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Growth performance, gut integrity and blood metabolites of laying hens fed pepper elder (*Peperomia Pellucida* (l.) kunth supplemented diets

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Abstract

The threat posed by the continuous application of antibiotics as growth promoters to poultry and humans who consume the products is too great to be under-estimated. The search for viable alternatives, such as phytonutrients has become necessary to keep the pace of healthy production and safe products to appreciable level. This study was therefore designed to evaluate the effect of Peperomia pellucida supplementation on performance, internal and external quality of the eggs, gut morphological indices, histopathological indices, haematological indices and lipid profile of laying hens in a 42-day feeding trial. Fifty-four 20-week-old Isa-brown laying hens were randomly assigned to three dietary treatments and replicated six times with three birds per replicate. Treatment 1 was a basal diet, while treatments 2 and 3 contained the basal diet with 0.5%, and 1% Peperomia pellucida powder, respectively. Performance indices were evaluated. On day 42, haematological indices, lipid profile, gut morphological indices, histopathological indices, internal and external quality of the eggs were examined following standard procedure. Data obtained were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$*. The results showed that feed intake, white blood cell, monocyte, basophil counts, high density lipoprotein, total cholesterol, low density lipoprotein, yolk width, albumen height, haugh unit, were significantly influenced by dietary treatments. Diets had no significant influence on the gut morphological indices. Histopathological observations of the ileum showed that no visible lesion in the ilea of the birds on both experimental diets and basal diet. The villi of hens fed with 1% Peperomia pellucida diet appeared slightly stunted. Conclusively, 0.5 % Peperomia pellucida dietary level was considered the bestlevel for laying hens as growth promoters thereby being a viable alternative to antibiotics.*

Keywords: *Peperomia pellucida*, Laying birds, Egg characteristics, Growth response, Blood profile

Running title: *Peperomia in laying bird's diet and performance*

Performances de croissance, intégrité intestinale et métabolites sanguins de poules pondeuses nourries avec des régimes supplémentés de sureau poivré (*Peperomia pellucida* (l.) Kunth)

Résumé

La menace posée par l'application continue d'antibiotiques comme stimulateurs de croissance aux volailles et aux humains qui consomment ces produits est trop grande pour être sous-estimée. La recherche d'alternatives viables, telles que les phytonutriments, est devenue nécessaire pour maintenir à un niveau appréciable le rythme d'une production saine et de produits sûrs. Cette étude a donc été conçue pour évaluer l'effet de la supplémentation en Peperomia pellucida sur les performances, la qualité interne et externe des œufs, les indices morphologiques intestinaux, les indices histopathologiques, les indices hématologiques et le profil lipidique des poules pondeuses dans un essai d'alimentation de 42 jours.

Cinquante-quatre poules pondeuses Isa-brown âgées de 20 semaines ont été assignées au hasard à trois traitements alimentaires et répétées six fois avec trois oiseaux par répétition. Le traitement 1 était un régime de base, tandis que les traitements 2 et 3 contenaient le régime de base avec respectivement 0,5 % et 1 % de poudre de Peperomia pellucida. Des indices de performance ont été évalués. Au jour 42, les indices hématologiques, le profil lipidique, les indices morphologiques intestinaux, les indices histopathologiques, la qualité interne et externe des œufs ont été examinés selon la procédure standard. Les données obtenues ont été analysées à l'aide de statistiques descriptives et d'ANOVA à α0,05. Les résultats ont montré que la consommation alimentaire, les globules blancs, les monocytes, le nombre de basophiles, les lipoprotéines de haute densité, le cholestérol total, les lipoprotéines de basse densité, la largeur du jaune, la hauteur de l'albumen, l'unité de hauteur, étaient significativement influencés par les traitements alimentaires. Les régimes alimentaires n'ont eu aucune influence significative sur les indices morphologiques intestinaux. Les observations histopathologiques de l'iléon n'ont montré aucune lésion visible dans l'iléon des oiseaux soumis au régime expérimental et au régime de base. Les villosités des poules nourries avec un régime à 1 % de Peperomia pellucida semblaient légèrement rabougries. En conclusion, un niveau alimentaire de 0,5 % de Peperomia pellucida a été considéré comme le meilleur niveau pour les poules pondeuses en tant que promoteurs de croissance, constituant ainsi une alternative viable aux antibiotiques.

