

Phytobiotic/Antimicrobial efficacy of spotted pumpkin (*Lagenaria breviflora*) pulp extracts on intestinal microbial, faecal oocyst, haematology and serum biochemistry indices of broiler chickens

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ADDITIONAL KEYWORDS

Antimicrobial.
Lagenaria breviflora.
Gut microbiota.
Faecal.
Broiler chickens.
Blood profile.

SUMMARY

Antibiotics have been used in the poultry industry for years either as prophylactic or therapeutic measures against different micro-organisms that affect the livestock. However, misuse or continuous use of antibiotics has led to the emergence of the antibiotics, residue and resistivity in livestock. Thus, this study evaluates the effect of spotted pumpkin pulp extract on gut microbiota, faecal and antimicrobial efficacy on the health of broiler chickens. A total of 240 days old broiler chicks were used for the experiment and were allotted into four (4) treatments of (4) replicates each. Aqueous extract of *Lagenaria breviflora* pulp was administered to the birds at a dosage of 0g/l (Control), 250g/l, 500g/l and 750g/l respectively for 5 days. All data obtained on gut microbial, faecal oocyst, haematological and serum biochemical parameters were subjected to one-way analysis of variance in a completely randomized design. Result obtained revealed that *Lagenaria breviflora* pulp extract had significant ($P < 0.05$) effect on Heterophils, basophils, monocytes, mean corpuscular haemoglobin, total protein, Albumin, Aspartate amino transferase and Alanine amino transferase at the starter phase. Results on intestinal microbial shows that there was no ($p > 0.05$) significant effect obtained on all the parameters measured but there was positive effect of *Lagenaria breviflora* pulp extract on birds administered 250g/l and 500g/l for the faecal oocyst. The levels of *Lagenaria breviflora* pulp extract had significant ($P < 0.05$) effect on Packed cell volume, haemoglobin, red blood cell, White Blood Cell, Lymphocytes and Mean Corpuscular Volume. All serum parameters measured were significantly ($P < 0.05$) influenced at finisher phase except Total cholesterol. It can be concluded that administering *Lagenaria breviflora* pulp extract at 250g/l or 500g/l eradicate/reduced the presence of oocyst in the gut of broiler chicken. *Lagenaria breviflora* pulp extract could be administered up to 750g/l of water as an antimicrobial without causing any deleterious effect on the health status of poultry birds.

Eficacia fitobiótica/antimicrobiana de los extractos de pulpa de calabaza manchada (*Lagenaria breviflora*) sobre los índices microbianos intestinales, ooquistes fecales, hematológicos y bioquímicos séricos de pollos de engorde

RESUMEN

Los antibióticos se han utilizado en la industria avícola durante años como medidas profilácticas o terapéuticas contra diferentes microorganismos que afectan al ganado. Sin embargo, el uso indebido o continuo de antibióticos ha provocado la aparición de residuos y resistencia a los antibióticos en el ganado. Por lo tanto, este estudio evalúa el efecto del extracto de pulpa de calabaza manchada sobre la microbiota intestinal, las heces y la eficacia antimicrobiana en la salud de los pollos de engorde. Para el experimento se utilizaron un total de 240 pollos de engorde de 240 días de edad, que se distribuyeron en cuatro (4) tratamientos de (4) réplicas cada uno. Se administró a las aves extracto acuoso de pulpa de *Lagenaria breviflora* en dosis de 0 g/l (control), 250 g/l, 500 g/l y 750 g/l, respectivamente, durante 5 días. Todos los datos obtenidos sobre los parámetros microbianos intestinales, ooquistes fecales, hematológicos y bioquímicos séricos se sometieron a un análisis de varianza unidireccional en un diseño completamente aleatorio. Los resultados obtenidos revelaron que el extracto de pulpa de *Lagenaria breviflora* tenía un efecto significativo ($P < 0,05$) sobre los heterófilos, basófilos, monocitos, hemoglobina corpuscular media, proteína total, albúmina, aspartato aminotransferasa y alanina aminotransferasa en la fase inicial. Los resultados sobre la microbiota intestinal muestran que no se obtuvo ningún efecto significativo ($p > 0,05$) en todos los parámetros medidos, pero sí se observó un efecto positivo del extracto de pulpa de *Lagenaria breviflora* en las aves a las que se administraron 250 g/l y 500 g/l en cuanto a los ooquistes fecales. Los niveles de extracto de pulpa de *Lagenaria breviflora* tuvieron un efecto significativo ($P < 0,05$) sobre el volumen celular empacado, la hemoglobina, los glóbulos rojos, los glóbulos blancos, los linfocitos y el volumen corpuscular medio. Todos los parámetros séricos medidos se vieron significativamente influenciados ($P < 0,05$) en la fase de acabado, excepto el colesterol total. Se puede concluir que la administración de extracto de pulpa de *Lagenaria breviflora* a 250 g/l o 500 g/l erradicó o redujo la presencia de ooquistes en el intestino de los pollos de engorde. El extracto de pulpa de *Lagenaria breviflora* se pudo administrar hasta 750 g/l de agua como antimicrobiano sin causar ningún efecto perjudicial para la salud de las aves de corral.

