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Research Article

Influence of Diets Supplemented with Carica Papaya and Chromolaena Odorata Leaf Meals on Performance, Blood Profile and Gut Integrity of Broiler Chickens

Omidiwura BRO[1*], Agboola AF[1], Omotosho OY[1], Mustapha-Olosho JA[1]

[1] Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Abstract

The awareness of the negative effects and ban of antibiotics makes it imperative to find effective natural alternatives, such as phytobiotics, to antibiotics to sustain the efficiency of current broiler chicken production. This study was carried out to investigate the effect of *Carica papaya* and *Chromolaena odorata* on growth performance, blood profile, gut microbial population, gut morphology and histopathology of broiler chicken. In a 42-day study, 200 one-day old Arbor Acre broilers were weighed and randomly allotted to 5 dietary treatments with 5 replicates having 8 birds in each group. The treatments were basal diet (negative control, NC), basal diet + 0.05% antibiotics (positive control, PC), NC + 3% *Carica papaya* (CP), NC + 3% *Chromolaena odorata* (CO) and NC + 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* in a completely randomised design. The growth performance indices were measured. On day 42, blood samples were collected, gut microbial population, gut morphology and histopathology were assayed following standard procedure. Data were analyzed using descriptive statistics and ANOVA at α0.05. The result showed that average daily weight gain (g/bird/day) of birds on NC, PC and combination of 1.5% CP + 1.5% CO leaf meals were significantly similar at the finisher phase. The feed intake of birds on NC and PC was higher than those on 3% CO and combination of 1.5% CP + 1.5% CO leaf meals. The dietary treatment had effect on feed conversion ratio in the finisher phase. The highest total *Lactobacillus* count was observed in 3% CO leaf meal diet, while the antibiotics diet (PC) had the highest *Escherichia coli* count, and the lowest total *Escherichia coli count* (3.28 cfu x10^5) was recorded for birds on combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meals. There were no significance differences in the gut morphological parameters (villus height crypt depth, epithelial wall thickness, epithelial cell) among all the treatments. The histopathology of the gut shows that only those fed 3% CP had eroded villi of the mucosa layer, while all other treatments show normal mucosa layer, moderate inflammatory infiltration of the gland and lamina propia except those fed combination of 1.5% CP + 1.5% CO. In conclusion, either *Carica papaya* or *Chromolaena odorata* leaf meal in broiler diet did not significantly affect any of the parameters of interest. However, the combination of the two at 1.5% inclusion in broiler diet had positive effect on beneficial gut microbial population.

Keywords: *Leaf Meal, Performance, Carcass Characteristics, Intestinal Microbiota, Blood Metabolites, Broiler Chickens.*

Introduction

Antibiotics have been used a long time ago as growth promoters because of its effectiveness in enhancing growth performance for poultry. But Since 2006, the European Union banned antibiotics as growth promoters because of its harmful effects on human health as a result of the drugs toxicity, residual effects and microbial resistance to these antibiotics [1,2]. The National Agency for Food and Drug Administration and Control (NAFDAC) [3] in 2018, also banned the use of antibiotics as feed additives, except when prescribed by a veterinarian. Research has been carried out to look for natural agents with similar beneficial effect as growth promoters, among the natural alternatives are phytogenic feed additives (PFA), PFA they are derived from plants, herbs and spices, and are less toxic. They have been very successful because of their positive effect on growth, improved immune system and reduced stress [4].

Chromolaena odorata (siam weed), – previously known as *Eupatorium odorata* is a perennial shrub which belongs to the family *Asteraceae*. The plant contains pharmacologically important phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds with essential antimicrobial activities [5]. *C. odorata* leaves are high in crude protein, which is rich in the essential amino acids [6]. Despite the presence of antinutritional factors in *C. odorata*, researchers have reported the plant as a useful plant in poultry production.

