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Phytogenic influence of *Mangifera indica L.* leaves and bamboo (*Bambusa vulgaris* S.) charcoal on gut microbial integrity and ammonia production in noiler chickens

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Abstract

This study was conducted to investigate the phytogenic influence of Mango leaves (Mangifera indica L). Leaf Meal (MI) and Bamboo (Bambusa vulgaris S.)Charcoal (BC)on gut microbial integrity and ammonia production in noiler chickens. A total of seventy-two Noiler chicken of both sexes with an average weight of 55.00±8.52g were allotted to four dietary treatments in a completely randomised design with each treatment having three replicates. The freshly harvested Mongo Leaves were air-dried and Bamboo Charcoal was prepared using standard procedure. The test ingredients were milled and incorporated into the bird's diet. Commercial broiler feeds were purchased and used as the basal diet. Birds in the first treatment (T1) were fed the basal diet only while those in the second treatment (T2) were fed combination of the basal diet and 0.5% MI, third treatment (T3) were fed the birds in the combination of the basal diet and 0.5% BC and birds in the fourth treatment (T4) were fed combination of the basal diet with 0.5% MI and 0.5% BC. Feed and water were given ad libitum. At day 56 of the feeding trial, faecal samples were collected for faecal nitrogen using Kjeldahl method, blood samples (5mL each) were collected for haematological parameters and three birds per replicate were sacrificed and the ileal content collected for the gut microbial count following standard procedures. The faecal nitrogen value of 9.83±0.75 in noiler chickens fed T2 was significantly higher than in other treatments while T4 recorded the lowest value (5.57±0.92). Significant differences (p<0.05)were also noticed in the Packed Cell Volume (PCV), Haemoglobin Counts, White Blood Cells (WBC) and the Platelets. The PCV value of 46.67±7.69 recorded in birds fed T2 was higher than others while 28.67±3.71 in T3 was the lowest. Birds fed T2 had the highest haemoglobin count (14.87±2.35) and least for those on T3(9.33±1.17). Also, noiler chickens fed T2 recorded the highest WBC count (286333.33±34671.47)while the lowest value(11716.67±3927.50) was observed in those fed T1. Similar trend was observed in platelet, where the highest value of 286333.33±34671.47 was recorded in birds fed T2 and the lowest (142000.00±27006.17) in those fed T1. There were no significant differences (p>0.05) in the gut microbial population across all the treatments. In conclusion, noiler chickens fed combination of 0.5% Mango leaf meal and 0.5% bamboo charcoal lowered the production of ammonia through the reduction of the faecal nitrogen , with no adverse effects on the microbial population in the gut and health of the chickens.

Keywords: Bamboo charcoal, mango leaves, noiler, ammonia, feed additives

Influence phytogénique de Mangifera indica L. feuilles et charbon de bois de bambou (Bambusa vulgaris S.) sur l'intégrité microbienne intestinale et la production d'ammoniac chez les poulets noiler

