Growth performance, haematology and intestinal histology of broiler chickens administered *Lagenaria breviflora* Roberty fruit juice

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Abstract

The use of botanicals to improve gut health and growth performance in broiler chickens is a current practice in the poultry industry. Christmas melon (Lagenaria breviflora) is one of the plants that has strong antimicrobial sensitivity, which is being explored as an alternative antibiotic growth promoter in broiler chickens. Growth performance, haematology and histological changes in the jejunum and ileum were studied in 120 Ross 308 broiler chickens aged 3-wk-old and weighting 0.15 ±SD kg. The chickens randomly divided into four equal treatments with three replicates of 10 birds and administered Lagenaria breviflora fruit juice. The treatments were 0.00, 2.50, 5.00 and 7.50 mL/4 litres of Lagenaria breviflora fruit juice in drinking water. The experiment was arranged in a completely randomized design (CRD). Feed and water were offered ad libitum for 42 days. Data were collected on body weight changes, feed intake, feed conversion ratio and haematological parameters. The jejunum and ileum were sectioned for histology. The data were subjected to one-way analysis of variance (ANOVA). The result indicated that growth parameters were significantly affected by the treatment ($p < 0.05$). The birds administered 2.5 mL of the LBFJ had the highest final body weight (2.58 kg), least total feed intake (4.33 kg) and best feed conversion ratio (1.75). Haematological parameters were significantly ($p < 0.05$) affected by the treatment. The mean PCV (35.00%), HB (12.87 g/dL), RBC (3.97 x 106 mm^3) and WBC (24.59 x 10^3 mm^3) values were significantly higher in birds administered LBFJ compared to control (30.6 g), (11.60 g/dL, (3.49 x 10^6 mm^3), and (21.63 x 10^3 mm^3), respectively. Lymphocyte count (65.00%) was significantly higher in birds administered LBFJ than the control (61.33%). Heterophil count (27.33%), and heterophil/lymphocyte ratio (0.42) were significantly lower in birds administered LBFJ compared to the control (61.33%), and (0.51) respectively. Jejunal and ileal histology showed normal morphological integrity. In conclusion, broiler chickens administered 2.5mL of LBFJ gave the best growth performance, while 5 and 7.5mL improved haematological parameters, humoral immune response and general well-being of the birds. Also, up to 7.5 mL of LBFJ did not derange the jejunal and ileal functional and structural, and functional integrity. It is therefore recommended that 2.5 mL of LBFJ is ideal for better growth performance, while up to 7.5 mL of LBFJ is considered safe for broiler chickens, and could be used when stronger immunity is needed, but at the expense of feed cost.

Keywords: Growth, Haematology, Histology, Broiler chickens

Performances de croissance, hématologie et histologie intestinale de poulets de chair ayant reçu du jus de fruit de Lagenaria breviflora Roberty

Résume

L'utilisation de plantes pour améliorer la santé intestinale et les performances de croissance des poulets de chair est une pratique courante dans l'industrie avicole. Le melon de Noël (Lagenaria breviflora) est l'une des plantes qui présente une forte sensibilité antimicrobienne,

actuellement étudiée comme alternative aux antibiotiques pour stimuler la croissance des poulets de chair. Les performances de croissance, l'hématologie et les changements histologiques dans le jéjunum et l'iléon ont été étudiés chez 120 poulets de chair Ross 308 âgés de 3 semaines et pesant 0,15 ± SD kg. Les poulets ont été répartis au hasard en quatre groupes de traitement égaux, avec trois réplicats de 10 oiseaux, et ont reçu du jus de fruit de Lagenaria breviflora. Les traitements étaient les suivants : 0,00, 2,50, 5,00 et 7,50 ml/4 litres de jus de fruit de Lagenaria breviflora dans l'eau de boisson. L'expérience a été organisée selon un plan complètement randomisé (PCR). La nourriture et l'eau ont été fournies à volonté pendant 42 jours. Des données ont été recueillies sur les variations de poids corporel, la consommation alimentaire, le taux de conversion alimentaire et les paramètres hématologiques. Le jéjunum et l'iléon ont été sectionnés pour l'histologie. Les données ont été soumises à une analyse de variance unidimensionnelle (ANOVA). Les résultats ont indiqué que les paramètres de croissance étaient significativement affectés par le traitement ($p < 0,05$). Les oiseaux ayant reçu 2,5 ml de LBFJ ont présenté le poids corporel final le plus élevé (2,58 kg), la consommation alimentaire totale la plus faible (4,33 kg) et le meilleur indice de conversion alimentaire (1,75). Les paramètres hématologiques ont été significativement ($p < 0,05$) affectés par le traitement. Les valeurs moyennes du PCV (35,00 %), de l'HB (12,87 g/dL), des RBC (3,97 x 106 mm3) et des WBC (24,59 x 10^3 mm3) étaient significativement plus élevées chez les oiseaux ayant reçu du LBFJ que chez ceux du groupe témoin (30,6 g), (11,60 g/dL, (3,49 x 106 mm3) et (21,63 x 103 mm3), respectivement. Le nombre de lymphocytes (65,00 %) était significativement plus élevé chez les oiseaux ayant reçu du LBFJ que chez ceux du groupe témoin (61,33 %). Le nombre d'hétérophiles (27,33 %) et le rapport hétérophiles/lymphocytes (0,42) étaient significativement plus faibles chez les oiseaux ayant reçu du LBFJ que chez ceux du groupe témoin (61,33 %) et (0,51) respectivement. L'histologie du jéjunum et de l'iléon a montré une intégrité morphologique normale. En conclusion, les poulets de chair ayant reçu 2,5 ml de LBFJ ont présenté les meilleures performances de croissance, tandis que les doses de 5 et 7,5 ml ont amélioré les paramètres hématologiques, la réponse immunitaire humorale et le bien-être général des oiseaux. De plus, jusqu'à 7,5 ml de LBFJ n'ont pas perturbé l'intégrité fonctionnelle et structurelle du jéjunum et de l'iléon. Il est donc recommandé d'utiliser 2,5 ml de LBFJ pour obtenir de meilleures performances de croissance, tandis que jusqu'à 7,5 ml de LBFJ sont considérés comme sans danger pour les poulets de chair et peuvent être utilisés lorsqu'une immunité plus forte est nécessaire, mais au détriment du coût de l'alimentation.