Carica papaya is an herbaceous perennial in the family *Caricaceae* grown for its edible fruit. It is one of the most popular and economically significant plants in the world [7]. *C. papaya* is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; minerals; the B vitamins and fibre. The leaf is rich in enzymes like papain and chymopapain, which aid digestion, prevents bloating and other digestive disorders. *Carica papaya* plays therapeutics role as an antioxidant, anti-inflammatory, analgesic, antimicrobial, anti-ulcer genic and gastro-protectant [8]. Therefore, the present study aimed to evaluate the beneficial effects of *Carica papaya* (pawpaw) and *Chromolaena odorata* (siam weed) leaf meals on performance, carcass characteristics, blood metabolites, gut microbial load, gut morphology and histopathology of broiler chickens.

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Unit Teaching and

Corresponding author: Omidiwura BRO, Department of Animal Science, University of Ibadan, Ibadan, Nigeria, Tel: +234-708-207-7886; Fax: +234-708-207-7886; E-mail: richardwura@gmail.com

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Research farm, University of Ibadan, Ibadan, Oyo state, Nigeria.

Preparation of the test ingredient

Fresh *Carica papaya* and *Chromolaena odorata* leaves were obtained from the farm premises. *C. papaya* and *C. odorata* leaves were air dried for 3 days. The dried leaves were blended, weighed and mixed with the diets in the right proportion as shown in Tables 1 and 2.

Experimental diets and management of birds

Two hundred (200) one-day old Arbor acre broiler chicks with initial average body weight of 41.6 g, sourced from a local hatchery (CHI farm) were randomly allotted to 5 dietary treatments consisting of 5 replicates of 8 birds per replicates in a completely randomized design. The birds were raised from day old to 42 days of age. Treatment 1 was the basal diet (0% leaf meal), Treatment 2 contained basal diet with 0.05% antibiotics, Treatment 3 contained basal diet with 3% *Carica papaya* leaf meal, Treatment 4 contained basal diet with 3% *Chromolaena odorata* leaf meal, Treatment 5 contained basal diet with combination 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meal. Experimental diets were given *ad libitum* and birds had free access to clean water. The experimental diets for both starter and finisher phases were formulated based on the nutritional requirement recommended by the NRC [9], using basically corn-soyabean meal with crude protein (CP) adjusted to 23% and 21% for starter and finisher phases respectively.

Data and sample collection

Performance indices: Performance parameters such as; weight gain, feed intake, feed conversion ratio, feed cost and percentage mortality were calculated. The initial weight was measured on day one of the experiment and subsequently and values were used to calculate weight gain. Feed Intake was calculated as the difference between amount of feed given and left over.

Table 1: Gross composition (g/kg) of broiler starter diets supplemented with *Carica papaya* and *Chromolaena odorata* leaf meal.

INGREDIENTS g/kg	Negative control (NC)	Positive control (PC)	NC + *Carica papaya* leaf	NC + *Chromolaena odorata* leaf	NC + *Carica papaya* + *Chromolaena odorata*
Corn	574.00	573.50	555.00	555.00	555.00
Soyabean meal	360.00	360.00	349.00	349.00	349.00
Fish meal	30.00	30.00	30.00	30.00	30.00
Soya Oil	7.00	7.00	7.00	7.00	7.00
Dicalcium phosphate	13.00	13.00	13.00	13.00	13.00
Broiler premix**	2.00	2.00	2.00	2.00	2.00
Limestone	8.00	8.00	8.00	8.00	8.00
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	2.00	2.00	2.00	2.00	2.00
Table Salt	2.00	2.00	2.00	2.00	2.00
Antibiotics	0.00	0.50	0.00	0.00	0.00
Pawpaw leaf meal	0.00	0.00	30.00	0.00	15.00
Siam weed leaf meal	0.00	0.00	0.00	30.00	15.00
TOTAL	1000	1000	1000	1000	1000
CALCULATED NUTRIENTS					
Crude protein	230.36	230.31	230.89	229.44	230.17
Energy ME*, kcal/kg	3087.72	3086.00	2992.82	2992.88	2992.85
Ether extract	43.91	43.89	42.84	43.07	43.07
crude fibre	38.04	38.03	40.03	40.35	40.35
calcium	8.51	8.51	8.78	8.82	8.82
Total phosphorus	6.93	6.93	6.95	6.90	6.90
Non-phytate P	3.54	3.54	3.56	3.67	3.62
Ca:NPP	2.40	2.40	2.46	2.41	2.44

