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Potentials of pepper elder *(Peperomia pellucida)* and humic acid as feed additives in noiler chicken production

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Abstract

Feed additives have become essential in feed formulation in the poultry industry. Amongst feed additives, Antibiotics Growth Promoter (AGPs) is one of the most widely used. The use of AGPs comes with its negative effects on not only poultry but humans that consume the products. The inadequacies that come with the use of antibiotics growth promoters (AGPs), has led to a need for a viable and sustainable alternative. Hence, this study was conducted to evaluate the effects of dietary inclusion of humic acid, Peperomia pellucida and their combinations on haematological and histomorphological parameters and microbial load in noiler chicken. A total of 72, one day old noiler birds from Amo Farm Sieberer Hatchery (AFSH) were randomly distributed into four dietary treatments with three replicates of six birds each in a completely randomized design in an experiment that lasted for 56 days. Treatment 1 was basal diet with no additive; treatment 2 was basal diet +0.1% humic Acid. Treatment 3 was basal diet +0.4% Peperomia pellucida, while treatment 4 was basal diet +0.1% humic acid +0.4% Peperomia pellucida. Haematological and histomorphological parameters as well as microbial load in the gut were measured. Data obtained were analysed with ANOVA while means were separated using Duncan's Multiple Range Test at $\alpha_{0.05}$. *Significant ($p \leq 0.05$) differences were observed in the heterophils count across treatments with T4 (31.00±4.36) having the highest heterophils count compared to T1 (27.33±2.52), T2 (26.33±3.21) and T3 (22.33±3.21). Statistically, there were no significant differences in the histomorphological parameters. There was significant ($p \leq 0.05$) decrease in the Total Heterotrophic Count (THC) and a significant ($p \leq 0.05$) increase in the total lactobacilli count across treatments. Birds fed with basal diet T1 (5.45±0.92) had significantly higher heterotrophic counts when compared with T2 (1.30±1.56), T3 (1.30±0.56) and T4 (1.80±1.27) while birds fed with T3 (17.53±5.35) had a significantly higher TLC when compared with T1 (9.07±2.56), T2 (15.40±3.08) and T4 (13.60±1.90). It can be concluded that humic acid and Peperomia pellucida can improve the gastrointestinal microflora, immune responses and general wellbeing of noiler. Hence, they can actively be used as an alternative to antibiotics growth promoters in maintaining gut and immune integrity in noiler chickens.*

Keywords: Humic acid, *Peperomia pellucida*, noiler, Antibiotics Growth Promoters (AGPs), feed additives

Potentiels de poivre aîné (peperomia pellucida) et acide humique comme additifs d'alimentation en production de poulet noiler

Résumé

Les additifs d'alimentation sont devenus essentiels dans la formulation d'aliments dans l'industrie de la volaille. Parmi les additifs alimentaires, le promoteur de croissances antibiotiques (PCA) est l'un des plus utilisés. L'utilisation d'AGPS vient avec ses effets