Mots-clés: Croissance, Hématologie, Histologie, Poulets de chair

Introduction

The quest for ethno-botanicals in humans and animal health care delivery systems is increasingly gaining relevance across the world, because they are considered as safer, more affordable and more eco–friendly (Baatsch *et al*., 2017; Gajender *et al*., 2023). Most medicinal plants are laden with secondary metabolites such as flavonoids, terpenoids, saponins and steroids, which makes them suitable as complementary and alternatives to the pharmaceutical antibiotics (Olakojo *et al*., 2024). *Lagenaria breviflora* Roberty also known as 'Christmas melon' and 'Tagir' by English and Yoruba people, respectively (Olakojo *et al*., 2024), and 'Egwusi ohia' and 'Ahenyi' by Abia and Enugu State people, respectively, is one of the

plants used in traditional medicine in different parts of Nigeria.

The phytochemical and GC-MS profiling of the leaf and fruit indicated the presence of steroid, flavonoids, phenols, alkaloids, carotenoid, saponin, oxalate, steroids and cucurbitacins (Balogun *et al.*, 2014; Adeyemi *et al.*, 2017), which are responsible for its antibacterial, antiviral, antifungal, anti-inflammatory and antioxidant effects (Ajani *et al*., 2015; Olakojo *et al*., 2024). Earlier research showed that the leaves and whole fruit of *Lagenaria breviflora* are potent in the treatment of Newcastle disease, coccidiosis, *Escherichia coli Salmonella gallinarium*, in poultry (Tomori *et al.*, 2007), and measles, gastrointestinal disorder, *Staphylococcus aureus* and as wound antiseptic in humans (Tomori *et al.*, 2007; Balogun *et al.*, 2014). Due to the directives by the World Health Organization (WHO) and European Medicines Agency (EMEA) to avoid pharmaceutical drugs in food animal production, because of resistance (Olokojo *et al*., 2024), research on *Lagenaria breviflora* expanded such that the leaf, whole fruit seed, mesocarp are being explored in a bid to develop an alternative antibiotic for humans and animals. Thus, the leaf and fruit of *L breviflora* have been used in such forms as methanolic fruit extract (Folorunsho *et al.*, 2019), ethanol leaf extract (Ajani *et al.*, 2015), cold water fermentation (Ekunseitan *et al.*, 2018), seed extract (Irivboje and Olufayo, 2023), hot water fruit extract (Egbeyale *et al.*, 2021) in animal experimental models.

However, conflicting research results have arisen from the various processing methods, ranging from liver and kidney damages to reduction and/or complete elimination of the potent phytoconstituents during processing. For instance, Banjo *et al*. (2013) reported reduced concentrations of flavonoids, terpenoids, tannins, and absence of saponins and cynogenic glucoside in sun–dried *Lagenaria breviflora* fruit extract while Adepegba and Abu (2016) reported absence of flavonoids, phenols, tannins and anthraquinine in sun–dried, oven–dried and hot water–soaked *Lagenaria breviflora* fruit.

Absence of research reports on the fresh whole fruit juice of *L breviflora* in animal models formed the basis for this study. Research reports indicated that *L breviflora* extract improved growth performance (Egbeyale *et al*., 2021), haematological and serum biochemical parameters in broiler chickens, and indicated that it has both haematic and therapeutic properties (Irivboje and Olufayo, 2023). Similarly, Olakojo *et al*. (2024) evaluated the antibacterial activity of *Lagenaria breviflora* whole fruit extract on broiler chickens and reported that it significantly inhibited the growth of *Salmonella spp* colony in the gut and also reversed hepatic and renal hepatomegaly, hepatic parenchymal infiltration, hepatic necrosis, nephritis and tubular necrosis of the kidney, which accompany bacterial infections.

However, while some research reports indicate that *Lagenaria breviflora* is safe at lower doses in rat (Ajani *et al.*, 2014), and chicken (Nworgu *et al.*, 2018; Egbeyale *et al.*, 2021; Irivboje and Olufayo, 2023), others reports on Wistar rats indicate potential nephrotoxicity and hepatotoxicity at higher doses (Elujoba and El-Alfy, 1986; Balogun *et al.*, 2014; Ajani *et al.*, 2015).

Due to lack of accurate understanding of the safety and toxicity level of fresh *Lagenaria breviflora* fruit juice, the need for this research is imperative. In this study, *Lagenaria breviflora* fruit is prepared in such a way as to retain maximum components as possible. The result of this study will provide useful data on its application to poultry farmers.