*ME- Metabolisable energy. **Vitamin A, 125,500,000 I.U; vitamin D3, 2,500,000 I.U; vitamin E, 40,000mg; vitamin K3, 2,000mg; vitamin B1, 3,000mg; vitamin B2, 5,500mg; niacin, 55,000mg; calcium pantothenate, 11,500; vitamin B6, 5000mg; vitamin B12, 25mg; choline chloride, 500,000mg; folic acid, 1000mg; biotin, 80mg; manganese, 120,000mg; iron, 100,000mg; Zinc, 80,000mg; copper, 8,500mg; iodine, 1,500mg; cobalt, 300mg; Selenium, 120mg; anti-oxidant, 120mg

Table 2: Gross composition (g/kg) of broiler finisher diets fed supplemented with *Carica papaya* and *Chromolaena odorata* leaf meal.

INGREDIENTS g/kg	Negative control (NC)	Positive control (PC)	NC + *Carica papaya* leaf	NC + *Chromolaena odorata* leaf	NC + *Carica papaya* + *Chromolaena odorata*
Corn	662.00	661.50	632.00	632.00	632.00
Soyabean meal	277.00	277.00	277.00	277.00	277.00
Fish meal	25.00	25.00	25.00	25.00	25.00
Soya Oil	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	15.00	15.00	15.00	15.00	15.00
Broiler premixes**	2.00	2.00	2.00	2.00	2.00
Limestone	7.00	7.00	7.00	7.00	7.00
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	2.00	2.00	2.00	2.00	2.00
Table Salt	2.00	2.00	2.00	2.00	2.00
Antibiotics	0.00	0.5.00	0.00	0.00	0.00
Pawpaw leaf meal	0.00	0.00	30.00	0.00	15.00
Siam weed leaf meal	0.00	0.00	0.00	30.00	15.00
TOTAL	1000	1000	1000	1000	1000
CALCULATED NUTRIENTS					
Crude protein	200.68	200.63	204.73	203.28	204.00
Energy ME*, kcal/kg	3143.11	3141.39	3040.14	3040.20	3040.17
Ether Extract	43.30	43.28	42.18	42.40	42.40
crude fibre	34.13	34.12	36.65	36.97	36.97
Calcium	8.11	8.11	8.40	8.44	8.44
Total phosphorus	6.81	6.81	6.88	6.83	6.83
Non-phytate P	3.71	3.71	3.76	3.86	3.81
Ca:NPP	2.18	2.18	2.23	2.19	2.21

*ME- Metabolisable energy. **Vitamin A, 125,500,000 I.U; vitamin D3, 2,500,000 I.U; vitamin E, 40,000mg; vitamin K3, 2,000mg; vitamin B1, 3,000mg; vitamin B2, 5,500mg; niacin, 55,000mg; calcium pantothenate, 11,500; vitamin B6, 5000mg; vitamin B12, 25mg; choline chloride, 500,000mg; folic acid, 1000mg; biotin, 80mg; manganese, 120,000mg; iron, 100,000mg; Zinc, 80,000mg; copper, 8,500mg; iodine, 1,500mg; cobalt, 300mg; Selenium, 120mg; anti-oxidant, 120mg

Carcass characteristics: At day 42, one bird per replicate was sacrificed by severing the jugular vein and eviscerated. The primal cuts and organ weights were taken and expressed as percentage of live weight.