PALABRAS CLAVE

Antimicrobiano.
Lagenaria breviflora.
Microbiota traqueal.
Fecal.
Pollos broiler.
Perfil sanguíneo.

INFORMATION

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INTRODUCTION

Poultry industry is one of the fastest means in achieving appreciable improvement in the nutritional standard of the populace because of its short generation interval and quick turnover rate (Smith, 2001; Ani and Okeke, 2011). Over the years, antibiotics have been used in the poultry industry for prevention and treatment of various diseases. However, the misuse or continuous use of antibiotics has led to the drug resistivity and drug residue in animal products. Nowadays, the use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance and their use has been banned or curtailed in many countries (Laxman, 2016). The negative impact of antibiotics on consumers of meat and poultry products has led to the ban on the use of antibiotics as growth promoters since 2006 by the European Union. The abuse and overuse of antibiotics have given rise to bacteria resistivity. Therefore, this has caused bacteria species to develop resistance to antibiotics (WHO, 2017). The reduced social acceptance and ban on antibiotics has necessitated Animal scientists and veterinarians to look inward for safe and natural alternatives such as plants leaves, extracts, stems and roots (phytobiotic) to replace antibiotics. Plants contain phytonutrients and phytochemicals (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides), which are referred to as secondary metabolites (Ogbe and Affiku, 2012). Plants' secondary metabolites are applied in nutrition and have pharmacologically active agents (Soetan and Oyewole, 2009).

The beneficial effects of phytobiotics are numerous including their antioxidative properties. However, studies have demonstrated that the antioxidant properties alone cannot explain these benefits (Halliwell et al., 2005; Martel et al., 2020). Phytobiotics have been shown to exert multiple effects, including anti-inflammatory, antimicrobial, anti-oxidative and metabolic-modulating effects (Qin S and Hou, 2017; Pandey et al., 2019; Martel et al., 2020). Therefore, there is need to explore antimicrobial alternatives that are safer and doesn't compromise the health of poultry species.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The experiment was carried out at the Poultry Unit of Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB). The areas lie on the Latitude 7°10' N and Latitude 3° 2' E. It is 76m above sea level and located in the tropical vegetation zone with an average temperature of 34.7° C (Google Earth, 2022).

SOURCE OF AND PREPARATION OF TEST INGREDIENT

Spotted Pumpkin (*Lagenaria breviflora*) fruit was purchased from an open market within Abeokuta metropolis, Ogun State. The fruit was rinsed in water, sliced into smaller parts and the pulp was separated from the bark. The weighed pulp was soaked in water for 72 hours at room temperature in order to give an aqueous extract.

EXPERIMENTAL BIRDS AND MANAGEMENT

A total of 240 days old chicks were purchased from a reputable hatchery in Ibadan, Oyo state. The pen was swept, washed and disinfected two weeks before the arrival of the chicks. The chicks were brooded in a well clean brooder house using gas brooder and electric bulb for 14 days. The chicks were fed with commercially produced broiler starter diet containing (23% CP and 2800kcal/kg/ME) at the starter phase and (19% CP and 3000Kcal/kg) at the finisher feed phase. Feed and water were provided *ad libitum*. The administration of the test ingredient started from the 1st day of arrival of the chicks.