négatifs sur non seulement de la volaille, mais également des humains qui consomment les produits. Les insuffisances fournies avec l'utilisation de promoteurs de croissance antibiotiques (PCAs) ont permis de nécessiter une alternative viable et durable. Par conséquent, cette étude a été menée pour évaluer les effets de l'inclusion alimentaire de l'acide humique, de la pepeperomia pellucida et de leurs combinaisons sur les paramètres hématologiques et histomorphologiques et la charge microbienne au poulet nilaer. Un total de 72 oiseaux noiler d'une journée d'Amo Farm Sieberer Hatchery (AFSH) ont été distribués au hasard en quatre traitements diététiques avec trois réplicats de six oiseaux chacun dans une conception complètement randomisée dans une expérience qui a duré 56 jours. Le traitement 1 était une alimentation basale sans additif; Le traitement 2 était une alimentation basale + 0,1% d'acide humique. Le traitement 3 était une alimentation basale + 0,4% de la pepeperomia pellucida, tandis que le traitement 4 était un régime alimentaire basal + 0,1% d'acide humique + 0,4% de pepperomia pellucida. Les paramètres hématologiques et histomorphologiques ainsi que la charge microbienne dans l'intestin ont été mesurés. Les données obtenues ont été analysées avec l'ANOVA tandis que des moyens ont été séparés à l'aide du test de plage multiple de Duncan à a0.05. Des différences significatives (p=0.0.05) ont été observées dans les hétéophiles comptant à travers les traitements avec T4 (31,00 ± 4,36) ayant le nombre de hétéophiles le plus élevé comparé à T1 (27,33 ± 2,52), T2 (26,33 ± 3,21) et T3 (22,33 ± 3,21). Statistiquement, il n'y avait pas de différences significatives dans les paramètres histomorphologiques. Il y avait une diminution significative (P=0.05) du nombre total hétérotrophique (NTC) et une augmentation significative (P=0.05) du nombre total de lactobacilli compter sur des traitements. Les oiseaux nourris avec une alimentation basale T1 (5,45 ± 0,92) avaient des comptes hétérotrophiques considérablement plus élevés par rapport à T2 (1,30 ± 1,56), T3 (1,30 ± 0,56) et T4 (1,80 ± 1,27) tandis que les oiseaux nourris avec T3 (17,53 ± 5,35) avaient un TLC nettement plus élevé par rapport à T1 (9,07 ± 2,56), T2 (15,40 ± 3,08) et T4 (13,60 ± 1,90). On peut conclure que l'acide humique et la pepeperomi apellucida peuvent améliorer la microflore gastro-intestinale, les réponses immunitaires et le bien-être général de Noiler. Par conséquent, ils peuvent être activement utilisés comme alternative aux promoteurs de croissance antibiotiques dans le maintien de l'intestin et d'une intégrité immunitaire dans des poulets nilaques.

Mots-clés: pepeperomiapellucida, noiler, promoteurs d'antibiotiques Croissance (PACs), Additifs alimentaires

Introduction

Feed additives have been used in poultry nutrition for many decades to increase production and maintain animal health (Alloui *et al.,* 2014). These feed additives have been added to poultry feed to combat some poultry health issue. Of all the feed additives, Antibiotics Growth Promoters (AGPs) is one of the most widely used. The use of AGPs comes with its negative effects on not only poultry but humans that consume by-products and products. In Nigeria, a ban has been issued by the National Agency for Food and Drug Administration and Control (NAFDAC) on antibiotics as growth promoters and mould inhibitors in animals feed with a recommendation for alternative healthy and approved methods to encourage the growth and wellbeing of animals (NAFDAC, 2018). The inadequacies that come with the use of antibiotics growth promoters (AGPs), has led to a need for a viable and sustainable alternative. Phytogenics/phytobiotics are one of the numerous alternatives to that can be used as

a growth promoter without negative effects on both human and animals. Alternatives like organic acids, yeast, prebiotics, probiotics, enzymes and plant extracts also called phytobiotics; which have developed new lines of research in animal nutrition have been utilized in animal diets. (Bedford, 2000; Wenk, 2003). Phytogenic feed additives (PFAs) are wide range of substances or compounds with botanical origin (Jacela *et al.,* 2010). PFAs have been reported to increase gut microbiota, improve growth and feed utilization (Hashemi and Davoodi, 2011). PFAs exhibit digestive, antioxidant, antimicrobial, antiparasitic and immunomodulating properties hence it can be used as an alternative to antibiotics in poultry production (Kuldeep *et al.,* 2014). Organic acids have been of immense help in poultry production by improving economic efficiency in poultry husbandry and it is becoming more acceptable by farmers and feed manufacturers. Organic acids in diet have improved digestibility and absorption of protein, minerals, and other nutrients (Kabir *et al.,* 2010). Organic acids are used in commercial feeds for antimicrobial activity and feed preservation (Wang *et al.,* 2009). Humic acid and *Peperomia pellucida have been identified to possess antimicrobial properties (Ceylan et al., 2003;* Zubair *et al.,* 2015). *Peperomia pellucida* (L.) Kunth is a wild type of plant native to Asia and America but widely distributed around the world. It is an herbaceous plant that belongs to the family Piperaceae. It is found in damp shaded habitat and thrives in loose humid soil during the rainy season. It is a naturally occurring weed (Schmelzer and Gurib-Fakim, 2008). *P. pellucida* contains pharmacologically important phtyochemicals such as tannins, saponin glycoside, alkaloids, cardiac glucosides, reducing sugars, steroid, terpenoid, phenolic compounds, flavonoid compound, α-amino acid, carbohydrate (Yi *et al.,* 2016), resin and inulin (Mensah *et al.,* 2009).Humic acid is an organic natural acid polymer that can be extracted from humus found in soil sediment or aquatic environment. Humic acid has been determined to improve animal performance (Yasar *et al.,* 2002; Ceylan *et al.,* 2003; Yoruk *et al.,* 2004). The use of humic acids in animal feeds brings a number of advantages for animal health and growth. Humic acid forms a protective layer on the mucous epithelium of the membrane and gas tract and stomach protecting it against infections and toxins (Kucukersan *et al.,* 2005). Humic acid just like other organic acid is important in immune system development especially the bursa of Fabricius and thymus, which are the major components of the avian immune system (Abdel-Fattah *et al.,* 2008; Ghazala *et al.,* 2011). The focus of this study is to use the combination of indigenous herb and organic acid as a viable alternative to antibiotic growth promoters that can serve as a feed additive to improve health gut integrity of noiler chicken thereby increasing meat production.

