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Effect of Fermented Lagenaria (Adenopus breviflorus) Fruit Extract on the Heamatological 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), aspatate 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 birds in control 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 $1.87x10^4$/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 ($353.00-314.50x10^3$/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 lagenaria (*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 Oridupo, 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 supernatant 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 hyperlipeamia 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 diaminetetra 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

Fruit extract Parameters	A VD	B V	C D	D (100ml) FLFE	E (200ml) FLFE	F (300ml) FLFE	± SEM
Packed cell volume (%)	40.00[a]	32.50[abc]	38.00[ab]	29.50[c]	32.00[bc]	33.00[abc]	1.18
Haemoglobin (%)	13.20[a]	10.70[ab]	12.60[a]	9.40[b]	10.70[ab]	10.70[ab]	0.43
Red blood cell ($x10^3$)	3.83[a]	3.64[ab]	3.82[a]	3.43[ab]	3.05[b]	3.67[ab]	0.10
White blood cell ($x10^4$)	1.42[b]	1.78[ab]	1.92[ab]	2.12[a]	1.37[b]	1.87[ab]	0.10
Platelet ($x10^3$)	271.50[bc]	188.50[d]	268.00[bc]	353.00[a]	314.50[ab]	221.50[cd]	15.60
Mean cell volume (fl)	104.44[a]	89.29[c]	99.48[b]	86.01[d]	104.92[a]	89.92[c]	1.84
Mean cell haemoglobin(pg/cell)	34.47[a]	29.40[c]	32.98[b]	27.41[d]	35.08[a]	29.16[c]	0.71
Mean cell haemoglobin concentration (%)	33.00[ab]	32.92[ab]	33.16[a]	31.86[c]	33.44[a]	32.42[bc]	0.14
Lymphocyte (%)	63.50	67.50	66.00	59.50	70.50	65.50	1.46
Heterocyte (%)	29.50[ab]	25.50[ab]	27.00[ab]	34.00[a]	22.50[b]	26.50[ab]	1.34
Monocytes (%)	2.50[b]	2.50[b]	4.00[a]	4.00[a]	2.00[b]	2.50[b]	0.25
Eosinophilis (%)	4.50[ab]	3.50[ab]	3.00[ab]	2.50[b]	5.00[a]	5.00[a]	0.32

a, b, c, d : means with different superscript on the same horizontal row differ significantly ($p<0.05$)
VD - 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) aspantate amino transminase (AST), alanine amino transminase (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 biochemical of broilers administered fermented lagenaria fruit extract

Fruit extract Parameters	A VD	B V	C D	D (100ml) FLFE	E (200ml) FLFE	F (300ml) FLFE	± SEM
Total serum protein(g/dl)	5.15	5.25	5.30	5.55	4.95	5.10	0.08
Albumin (g/dl)	1.25	1.25	1.25	1.50	1.20	1.20	0.05
Globulin (g/gl)	3.90	4.00	4.05	4.05	3.75	3.90	0.05
Creatinine (mg/dl)	272.50[b]	402.50[ab]	327.50[ab]	424.00[a]	449.50[a]	379.00[ab]	20.98
Glucose (mg/dl)	271.50[bc]	188.50[d]	268.00[bc]	353.00[a]	314.50[ab]	221.50[cd]	15.60
Aspartate amino	178.00[b]	190.50[b]	186.50[b]	231.00[a]	192.00[b]	197.00[b]	4.80
Alanine amino Transminase (iμ/L)	29.50[ab]	32.00[ab]	24.00[b]	35.00[ab]	40.00[a]	29.50[ab]	1.79
Alkaline phosphates (iμ/L)	237.50	370.50	268.50	222.50	241.50	245.00	19.86
Blood urea nitrogen (iμ/)	14.50	14.60	15.30	14.70	15.70	13.40	0.35
Cholesterol (mg/dl)	78.50[a]	42.00[c]	46.00[bc]	42.50[c]	68.50[ab]	89.00[a]	5.09

abc: means with different superscript on the same horizontal row differ significantly ($P<0.05$); VD - 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 session of the year and the methanol extracts of FLFE contain some secondary metabolites such as alkaloid, tannins, terpreniod, 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 Oyedeji *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 (Orisakeye 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 and the bird 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) ($x10^3$/ml) 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 ($3.0x10^6$L) 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 Ekunseitan *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. Orisakeye and Johnson (2015) reported that pharmacological study of *Lagenaira breviflora* revealed its anti-implantation, anti-bacterial, antiulcer, anti-inflamatory, antioxidant, miracidal and cercidal 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 3days 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.