Gut microbial load: The ileal digesta samples of one bird per replicate was collected in sterile syringe and taken to laboratory. A ten-fold serial dilution was carried out by transferring 1ml from the gut solution into 9ml of sterile water. This was done to the desired dilution factor of 10^{-4}. 1ml from 10^{-4} dilution factor was dispensed into respective labeled sterile plates, using a sterile syringe. Afterwards, approximately 15ml of the cooled culture media were poured into each of the plates containing the inoculated samples respectively. To examine the Total heterotrophic count (nutrient agar, incubated aerobically 28h); *Lactobacilli* (DeMan Rogosa Sharpe agar, incubated anaerobically 48h); *Escherichia coli* (Eosin methyl blue agar, incubated aerobically 24h); *Coliformi* (MacConkey, incubated aerobically, 24h). After 48 hours the plates were observed for bacteria and colonies were counted.

Gut Morphology: From the birds sacrificed and eviscerated, the longitudinal sections of the ileal tissue were cut and tissues from each group of birds in replicates were preserved in labeled specimen bottles containing buffered formalin solution and later processed for morphological measurements.

Histopathological Examination: Ileal tissue sections were transfer into specimen bottles containing 10% formalin for preservation. Then normal haematoxylin and eocin standard procedures were performed according to the method of Iji *et al.,* [10]. The tissues were processed and slides prepared were viewed under a microscope and photomicrograph was captured using a motic camera. The photomicrographs were transferred to the computer for the further pathologic reading.

Blood Parameters Measurements

At the end of 42 days, blood samples were collected from the jugular veins of one bird per replicate into two vaccutainer tubes for each broiler chicken, one containing Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological analysis and the other without EDTA for serum analysis. Red blood cell (RBC) and White blood cell (WBC) were determined using Neubauer haemocytometer, after the appropriate dilution. Differential leukocyte counts were performed using the oil immersion objective examination of blood films stained with the modified Romanovsky's Giemsa stain. Packed cell volume (PCV) was determined as described by Wintrobe [11], using Wintrobe haematocrit method. Haemoglobin concentration was determined by a cyanmethaemoglobin method using Drabkin's solution as diluent. Platelets were determined by phase microscopy method of Brecher and Cronkite.

Other Serum Biochemical Parameter: The biuret method was utilized in the determination of the total protein fraction while the serum albumin was subjected to the direct colorimetric method as described by Peters *et al.* [12]. Serum creatinine was determined using the principle of Jaffe reaction as described by Bonsnes and Tausslay [13], while serum urea was determined by the kit (Quinica Clinica Spam), the Uricase method as described by Wootton [14].

Statistical Analysis: Data obtained were analysed by descriptive statistics and Analysis of Variance (ANOVA) procedure of SAS at α0.05. Mean differences were separated using Duncan Multiple Range Test (DMRT).

Results and Discussion

Growth performance of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets

The results of the growth performance of birds fed experimental diets for the starter phase is presented in Table 3. Final weight, average weight gain, average daily weight gain , feed intake, feed intake, feed cost/kg, feed cost/kg/bird and percentage mortality were significantly influenced ($p<0.05$) by the dietary treatments. The average daily weight gains of birds on control diet and 0.05% antibiotics significantly ($p<0.05$) higher than those on 3% *Carica papaya*, 3% *Chromolaena odorata* and combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meals. There was no significant difference ($p>0.05$) in the feed conversion ratio and feed cost/kg weight gain.

The result on growth performance of broiler chicken on experimental diets at the finisher phase is shown in Table 4. Results show that the effect of dietary treatments on Initial weight (b/bird), final weight (g/bird), average weight gain (g/bird), average daily weight gain, feed conversion ratio, feed cost/kg, feed cost/kg weight gain and percentage mortality was significant ($p<0.05$). The final weight (g/bird) of birds on control diet and 0.05% antibiotics were similar and higher from birds on 3% *Carica papaya*, 3% *Chromolaena odorata* and combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* leaf meals. Average daily weight gain of birds fed control diet, 0.05% antibiotics and combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meal was similar. The feed conversion ratio of birds on 3% *Carica papaya* and combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* leaf meals was similar to those on the other experimental diets.

Table 3: Growth performance of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets (starter phase).