Mots-clés: *Peperomia pellucida*, Oiseaux pondeurs, Caractéristiques des œufs, Réponse à la croissance, Profil sanguine

Introduction

Antibiotics as growth promoters have helped to maintain healthy birds and boost productivity and performance, but its adverse effects on birds and humans cannot be ignored (Hughes and Heritage, 2004). Hence, the use of antibiotics as growth promoters was banned in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC, 2018).

In search of viable alternatives to increase animal productivity, the ethnomedicinal plant like *Peperomia pellucida (Pepper elder) shows a great potential because of its antimicrobial, anticancer, anti-inflammatory and antioxidant properties (Wei et al., 2011). Peperomia pellucida* (L.) Kunth is a slender, shallow-rooted herb that can be wild or cultivated. It is an annual plant usually growing to a height of about 6 to 18 inches, it is characterized by its glossy, light-green fleshy leaves, and succulent stems that root at the nodes and fruiting spikes with dot-like seeds (The Green Institute, 2020). This plant is mainly

distributed in the Neotropics, Africa, Southeast Asia, and Australia and employed in the treatment of a variety of health conditions such as abscesses, abdominal pain, skin sores, conjunctivitis, measles (Alves *et al*., 2019).

Several studies have also described its antimicrobial, cytotoxic, antidiabetic, antihypertensive, anti-inflammatory, antioxidant, *anti-glycation, gastroprotective* antihyperglycemic, *antiosteoporotic, immunostimulatory*, anti-angiogenic activities and a variety of other bioactivities (*Arrigoni-Blank et al., 2004; Khan et al., 2008; Oloyede et al., 2011; Hamzah et al., 2012; Zubair et al., 2015; Lee et al., 2016; Okoh et al., 2017; Ngueguim et al., 2017; Narayana et al., 2018;* Alves *et al*., 2019; *Men et al., 2022;* Ho *et al*., 2022a,b).

Similarly, studies have also shown that Peperomia pellucida contains alkaloids, polyphenols, flavonoids, and essential oils (Silva et al., 1999; Usman and Ismaeel, 2020) which are important groups of plants' secondary metabolites that are not essential for plant survival but are significant for their defense, especially against microbial pathogens (Fialova *et al*., 2017). These compounds have been approved for their antimicrobial activities in animal nutrition and human medicine (Fialova *et al*., 2017; Omidiwura *et al*., 2022).

Against this background, this study aimed at evaluating the effects of *Peperomia pellucida* supplemented diets on growth performance, internal and external attributes of eggs, gut morpho-pathological indices and blood profile of laying hens.

Materials and Methods

Experimental Site

The experiment was conducted at the Teaching and Research Farm Poultry Unit, University of Ibadan, Oyo state, Nigeria.

Experimental Diets

Peperomia pellucida plants were hand plucked, air-dried at room temperature, processed into a fine powder, and stored in sterile containers until needed for use. The basal diet used was a standard commercial bagged layers' mash containing: Crude protein-16.5%, Calcium-3.6%, Available phosphorus-0.45%, and Metabolizable energy-2,500Kcal/kg in accordance with NRC (1994) guidelines. Treatment 1 was the control diet (basal diet), not supplemented with *Peperomia pellucida*. Treatment 2 had basal diet + 0.5% *Peperomia pellucida* while Treatment 3 was a basal diet + 1.0% *Peperomia pellucida*.

Management of Birds

Fifty-four 20-week old Isa brown hens were sorted by body weight and randomly distributed into three dietary treatments with six replicates comprised of three hens each in a completely randomized design. The birds were housed in a battery cage in a semi-controlled environment. The birds had access to 100 g of experimental diet per bird per day and water was supplied *ad libitum* during the study period which lasted for six weeks.

Data collection

Feed intake

Each replicate was supplied 100 g of feed in the morning and 200 g of feed in the afternoon making a total of 300 g of feed per replicate (3 birds) per day. Feed leftover was measured at the end of each week. Weekly feed intake was calculated by subtracting the leftover feed from the feed supplied. Daily feed intake was calculated according to standard procedure.

Weight gain

The birds were weighed weekly with the use of a sensitive weighing scale to determine accurately the birds' weight. The weight gain was thereafter calculated according to standard procedure.