Résumé

Cette étude a été menée pour étudier l'influence phytogénique de la farine de feuilles de Mangifera indica L. et du charbon de bois de bambou (Bambusa vulgaris S.) sur l'intégrité microbienne intestinale et la production d'ammoniac chez les poulets noiler. Un total de soixante-douze (72) poulets noiler des deux sexes d'un poids moyen de 55,00 ± 8,52 g ont été répartis en quatre traitements diététiques dans une conception entièrement randomisée, chaque traitement ayant trois répétitions. Les feuilles de Mangifera indica L. (M.I) ont été séchées à l'air et le charbon de bois de bambou (BB) a été broyé et incorporé dans le régime alimentaire de l'oiseau à des niveaux variables. Des aliments commerciaux pour poulets à griller ont été achetés et utilisés comme régime de base. Les oiseaux du premier traitement (T1) ont été nourris uniquement avec le régime de base. Les oiseaux du deuxième traitement (T2) ont reçu une combinaison de l'alimentation de base et de 0,5 % de Mangifera indica L. Les oiseaux du troisième traitement (T3) ont reçu une combinaison de l'alimentation de base et de 0,5 % de charbon de bambou. Les oiseaux du quatrième traitement (T4) ont reçu une combinaison du régime de base avec 0,5 % de Mangifera indica L. et 0,5 % de charbon de bambou. La nourriture et l'eau ont été données ad libitum et à la fin de l'essai d'alimentation de quarante-deux (42) jours, des échantillons fécaux ont été prélevés pour la production d'ammoniac en utilisant la méthode Kjeldahl, des échantillons de sang ont été prélevés pour les paramètres hématologiques et trois (3) oiseaux par répétition ont été sacrifié pour la numération microbienne intestinale en disséquant la jonction iléo-caecale. Il y avait des différences significatives ($p<0,05$) dans l'azote fécal à travers tous les traitements. Le traitement T2 a montré la valeur la plus élevée (9,83 ± 0,75) et le traitement T4 a montré la valeur la plus basse (5,57 ± 0,92). Des différences significatives ($p < 0,05$) ont également été observées dans l'hématocrite (PCV), le nombre d'hémoglobines (Hb), les globules blancs (GB) et les plaquettes. La valeur la plus élevée (46,67 ± 7,69) de l'hématocrite (PCV) a été enregistrée dans le traitement T2 tandis que la valeur la plus basse (28,67 ± 3,71) a été enregistrée dans le traitement T3. Le traitement T2 a montré le taux d'hémoglobine le plus élevé (14,87 ± 2,35) tandis que le traitement T3 a enregistré la valeur la plus faible (9,33 ± 1,17). Le traitement T2 a enregistré le nombre de globules blancs le plus élevé (286333,33±34671,47) tandis que la valeur la plus faible (11716,67±3927,50) a été observée dans le traitement T1. La même tendance a également été observée dans les plaquettes, la valeur la plus élevée (286333,33±34671,47) a été enregistrée dans le traitement T2 tandis que la valeur la plus faible (142000,00±27006,17) a été observée dans le traitement T1. Il n'y avait pas de différences significatives ($p>0,05$) dans l'activité microbienne intestinale entre tous les traitements. On peut conclure que les poulets noiler nourris avec la combinaison de 0,5 % de feuilles de Mangifera indica L. et de 0,5 % de charbon de bambou réduisent la production d'ammoniac, n'ont aucun effet néfaste sur la santé du poulet et n'ont également aucun effet sur les activités microbiennes dans l'intestin des poulets.

Mots-clés: Charbon de bambou, feuilles de manguier, noiler, ammoniac, additifs alimentaires

Introduction

Ammonia production in poultry houses are mainly due to high protein formulated chicken diets. The chickens have no storage mechanisms for amino acids consumed beyond the requirement for protein synthesis, so the excess amino acids are deaminated and the derived nitrogen is excreted in the urine mainly as uric acid (80%), ammonia (10%) and urea (5%), (Goldstein and Skadhauge, 2000). However, according to Patrick and Glasswell (2015), the large amount of uric acid excreted is readily converted to ammonia through a chain of reactions catalyzed by enzymes namely *uricase* and *urease* which are present in the manure. Ammonia has adverse effects particularly to the nasal cavity and eyes of affected chickens due to the gas alkalinity and corrosiveness (Bahl and Bahl, 2004). When in contact with nasal moisture, NH_3 reacts with the moisture and forms a corrosive basic solution. The aqueous ammonium solution formed corrodes the respiratory lining of the affected chicken consequently weakening immunity in the respiratory system making the animal susceptible to bacterial infections especially *E.coli* (Patrick and Glasswell, 2015). Not only does indoor ammonia pollution affect the chickens but also pose a risk to the health of the agricultural workers in these facilities. Ammonia emissions from poultry houses cause environmental problems such as acid precipitation, fine particulate matter formation (particulate matter with an aerodynamic diameter less than 10 μm) and nitrogen deposition in aquatic systems (Moore *et al*., 2008). Because ammonia emission is harmful to poultry birds, care takers and the environment at large, several strategies have been employed to minimize NH_3 volatilization into the environment (Moore *et al.,* 2008). These strategies include NH_3 gas absorbers, enzyme inhibitors, feed manipulation and other litter additives such as aluminium sulphate and sodium bisulphate. The limitations to these strategies are enormous part of which includes the cost of setting up and maintenance of the gas absorbers. There is a need for a suitable alternative that is cheap and readily available. Plants are considered as largely complicated chemicals factories which can turn the relatively simple ingredients of air and water into so many compounds including liquids and oils (Mabey, 1997). Mango (*Mangifera indica* L.*)* is one of the most popular of all tropical fruits. Mangiferin, being a polyphenolic antioxidant and a glucosylxanthone present in mango, has been reported to have antimicrobial activities that help in the reduction of harmful pathogens in the gut of animals (Pallab, 2004). Bamboo Charcoal is a non-digestible phytogenic material. Its adsorptive capacity makes it an excellent means of preventing the absorption of toxins into the body from the digestive tract (Gyo Moon Chu *et al.,* 2013).