Parameters	T1	T2	T3	T4	T5	SEM	P Values
Initial weight (g/bird)	41.60	41.52	41.42	41.20	41.70	0.23	0.96
Final weight (g/bird)	595.38^{a}	603.92^{a}	517.58^{b}	508.54^{b}	467.28^{b}	10.13	0.0013
Average weight gain (g/bird)	553.78^{a}	562.40^{a}	476.16^{b}	467.34^{b}	425.58^{b}	10.12	0.0013
Average daily weight gain (g/bird/day)	26.37^{a}	26.78^{a}	22.67^{b}	22.25^{b}	20.27^{b}	0.48	0.0013
Feed intake (g/bird)	1299.10^{a}	1225.80^{a}	1054.10^{ab}	812.50^{b}	861.30^{b}	40.11	0.0030
Daily feed intake (g/bird/day)	61.86^{a}	58.37^{a}	50.20^{ab}	38.69^{b}	41.01^{b}	1.91	0.0030
Feed conversion ratio	2.35	2.17	2.36	1.74	2.09	0.13	0.54
Feed cost/kg (₦)	179.14^{b}	182.33^{a}	179.13^{c}	179.13^{c}	179.13^{c}	0.0010	0.00
Feed cost (₦/kg/bird)	11.08^{a}	10.64^{a}	8.99^{ab}	6.94^{b}	7.35^{b}	0.34	0.003
Feed cost/kg weight gain (₦/kg)	420.45	395.84	422.67	312.03	374.69	22.55	0.54
Percentage mortality (%)	13.33^{b}	10.00^{b}	16.67^{a}	3.33^{c}	3.33^{c}	2.75	0.046

^{ab} Means on the same row with different superscript are significantly ($p<0.05$) different, SEM- Standard Error of Mean, T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

Table 4: Growth performance of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets (finisher phase).

Parameters	T1	T2	T3	T4	T5	SEM	P Values
Initial weight (g/bird)	595.38^{a}	603.92^{a}	517.58^{b}	508.54^{b}	467.28^{b}	10.13	0.0013
Final weight (g/bird)	1562.56^{a}	1600.28^{a}	1124.54^{bc}	994.18^{c}	1230.62^{b}	30.49	0.0001
Average weight gain (g/bird)	967.20^{a}	996.40^{a}	607.00^{bc}	485.60^{c}	763.30^{ab}	35.51	0.0006
Average daily weight gain (g/bird/day)	46.06^{a}	47.45^{a}	28.90^{bc}	23.13^{c}	36.35^{ab}	1.69	0.0006
Feed intake (g/bird)	2219.80	2086.10	1762.60	1613.50	2346.50	150.40	0.51
Daily feed intake (g/bird/day)	105.70	99.34	83.93	76.83	111.74	7.16	0.51
Feed conversion ratio	2.31^{b}	2.10^{b}	3.04^{ab}	3.36^{a}	2.93^{ab}	0.14	0.046
Feed cost/kg (₦)	165.37^{c}	167.91^{a}	167.52^{b}	167.52^{b}	167.52^{b}	0.010	0.00
Feed cost (₦/kg/bird)	17.48	16.68	14.06	12.87	18.72	1.20	0.53
Feed cost/kg weight gain (₦/kg)	382.59^{b}	352.79^{b}	508.74^{ab}	562.73^{a}	490.22^{ab}	22.85	0.043
Percentage mortality (%)	28.00^{a}	3.33^{c}	3.33^{c}	10.00^{b}	29.33^{a}	5.33	0.035

^{abc} Means on the same row with different superscript are significantly ($p<0.05$) different, SEM- Standard Error of Mean, T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

The result revealed that inclusion of *Carica papaya* and *Chromolaena odorata* leaf meals in the diet of broilers did not improve the body weight gain of the birds at that inclusion level. This result corroborates the findings of by Haruna and Odunsi [15], who fed broiler chickens crude pawpaw latex. Similarly, Akinmutimi and Akufo [16] reported that increasing dietary inclusion level of *Chromolaena odorata* do not support good growth performance, carcass quality and organ weight in grower rabbits. The cost of feed per kilogram was higher in the 0.05% antibiotic starter diet (₦182.33). This could possibly be as a result of price of antibiotics since other feed additives used were harvested and processed locally.