Materials and methods

Location of the study

The experiment was conducted at Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike.

Experimental birds and management

A total of 120 Ross 308 broiler chicks were used for the study. The birds were purchased at day-old from a reputable Farms in Ibadan,

Nigeria and raised in deep litter pens under standard conditions. The birds were fed compounded starter diet containing 23.11CP and 2862.95 kcal/kg metabolizable energy from 0-4 weeks and then a finisher diet containing 20.13 CP and 2907.15 kcal/kg metabolizable energy from 5-8 weeks of age as presented in Table 1. The birds were vaccinated as recommended by the National Veterinary Research Institute, Nigeria. At 2-weeks of age, the birds were randomly allotted to 4 treatments (T_1, T_2, T_3 and T_4) in a completely randomized design (CRD). Each treatment had 30 birds in 3 replicates with 10 birds.

Sourcing/preparation of the test ingredient

Fresh *Lagenaria breviflora* fruits were harvested at Michael Okpara University of Agriculture premises. An aliquot (1.5 kg) of the fruits was weighed out with an electronic balance (XY2000 - 2C, PEC MEDICAL, China), washed with clean water and sliced into pieces with a sharp kitchen knife, and then blended with an electric blender to form the juice. The juice was poured into a clean coffee sieve to remove chaffs. The sieved juice was transferred into a clean 1 litre plastic gallon and stored in a refrigerator at 4 ^{0}C. Fresh preparations were made after every 4 days till the end of the experiment.

Administration of the LBFJ

Administration of the fruit juice started at week-three of age. Aliquots (0, 2.5, 5 and 7.5 mL) of the juice were taken with a syringe into separate drinkers containing 4 litres of water. The mixtures were homogenized and administered to T_1 (control), T_2, T_3 and T_4 daily for 42 days, respectively.

Experimental design

One hundred and twenty birds were randomly divided into equal treatments in a completely randomized design (CRD) with 3 replicates of 10 birds. The experimental model is as shown below:

$Y_{ij} = \mu + T_i + e_{ij}$

Where

Y_{ij} = Single observation on each treatment.

μ = Overall mean

T_i = Effect of the of treatment

e_{ij} = Random error

Data collection

Growth parameters

Body weight: The birds were weighed at the beginning of the experiment and subsequently on weekly basis with a top loading 20 kg CAMRY brand with 50 g sensitivity scale. Data on growth were collected as follows:

Initial body weight: Weight at the beginning of the experiment (kg).

Final body weight: Weight at the end of the experiment (kg).

$$Weight\ gain\ (kg) = Final\ live\ body\ weight - Initial\ \ live\ body\ weight$$

$$Average\ daily\ weight\ gain\ (g) = \frac{Total\ weight\ gain}{Number\ of\ experimental\ days}$$

$$Average\ feed\ intake\ (g) = \frac{Total\ feed\ consumed}{Number\ of\ birds}$$

$$Average\ daily\ feed\ intake\ (g) = \frac{Total\ feed\ consumed}{Number\ of\ birds\ \times Number\ of\ experimental\ days}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Total\ feed\ itake}{Total\ weight\ gain}$$

Mortality (%) = Number of birds at the beginning of the experiment – Number of birds at the end of the experiment.

Blood sampling and analysis

At the end of the experiment, six birds were randomly selected from each treatment, two birds from each replicate for haematology. In each treatment, 2mL of blood was taken from the jugular vein between the hours of 8am and 10am with a syringe and dropped in sample bottle containing ethylenediaminetatracetic acid. The blood samples were assessed

immediately after collection. The PCV was determined by microhaematocrit method as described by Schalm *et al.* (1975) with microhaematocrit centrifuge and reader (Hawksley and Sons, England). Haemollobin (Hb) was determined using standard haemoglobinometer (Marienfold, Germany). Red blood cells and total white blood cells were measured with a haemocytometer using Natt and Herrick's solution as diluting fluid ccording to (Campbell, 1994). The smears for white blood cell differential count were prepared and stained by the Leishman technique and enumerated by the battlement counting method (Thrall and Weiser, 2002). The Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated according to Schalm *et al.* (1975).

Histological procedure

The intestinal histology was carried out on the previously used for growth and haematological studies. Three birds were selected (one bird per replicate) and sacrificed with a sharp knife. The intestine was harvested with a surgical blade. Small samples (3cm) of the jejunum (the midpoint between the entry of common bile duct and the Meckel's diverticulum) and ileum (from the Meckel's diverticulum to ileocecal junction) were cut with surgical scissors and properly flushed with 0.9% physiological saline to remove digester artifacts. The samples were put in separate sample bottles containing formalin. Histological procedures for processing the intestinal sections were done according to Clayden (1967). The slides were captured with a digital camera (Motic image camera Moti-Cam) connected to a laptop computer at x100 magnification.

Data analysis

The data collected were subjected to one-way analysis of variance (ANOVA) using SPSS software package (Version 16). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955). Significantly different means were accepted at 5% confidence levels.