EXPERIMENTAL DESIGN

Birds were randomly allotted to four treatment groups T1, T2, T3 and T4 having 60 birds per treatment which was further sub-divided into four (4) replicates of ten (10) birds each. Birds in treatment 2, 3 and 4 were given aqueous extract of *Lagenaria breviflora* pulp extract at a dosage of 250g, 500g and 750g/liter respectively for 5 days after which natural vitamin (pineapple) was blended and administered at a dosage of 0.1 liter to 1 liter of water given for 2 days. The administration of *Lagenaria breviflora* pulp extract was done every other week. Birds in treatment 1 served as the control where a conventional drug (antibiotics) was administered. Standard vaccination procedure was observed for all birds.

DATA COLLECTION

BLOOD COLLECTION

At the end of starter phase (28 days of age) and at the end of finisher (49 days of age) phase, blood samples were collected with 5ml syringe through the wing web vein from five birds per replicate. The blood samples were emptied into EDTA and plain bottles for the determination of haematological indices and serum biochemical analysis.

FAECAL SAMPLE AND GUT CONTENT COLLECTION

Faecal samples were collected on the 44th day of the experiment, which was used in determining bacterial load, oocyst and egg worm count. Faecal samples were collected from each replicate with the aid of swab sticks. Bacteria count was then counted using a colony counter while oocyst and egg worm count was determined using the MacMaster method (Maff, 1986).

On the 49th day of experiment five (5) birds were selected at random from each of the replicate group for the evaluation of intestinal microbes. The birds were fasted for 12 hours before slaughtering, after which they were eviscerated immediately. The intestinal content from the caecal was collected separately into a sterile glass container for the identification of microbial population.

STATISTICAL ANALYSIS

All data collected were subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) using SAS (SAS, 2012). Significant ($P < 0.05$) different means were separated using Duncan Multiple Range test of the same statistical model.

RESULTS AND DISCUSSION

The effect of *Lagenaria breviflora* pulp extract on haematological parameters of broiler chicken at starter phase is shown in **Table 1**. The levels of *Lagenaria breviflora* pulp extract significantly ($p < 0.05$) influenced heterophils, Basophils, monocytes and mean corpuscular haemoglobin (MCH).

Table 1. Effect of *Lagenaria breviflora* pulp extract on the haematological parameters of broiler chicken at starter phase (Efecto del extracto de pulpa de *Lagenaria breviflora* sobre los parámetros hematológicos de pollos de engorde en la fase inicial).

Parameters	Control	250g/L	500g/L	750g/L	SEM	P-Value
Packed cell volume (%)	30.00	35.50	35.50	38.50	1.69	0.39
Haemoglobin (g/dl)	10.25	12.20	12.20	13.30	0.58	0.34
Red blood cell ($\times 10^{12}/L$)	3.12	3.72	3.73	3.65	0.16	0.53
White blood cell ($\times 10^9/L$)	10.20	12.40	9.20	11.70	0.60	0.24
Heterophils (%)	27.00 ^b	38.50 ^a	32.50 ^{ab}	34.00 ^{ab}	1.69	0.04
Lymphocyte (%)	67.00	59.00	62.50	63.00	1.42	0.29
Eosinophils (%)	1.00	0.00	1.50	1.00	0.21	0.06
Basophils (%)	2.00 ^a	1.50 ^a	0.50 ^b	0.50 ^b	0.22	0.01
Monocytes (%)	3.00 ^a	1.00 ^b	3.00 ^a	1.50 ^b	0.30	0.02
MCV (fl)	10.40	10.76	10.66	10.96	0.79	0.70
MCH (pg)	32.94 ^b	32.76 ^b	32.97 ^b	35.22 ^a	0.33	0.03
MCHC (g/dl)	34.13	34.38	34.36	34.64	0.81	0.17

^{a,b} -means in the same row with different superscripts is significantly different ($p < 0.05$)