Materials and methods

Experimental site

The research was carried out at the Poultry Unit of University of Ibadan Teaching and Research Farm, Ibadan Oyo State, Nigeria.

Test materials

Green Mango Leaves and Bamboo stems were sourced for in areas around Ibadan, Oyo State. The leaves were air dried for 21 days and ground into powdery form and the bamboo charcoal was prepared by heating the bamboo stems (*Bambusa vulgaris* S.) in the absence of air. The charcoals formed were crushed into fine grains.

Experimental design and birds

The experiment was conducted for a 56-day feeding period using a total of 72-day old noiler birds which were allotted to the four dietary treatments in a completely randomized design with each treatment having three replicates at the start of the experiment.

Experimental diets and groups

Commercial broiler feeds were purchased and used as the basal diet. Broiler starter feeds were fed to the chickens form day 1-30 and broiler finisher feeds were fed from day 31-56.

The dietary Treatments were;

(i) Treatment T1 (basal diet (control)
(ii) Treatment T2 (basal diet + 0.5% *Mangifera indica*)
(iii) Treatment T3 (basal diet + 0.5% Bamboo Charcoal)
(iv) Treatment T4 (basal diet + 0.5% *Mangifera indica* + 0.5% Bamboo charcoal)

Data and sample collection

Faecal nitrogen

Chicken excreta were collected at the end of the experiment and taken for laboratory analysis at University of Ibadan Animal Science laboratory. The excreta of approximately 500g were collected from each poultry house and were kept in clearly labelled sealable polythene bags under low temperatures and Faecal nitrogen analysis analysis was conducted using Kjeldahl method (Kjeldahl, 1883)

Haematological indices

One millilitre (1mL) of blood in vials with 2mg ethylenediaminetetraacetic acid (EDTA) was collected from the jugular vein of the sampled birds (3 birds per replicate). 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 were determined by the method described by Emiola *et al.* (2013).

Gut microbial load

Three birds per replicate were sacrificed for microbial count. The ileum was removed. About 2 cm segment from the last two-third portion of the ileum from the ileo-caecal junction was dissected. Microbial counts were done using methods described by Barrow and Feltharn (1993). Culture media were prepared according to manufacturers' specifications. The standard plate count technique was used in the microbial load determination. One millilitre of the digesta was used for serial dilution in sterile 15mL test tubes, containing 9mL of 0.1% sterile peptone water and vortex. Serial dilution of digesta was made to 10^{-3}dilution level. One ml of the dilution was delivered by pipetting on Plate count agar and Mac Conkey agar and incubated at 37^0C for 18 – 24 hours. Discrete colonies on plates were counted using a colony counter and counts were estimated in log10CFU/mL.

Statistical analysis

The data was analyzed using SAS (2012) 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= 0.05.