Table 1: Composition of poultry chicken starter experimental diets

Ingredients Composition (%)	Starter	Finisher
Maize	48.00	55.00
Soya bean meal	30.00	26.00
Palm kernel cake	10.20	10.20
Lysine	0.20	0.20
Methionine	0.10	0.10
Bone meal	5.00	2.00
Fish meal	3.00	3.00
Common salt	0.25	0.25
Vitamin/mineral premix	0.25	0.25
Total	100.00	100.00
Calculated composition		
Crude protein (CP) (%)	23.11	20.13
Metabolizable energy (ME)(Kcal/kg)	2862.95	2907.15

Starter and finisher premix to supply: Vitamin A (10,000mg), vitamin D (2,000mg), vitamin E (10mg), vitamin K_3 (2,000mg), vitamin B_{12} (10,000mg), folic acid (1,000mg) pantothenic acid (10,000mg), Niacin (26,000mg), biotin (100,000mg) choline (150000mg), antioxidant (125000mg), Manganese (10,000mg), Zinc (50,000mg), Cobalt (250mg), Iron (40,000mg), Copper (6,000mg), Iodine (5000mg), Selenium (100mg).

Results

Growth performance of broiler chickens administered LBFJ

The result shows significant ($p < 0.05$) effect of treatment on some growth parameters measured. Final body weight (2.58 kg) and average daily weight gain (49.66g) were significantly higher in birds administered 2.5 ml of the LBFJ and least (2.08 kg) and (39.46 g) in birds administered higher dose (7.5 ml) of the LBFJ. Average feed intake (4.70) and average daily feed intake (95.92 g) were higher in bird administered 7.5 mL of the LBFJ, respectively. Feed conversion ratio was least (1.78) in birds on 2.5 mL of the LBFJ. Percentage mortality did not vary ($p > 0.05$) between the control and the treated birds (Table 2).

Table 2: Growth performance of broiler chickens administered LBFJ

Parameters	T1	T2	T3	T4	SEM	P-value
Initial weight (kg)	0.15	0.15	0.14	0.15	0.00	0.693
Final weight gain (kg)	2.28^{c}	2.58^{a}	2.42^{b}	2.08^{d}	0.06	0.000
Weight gain (kg)	2.00^{c}	2.43^{a}	2.28^{b}	1.93^{d}	0.06	0.000
Average daily weight gain (g)	40.82^{c}	49.66^{a}	44.46^{b}	39.46^{d}	1.25	0.000
Average feed intake (kg)	4.56^{c}	4.33^{d}	4.61^{b}	4.70^{a}	0.04	0.000
Average daily feed intake (g)	92.99^{c}	88.43^{d}	94.15^{b}	95.92^{a}	0.83	0.000
FCR	2.27^{b}	1.78^{d}	2.12^{c}	2.43^{a}	0.07	0.000
Mortality (%)	0.00	0.33	0.00	0.28	0.10	0.594

Means along the same row with different superscripts are significantly ($p<0.05$) different. SEM= standard error of mean, FCR= feed conversion ratio.

Haematological parameters of broiler chicken administered LBFJ

The result shows significant differences ($p < 0.05$) only on haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC) and total white blood cell count (WBC). The Hb (12.87 g/dL), PCV (35.00%), RBC (3.97×10^{6} mm^{3}), and WBC (24.59×10^{3} mm^{3}) values were statistically the same in birds administered LBFJ, but significantly lower in the control group. The mean MCV, MCH and MCHC values did not vary ($p > 0.05$) between the control and the birds administered LBFJ (Table 3).

Table 3: Haematological parameters of broiler chicken administered LBFJ

Parameters	T_1	T_2	T_3	T_4	SEM	Pvalue
Hb (g/dL)	11.60^{b}	12.30^{a}	12.57^{a}	12.87^{a}	0.16	0.03*
PCV (%)	30.67^{b}	33.33ab	34.00^{a}	35.00^{a}	0.63	0.04*
RBC($\times 10^{6}$mm^{3})	3.49^{b}	3.76ab	3.87^{a}	3.97^{a}	0.07	0.02*
WBC($\times 10^{3}$mm^{3})	21.63^{c}	23.04^{b}	23.73ab	24.59^{a}	0.37	0.02*
MCV (*fl*)	87.95	88.72	87.92	88.19	0.23	0.61
MCH (*pg*)	38.22	33.10	32.55	32.47	1.35	0.08
MCHC (g/dL)	43.44	36.92	36.99	36.77	1.54	0.08

Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM = standard error of mean, Hb = haemoglobin, PCV = packed cell volume, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, TWBC = total white blood cells count, MCHC = mean corpuscular hemoglobin counts.

White blood cell differentials of broiler chickens administered LBFJ

The result shows significant differences ($p < 0.05$) only on lymphocytes, heterophils and heterophil/lymphocyte ratio. Lymphocyte count (65.00%), was significantly higher in the treated groups than the control (61.33%), while heterophil count (27.33%) and Heterophil/lymphocyte ratio were statistically the same in birds administered LBFJ (0.04), but significantly higher in birds fed control diet (31.33%) and (0.51) respectively. Monocyte

and eosinophil count did not vary ($p > 0.05$) between the control and birds administered LBFJ (Table 4).

Table 4: White blood cell differentials of broiler chickens administered LBFJ

Indices	T_1	T_2	T_3	T_4	SEM	P-Value
Lymphocytes	61.33^{b}	63.00ab	64.00^{a}	65.00^{a}	0.53	0.03^{*}
Heterophils	31.33^{a}	29.00^{b}	28.00^{b}	27.33^{b}	0.51	0.03
Heterophil/lymphocyte ratio	0.51^{a}	0.46^{b}	0.44^{b}	0.42^{b}	0.04	0.01
Monocyctes	5.67	6.00	5.67	5.33	0.19	0.63
Eosinophils	1.67	2.00	2.33	2.33	0.15	0.31

Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM = standard error of mean, Hb = haemoglobin, PCV = packed cell volume, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, TWBC = total white blood cells count, MCHC = mean corpuscular hemoglobin counts.