RERERENCES

Adedapo A., Adewuyi T. and Sofidiya, M. (2013). Phytochemistry, anti-inflammatory, and analgesic activities of the aqueous leaf extract of *Lagenaira breviflora* (*Cucurbitaceae* in laboratory animal, *Rev.boil trop.Vol*.61 (19), Pp.281-290.

Adedapo A.A. and Bankole B.O. (2011). Ethnoveterinary in some selected farms in Ibadan, Nigeria. Recent Prog. *Med. Plant*.31:305-316.

Adedapo, A.A., Mogbojuri, O.M. and Emikpe, B.O. (2009). Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. *Journal of Medicinal Plants Research* (8): 586-591. Available: http//:www.acadmicjournals.org/JMPR.

Aderemi, F.A. (2004). Effect of replacement of wheat bran with cassava root sieviate supplement or unsupplemented with enzymes on the haematology and serum biochemistry of pullet chicks. *Tropical Journal of Animal Science.* 7:147-153.

Ahmad I., Aqil F., and Owais M. (2006). Modern phytomedicine, turning plants introduces Willey.VCH Verlag GMBH and Co KGOA, Weinueim.

Ajayi G.O., Awajo N.C. and Abule L.E.(2002). Themiracidal and cercavicidal activity of the methanolic extract of lagenaira breviflora Robert family *cucurbitaceae* fruit *schistomamansoni*. Niger O.J. Hosp. Med., 12:57-59

Awotuyi E.K. (1999). A study of baseline hematological value of domestic and commercial chicken in Ghana, Bull *Animal production Africa* 38:453-458.

Ayoola G.A, H.N.E Coker, S.A. Adesegun, A.A. Adepoju, Bello K., Obaweyo E.C., Ezenni O.S. and Atoneboyoila T.O (2008). Phytochemical screening and Antioxidant activities of some selected medicine, plants used for malaria Therapy in south western Nigeria. *Trop .J. Pharm, Res. 7:1019-1-024.*

Balogun M.E., Ajayi, Oji, O.J. Besong, E. Fibarrs, Bello,Folwiyo, M.A.(2014). Toxicological and Biochemical Studies of ethanolic fruit extract of *Adenopus breviflora* Roberty) in male Albino Wister Rats. *American Journal of phytomedicene and clinical therapeutics. Home > Vol.* 2, No 9:Balogun.

Bamigbose, A.M., and Niba, A.T. (1998). Performance of broiler chicken fed cotton seed cake in starter and finisher rations. In: Proc. of 3rd Ann. Conf. of ASAN. held at Airport Hotel, Ikeja-Lagos on Sept. 22-24:84-87

Bamishaiye E., Muhammad N. and Bamishaye, O. (2009). Hematological parameters of Albino rats feed on tiger diet. *The internet journal of nutrition and wellness*, 10(1).

Blacks, L.K. (1998). 12 Floral status of buxa tiger reserve west Bengal. Plant diversity in the tiger reserve of Indians 71.

Burkill H.M. (1995). The useful part of west Tropical Families. *J-Rajal Botanical Garden Kew*; 3:45.

Bush, B.M. (1991). Interpretation of laboratory results for small animal clinicians. Blackwell scientific publications, Oxford (11): 25-34.

Chineke, C.A., Ologun, A.G. and Ikeobi, C.O.N. (2006). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences,* 9:2012-2106.

Choladda, V.C. (2012). Mean Corpuscular Volume (MCV). Emedicine.medscape.com/article2085.

Church, D.C and Poud W.J. (2003): Basic animal nutrition and feeding. 3rd ed.john wildey and sons.15-48.

Coles, E.H. (1986). Veterinary clinical pathology 4th ed E.B. Saunder Company, Philadelphia and London. 72-163. USA 22-23

Cowan M. M. (1999). Plant product as anti microbial agents. Clinical microbiology reviews, 12(4): 564-582.

Craig G.M. Newman D.J. and Snader K.M. (1999). Natural products in drug discovering and development. *Journal Nat Prod*.60:52-60. PMID: 9014353.

Edoziem, J.C. and Switter, B.R. (1997). Effect of dietary protein, fat and energy on blood haemoglobin and haematocrit in rat. *Jouranl of Nutrition,* 107: 1016-1021.

Egbe-Nwiyi T. N., Nwaosu S.C. and Salami H.A. (2000). Heamatological value of apparently healthy sheep and goat as influence by age and sex in arid zone in Nigeria. *African Journal of biomedical Research,* (32): 109-115.