MCV- Mean corpuscular volume

MCH- Mean corpuscular haemoglobin

MCHC- Mean corpuscular haemoglobin concentration

The result obtained on Heterophils at starter phase corroborate the findings of Ekunseitan et al 2017 who reported significant ($p < 0.05$) difference on heterophils when *Lagenaria breviflora* fruit extract was administered to laying birds. Heterophils levels obtained in this study was decreasing as the levels of *Lagenaria breviflora* pulp extract increases. The decreasing values obtained for these parameters might be due to some presence of bioactive components in the test ingredient. Basophils levels were significantly ($p < 0.05$) influenced by the levels of *Lagenaria breviflora* pulp extract. Higher basophils were obtained for birds administered 0 and 250g/l of *Lagenaria breviflora* pulp extract compared to what was obtained from birds on 500g/l and 750g/l of *Lagenaria breviflora* pulp extract. This result is contrary to the findings of Ekunseitan et al 2007 who reported no significant ($p > 0.05$) difference in basophils of laying birds administered *Lagenaria breviflora* fruit extract. Lower basophils obtained at 500g/l and 750 g/l might be attributed to higher dosages of the pulp extract administered at the early stage of growth as basophils has been reported to protect the body against infection, parasites, pathogens and cancer cells. Higher monocytes mean values were obtained from birds

administered 0g/l and 500g/l of *Lagenaria breviflora* pulp extract compared to lower mean values obtain for birds on 250g/l and 750g/l of *Lagenaria breviflora*. The result obtained on monocytes in this study corroborates the findings of Ekunseitan et al 2007, Nworgu et al 2018 who reported significant difference in monocytes levels when *lagenaria breviflora* fruit extract was administered to broiler birds. The lower mean values of monocytes at 250g/l and 750g/l might be a pointer that birds under this treatment might be susceptible to infection, although all mean values were within the ranges of normal monocytes in broiler chickens. Higher mean corpuscular haemoglobin was highest in birds administered 750g/l of *Lagenaria breviflora* pulp extract compared to other treatment groups that had lower statistical similar mean values, the result obtained above agrees with the findings of Nworgu et al 2018 who reported significant difference in mean corpuscular haemoglobin of broiler chickens. Higher mean corpuscular haemoglobin values obtained at 750g/l of *lagenaria breviflora* pulp extract might be attributed to some bioactive substances that aided the continuous production of haemoglobin in the circulatory systems of the chickens.

Results obtained on serum biochemistry indices at the starter phase is shown in **Table 2**. All parameters measured were significantly ($p < 0.05$) influenced by the levels of *Lagenaria breviflora* pulp extract except globulin, creatinine and total cholesterol. Higher total protein was obtained from birds administered *Lagenaria breviflora* pulp extract compared to the control groups that recorded the least. The higher protein content obtained in this study can be attributed to the higher protein in *Lagenaria breviflora* pulp extract. This result supports the findings of Aladekoyi et al 2020 who reported higher protein content in fermented *Lagenaria breviflora* root when the proximate composition was carried out. Higher Albumin levels were obtained for birds administered *Lagenaria breviflora* pulp extract compared to the control birds. The higher albumin obtained can be attributed to the presence of some phytochemicals present in the test ingredient which helps in maintaining fluid from leaking out of blood vessels into other tissues and capable in stabilizing and maintaining immune functions and system of the birds as all mean values obtained in this study were within the normal range for healthy chicken. This result is contrary to the findings of Ekunseitan et al 2007 who reported no significant difference in the albumin of laying birds when *Lagenaria breviflora* was administered.

Aspartate amino transaminase (AST) was significantly higher in birds administered *Lagenaria breviflora* pulp extract compared to the control groups. AST was increasing as the levels of the test ingredient increases. Higher AST obtained in this study might be attributed to higher dosages of *Lagenaria breviflora* pulp extract as the birds are just advancing in age and the liver is trying to adjust to the bioactive substance in the test ingredient. Results obtained on Alanine amino transferase (ALT) shown higher mean values at 250g/l and 750g/l of *Lagenaria breviflora* pulp extract compared to the control groups where the least was reported. The result obtained on ALT in this study were within the normal range for healthy chicken, higher ALT obtained from birds on the test ingredient could be attributed to higher dosages of the test ingredient that might have cause some impairment on liver functions.

Table 2. Effect of *Lagenaria breviflora* pulp extract on the serum indices of broiler chicken at starter phase (Efecto del extracto de pulpa de *Lagenaria breviflora* sobre los índices séricos de pollos de engorde en la fase inicial).