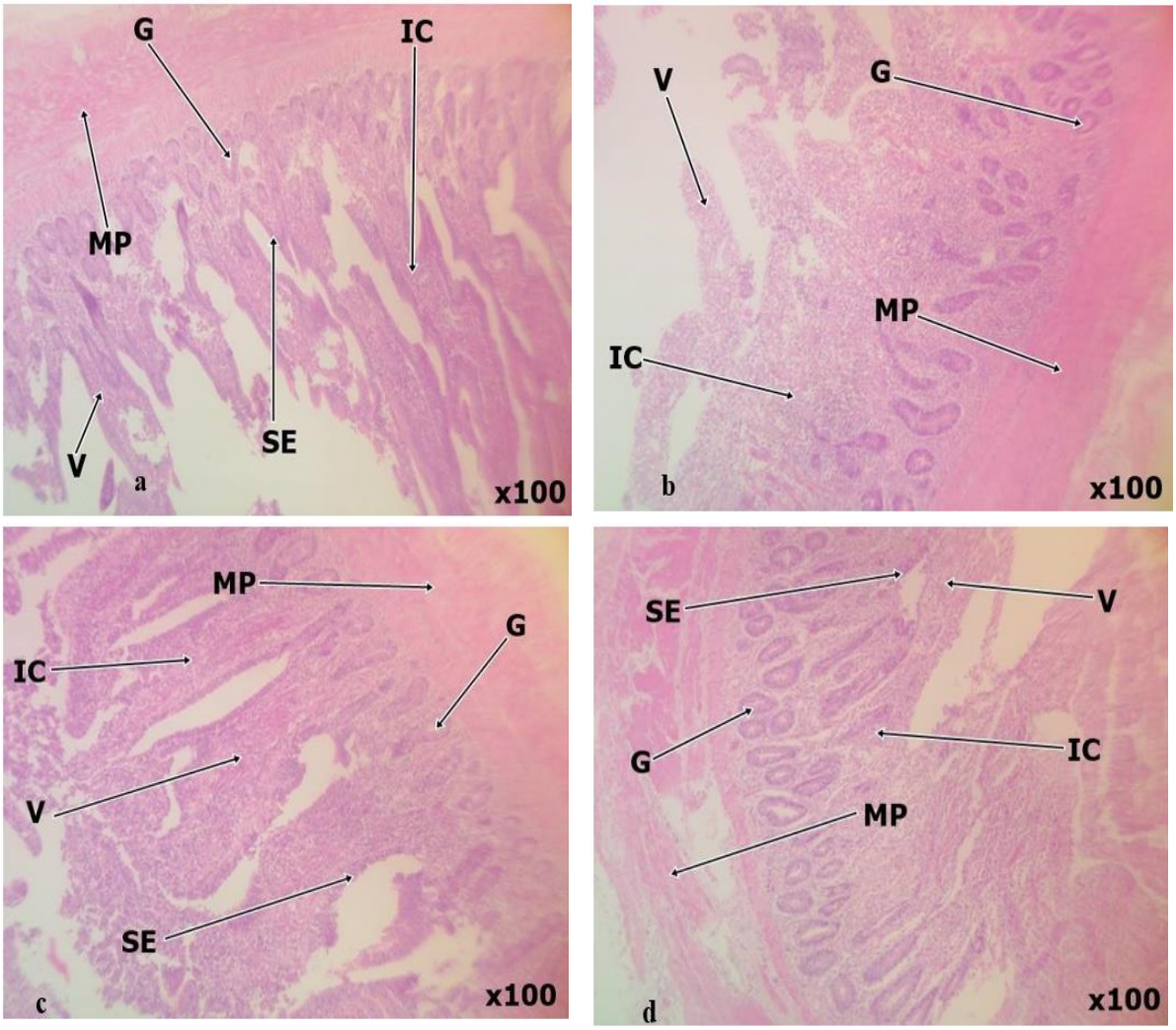


Figure legend: V= Villus, SE=Epithelium, G= Sub mucosal gland, MP= Muscularis propria, IC= Inflammatory cells

Figure 1: Photomicrographs (a-d) of the jejual sections of broiler chickens administered LBFJ

(a) Shows the jejunal section of broiler chickens administered 0.00 mL of LBFJ. There are orderly differentiated simple columnar epithelium with extensive denudation of the surface epithelium. There are overlying muscularis mucosa and muscularis propria. The sub mucosa contains tubular glands are lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, occasional neutrophils and scant eosinophils. H & E x100. **(b)** Shows the jejunal section of broiler chickens administered 2.50 mL of LBFJ. There are orderly differentiated simple columnar epithelium and extensive denudation of the surface epithelium. There are overlying muscularis mucosa and muscularis propria. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, occasional neutrophils and scant eosinophils. H & E 100. **(c)** Shows the jejunal section of broiler chickens administered 5.00 mL of LBFJ. There are orderly differentiated simple columnar epithelium and extensive denudation of the surface epithelium. There are overlying muscularis mucosa and muscularis propria. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, occasional neutrophils and scant eosinophils. H & E 100. **(d)** Shows the jejunal section of broiler chickens administered 7.50 mL of LBFJ. There are orderly differentiated simple columnar epithelium and extensive denudation of the surface epithelium overlying muscularis mucosa and muscularis propria. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, occasional neutrophils and scant eosinophils. H & E x100.