Results and discussion

Faecal nitrogen of noiler chickens fed Mangifera indica L. (M.I) leaves and bamboo charcoal (B.C)

The faecal nitrogen of noiler chicken fed *Mangifera indica* L. leaves (M.I) and Bamboo Charcoal (B.C) is shown in Table 1. There were significant differences (P<0.05) in the means of all the treatments. Birds in treatment T2 recorded the highest level of faecal nitrogen (9.83±0.75) and birds in treatment T4 recorded the lowest level (5.57±0.92). The higher faecal nitrogen recorded in treatment T2 could be due to the presence of an anti-nutritional factor called tannin which is present in the mango leaves. According to Glick and Joslyn (1970), a dietary tannin concentration of 0.5% has been reported to decrease nitrogen retention and cause 5% mortality rate in rat. Thu *et. al***.** (2009) reported that B.C significantly reduced total ammonia nitrogen (TAN) excretion in puffer fish while the nitrogen retention in fish muscle was significantly increased in fish groups fed B.C diets than those in the control group. These results suggested that dietary B.C could decrease the Total Ammonia Nitrogen (TAN) excretion by increasing carcass nitrogen retention in tiger puffer fish. Similar trend was also noticed in this result where the treatment with the bamboo charcoal inclusion showed lower faecal nitrogen when compared with the control and the treatment with mango leaves. However, no research was conducted on the carcass to check for the carcass nitrogen retention so as to validate this result.

Table 1: The faecal nitrogen of noiler chicken fed *Mangifera indica* L. leaves and bamboo charcoal (B.C)

Parameters	Basal diet	*0.5% Mangifera indica*	*0.5% Bamboo Charcoal*	*0.5% Mangifera indica + 0.5% Bamboo charcoal*
Nitrogen	7.63 ± 0.87^{ab}	9.83 ± 0.75^{a}	6.70 ± 0.78^{b}	5.57 ± 0.92^{b}

Values are Means± Standard Error

a, b: means in the same row with different superscript are significantly different ($p\leq0.05$).

Haematological parameters of noiler chickens fed Mangifera indica L. Leaves and bamboo charcoal

'Haematological Parameters of noiler chickens fed Mangifera indica L. (MI) leaves and Bamboo Charcoal'

There were no significant differences (P>0.05) among the means of the Red Blood Cell (RBC), Lymphotes (LYM), Hetrophils (HET), Monocytes (MON), Eosinophil (EOS), Basophil (BAS) in all the treatments. Significant differences (P<0.05) were noticed among the means of the Packed Cell Volume (PCV) in all the treatments. Birds fed treatment T2 had the highest level (46.67±7.69) when compared to all other treatment. According to Bounous and Stedman (2000), the normal ranges of the haematological parameters in chickens are PCV: 22-35 %, Hb: 7-13 g/dl, RBC: 2.5-3.5 x10^6 µl and WBC: 12-30 x 10^3 µl. The Packed Cell Volume (PCV) of noiler chicken in treatment T2 (46.67±7.69) falls above the normal range and this shows a sign of dehydration, a disorder called polycythemia that causes the bone marrow to produce many red blood cells. The excess Red Blood Cell thickens the blood, slowing its flow causing serious problems, such as blood clots. Also, the means of the Haemoglobin (Hb) showed significant differences (P<0.05) across the treatments. Furthermore, Bounous and Stedman (2000) stated that the normal ranges of the haematological parameters in chickens are PCV: 22-35%, Hb: 7-13g/dl, RBC: 2.5-3.5 x10^6µl and WBC: 12-30 x 10^3µl. Birds fed Treatment T2 showed the highest haemoglobin concentration (14.87±2.35) among all the treatments, which fell above the normal range in poultry. Significant differences (P<0.05) was also observed among the means of the White Blood Cells (WBC) in the treatments. The highest level of White Blood Cells was noticed in treatment T2 (19750.00±1476.76). According to Osman *et al.* (2004), the White Blood Cells protect the body from pathogen and build up immunity. Ologhobo *et al.* (1986) observed that an increase in WBC count above the normal range is an indication of the presence of exogenous substances and foreign bodies in the body of an animal. Due to the high level of White Blood Cells Chickens in treatment T2 will have the highest resistance to diseases.