Ekunseitan, D.A., Abiola, S.S., Oluwatosin, O.O.., Sogunle, O.M., Adeleye, O.O. Egbeyale, L.t., and Iyasere (2017). Health status of laying birds administered extracts of *Lagenaria breviflora* manged under two housing systems. *Canadian Journal of Animal Science* (97): 154-164.

El –Mahmood A.M, Doughari J.H. and Lafan N. (2008). Anti microbial screening of stem bark extracts of *Vitellariaparadoxa* against some enteric pathogenic microorganism . *African journal of pharmacy and pharmacology* vol . 2 (5). Pp 089-094.

Elujoba, A.A. Fell, A.F. Linley P.A. (1991). Chromatography and spectroscopic analysis of bound and unbound phenolic acids in *Lagenaria breviflora fruit. J. Phamaceut Biomed anal*. 9(9): 711-715.

Epstein, F.H. and Hsia C.C.W. (1998). Respiratory function of haemoglobin. *New England Journal of Medicines,* 338 (4): 2349-247.

Esonu, B.O., Emenalom, O.O. Udedibie, A.B.I., Herbert, U., Ekpor, C.F., Okoli, I.C., and Iheukwumere, F.C. (2001). Performance and blood chemistry of weaner pigs fed raw mucana bean (velvet) meal. *Tropical animal Production Investigation* 4:49-54.

Esuoso, K.O. and Bayer, E. (1998). Chemical Composition and Potentials of some tropical underutilized biomass. Note 11 *Adenopus breviflours and Cucmeropsis edulis. Rivista-Italiana Sostanze Grasse* 95:191-196.

Etim N.N. (2010). Physiological and reproductive responses of rabbit foes to *Aspiliaafricana*. M.sc Thesis. Department of Animal Breeding and physiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Dairo, F.A.S., and Ogunmodede, B.K. (2000). Performance and some blood metabolites of broiler chickens fed fermented copra meal based diets *Nig. Poult. Sci. J. Vol.* 1 61-74.

Doumas, B.T., Watson W. and Biggs, H.G. (1971). Albumin standard and the measurement of serum albumin with bromocresol green. Clinical chimica 31:896.

Fasola T.R. and Egunyomi A. (2005). Nigerian usage of bark in phytomedicine. Ethnobotany Res APP. 3: 73-77.

Halle, P.D. Umoh, J.U., Saidu, L. and Abdu, P.A. (1998). Disease of Poultry in Zaria, Nigeria. A ten years analysis of clinic records. *Nigeria Journal of Animal Production* Vol. 25 91): 88-92.

Holder N.N and Rej R. (1983). Alanine transminase. In: methods of enzymatic analysis 3rd ed. Bergineyer. H.U.J. Bergemyer and M. Gassi. (eds.) Weinhein Vela genemie: 380-401.

Iyayi, E.A.A. and Tewe, O.O. (1998). Serum total protein, urea and creatine levels of indices of quality of cassava diet for pigs. *Tropical Veterinary* 36:59-67.

Izunobi, N.D. (2002). Poultry husbandry: an integrated approach for tertiary student extension agents policy maker and farmers. Ihiala (Nigeria), NADS Publishers.

Jain, N.C. (1993). Essentials of Veterinary haematology. Lea and Ferbeiger, Pennsylvania, PA:7.

Jain, N.C. (1986). Schalms Veterinary Haematology. 4th ed. *Lea and Febiger, Philadelphia USA*. 34-50.

Kalimuthu K., Vijayakumar S. and Senthilkumar R. (2010). Antimicrobial activity of the bio diesel plant, *jatropha curcas L., International journal of Pharma and Bio-sciences.*

Kekeocha, C.C. (1985). Poultry Production hand book. 1st ed. *Publ. Pfizer corp and Macmillan.* 92-110.

Kerr, G.R., Lee, E.S., Lan, E.K., Larimor, R.J., Randall, E., Forthofer, R.N., Davis, M.A. and Magnetism, A. (1982). Relationships between dietary and biochemical measures of nutritional status. *American Journal of clinical nutrition*. 35:294-308.

Khan, T.A. and Zafar, F. (2005). Haematological study in response to varying doses of estrogen broiler chicken. *International Journal of Poultry Science* 4(10): 748-751.

Kohn R.A., Allen M.S. (1992). Enrichment of protoclysis activity of nitrogen in preparation from the rumen for in-vitro studies. *Amin. Feed sci. Tech* 52:1-4.

Larry E.N. (1993). Broiler feeding and management. *Poultry, int*. 32(1): 70.