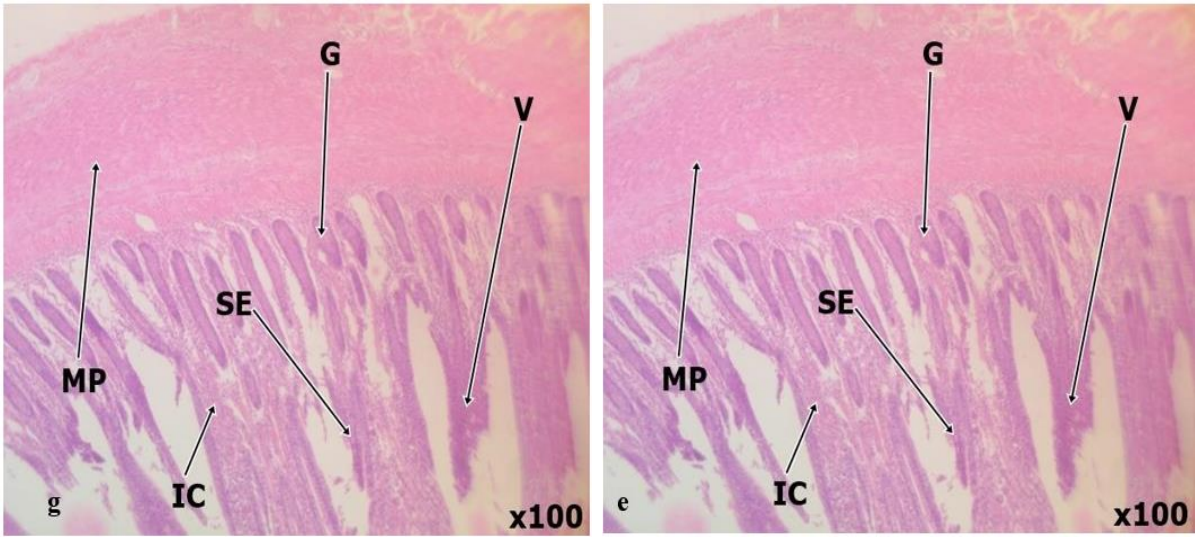
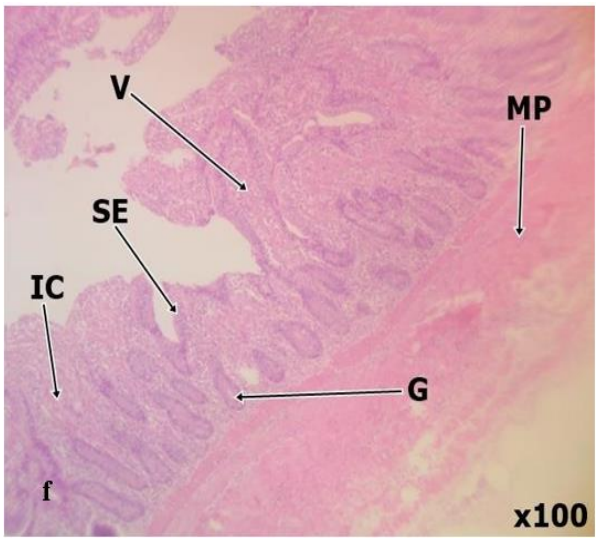
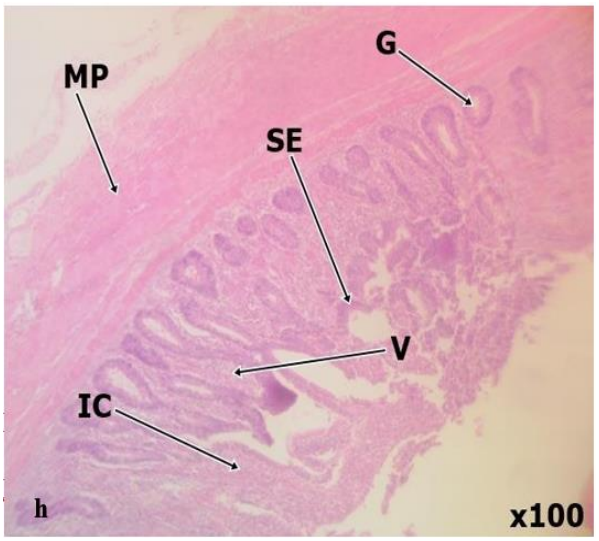


Figure 2: Photomicrographs (e-h) of the ileal sections of broiler chickens administered LBFJ

Figure legend: V= Villus, SE=Epithelium, G= Sub mucosal gland, MP= Muscularis propria, IC= Inflammatory cells.





(e) Shows the ileal section of broiler chickens administered 0.00 ml of LBFJ. There are luminal cells with orderly differentiated simple columnar epithelium and prominent villi. There are epithelium surface, overlying muscularis mucosa and muscularis propria. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, scant eosinophils and occasional neutrophils. H & E x100. **(f)** Shows the ileal section of broiler chickens administered 2.50 mL of LBFJ. There are orderly differentiated simple columnar epithelium and prominent villi. Epithelial surface, overlying muscularis mucosa and muscularis propria are present. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, scant eosinophils and occasional neutrophils. H & E x100. **(g)** Shows the ileal section of broiler chickens administered 5.00 mL of LBFJ. There are orderly differentiated simple columnar epithelium and prominent villi. Epithelial surface, overlying muscularis mucosa and muscularis propria are present. H & E x100.