Table 2: Haematological parameters of noiler chickens administered *Mangifera indica* leaves and bamboo charcoal

Parameters	Basal diet	*0.5% Mangifera indica*	*0.5% Bamboo Charcoal*	*0.5% Mangifera indica + 0.5% Bamboo charcoal*
PCV (%)	29.33±3.48[b]	46.67±7.69[a]	28.67±3.71[b]	32.33±1.45[ab]
Hb (g/dl)	9.43±1.20[b]	14.87±2.35[a]	9.33±1.17[b]	9.67±1.04[b]
RBC (x10^{6}uL)	2.82±0.54[a]	3.97±0.31[a]	2.82±0.51[a]	3.57±0.15[a]
WBC (x10^{3}ul)	11716.67±3927.50[b]	19750.00±1476.76[a]	15400.00±765.40[ab]	117383.33±363.24[ab]
PLATELET	196666.67±37172.27[ab]	286333.33±34671.47[a]	142000.00±27006.17[b]	205000.00±46808.12[ab]
LYM (%)	59.00±4.36	70.67±3.38	60.33±4.33	66.00±1.53
HET (%)	33.33±3.53	22.00±2.89	32.00±5.29	26.00±1.53
MON (%)	3.00±0.58	2.67±0.33	3.67±0.33	3.33±0.67
EOS (%)	4.33±0.33	4.67±1.45	4.67±0.88	3.33±0.33
BAS (%)	0.33±0.33	0.00±0.00	0.33±0.33	0.33±0.33

Values are Mean±Standard Error.
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, EOS: Eosinophils, BAS: Basophils.

The microbial activity of noiler chickens fed Mangifera indica L. leaves and bamboo charcoal

The microbial activity of noiler chickens fed *Mangifera indica* L. leaves (M.I) and Bamboo Charcoal (B.C) is shown in Table 3. There were no significant differences (P' 0.05) between the Total Heterotropic Count (THC), Total Coliform Count (TCC), Total Escherichia Count (TEC) and Total Lactobacilli Count (TLC) across the treatments. The Total Coliform Count showed no significant differences (P>0.05) in all the treatments. Treatment T2 showed an elevated microbial count when compared with the control and this may be attributed to the fact that, Coliform bacteria are gram-negative bacteria. Various research work has established that Gram positive bacteria are more susceptible towards plants extracts when compared to Gram negative bacteria (Oboh *et al.,* 2007; Nair and Chanda, 2007; Costa *et al.,* 2008; Khan *et al.,* 2008). The cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is a multi-layered structure (Essawi and Srour, 2000). The complex nature of cell wall structure of Gram negative bacteria may inhibit the passage of the active compound through the Gram negative cell wall. No significant differences (P>0.05) was observed in the Total *Escherichia coli* count across the treatments. Naka *et al.* (2001) reported that activated charcoal has the capacity to absorb verotoxin produced by *Escherichia coli*. The findings of this study corroborate the results of Naka *et al.* (2001). Although, the charcoal used for this study was not activated but the absorption ability of the bamboo charcoal was also noticed when the values of the bacteria count in the control was compared with Treatment T3. A higher value of *E.coli* count was recorded in Treatment T2, which was not in alliance with what Shengan *et al.* (2018) reported when the effect of Quercetin on cecal microbiota in Arbor Acres broilers was investigated. Quercetin is a ubiquitous flavonoid found in mango leaves extract which is known to have antibacterial effects. It was reported that quercetin significantly decreased the copies of *Pseudomonas aeruginosa* (P<0.05), *Salmonella enterica serotype Typhimurium* (P<0.01), *Staphylococcus aureus* (P<0.01), and *Escherichia coli*