Feed conversion ratio

The feed conversion ratio was calculated by dividing the feed intake per bird per week by average egg weight per week multiplied by number of eggs laid per bird per week.

Feed Conversion Ratio= Feed intake per week (g)/ Average egg weight per week x Number of eggs laid per week

Egg weight

Eggs were collected from each replicate daily throughout the experimental period to evaluate the egg weight using a sensitive weighing scale.

Hen day production

Hen day production was obtained by dividing the number of eggs collected per week by number of days in a week multiply by numbers of birds per treatment.

Hen-day production (%) = Total Number of eggs collected per week x 100 / Number of days in a week x Number of birds

Egg quality evaluation

At day 42 of the experiment, the egg quality parameters were measured. Egg length and width were measured using Vernier caliper. The width was measured as the distance between the broad and narrow ends of the eggs. Egg Shape Index was calculated as the percentage of the egg width to the egg length.

Egg Shape Index =Width of egg (mm) x 100 / Length of egg (mm)

Yolk height and yolk width were measured using a vernier caliper. Albumen height was measured with tripod micrometer after the egg was gently broken and the maximum height was taken. Yolk index was estimated from ratio of yolk height to yolk width. Yolk colour was determined with a yolk colour fan scale (1 to 15). Haugh unit, which is a relationship between egg weight and height of thick albumen surrounding yolk was calculated using the values obtained from the egg weight and albumen height. This can be expressed as:

$HU = 100 \log [H + 7.57 - 1.7W^{0.37}]$

Where, H = Albumen Height (mm) and

W = Weight of the egg (g)

Histomorphological indices

At day 42 of the experiment, two birds were randomly selected and slaughtered from each treatment. The ileum (the portion of the small intestine extending from the Vitelline diverticulum to read a point of 40mm proximal to the ileocaecal junction) was removed for for ileal morphological measurements. The ileal samples were collected, prepared and stained with standard hematoxylin-eosin solution and examined by light microscopy and photomicrographs at x40, x100, and x400 magnifications for the morphometrical measurements of crypt, villi, and epithelial parameters as well as histopathological assay.

Blood parameters

At day 42 of the experiment, blood samples were collected from the jugular vein of two birds per treatment, using a sterile syringe into two vacutainer tubes for each bird, one containing Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological analysis and the other without EDTA for lipid profile assay. Red blood cell (RBC), White blood cell (WBC) Packed cell volume (PCV) , Haemoglobin concentration and Platelets were determined following standard procedures.

Serum biochemical indices

Total serum cholesterol, triglycerides, and High Density Lipoprotein (HDL) were assayed by the method of Roschlan *et al*. (1974). Very Low Density lipoproteins (VLDL) were estimated as (triglycerides/5) (Friedewald *et al*., 1972), while low density lipoproteins (LDL) were estimated using Friedewald equation [LDL= Total cholesterol – (HDL-Triglycerides/5)].

Lipid profile

Three eggs per replicate were prepared according to the procedure described by Elkin and Rogler (1990) for egg yolk cholesterol quantification. Total cholesterol, High-Density Lipoprotein (HDL), and total triglyceride concentration in the egg yolk were determined using the respective cholesterol assay kit. Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were calculated.Using two hens per replicate, the serum cholesterol level was determined using serum cholesterol kit (Cell Biolabs' HDL and LDL/VLDL Cholesterol Assay Kit) according to the procedure described by Siedel *et al*. (1981). At the end of the experiment, two hens per replicate were sacrificed and breast meat samples were collected for analysis of cholesterol level in meat. Approximately 2 g of sample was saponified according to a modified version of the method described by Stewart *et al*. (1992).

Chemical analysis

Proximate analysis of the experimental diets was according to the methods described by AOAC (2005).

Statistical analysis

Data obtained were analyzed using descriptive statistics and ANOVA, using completely randomized design of Statistical Analysis System (SAS, 2008). The treatment means were separated using Duncan's Multiple Range Test (Duncan, 1955) of the same software package to test significant differences ($p = 0.05$) among the means.