Parameters	Control	250g/L	500g/L	750g/L	SEM	P-Value
Total Protein	55.80 ^b	70.00 ^a	73.35 ^a	67.45 ^a	2.23	0.03
Albumin (g/dl)	32.80 ^b	41.65 ^a	40.40 ^a	38.55 ^a	1.21	0.02
Globulin (g/dl)	25.50	28.35	27.95	28.90	2.12	0.66
Creatinine (mg/dl)	0.85	0.80	0.70	0.95	0.03	0.10
AST (u/L)	100.50 ^c	115.60 ^b	136.10 ^a	146.35 ^a	8.95	0.04
ALT (u/L)	12.50 ^c	20.10 ^a	14.80 ^b	22.80 ^a	1.60	0.03
Total Cholesterol (mg/dl)	96.70	82.15	92.75	91.85	3.26	0.25

^{a,b,c} -means in the same row with different superscripts is significantly different ($p < 0.05$)

AST=Aspartate amino transaminase

ALT=Alanine amino transferase

Results obtained on intestinal microbial count is shown in **Table 3**. Results obtained on intestinal microbial count revealed no ($P > 0.05$) significant difference in all the parameters measured. Total bacteria count increases as the level of *Lagenaria breviflora* pulp extracts increases across all treatments groups. The highest total fungi count was found in broiler chickens administered 500g/l of *Lagenaria breviflora* pulp extracts, while the lowest total fungi count was found in broiler chickens administered given 250g/l of *Lagenaria breviflora* pulp

extracts. Given that *Lagenaria breviflora* contains tannin as an active ingredient, the result obtained might be due to presence anti-microbial activity of the test ingredient (Ekunseitan et al., 2016). This provides more evidence in favor of the in-vivo studies using *Lagenaria breviflora* extract on particular pathogenic species as reported by Ekunseitan et al., 2016.

Table 3. Effect of *Lagenaria breviflora* pulp extracts on the intestinal microbial in broiler chicken (Efecto de los extractos de pulpa de *Lagenaria breviflora* sobre la microbiota intestinal en pollos de engorde).

Parameters	Control	250g/l	500g/l	750g/l	SEM	P-value
TBC	4.02	3.57	3.86	4.50	1.61	0.52
TCC	0.36	0.28	0.60	0.26	0.32	0.29
TFC	1.70	1.52	2.75	2.41	1.02	0.69

TBC- Total bacteria count

TCC- Total coliform count

TFC- Total fungi count

Results obtained on bacteria, fungi and coliform isolates on intestinal microbial is shown in **Table 4**. Results on bacteria isolates shows that birds in the control groups and birds administered 750g/l of *Lagenaria breviflora* pulp extracts has higher population of pathogenic bacteria (*Streptococcus lactics*, bacteria

macerans, *bacteriodesamylophilus*) while birds administered 250g/l of *Lagenaria breviflora* pulp extracts and 500 g/l of *Lagenaria breviflora* pulp extracts has higher population of beneficial bacteria (*Micrococcus varain*, *Micrococcus accidophilus*, *Lactobacillus acidophilus*, *Selenomonas ruminatium*).

Table 4. Bacteria, fungi and coliform isolates present in the intestinal microbial of broiler chicken (Bacterias, hongos y aislados coliformes presentes en la microbiota intestinal de pollos de engorde).

