskayeye and Johnson, 2015).

Ekunseitan et al., (2017) concluded that bird dosed 200g L. breviflora had higher PCV, Hb and RBC compared to 0 and 300g dosed unlike the birds placed on 300 g that had declined PCV and RBC. These authors noted that increased *L. breviflora* administration resulted in increased serum glucose (GL) except at 16 weeks in lay. Serum glucose in the present study varied from 272mg/dl in control to 449.50mg-dl for birds served 200ml FLFE. The GL reported here is higher than the report of Ekuseitan et al., (2017) (211.67-212.88mg/dl) except for control. The PCV (29.50-40.00%), Hb (9.40-13.20%) and RBC (3.05-3.83) (x10^12/L) are higher than the values reported by Ekunseitan et al., (2017) (28.05-29.65%, 9.50-9.88% and 2.36-2.98(x10^12)/L respectively. However, the values of Hb (9.0-13.0%), RBC (3.0x10^12) and PCV (30.0-40.0%) reported by MVM (1986) are in harmony with the present study. However, PCV and HB in this study were within the range by Jain (1993) whose values were 22-35.00% for PCV and 7-13% for Hb for birds. The increased in PCV in this study can be attributed to the elevation in both red and white blood cell indices. Variation of the hematological and serum biochemical value could be due to the quality of diets used and the effect of best ingredients. In the present study no fibre feed source/ingredient was used. Increase in LY in all the treatments is an indication of enhanced immunological well being of the birds. The TCP components of birds served 100-300ml FLFE are similar to that of control. Saba et al., (2009) reported that increased blood proteins are mainly created by hepatocytes with the assumption that the comparable values of these protein components can be a direct influence of *Adenopus breviflorus*.

The birds served 100-300ml FLFE had elevated glucose level of 53.21% (379.00-449.50mg/dl) compared to control (272.50mg/dl). This result agrees with the reports of Ekuseitan et al., (2015) and Saba et al., (2010) as these authors reported high glucose values when laying chickens and Wistar rats were served different doses of *Adenopus breviflorus* fruit extract. Esuoso and Bayer, (1998) affirmed that the fruits of *Adenopus breviflorus* have high amino acids content which may led to increased gluconegensis through the intermediate metabolic pathways (Lehninger et al., 1993). The ALT and AST in this study for the birds offered 100-300ml FLFE were 35.00-40.00iu/L and 192.00-231.00iu/L, respectively and these values correspond with values of the birds in control (29.50 and 179.00iu/L), respectively.

The ALT is known to increase in liver disease (Adedapo et al., 2009 and Bush, 1991). However, based on the values of the blood parameters and survival rate of 95-100% of the birds, liver damage could not be suggested. Oriskayeye and Johnson (2015) reported that pharmacological study of *Lagena ala breviflor a* revealed its anti-implantation, anti-bacterial, antiulcer, anti-inflamatory, antioxidant, miricalid and cercidial activity and anti-fertility and that its phytoconstituents include triterpenoids, curcubitacin, phenolic acid, vanillic acids and...
ferulic acid. *Lagenaria breviflora* is commonly used to nutritionally support the immune system when stressed such as during viral fungi, candida infections and inflammations (Ekuseitan et al., 2016). Ekunseitan et al., (2017) concluded that the use of extract of *L. breviflora* up to 300g per 4 litres of water had no deleterious effect on the health of the birds when raised on modified housing systems.

**Conclusion**
The FLFE is rich in phytochemicals and low in fibre and protein. The birds served 100-300ml FLFE had reduced concentration of Hb of 21.25% compared to the control and they have elevated concentration of WBC of 25.82% with reverence to control, but have elevated glucose level. The broiler chickens tolerated 100-300ml FLFE which was served at 3 days interval for six weeks with the survival rate of 95-100%.The values of the hematological and serum biochemical values of the birds served 100-300ml of FLFE in 3 days interval were not harmful and toxic to the broiler chicken but rather improved the health of the birds. The birds served 100-300ml FLFE and elevated glucose level of 53.21% (379.00-449.50mg/dl) compared to control (272.50mg/dl).

**Recommendation**
Broiler chickens can tolerate 100-300ml FLFE for improved blood formation. However, 100ml FLFE in 250ml of water is recommended to be served to broiler chickens at 3 days interval.

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pesticides from the Neem tree (Azadirachta Indica) and Caher tropical plants. CRC Press, Boca Raton, Florida, U.S.A. P. 437-482.