Results

Performance characteristics of birds fed Peperomia pellucida supplemented diets

The performance characteristics of laying birds fed *Peperomia pellucida* supplemented diets is shown in table 1. Average egg weight, feed conversion ratio, hen day production, final body weight and weight gain did not differ significantly in birds fed *Peperomia pellucida* supplemented diets compared to the control birds. However, the feed intake was significantly ($P<0.05$) influenced by *Peperomia pellucida supplementation. Birds on 0.5% Peperomia pellucida supplemented diet had the highest feed intake.*

Table 1. Performance characteristics of laying hen fed with *Peperomia pellucida* supplemented diets

	Peperomia pellucida inclusion* levels *(%)				
Parameters	**0%**	**0.5%**	**1%**	**SEM**	**P -Value**
Feed intake (g/hen/day)	732.76[b]	763.82[a]	734.76[a]	5.94	0.003
Average egg weight (g)	15.59	17.39	19.52	1.54	0.228
Feed conversion ratio	13.25	13.78	12.81	0.47	0.367
Hen day production (%)	77.62	77.14	65.24	6.02	0.287
Initial body weight (g/hen)	1564.00	1658.83	1557.17	52.80	0.341
Final body weight (g/hen)	1595.00	1676.50	1569.50	43.25	0.221
Weight gain (g/hen)	31.00	17.67	12.33	45.56	0.957

Means along the row with different superscripts are significantly ($P<0.05$) different

Internal and External Egg Attributes of Laying Hens fed Peperomia pellucida supplemented diets

The result of the supplementation of layers' diet with *Peperomia pellucida* on internal and external qualities of eggs is presented in table 2. No remarkable difference (P>0.05) was observed in the egg weight, egg length, egg width, egg shape index, shell weight, yolk weight, yolk height, yolk colour, albumen width, and albumen width. However, yolk width was significantly (p<0.05) influenced by dietary treatments. Yolk width for birds on control diet and 0.5% *Peperomia pellucida supplemented* diets were significantly (P<0.05) higher than those on 1.0% *Peperomia pellucida supplemented* diet. Albumen height and haugh unit of 0.5% and 1.0% *Peperomia pellucida supplemented* diets were similar but significantly lower (p<0.05) than the control diet.

Table 2. Internal and external qualities of commercial eggs of Isa brown layers fed *Peperomia pellucida* supplemented diets

	Peperomia pellucida inclusion				
Parameters	**0%**	**0.5%**	**1.0%**	**SEM**	**P value**
Egg weight (g)	55.67	55.67	57.67	1.68	0.600
Egg length (cm)	5.41	5.34	5.53	0.08	0.290
Egg width (cm)	4.28	4.30	4.34	0.18	0.800
Egg shape index (cm)	0.80	0.80	0.78	0.12	0.600
Shell weight (g)	6.83	6.83	6.50	0.36	0.810
Yolk weight (g)	12.50	12.83	13.00	0.43	0.710
Yolk height (cm)	4.13	4.53	4.05	0.61	0.210
Yolk width (cm)	3.97^{a}	4.06^{a}	3.65^{b}	0.09	0.010
Yolk colour	2.83	3.50	3.83	0.51	0.420
Albumen height(cm)	6.74^{a}	5.42^{b}	5.50^{b}	0.41	0.070
Albumen width (cm)	5.44	4.79	5.08	0.26	0.240
Haugh unit (%)	82.90^{a}	72.78^{b}	73.44^{b}	3.08	0.060

Means along the row with different superscripts are significantly (P<0.05) different

Gut Histomorphological Indices of Laying Hens fed Peperomia pellucida Supplemented Diets

The result of the gut histomorphology of laying hens fed *Peperomia pellucida* supplemented diets is presented in table 3. Diets had no significant influence on the villus height, villus width, crypt depth, crypt width, epithelial height and goblet cell density.

Table 3. Gut histomorphological indices of laying hens fed *Peperomia pellucida* supplemented diets

	Peperomia pellucida inclusion				
Parameters (µm)	**0%**	**0.5%**	**1%**	**SEM**	**P Value**
Villus height	761.70	758.80	603.30	120.38	0.618
Crypt depth	112.84	85.31	109.66	51.16	0.919
Villus width	75.00	78.02	84.68	7.74	0.960
Crypt width	22.97	22.18	18.22	1.90	0.306
Epithelial height	20.65	27.89	25.93	2.50	0.253
Goblet cell density	0.01	0.01	0.01	0.01	0.252

Histopathological Readings of birds fed with Peperomia pellucida Supplemented diets

The result of the histopathological readings of the ileum of birds fed *Peperomia pellucida* supplemented diets is presented in Plates 1-3 using **microscopic images.** Plates 1, 2 and 3 showed the sample partitioning of ileum of birds fed 0, 0.5 and 1% *Peperomia pellucida* supplemented diets, respectively. It was observed that the glands at the submucosa appear slightly dense with reduced acini lumen for birds fed 0% *Peperomia pellucida* supplemented diet. While the photomicrographs of the ileum of birds on 0.5 % and 1% *Peperomia pellucida* supplemented diets revealed no evidence of lesion on the ileum, but the villi of birds fed 1% *Peperomia pellucida* supplemented diet appeared slightly stunted.