Materials and methods

Experimental site

The study was carried out at the Animal Project Venture (Layers unit), Teaching and Research farm, University of Ibadan.

Test materials

Fresh samples of *Peperomia pellucida* leaves were harvested, sorted, cleaned, cut into smaller sizes and air dried at room temperature. Air dried leaves were ground using a pre-cleaned domestic blender. Powdered samples were stored in air tight plastic bottles and used in batches for feeding. Humic acid used was HumicVet product of UCORP.

Experimental design and birds

The experiment was conducted for a 56-day feeding period using a total of 72 noiler birds. The birds were randomly assigned to

four dietary treatments at the start of experiment. The four treatments groups consist of three replicate pens of six chicks, each resulting in 18 birds per treatment.

Experimental diets and groups

The dietary groups were basal diet (control),basal diet + 0.1% humic acid, basal diet + 0.4% *Peperomia pellucida*, and basal diet + 0.1% humic acid + 0.4% *Peperomia pellucida*.

Data and sample collection

Histomorphology of the small Intestine

Three birds per replicate pen were euthanized at the end of the experiment on day 56. The entire GIT tract was removed aseptically before separating into sections of duodenum, jejunum, ileum, caecum and colon. The small intestinal segments (duodenum, jejunum and ileum) were processed for histomorphological analysis according to Haldar *et al*. (2011). Segments measuring 2cm in length from the mid-points of the duodenum, jejunum, and ileum was cut, flushed with cold saline, fixed in 10% buffered formalin, and stained with hematoxylin-eosin. Histological sections were examined with a phase contrast microscope coupled with a deconvolution imaging analysis system. Villus height (VH, from the tip of the villus to the top of the lamina propria), villus width, epithelial height, crypt depth (CD), from the base to the region of transition between the crypt and villus), and the thickness of the muscularis mucosae in the duodenum, jejunum, and ileum were determined. Measurements of 5 complete villi for VH and associated crypts for CD was taken from each segment, and the average of these values was used for statistical analysis.

Gut microbiota composition

The gut contents were extracted using a syringe and stored at 4˚C for determination of gut microbiota. Each gut digesta homogenate was serially diluted. Coliforms were cultured using MacConkey Agar, E. coli was cultured using Eosin Methylene Blue (EMB) agar. Nutrient agar was used for routine media of total bacteria counts. *Lactobacillus spp*. was cultured using Lactobacillus MRS agar *Pseudomonas aeruginosa* was cultured using Cetrimide agar. Agar was introduced using the pour plate method into plates (petri-dish) containing 1ml of diluted digesta samples, swirled clock wisely and incubated at 37˚C for 24h. Visible colonies were enumerated using a colony counter and the results were expressed as log10 Colony Forming Units per mL (log10 CFU/mL) of gut digesta.