Leeson S.D., Lewus B. and Shrimpton D.H. (1973). Multiple linear regression equations for the prediction of food intake in laying fowl. *Brit poult. Sci* 14: 595.

Lehninger, A.L., Nelson, D.L. and Cox, M.M (1993). The citric acid cycle. Principles of biochemistry. Worth Publishers. New York, NY: 598-611.

Linton, A.H. (1982). Antibiotics in Veterinary Practice. *Brit. Med. J.* 4:11-31.

March B.E., and Biely J. (1969). The effect of energy supplied from the diet from environment heat on the response of chicks to different level of dietary lysine. *poult. Sci*. 51: 665-670.

Mass A.C., Sataikamp H.W., Verschuwr M.A. and Eehern N. V. (1993). *Poultry production in the tropics.1st ed :32-35.*

Maton A., Jean H. Charles W., Mclaughlin S.J., Maryama Q. W., David L. and Wright J.D. (1993). Human biology and health. Eaglewoord Cliffs New Jersey, USA. Prentice Hall.

Maxewell E. (2013). Haematology day in the like RCPA Foundation Education + Research.

Mc Donald P., Edwards R.A and Greenhalyh J.F.D. (1994): Evaluation of food in animal nutrition 4th ed . Longman scientific and Technical England. 260-283.

McNab J.M and Shannon D.W.F. (1992): The effect of different level of maize germ meal on the performance of broiler. *Brit poult.sci* 13:441-447.

Morimoto Y., Maudu P. and Fujimaki H. (2005). Diversify of landrace of the white flowered ground (*Lagenarias, ceraria*) and its wild life in Kenya: fruit and seed morphology. *Genet Res Evol.,* 53:737-747.

NRC (1994). National Research Council nutrition requirement of poultry 9th ed. National Edition. Washington D.C. USA.

Nworgu, F.C., Adebowale C.A., Oredein A.O. and Oni A.(1997). Water leaf *(Talinumtrangtulare)* leam leaf as a protein supplement for broiler chicken prospect and economics of broiler production using two plant protein source. *Trop. J. Anim. Sci.* 2(1): 159-166.

Nworgu, F.C. and Egbunike, G.N. (2013). Water leaf (Talinum triangulare) leaf meal as protein supplement for broiler chicks. Proceedings of the 4th *Nigeria International Poultry Summit, Abeokuta,* 17-21 February, 2013; Pg. 68-73.

Nworgu, F.C., Hamizat, R.A.,Odunla, O.A. and Arasi, M.A (2003) : Performance and some heamatological indices of cockrel chicks feed cocoab (Theobroma cacao) Rod husk. ASSETS series A 3 (2): 11-21.

Obi, I.U. (1990). The Tropical Agriculturalist; Poultry. The Macmillian Press Limited, London United Kingdom. Pg. 30-91.

Ogunfowora O.B., Fetuga B.L. and Ademosun A.A., (1975). Surveys of the livestock feed situation in Nigeria . Report to the livestock department, Lagos November, 1975: 1-4.

Olomu, J.M. (1976). Optimum protein and energy level for broiler chicks in a tropical environment. *Nig. J. Anim. Prod.* 4(1): 239-256.

Oluyemi J.A. and Robert F.A. (1999). Poultry production in warm wet climate 1st ed Macmillan press ltd. London 22-31.

Omoruyi, S.A. Orhue, U.X., Akerobo, A.A. and Aghimien, C.I. (1999). Prescribed Agricultural Science for Senior Secondary Schools. Benin city (Nigeria): Idodo umeh publishers Ltd.

Onyeyili, P.A., Egura, G.O., Jibike, G.I., Pepple, D.J. and Chaegbulam, J.O. (1991). Seasonal variations as haematological indices in the grey-breasted Guinea fowls (Numida meleagris Gallata palla). *Nig. J. Anim. Prod*. 18 (2): 108-111.

Oridupa O.A., Saba A.B. Sulaiman L.K. (2011). Preliminary report on the anti-viral activity of the ethionic fruit extract of *lagenaira breviflora* Roberts on new castle disease virus. *Trop. Vet* 29 (1):22-33.

Orisakeye, O.T and Johnson, J. (2015). Phytochemical and Pharmacological activities of *lagenaria breviflora:* A Review. *Jacobs Journal of Medicinal Chemistry* 1 (1): 1-4.

Oyedeji, K.O. Adurodija, M.W. Adeleye, A.S and Abidoye, D. (2015). Effect of ethanol extract of *Adenopus breviflorus* (Roberty) on haematological and plasma biochemical parameters in male Wistar rats. *International Journal Pharmastical Sci. Rev. Res.* 35 (2): 36-40.