(h) Shows the ileal section of broiler chickens administered 7.50 mL of LBFJ. There are orderly differentiated simple columnar epithelium and prominent villi. Epithelial surface, overlying muscularis mucosa and muscularis propria are present. The sub mucosa contains tubular glands lined by columnar cells. The stroma is infiltrated by florid population of lymphocytes, plasma cell, scant eosinophils and occasional neutrophils. H & E x100.

Discussion

The significantly higher final body weight, daily weight gain, least mean feed intake and best feed conversion ratio (FCR) recorded in birds on low dose (2.50 mL) of LBFJ in this study is consistent previous studies on broiler chickens administered 200mg/kg Yucca extract (Su *et al*., 2016); broiler chickens administered 90mL of *Moringa oleifera* leaf extract in 1litre of water (Alabi *et al*., 2017); broiler chickens fed diets supplemented with low doses (0.50%), (1.00%) and (1.5%) of red ginger and turmeric mixtures (Tuti *et al*., 2020). This result is in contrast to Ekunseitan *et al*. (2018) in Yaffa Brown layers, but partly agrees with Egbeyale *et al*. (2021), who reported better F CR, without a corresponding increase in final weight gain in broiler chickens administered lower dose (75.00 g/L) of *Lagenaria breviflora* fruit extract. Although the reduced body weight gain in broiler chickens fed diets supplemented red ginger and turmeric mixtures were attributed to low feed intake due to the characteristic sharp taste and odor of atsiri oil of ginger, in this study, feed intake increased with a decrease in weight gain as dose increased. The scenario in this study also disagreed with Egbeyale *et al*. (2021) who attributed reduced weight gain in broiler chickens to reduced water intake due to bitter

taste of essential oil in *Lagenaria breviflora* fruit.

The reasons for the differences between the result of this study and those of Egbeyale *et al*. (2021) could in addition to variations in dose, be due to loss of flavonoids, saponins and terpenoids in the course of processing. Earlier research reports by Adepegba and Abu (2016) and Banjo *et al*. (2013), showed that the concentrations of flavonoids, tannins and terpenoids in *Lagenaria breviflora* fruit could either be reduced or completely eliminated by sun-drying, oven-drying and hot water-soaking. Alabi *et al*. (2024) reported improved intestinal integrity, better feed conversion ratio in broiler chickens fed diet rich in alkaloids. Also, broiler chickens fed diets rich in steroids, saponins and polyphenols had higher body weight gain, feed efficiency (Su *et al*., 2016). On the other hand, Qin *et al.* (2022) reported that dietary flavonoids inhibit virulent factors and inflammation; improved feed conversion ratio and body weight gain.

In this study, fresh *Lagenaria breviflora* fruit juice was used, while Egbeyale *et al*. (2021) used hot water fermented *Lagenaria breviflora* fruit, which could have limited the bio-availability and pharmacological activities of some antimicrobial, antioxidant and anti-inflammatory compounds in the experimental birds. The significantly higher body weight and better feed conversion ratio recorded at low dose (2.50 mL) of *Lagenaria breviflora* is consistent with earlier reports on broilers, pigs and mice subjected to in feed sub- therapeutic doses of antibiotics (Cox *et al*., 2014). Krishnan *et al*. (2015) attributed body weight gain induced by sub-therapeutic doses of antibiotics to increased nutrient extraction and modulation immune of specific metabolic pathways microbial metabolites (butyrate, acetate, and propionate), which improve the nutritional status of the host. Hossan *et al*. (2018) attributed improvement in growth and feed conversion ratio in birds treated with antibiotics to reduced weight and length and of the intestine, which shortens the transit time of the digesta in the gastrointestinal tract.

The reasons for the result obtained in this study in birds administered 2.50 ml of LBFJ could be attributed to better gut microbiome stability, resulting in improved intestinal health and nutrient absorption. This result is in agreement with Dibner and Richards (2005) and Juan *et al.* (2019) for an ideal antimicrobial, and with Tomori *et al.* (2007) and Olakojo *et al*. (2024), who reported dose dependent antibacterial potentials of *Lagenaria breviflora*.

The significantly lower body weight recorded in birds administered 7.50 mL of LBFJ is consistent with Tuti *et al*. (2020) who reported decreased weight gain in broiler chickens fed diet containing higher dose (1.50%) of red ginger and turmeric mixture. The result of this study is in line with Murphy *et al*. (2013) who reported that high-dose antibiotic treatment that results in substantial reduction of microbial population can lead to weight loss in mouse, indicating that modulation of the microbiota is more important than removing the bulk of microbiota. The result of this study appears to suggest that 7.50 mL of LBFJ is a therapeutic dose that could knock down gut microbiota. The decrease in body weight in birds administered 7.50 mL of LBFJ could be due to loss of calories from microbial metabolites, which the birds could not synthesize, but depended on knocked down beneficial gut microbes.