Birds fed combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* had the highest average daily weight gain and lowest feed conversion ratio among the leaf meals diets at the finisher phase, although statistically there was no significant difference. This could

suggest that *Carica papaya* and *Chromolaena odorata* leaf meals had positive combined effect on final weight, average daily weight gain and feed conversion ratio of the birds. Unigwe *et al.* [17], observed progressive numerical increase in average daily weight gain, average daily feed intake and feed conversion ratio of broiler birds fed sun dried pawpaw leaf meal as the level of inclusion increases. Broiler chickens on 3% *Chromolaena odorata* leaf meal finisher diet had the lowest feed intake but statistically similar to those on 3% *Carica papaya* and combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meals. Donkoh *et al.* [18] concluded that *Chromolaena odorata* leaf meal addition to broiler diets negatively affects the performance of the bird as shown by decreased feed intake, growth and water consumption. The cost of feed per kilogram weight gain (₦562.73) was higher in the 3% *Chromolaena odorata* and it is similar to those on 3% *Carica papaya* and 3% *Chromolaena odorata* leaf meal diets, this could be as a result of higher values of feed conversion ratio recorded. This was in contrast to the findings of Ekenyem *et al.* [19], which observed least feed cost/kg weight gain of ₦52.08 in finisher broilers that had the highest inclusion level of *Chromolaena odorata* leaf meal.

Relative carcass and organ weights of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets

The carcass and organ weights of birds are shown in Table 5. Dietary treatment effect on Live weight, breast, wings, drumstick, shank, liver and heart weight (as percentage of live weight) were significant ($p<0.05$) which contrast findings of Bonsu *et al.* [20] who stated that the overall carcass characteristics of broilers were not influenced by the inclusion of *Chromolaena odorata* leaf meal in the diets. No significant influence was observed on weights of head, neck, back, thighs, full gizzard, abdominal fat, spleen, pancreas and bursa of fabricius (as percentage of live weight). The biggest liver was recorded in birds on the combination of *Carica papaya* and *Chromolaena odorata* while the smallest was observed in birds fed control diet.

Effect of experimental diets on live weight, breast, wings, drumstick, shank, liver and heart weight (expressed as percentage of live weight) was significant. The liver is a major detoxification organ in the body, increase in liver activities leads to increased liver weight. The liver weight was bigger in combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* than control diet this could be due to the presence of anti- nutritional factors in the combination of *Carica papaya* and *Chromolaena odorata* leaf meals.

Table 5: Relative carcass and organ characteristics of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets.

Parameters (%)	T1	T2	T3	T4	T5	SEM	P Values
Live weight	1562.56^{a}	1600.28^{a}	1124.54^{bc}	994.18^{c}	1230.62^{b}	30.49	0.0001
Head	2.92	2.84	3.56	3.57	3.69	0.14	0.19
Neck	3.63	4.88	4.53	5.54	5.48	0.29	0.25
Breast	21.84^{ab}	25.37^{a}	23.26^{ab}	20.45^{b}	21.49^{ab}	0.57	0.10
Back	15.28	16.40	16.46	15.75	17.04	0.58	0.89
Wings	7.63^{b}	9.25^{ab}	8.92^{ab}	9.82^{a}	10.13^{a}	0.26	0.058
Drumstick	9.58^{b}	12.13^{a}	11.69^{ab}	10.82^{ab}	10.44^{ab}	0.31	0.11
Thighs	8.83	9.56	10.02	10.03	11.73	0.55	0.56
Shank	4.00^{b}	5.14^{a}	5.14^{a}	5.49^{a}	5.53^{a}	0.13	0.070
Full Gizzard	3.63	3.71	3.96	3.49	4.00	0.15	0.78
Abdominal fat	0.81	0.94	0.57	0.36	0.58	0.10	0.43
Liver	2.15^{b}	2.52^{ab}	2.55^{ab}	2.63^{ab}	2.77^{a}	0.077	0.18
Spleen	0.11	0.13	0.12	0.11	0.13	0.010	0.96
Heart	0.49^{b}	0.64^{a}	0.57^{ab}	0.64^{a}	0.64^{a}	0.021	0.11
Pancreas	0.27	0.31	0.29	0.30	0.34	0.016	0.73
Bursa of fabricius	0.25	0.24	0.25	0.15	0.21	0.014	0.20