Haematological indices

1ml of blood in vials with 2mg ethylenediaminetetraacetic acid (EDTA) was collected from the jugular vein of sampled birds. The Red Blood Cell, White Blood Cells, Haemoglobin, Packed Cell Volume, monocytes, and neutrophils were determined by method described by Ewuola and Egbunike (2008). The mean corpuscular haemoglobin and mean corpuscular volume was determined by the method described by Emiola *et al*. (2013).

Statistical analysis

The data was analyzed using SAS 9.2 (2008) software package. Repeated measures ANOVA were used to compare differences among treatments. Means were separated using New Duncan Multiple Range (NDMR) test. Values were considered statistically significant at $P \leq 0.05$.

Results

Haematological indices of Noiler chicken administered Peperomia pellucida (PP) and Humic acid (HA)

Presented in Table 1 are the haematological parameters of noiler chickens administered *Peperomia pellucida* (PP) and humic acid (HA). The result showed there were no significant differences in the means of the

packed cell volume (PCV), haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), platelet, lymphocytes, monocytes, eosinophils, and basinophils. This can be an indication of the tolerance level of the quantity of HA and PP in their diets. Heterophils in T4 was significantly higher ($p \leq 0.05$) compared to other treatments. Heterophils have an important role in the immune system of poultry (Genovese *et al*., 2013) and they are the first guard of defense against pathogenic microorganisms (Bennoune*et al*., 2009). Combination of HA and PP in the diet gave the highest heterophils and this is an indication of the immunostimulatory effect they both can have on birds. The immunostimulatory effects of these feed additives can improve the innate and acquired/adaptive immune responses (Genovese *et al*., 2013). These heterophils, stimulates activity which may protect against bacterial pathogens and reduce mortality during acute infection (Islam *et al*., 2005). Hence, they can actively be used as an alternative to Antibiotics Growth Promoters (AGPs) in maintaining immune integrity and healthy food producing animals.

Histomorphology parameters of noiler chicken administered Peperomia pellucida (PP) and Humic acid (HA)

There were no significance differences in the means of Epithelial height (EH), Villous height (VH), Crypt depth (CD), Villous width (VW) and Muscular wall thickness (MWT). Crypth depth, Epithelial and Villous heights increased along the treatments with T1 having the least and T4 having the highest. The evidence of the reduction in the proliferation of potentially harmful microorganisms is seen in the Muscular Wall Thickness (MWT) of birds fed HA and PP as they had less thick muscular walls in relative to birds fed control diet. Modulation of indigenous bacteria and the prevention of proliferation of pathogenic bacteria by these feed additives can improve health status, immune system and performance of birds (Ravindran, 2006).

Table 1: Haematological paramete rs of noiler chickens administered *Peperomia pellucida* (PP) and Humic acid (HA)

Parameters	T1	T2	T3	T4
PCV (%)	36.67±2.31	35.33±2.08	35.33±6.43	39.67±16.86
Hb (g/dL)	11.53±0.64	11.43±0.74	11.67±2.19	12.60±4.87
RBC($x10^6$uL)	3.78±0.11	3.63±0.17	3.71±0.28	3.65±0.58
WBC($x(10^3$ul)	18700±614.41	18466.67±448.14	17683.33±1990.18	18716.67±2518.10
PLATELET	181666.67±8504.90	179000.00±78581.17	172333.33±30892.29	227666.67±92115.87
LYM (%)	67.00±1.00	67.33±2.08	69.33±2.89	64.67±9.29
HET (%)	$27.33±2.52^{ab}$	$26.33±3.21^{ab}$	$22.33±3.21^{b}$	$31.00±4.36^{a}$
MON (%)	3.33±0.58	2.33±0.58	3.00±1.00	2.67±0.58
EOS (%)	3.00±1.00	3.67±1.53	4.67±2.08	4.67±0.58
BAS (%)	0.33±0.58	0.33±0.58	0.33±0.58	0.33±0.58

The values in the same row with different superscript are significantly different ($p \leq 0.05$). PCV: Packed cell volume, Hb: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, LYM: Lymphotes, HET: Heterophils, MON: Monocytes, EO: Eosinophils, BA: Basophils.