Treatments	Bacteria isolate	Coliform isolate	Fungi isolate
CONTROL	<i>Pseudomonas auginosa</i> , <i>Staphylococcus aureus</i> , <i>Micrococcus acidophilus</i> , <i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Bacteria macerans</i>	<i>Escherichia</i> <i>Coli</i>	<i>Aspergillus</i> <i>Niger</i> , <i>Fusarium</i> <i>Oxysporum</i> , <i>Penicillium</i> <i>Oxysporium</i>
250g/l	<i>Micrococcus varian</i> , <i>Micrococcus accidiophilus</i> , <i>Streptococcus faecium</i> , <i>Proteus mirabis</i> , <i>Succinimonas amyloctica</i>	<i>Escherichia</i> <i>Coli</i>	<i>Penicillium</i> <i>Oxysporium</i> , <i>Fusarium</i> <i>Oxysporium</i>
500g/l	<i>Selenomonas ruminatum</i> , <i>Enterobacter aerogens</i> , <i>Bacillus macerans</i> , <i>Micrococcus acidophilus</i> , <i>Lactobacillus</i> <i>Acidophilus</i>	<i>Escherichia</i> <i>Coli</i>	<i>Aspergillus</i> <i>Niger</i> , <i>Penicillium</i> <i>Oxysporium</i>
750g/l	<i>Enterobacter aerogens</i> , <i>Bacteriodes amylophilus</i> , <i>Proteus mirabilis</i> , <i>Proteus vulgaricus</i> , <i>Ruminicoccus</i> <i>Albus</i> , <i>Bacteria macerans</i> , <i>Streptococcus lactics</i>	<i>Escherichia</i> <i>Coli</i>	<i>Fusarium</i> <i>Oxysporum</i> , <i>Saccharomyces</i> <i>Cerevisae</i> , <i>Aspergillus</i> <i>Niger</i>

Results obtained on faecal oocyst of broiler chicken is shown in **Figure 1**. Egg count revealed that birds in control group had over 1200 EPG and 0 oocyst counts while the broiler chicken administered 250g/l and 500g/l of *Lagenaria breviflora* pulp have no egg count and no oocyst count. The difference obtained in this graph may be related to the high concentration of saponins, flavonoids, and tannins in the extracts of

Lagenaria breviflora pulp extracts, which was capable of causing oxidative stress on the worms, thereby eradicating the worms from the birds this results supports the findings of John et al., 2009 that reported positive effect of helminthic property of cassia tora leaves extracts owing to its phytochemicals interfering with the glycoprotein of the cuticle and leading to the worms death.

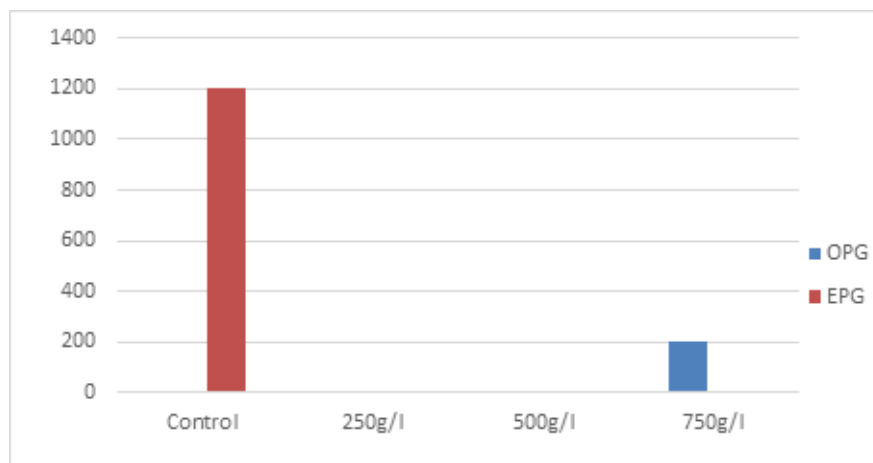


Figure 1. Results obtained on faecal oocyst of broiler chicken (Resultados obtenidos en oocistos fecales de pollos de engorde).

The effect of *Lagenaria breviflora* pulp extract on haematological parameters at the finisher phase is shown in **Table 5**. The levels of *lagenaria breviflora* pulp extract significantly ($p < 0.05$) influence Packed cell volume, Haemoglobin, Red blood cell, White blood cell, Lymphocytes and Mean corpuscular volume across the treatment groups. The result obtained on packed cell volume, red blood cell and haemoglobin shows that higher packed cell volume, red blood cells and haemoglobin was recorded from the control groups compared to other treatments groups. This result is in agreement with the findings of Nworgu et al 2018 who reported significant difference in pack cell volume, red blood cells and haemoglobin of broilers in the control groups compared to *Lagenaria breviflora* treated groups.