Other reasons for body weight loss in the birds administered high dose (7.50 mL) could be attributed to hypocholesterolemic, and hypolipidaemic effects of the LBFJ, which counteracted the path ways for body weight gain. Earlier reports of Ajani *et al.* (2014) and Adeyemi *et al*. (2017) indicated that *Lagenaria breviflora* fruit contains saponins and alkene linoleic acid, which have hypolipidaemic and hypocholesterolemic properties. The probable mechanisms by which hypolipidaemic and hypocholesterolemic compounds could increase feed intake, and reduce body weight gain have been postulated. Wallace *et al.* (2010) attributed weight reduction due hypolipidaemia and hypocholesterolemia with photobiotics to decreased serum concentrations

cholesterol and triglycerides through biliary excretion, and inhibition of cholesterol and lipid uptake by the liver and muscle tissues. Tzeng and Liu (2013) reported that it is by inhibition adipogenesis, and suppression of accumulation of cytoplasmic oil droplet, and by emulsification the fat globules in the adipocytes, and reduction the level of fatty acid synthesizing enzyme. Akinyemi *et al*. (2016) reported that it is by inhibition of arginase activity. Mahmoud and Elnour (2013) reported that ginger reduced body weight by increasing catalase level.

The reason for the decreased body weight obtained in this study at the highest dose (7.50 mL) could be attributed to induction of negative anabolic effects through path ways that decreased glucose, cholesterol and lipid uptake by the muscle and liver tissues. There could also have been a reduction in fatty acid synthase and inhibition of arginase activity as well as a suppression of adipogenesis at 7.50 mL dosing.

The higher mean feed intake value recorded in birds administered 7.50 mL of the LBFJ suggests that the endogenous enzyme secretions, which control appetite and feed intake are hyper- activated by the active ingredients in the LBFJ with increase in dose. This result is in consonance with earlier report of Wenk (2005) that herbs, spices and their extracts can stimulate feed intake and endogenous secretions. The dose dependent effect of LBFJ on growth performance recorded in this study is in line with earlier reports of Nath *et al*. (2023) on probiotics,

The significantly higher Hb, PCV and WBC recorded in this study in birds administered LBFJ agrees with the report of Irivboje and Olufayo (2023) on broiler chickens administered *Lagenaria breviflora* fruit extract, and Islam *et al*. (2025) on broiler chickens fed diet treated with antibiotics, probiotics, and acidifier, but contrasted to the report of Nworgu *et al*. (2018) on broiler chickens administered *Lagenaria breviflora* fruit extract. The reasons for the disparity could be due to variations in the processing method, dose of administration, time /and season at which the fruits were harvested. While Nworgu *et al*. (2018) used fermented fruit extract, in this study, fresh fruit juice was used. Aster (2004) reported that an increase in PCV is an indication of a corresponding increase in RBC, which is an indication of good blood level. This scenario is in agreement with the result obtained in this study. The significantly higher Hb and PCV values in birds administered LBFJ in this study agrees with the report of Irivboje and Olufayo (2023) that *Lagenaria breviflora* fruit improved haematological parameters. The reason for the high Hb and PCV values recorded in this study could be attributed to effect of the LBFJ on the gut, which reduced inflammation and subclinical infection and stress; improved nutrient absorption and utilization, and promoted red blood cell production and other blood parameters. The significantly higher lymphocyte count observed in birds administered LBFJ could be attributed to increased humoral immune response and antibody formation. This is because monocyte and eosinophil counts in the treated groups did not differ from the control. This indicates absence of inflammation, pathogenic inversion as well as hypersensitivity reactions (Terry, 2012). Since the lymphocyte / heterophil ratio did not increase in birds administered LBFJ across the treatments, it indicates absence of stressors due to the LBFJ administration.

The result of this study indicates that the levels – 2.50 – 7.50 mL of LBFJ used in this study did not derange the haematological profile of the experimental bird, indicating that up to 7.50 ml of LBFJ is safe for broiler chickens.

The jejunal and ileal histo-pathological studies on the birds administered 2.50, 5.00 and 7.50 ml of LBFJ did not vary with the control. This means that the administration of LBFJ at 2.50 ml, 5.00 mL, and 7.50 mL of LBFJ did not result in any pathological derangement of the structural and functional integrities of the jejunum and the ileum. The result of this study is in line with the report of Xue *et al*. (2017) that a healthy gut is vital for the digestion of

feed and absorption of nutrients required for growth and body maintenance. Although histo-morphomatry data were not collected on jejunum and ileum, inference from the growth performance suggests marked improvements in the absorptive indices, and structural integrity of the treated birds. This is in line with Abou-Ashour *et al*. (2021) who reported decreased inflammation of the intestinal mucosa, leading to increased villi height, gastric secretion, digestion and absorption of nutrients, and intestinal health; in broiler chickens fed diet containing 5% acetic acid. This result is also in consonance with the report of Ivana *et al*. (2019) that propolis pollen extract improved nutrient absorptive index in broiler chickens.

Conclusion

In conclusion, daily administration of 2.50 mL of LBFJ gave higher final body weight and least feed conversion ratio. Administration of more than 2.50 mL of LBFJ compromise body weight gain, but improved haematological parameters and humoral immune response at the expense of feed cost. Administration of up to 7.50 mL of LBFJ did not cause any adverse effect on the jejunal and leal functional and morphological integrities, as well as the general well-being of broiler chickens.

Recommendation

It is therefore recommended that administration of LBFJ for the purpose of increase in body weight should not exceed 2.50 mL in broiler chickens. Up to 7.50 mL daily administration of LBFJ is recommended where stronger immunity required, but at the expense of feed cost.

acknowledgement

The authors are sincerely grateful to Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike, for providing the facilities for this research.

Conflict of Interest

There no conflicts of interest.

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Date received:26th January, 2026
Date acceptance: 3rd March, 2026