Table 2: Histomorphology parameters of noiler chicken administered *Peperomia pellucida* (PP) and Humic acid (HA)

Parameters	T1	T2	T3	T4
EH	25.37±2.70	27.55±4.30	30.09±4.21	32.09±1.76
VH	550.24±88.24	604.22±181.50	609.78±183.45	655.73±89.20
CD	120.09±18.01	127.39±47.30	153.65±58.06	129.10±43.71
VW	101.34±16.04	93.93±11.28	89.43±6.64	114.25±19.08
MWT	158.94±75.48	128.15±43.20	149.65±51.06	148.00±66.09

The values in the same row with different superscript are significantly different ($p \leq 0.05$).EH: Epithelial height, VH: Villous height, CD: Crypt depth, VW: Villous width, MWT: Muscular wall thickness.

Gut microbial load of noiler chicken administered Peperomia pellucida (PP) and humic acid (HA)

There were no significant differences in the means of Total Coliform Count (TCC) and Total *Escherichia coli* count. There were significant ($p \leq 0.05$) differences in the Total Heterotrophic Count (THC) and Total *Lactobacilli* count (TLC). Birds fed basal diets had the highest THC when compared to other treatments. Birds fed with T3 had a significantly higher TLC when compared with the other treatments. The result of the study showed that humic acid and *Peperomia pellucida* had very high Total *Lactobacilli* count (TLC) compared to the control and this is an indication that either these additives are used singly or combined, they can improve and regulate the microflora in the gut of birds. Their use as Antibiotics Growth Promoters (AGPs) would not cause any deleterious imbalance in the intestinal microflora of birds. Humic acid and *Peperomia pellucida* also had the least Total Heterotrophic count (THC) when compared with the control. Reduction in the THC is assumed to be due to the suppression of potentially pathogenic microorganisms in the gastrointestinal tract (Pascaul *et al*., 1999; Burt, 2004 and Si *et al*., 2006). Lowering of the intestinal pH causes a cellular dysfunction and competitive exclusion of pathogenic microorganisms (Kabir, 2009; Hajati, 2018). Total *Escherichia* count (TEC) and Total *Coliform* count (TCC) were low in birds fed Humic acid and *Peperomia pellucida*. In particular, birds fed humic acid based diet had the least TEC (0.87±1.07) compared to all other diets. Humin Tech (2004) validated this result with a conclusion that humic acid improves the animal's defenses against pathogens such as *E. coli*. This is an indication of the antibacterial activity of both additives thereby hindering the proliferation of potentially harmful microorganisms. Proliferation of these organisms leads to a thickening of muscular cell wall and makes absorption of nutrients difficult.

Table 3: Gut microbial load of noiler chicken administered *Peperomia pellucida* (PP) and humic acid (HA)

Parameters	1	2	3	4
THC($\times 10^7$cfu/mL)	5.45±0.92^{a}	1.30±1.56^{b}	1.30±0.56^{b}	1.80±1.27^{b}
TCC($\times 10^5$cfu/mL)	12.77±5.64	9.47±10.15	9.13±3.06	11.00±7.45
TEC($\times 10^5$cfu/mL)	9.07±7.71	0.87±1.07	5.83±7.34	6.60±7.71
TLC($\times 10^5$cfu/mL)	9.07±2.56^{b}	15.40±3.08^{ab}	17.53±5.35^{a}	13.60±1.90^{ab}

The values in the same row with different superscript are significantly different ($p \leq 0.05$). THC: Total Heterotrophic count, TCC: Total Coliform count, TEC: Total *Escherichia coli* count, TLC: Total *Lactobacilli* count.

Conclusion

The study showed that humic acid and *Peperomia pellucida* can be used as feed additives. Their addition in diets can serve as an alternative to antibiotics growth promoters (AGPs) without having negative effects on the overall health of the birds. This research suggests that humic acid and *Peperomia pellucida* can be used to increase beneficial lactic acid bacteria (LAB) in noiler. The study also justified the ethno-botanical and pharmacological usage of the test ingredients. From this study, it can be inferred that humic acid and *Peperomia pellucida* cannot only be used at sub-therapeutic level but for both prophylaxis and therapeutic treatment. Tolerance level of birds to the percentage inclusion of humic acid and *Peperomia pellucida* used in this study is an indication that both test ingredients can still be used at increased levels without residual and toxic effects on birds.

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