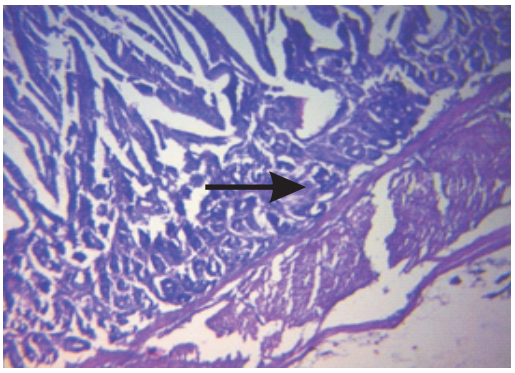

Plate 1: Histopathological Readings of birds fed with 0% *Peperomia pellucida supplemented* diets

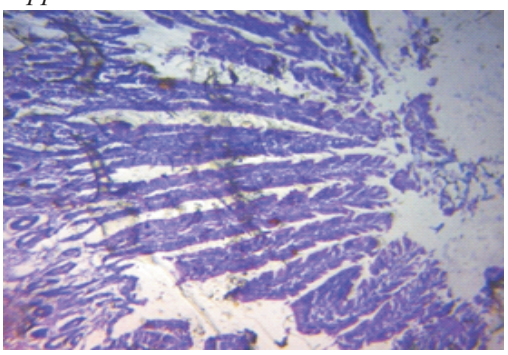

Plate 2: Histopathological Readings of birds fed with 0.5% *Peperomia pellucida supplemented* diets

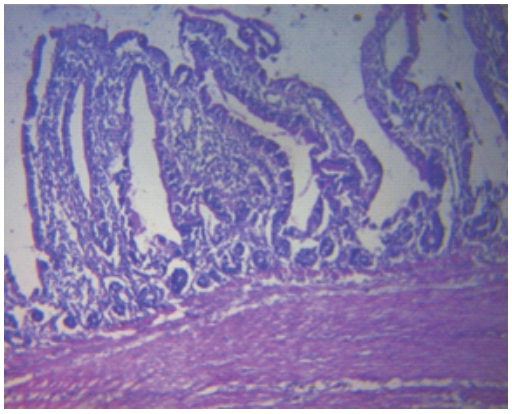

Plate 3: Histopathological Readings of birds fed with 1% *Peperomia pellucida supplemented* diets

Haematological indices of birds fed with Peperomia pellucida supplemented diets

The result of haematological indices of laying **birds fed** *Peperomia pellucida* supplemented diets is presented in Table 4. There were no significant differences ($P>0.05$) observed in the Packed Cell Volume, Haemoglobin, Red Blood Cell, Platelets, Lymphocyte, Heterophil, Eosinophil and Heterophil to Lymphocyte ratio of birds on dietary treatments. However, White Blood Cell (WBC), Monocyte and Basophil counts of birds on experimental diets were significantly ($P<0.05$) influenced. The WBC counts of birds on 1.0% *Peperomia pellucida* diet were significantly higher than those on the control diet. Monocyte count of birds on the control diet was significantly higher than those fed *Peperomia pellucida* supplemented diets, though similar to those on 1.0% *Peperomia pellucida* supplemented diet. However, basophil count was highest (0.50%) in hens on 1.0% *Peperomia pellucida* diet while the least count was recorded in the control diet and 0.5% *Peperomia pellucida* diet.