The reduced packed cell volume, red blood cells and hemoglobin obtained in this study at finisher phase might be attributed to iron deficiency and bitterness of *lagenaria breviflora* pulp extract that reduced water intake during the period of administration especially at higher dosages. Although, all these parameters were within normal range for healthy chickens which means the test ingredient did not impair availability that can cause anaemic conditions. White blood cells and lymphocytes obtained for the control groups was significantly ($p < 0.05$) higher than other treatments groups, the result obtained on white blood cells is contrary to the findings of Irvboje and Olufayo 2023 who reported

higher white blood cells in broiler chickens administered *lagenaria breviflora* compared to the control groups. Lymphocytes obtained in this study supports the findings of Irvboje and Olufayo 2023 who reported higher lymphocytes for the control groups compared to other treatments groups. These findings shows that *Lagenaria breviflora* is capable as an immuno-modulatory agent regulating and maintaining immune functions of the birds. The mean values obtained in these studies are within the range for normal healthy chicken.

Mean corpuscular volume was significantly ($p < 0.05$) influenced by the levels of *Lagenaria breviflora* pulp extract. Higher mean corpuscular volume (HMCV) was obtained from birds administered *Lagenaria breviflora* pulp extract compared to the control group that recorded the least. This result supports the findings of Nworgu et al 2018 who reported significant difference in Mean cell volume (MCV) of broilers administered fermented *Lagenaria* fruit extract at 200ml. These results showed that birds administered *Lagenaria breviflora* pulp extract have enough oxygen supplied to their tissues and organs at the finisher phase. This further shows that better sizes of red blood were obtainable from the treatment group compared to the control groups.

Table 5. Effect of *Lagenaria breviflora* pulp extract on haematological parameter of broiler chicken of broiler chicken at finisher phase (Efecto del extracto de pulpa de *Lagenaria breviflora* sobre los parámetros hematológicos del pollo de engorde depollo de engorde en la fase de acabado).

Parameters	Control	250g/L	500g/L	750g/L	SEM	P-Value
Packed cell volume (%)	30.50 ^a	28.50 ^b	25.50 ^c	20.50 ^d	1.43	0.00
Haemoglobin (g/dl)	10.50 ^a	8.60 ^b	8.25 ^b	6.53 ^c	0.54	0.00
Red blood cell (x10 ¹² /L)	3.22 ^a	2.70 ^b	2.37 ^c	1.93 ^d	0.17	0.00
White blood cell (x10 ⁹ /L)	16.31 ^a	16.03 ^b	13.83 ^d	15.61 ^c	0.36	0.00
Heterophils (%)	36.33	31.83	31.67	30.83	1.55	0.35
Lymphocyte (%)	63.00 ^a	60.50 ^{ab}	57.50 ^{bc}	55.00 ^c	1.18	0.01
Eosinophyte (%)	2.50	4.00	5.00	3.00	0.49	0.34
Basophiles (%)	0.00	1.00	0.00	0.00	0.16	0.00
Monocytes (%)	3.00	2.00	3.00	2.00	0.18	0.00
MCV (fl)	94.87 ^b	105.77 ^a	107.82 ^a	106.21 ^a	2.08	0.03
MCH (pg)	32.67	31.91	34.89	33.83	0.53	0.21
MCHC (g/dl)	34.47	30.19 ^a	32.36	31.86	0.73	0.24

^{a, b, c, d} -means in the same row with different superscripts is significantly different (p<0.05)

MCV=Mean corpuscular volume

MCH=Mean corpuscular haemoglobin

MCHC=Mean corpuscular haemoglobin concentration

The effect of *Lagenaria breviflora* pulp extract on serum biochemistry indices of broiler chickens is shown in **Table 6**.

Levels of *lagenaria breviflora* pulp extract (p<0.05) significantly influenced all parameters measured except total cholesterol. Total protein was significantly (p<0.05) higher in birds administered 750g/l of *Lagenaria breviflora* pulp extract (2.67 ± 0.35) compared to (2.36 ± 0.02) obtained from birds administered 250g/L which recorded the least value. This result corroborates the findings of Ekunseitan et al 2017 who reported significant difference in total protein of laying birds administered 300g/4L of *Lagenaria breviflora* at 5week in lay. This result might be attributed to higher dosage and fermentation process that occurred during processing of the test ingredient as it has been reported that *Lagenaria breviflora* contains protein. Result obtained on Albumin showed that birds administered 500g/l of *Lagenaria breviflora* pulp extract had higher albumin mean values compared to (0.87 ± 0.01) obtained for birds on the control groups. This results further support the findings of Ekunseitan et al 2017 but contrary to the findings of Nworgu et al 2018 who reported no (p>0.05) significant difference in Albumin of broiler chickens administered *Lagenaria breviflora* fruit extract. The result shown that phytochemicals present in the test ingredient helped in maintenance of appropriate osmotic pressure, binding and transport of various