(P<0.01). The variation in the results may be due to the difference in the breed of animals used. Total Lactobacilli Count has the highest value in Treatment T2 and the lowest value was recorded in the control treatment T1. The absorption ability of activated charcoal against *Lactobacillus acidophilus* showed that activated charcoal showed lower binding capacity to the normal bacterial flora (Naka *et al.,* 2001). This is also in accordance with the result reported in this study. The bacteria count for treatment T3 which contains Bamboo charcoal showed higher value than the control treatment T1. A higher count of Lactobacilli was observed in Treatment T2 this could be due to the presence of quercetin. A study conducted by Shengan *et al.* (2018), to investigate the effect of quercetin on cecal microbiota on Arbor Acre (AA) broiler chickens in vivo showed an increased value of Lactobacilli when compared with the control. Quercetin significantly decreased the copies of *Pseudomonas aeruginosa* (P<0.05), *Salmonella enterica serotype Typhimurium* (P<0.01), *Staphylococcus aureus* (P<0.01) and *Escherichia coli* (P < 0.01) but significantly increased the copies of *Lactobacillus* (P < 0.01), *Bifidobacterium* (P< 0.01).

Table 3: Microbial activity of noiler chickens administered *Mangifera indica* L. leaves and bamboo charcoal

Parameters	Basal diet	*0.5% Mangifera indica*	*0.5% Bamboo Charcoal*	*0.5% Mangifera indica + 0.5% Bamboo charcoal*
THC ($\times 10^7$cfu/mL)	0.85±0.05	1.10±0.80	1.07±0.33	0.97±0.49
TCC ($\times 10^5$cfu/mL)	4.50±1.22	6.37±4.39	4.27±0.64	4.23±1.15
TEC ($\times 10^5$cfu/mL)	0.93±0.03	0.95±0.55	0.80±0.25	0.47±0.09
TLC ($\times 10^5$cfu/mL)	4.43±0.88	9.90±9.0	6.15±0.75	7.80±2.70

Values without superscripts show no significant differences
THC: Total Heterotrophic count, TCC: Total Coliform count, TEC: Total Escherichia coli count, TLC: Total Lactobacilli count

Conclusion and recommendation

The findings of this study showed that noiler chickens in Treatment T2 (fed 0.5% *Mangifera indica* L. supplementation) produced higher faecal nitrogen due to the presence of an anti-nutritional factor called Tannin. Also, Noiler chickens in Treatment T3 (fed 0.5% Bamboo Charcoal) showed lower faecal nitrogen compared to the chickens in Treatment T2 (fed 0.5% *Mangifera indica* L.) due to the absorption characteristics of charcoal. The combination of the two test ingredients resulted to low faecal nitrogen when compared with other treatments. This showed that the ammonia production in this treatment will be lowered. Birds in Treatment T2 (fed 0.5% *Mangifera indica* L.) showed higher Packed Cell Volume (PCV), Haemoglobin Concentration (Hb) and White Blood Cells (WBC) which is above the normal range in poultry and Birds in Treatment T3 (fed 0.5% Bamboo Charcoal) has no effect on the Packed Cell Volume (PCV), Haemoglobin Concentration (Hb) and White Blood Cells (WBC). Furthermore, birds fed the combination of the two test ingredients gave an appreciable result which fell between the normal haematological ranges for poultry. *Mangifera indica* L. and Bamboo Charcoal no effect on the gut integrity of the birds. From the research findings, it is therefore recommended that the inclusion of 0.5% *Mangifera indica* L. leave meal and 0.5% Bamboo Charcoal powder into noiler feeds has the ability to reduce faecal ammonia production, has no negative effect on the haematological parameters and also no effect on the gut microbial integrity of the chickens. Further researches should therefore be conducted to evaluate the carcass for nitrogen retention in birds fed diets with Bamboo Charcoal inclusion. Also, the gut microbial integrity of birds fed *Mangifera indica* L. leaf meal and Bamboo Charcoal at a higher inclusion rate should be investigated.

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