Oyekunle, M.A. and Owonikoko, M.O. (2002). Antimicrobial drug usage for poultry production within a local government area in Ogun State. *Nig. J. Anim. Prod.* 29(1): 113-120.

Payne, G.C(1967). Practical aspect of environm,ental temperature for laying hen. *World poult. Sci.J.*22-126.

Reinhold, J.G. (1953). Standard method of clinical chemistry, Reiner, I. (ed) Academic press New York. 88.

Renkema, J.A. (1992). Economy aspect of nutrition relationship to environment and production of quality eggs and broilers. In: procs. Vol. 2 XIX world's poultry congres. Amsterdam, the Nertherland Sep. 20-24th, 1992. Ponser and Looiger, Wagenineger. Edited by Roel Muider. 97-99.

Rodkey, F.L. (1969). Globulin direct determination. Clincal chemistry 11: 486.

Rose, S.P. (1997). Principle of Poultry Sciecne, 2nd edition, *Wallingford. Oxon, CAB International* 135.

Ross, J.G., Christien, C., Helliday, W.G. and Jorus, R.M. (1978). Haematological and blood chemistry. Comparism value for clinical pathology in Poultry Veterinary record 102:29-31.

Saba, A.B., Oridupa, O.A., Oyagbemi, A.A. and Alao, O.E. (2010). Serum biochemical changes accompanying prolonged administration of ethanolic extract of whole fruits of *Lagenaria breviflora* (Benth)Roberty in Wistar rats. *African Journal Biochemistry* 9 (42): 7128-7133.

Sadre, N.L., Deshpande, V.Y., Mandika, K.N. and Nandal, D.H. (1984). Male antifertility acitivty of Azadirachta Indica A. Juss (Neem) in different specieis. In:Schumttereitt and Ascher KRS. Eds. Natural

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

Sainsbury D. (1995). Poultry health and management. 3rd ed. *Black Well Sci.Ltd.London*.22-31.

Schalm O.W., Jain N.C. and Card E.J. (1975). Veterinary Haematology. 3rd ed.lea and Febigerphiladephia 51-81.

Shannon, D.W.F and Brawn, W.O. (1969). The period of adaptation of the fasting metabolic rate of the common fowl to an increase in environmental temperature from 22^0C to 28^0C. *Brit Poult. Sci.* 10:13.

Shu Y.Z. (1998). Recent Natural based drug development :A Pharmaceuticalb industry perspective. *Journal Nat Prod*. 1998: 61-1053-107. PMID: 9722499.

Sonaiya E.B. (1995). Family poultry and food security. Research requirement in science Tech. Socio-economics. Phytocchemical screening of pignut (jatrophascucuras Linn) on some pathogenic bacteria. *J. mel Plants Res.* 5(7):1261-1264.

Sturkie, P.O., Hazel, W. and Wood, R. (2000). Avian physiology, 3rd edition New York. *NY Spinger Vallock. New York. Inc*. 54-60.

Tomori O.A., Saba A.B. and Dada-Adegbola H.O.(2007). Antibacterial activity of ethanolic extract of Whole Fruit of *lagenairia breviflora* Robert. J. *Annual Vet. Advances*. 6(5):752-757.

Wihrauch T.L. and Feter B. B. (1994). Fruit and vegetable by products as sources of oil.

Wikihow (2013). How to study haematology. Wikihow cited by Yemi, Oyo, B. R. Flickely, Teresa and others. Available at www.wikiho.cp>home>categories>educationandcommunications>subjects>science

Wikipedia (2013a). Haematology. Available at: en.wikipedia.org/wiki/haematology.

Wikipedia (2013d). Mean Corpuscular Volume. Available at: enwiukipedia.,org/wiki/mean-corpuscular volume.

Willianson F. E. and Payne K. A (1998). A Handbook on Poultry Nutritional Requirement. *Macmillian press Ltd. Pp. 14-20*

Yasuguki, M., Patrick, M., Hiroshi, F., Hirojo, M. (2005). Diversity of landraces of the white flowered guard *(Lagenariasiceraria)* and its wild relatives in Kenya *Genet. Res. Crop E.* Vol. 52:737-747.

Zunszain, P.A., Ghuman, J., Komatu, T., Tsuchida, E. and Cury, S. (2003). Crystal structural analysis of human serum albumin completed with hemin and fatty acid. Biol. 3:6. Doi: 10.1186/1472-6807-3-6. PMC 1661363. PMID 12846933