^{abc} Means on the same row with different superscript are significantly ($p<0.05$) different, SEM- Standard Error of Mean, T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

Gut microbial population of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets

The gut microbial load of broilers fed experimental diets is presented in Table 6. The Total heterotrophic count (THC x10^5) was significantly higher in the control diet in comparison to other treatments. Total Coliform count (TCC x10^5) showed similarities ($p>0.05$) between birds on 3% *Carica papaya* and 3% *Chromolaena odorata* leaf meals diets. There was significant difference ($p<0.05$) in the Total *Escherichia coli* count across the treatments. Highest *Escherichia coli* count (23.42 x 10^5) was recorded in antibiotic diet while least (3.28 x 10^5) was observed in the combination of *Carica papya* and *Chromolaena odorata* leaf meals. Total *Lactobacillus* count of all treatments differed significantly ($p<0.05$) and 0.05% antibiotics had least Total *Lactobacillus* count while highest TCC counts was recorded in *Chromolaena odorata* diet.

The inclusion of 3% *Chromolaena odorata* leaf meal in the diet increased the gut *Lactobacillus* count this implies that the presence of *Chromolaena odorata* in the diet stimulated the proliferation of *Lactobacillus.* This is in agreement with Jiwuba *et al.* [21] who posited that *Chromolaena odorata* leaf meal diets at inclusion levels of 2, 4 and 6% have the ability to stimulate *Lactobacillus* growth. However, birds on 0.05% antibiotic diet recorded the lowest value of *Lactobacillus* count. This indicates that antibiotics in the diet had negative effect on beneficial microbes in the gut of broilers. Birds on the combination of *Carica papya* and *Chromolaena odorata* leaf meal diets had lower level of TCC and TEC than those on the individual treatment. The result of this study supported the reports of Omidiwura [22], that methanol-extracted pawpaw leaf can be used as alternatives to synthetic antibiotic in livestock production because it is effective against pathogenic microbial organisms.

Ileal morphology and histopathology of broilers chicken fed with *Carica papaya and Chromolaena odorata* leaf meal supplemented diets

Result obtained for ileal morphology (Table 7) shows no significant ($P>0.05$) difference in villus height, villus width, crypt depth, epithelial wall thickness and epithelial cell. The ileal morphology result shows no significant difference, which agrees with Peric *et al.,* [] (2010) that reported no significant difference in the gut morphology of broiler chickens fed phytogenic additives but contradict Wealleans *et al.* [23] who reported significant difference in the villus height and crypt depth of broiler chickens fed *Bacillus* probiotics.

As indicated on plates 1-5, for the histopathology of the ileum, it was observed that only 3% *Carica papaya* diet has eroded villi of the mucosa layer. All other treatments showed moderate inflammatory infiltration of the gland and lamina propia except combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* leaf meal diet which shows severe to chronic infiltration of inflammatory cells. The result showed that the inclusion of *Carica papaya and Chromolaena odorata*

Table 6: Gut microbial population of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets.

Parameters (Cfu x 10^5)	T1	T2	T3	T4	T5	SEM	P Values
THC	73.20^{a}	23.64^{d}	27.22^{c}	48.78^{b}	14.02^{e}	0.052	0.0001
TCC	41.16^{a}	39.20^{b}	35.70^{c}	35.10^{c}	25.64^{d}	0.096	0.0001
TEC	9.20^{c}	23.42^{a}	13.66^{b}	6.24^{d}	3.28^{e}	0.050	0.0001
TLC	17.98^{c}	3.06^{e}	14.12^{d}	27.12^{a}	21.24^{b}	0.046	0.0001

^{abcde} Means on the same row with different superscript are significantly ($p<0.05$) different, SEM- Standard Error of Mean, THC- Total heterotrophic count, TCC- Total coliform count, TEC- Total Escherichia coli count, TLC- Total Lactobacillus count. T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

Table 7: Ileal morphology of broilers chicken fed with *Carica papaya and Chromolaena odorata* leaf meal supplemented diets.