Table 4. Haematological indices of laying hens fed *Peperomia pellucida* supplemented diets

	***Peperomia pellucida inclusion* levels (%)**				
Parameters	**0%**	**0.5%**	**1%**	**SEM**	**P value**
PCV (%)	25	25	27.67	1.07	0.161
Hb (g/dL)	8.1	8.25	9.17	0.35	0.104
RBC ($x10^6$uL)	2.45	2.69	3.02	0.26	0.336
WBC ($x10^6$uL)	14.17^b	14.37^{ab}	15.50^a	43.89	0.074
PLATELET ($x10^4$uL)	14.75	15.55	16.38	15.79	0.769
LYM (%)	60.83	58.67	59.67	2.15	0.779
HET (%)	34.67	35.17	33.17	2.12	0.789
MON (%)	3.67^a	2.50^b	2.83^{ab}	0.33	0.062
EOS (%)	2.5	3.67	3.83	0.68	0.346
BA (%)	0.00^b	0.00^b	0.50^a	0.13	0.022
H:L	0.58	0.60	0.57	0.06	0.907

Means on the same row with different superscripts are significantly (P<0.05) different.
PCV - packed cell volume, Hb - Haemoglobin, RBC - red blood cell, WBC - white blood cell, LYM=lymphocyte, HET=heterophils, MON=monocytes, EOS=eosinophil, BA=basophil, **H:L** – heterophils to lymphocyte ratio

Lipid profile of birds fed with Peperomia pellucida supplemented diets

The result of lipid profile of laying birds fed ***Peperomia pellucida*** supplemented diets is shown in Table 5. Diets had no influence on triglyceride and very low-density lipoprotein (VLDL). However, high density lipoprotein (HDL), total cholesterol (TC) and low-density lipoprotein (LDL) were significantly (P< 0.05) influenced by the dietary treatments. High density lipoprotein and total cholesterol of birds placed on 1.0% *Peperomia pellucida* diet were significantly (P<0.05) higher than those fed with 0.5% *Peperomia pellucida supplemented* diet. Low density lipoprotein values of birds that received the control diet were similar to those on *Peperomia pellucida* supplemented diets.

Table 5. Lipid profile of laying hens fed *Peperomia pellucida* supplemented diets

	***Peperomia pellucida inclusion* levels (%)**				
Parameters	**0%**	**0.5%**	**1%**	**SEM**	**P value**
HDL (mg/dL)	8.68^{ab}	6.46^b	10.07^a	1.04	0.058
Total cholesterol (mg/dL)	87.78^{ab}	83.06^b	96.65^a	3.04	0.019
Triglyceride (mg/dL)	284.65	296.78	294.77	7.60	0.499
VLDL (mg/dL)	56.93	59.34	58.95	1.52	0.500
LDL (mg/dL)	22.17^{ab}	27.63^a	17.26^b	2.03	0.009

Means on the same row with different superscripts are significantly (P<0.05) different, HDL=High Density Lipoprotein, VLDL=Very Low Density Lipoprotein, LDL=Low Density Lipoprotein

Discussion

The results of this present study showed that *Peperomia pellucida supplemented* diets did not have a significant effect on the performance of laying hens except for improving feed consumption. The average egg weight, feed conversion ratio, hen day production, initial body weight, final body weight and weight gain were not significantly influenced across the treatments. This result is in agreement with the findings of Agboola *et al.* (2016) who reported no significant differences in egg production, egg weight, weight gain, feed intake, and feed conversion ratio of birds fed with dietary oils. Conversely, in view of the entire experimental period, an increasing trend for feed intake was observed in birds fed *Peperomia pellucida supplemented* diets. This reaction to the *Peperomia pellucida* may support this proposition that as the birds mature the taste buds may have developed and grown-up birds have twice the number of taste buds of young birds, consequently, better sensing of sharp-tasting of *Peperomia pellucida* (Whittow, 1999).

The results of this present study showed that *Peperomia pellucida* supplemented diets did not have a significant effect on the egg weight, egg length, egg width, egg shape index, shell weight, yolk weight, yolk height, yolk colour, albumen width, and albumen width. However, the yolk width, albumen height and haugh unit were significantly influenced. Birds fed *Peperomia pellucida* supplemented diet experienced a lower haugh unit and albumen height compared to the control diet but still fell within the best egg quality classification. The higher the yolk index and haugh unit, the more desirable the egg quality (Fayeye *et al*., 2005). The range of haugh unit observed in this study was 72.78-82.90, which is within the best egg quality classification. The higher the value of the Haugh unit, the better the quality of eggs, which are classified according to the United States Department of Agriculture (USDA) as AA (100 to 72%), A (71 to 60%), B (59 to 30%) and C (below 29%) (USDA, 2000). Oluyemi and Robert (2000) also reported that unit score of 72 and above has been graded as the best quality. This showed that, the quality of the egg regarding the Haugh unit in the current study was under AA grade.