substances like hormones in blood. Globulin values obtained in this study is contrary to the findings of Nworgu et al 2018 who reported no (p>0.05) significant difference in the globulin of broiler chickens administered *Lagenaria breviflora* fruit extract. This result though significant showed that liver functions, blood clotting activities and immune response of the birds administered the test ingredient was not compromised as the values were within the normal range for healthy chickens. Creatinine levels were significantly (p<0.05) higher in birds administered 250g/l compared to mean values obtained from birds on 750g/l of *Lagenaria breviflora*. This result support the findings of Nworgu et al 2018 and Ekunseitan et al 2017 that reported significant difference in creatinine levels in broilers and pullets' birds. This result further confirms that *Lagenaria breviflora* pulp extract at higher dosages does not have adverse effect on the kidney functions of growing birds as lower creatinine levels were obtained at higher inclusion levels, this results further corroborate the pattern reported by Ekunseitan et al 2017 who reported lower creatinine levels at higher inclusion rate compared to the control groups.

Aspartate amino transaminase (AST) was significantly (p<0.05) higher in control birds and birds administered 500g/l of *Lagenaria breviflora* pulp extract compared to lower values obtained in birds administered 250g/l of *Lagenaria breviflora* pulp extract. This

result supports the findings of Nworgu et al 2018 who reported significant ($p<0.05$) difference in broiler chickens administered fermented *Lagenaria breviflora* fruit extract. This result showed that better liver functions was observed at lower inclusion levels. Alanine amino transferase (ALT) was significantly ($p<0.05$) higher in birds administered 500g/l compared to what was obtained in birds administered 250g/l of *Lagenaria breviflora* pulp extract. This result is contrary to the findings

of Nworgu et al 2018 and Ekunseitan et al 2017 that reported no significant ($P>0.05$) difference in broiler and pullet chickens at 8 weeks of age. The significant difference obtained might be due to liver impairment functions that allowed higher ALT to be released into the system from birds administered 500g/l of the test ingredient and this further confirms the reason why higher AST was observed from the same group.

Table 6. Effect of *Lagenaria breviflora* pulp extract on the serum indices of broiler chicken at finisher phase (Efecto del extracto de pulpa de *Lagenaria breviflora* sobre los índices séricos de pollos de engorde en fase de acabado).

Parameters	Control	250g/L	500g/L	750g/L	SEM	P-Value
Total Protein	2.42 ^b	2.36 ^c	2.40 ^{bc}	2.67 ^a	0.46	0.00
Albumin (g/dl)	0.87 ^c	0.82 ^d	1.27 ^a	1.02 ^b	0.65	0.00
Globulin (g/dl)	1.58 ^b	1.55 ^b	1.12 ^c	1.66 ^a	0.79	0.00
Creatinine (mg/dl)	0.87 ^b	0.92 ^a	0.69 ^c	0.61 ^d	0.47	0.00
AST (u/L)	132.44 ^a	113.61 ^d	132.99 ^a	128.78 ^c	2.97	0.00
ALT (u/L)	19.32 ^c	15.65 ^d	21.16 ^a	20.66 ^b	0.81	0.00
Total Cholesterol (mg/dl)	61.36	69.74	87.19	78.35	3.63	0.25

^{a,b,c} -means in the same row with different superscripts is significantly different ($p<0.05$)

AST=Aspartate amino transaminase

ALT=Alanine amino transferase

CONCLUSIONS

Administration of *Lagenaria breviflora* extract at 250g/l and 500g/l improved the overall health performance of broiler birds as the immune system of the broiler chickens was not affected by the pulp extract both at the starter phase and finisher phase. Administration of *Lagenaria breviflora* extract at 250g/l and 500g/l greatly eradicate/suppress faecal oocyst of broiler chickens.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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