Gut morphology indices such as the villus height, villus length, crypt depth, villi width and epithelial thickness were measured in the ileum of laying hen across the treatments. There were no observable differences for all the measured indices in this study. This is in line with the findings of Omidiwura *et al.* (2022) who reported that 0.4% *Peperomia pellucida supplemented* diet had no influence on the gut morphology indices of birds. It is equally in line with the findings of Catala-Gregori *et al.* (2008) who established that dietary supplementation with plant extracts had no significant effects on intestinal cell morphology.

The histopathological readings of the ileum of birds in this study show that birds fed *Peperomia pellucida* supplemented diets had no lesion on the ileum. This is a reflection of the gastroprotective activity of *Peperomia pellucida.* The villi of the birds fed 1% *Peperomia pellucida* supplemented diet appeared slightly stunted. Previous Studies have shown that *peperomia pellucida* plant is a gastroprotective agent (Roslida and Aini, 2009; Reza *et al.,* 2022) and this is traced to the metabolites contained in the plant. It contains secondary metabolites in the form of flavonoids, tannins, saponins, triterpenoids and steroids (Rachmawati and Rantelino, 2018).

The hematological parameters in Isa brown birds can vary, depending on a number of factors including age, sex, health status and nutrition (Oluyemi and Robert, 2000).

However, the results of the hematological parameters of this research are in range with the general reference ranges for hematological parameters in healthy adult birds. There were no significant differences in the results of packed cell volume, haemoglobin, red blood cell, platelet, lymphocytes, eosinophils, heterophils and heterophils-lymphocytes ratio. This corresponds to the findings of Omidiwura *et al.* (2022), who reported that 0.4% *Peperomia pellucida supplemented* diet had no influence on the haematological parameters of birds. Contrary to the findings above, the white blood cell, monocyte, and basophil values in the present study were influenced by *Peperomia pellucida supplementation at different levels although still within acceptable level.* White blood cells protect the body from damage by bacteria, viruses, parasites toxins and tumor cells. High level of white blood cells can be a response to protect the body against the pathogens or immunostimulation. White blood cell profile is the relative proportion of five white blood cell types: lymphocytes, monocytes, eosinophils, basophils and heterophils (Davis *et al.,* 2008). A significant increase in heterophils, and lymphocyte is an indication of infection, inflammation or toxin ingestion. Chickens with low H/L ratio are superior to the chickens with high H/L ratio in survival, immune response, and resistance to diseases (Minias, 2019; Thiam *et al*., 2022). Elevations of eosinophils in the blood is an indication of infestation of parasitic worms. Haemoglobin and red blood cell transport oxygen in the tissues of laying hens (Etim, 2014). *Peperomia pellucida* supplemented diets have no negative impact on the transportation of oxygen into the tissues of laying hens.

Although still within the acceptable range, birds fed 0.5% *Peperomia pellucida* supplemented diet experienced a significantly decreased in HDL and Total cholesterol. This is in line with the result of Chasanah *et al*. (2016) who reported that ethanol extract of *Peperomia pellucida* at a dose of 300 mg/kg body weight white rat can lower total cholesterol significantly. *Hamzah et al.* (2012) equally reported that diabetic rats on diets supplemented with *Peperomia pellucida* experienced a significant decrease in total serum cholesterol, triglycerides and LDL compared to the untreated diabetic rats. Additionlly, Alfonso and Riego de Dios (2015) also reported that *Peperomia pellucida* crude extract significantly decreased the total cholesterol level of hypercholesterolemic induced Sprague Dawley rats.

Conclusion

Peperomia pellucida inclusion up to 1% had no significant effect on performance parameters, internal and external attributes of commercial eggs and gut morphological indices of laying birds except for the feed intake and yolk width. However, it did not elicit any deleterious effect on the parameters measured, haematological indices and lipid profile. In histopathological aspect of the research, 0.5% inclusion of the experimental diet was considered to be the safest for the laying hens.

Therefore, *Peperomia pellucida* up to 0.5% inclusion can be used as viable alternative to antibiotics administered as growth promoters.

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