Parameter	T1	T2	T3	T4	T5	SEM	P-value
Vilus height	864.3	668.6	872.3	687.1	711.2	33.058	0.2038
Vilus width	100.71	99.23	138.83	110.76	98.96	4.646	0.0942
Crypth depth	205.16	111.4	142.01	118.37	96.8	14.81	0.2358
EpitheliumWall thickness	255.75	267.65	228.92	243.063	306.37	14.651	0.5463
Epithelium	28.17	26.35	31.74	27.09	27.53	1.37	0.744

Values with different superscript are significantly ($p<0.05$) different. T1- Basal diet feed (negative control (NC) diet), T2-Basal diet feed +3.0% Neomycin sulphate (positive control (PC) diet), T3-Negative control + 3% Pawpaw leaves, T4-Negative control + 3% *Chromolaena odorata* leave and T5-Negative control + 1.50% *Carica papaya* and 1.5% *Chromolaena odorata*.

leaf meal in the diet of the birds has no effect on the histopathology of the ileum section of the bird as compared to those fed the negative and positive control diet. Agboola and Adenuga [24] reported that higher inclusion level of heat treated jatropha seed cake had a negative effect on the histopathology liver and bursa of fabricus of japanese quails. Figure 1-5.

Heamatological indices and serum biochemical parameters of broiler chickens fed *Carica papaya* and *Chromolaena odorata*

The result of haematological indices of broiler birds fed experimental diets is presented in Table 8. There was no effect of dietary treatments on all the parameters measured except red blood cell (RBC) count. There was a significant difference between RBC count of birds on 0.05% antibiotics and combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* leaf meal diets. The haematological parameters are important indices that reflect the physiological state of the individual animal, the ability to interpret the blood profile in healthy and diseased conditions is one of the primary objectives of haematological studies [25].

The packed cell volume (PCV) and haemoglobin (Hb) of birds

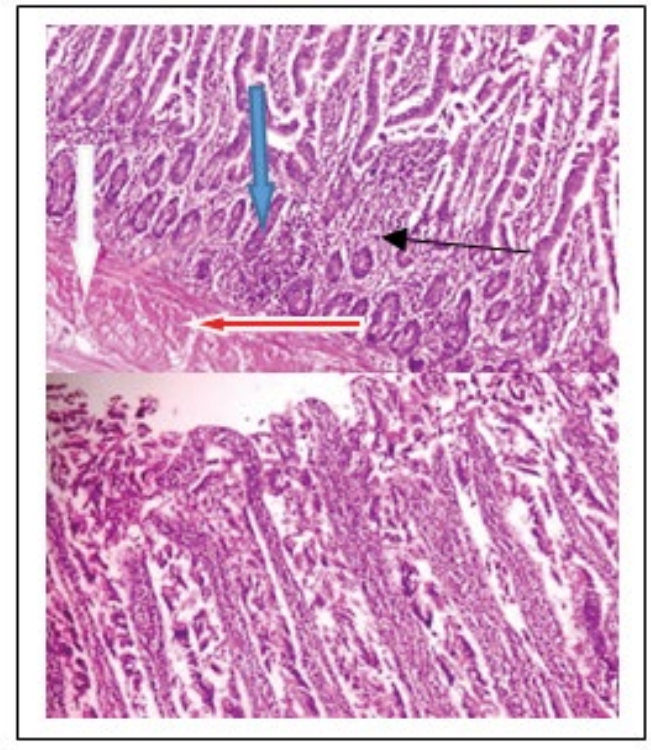

Figure 1: Plate 1- Birds fed on basal (control diet). Photomicrograph showsnormal mucosa layer with normal villi (white arrow), the glands and lamina propria show moderate infiltration of inflammatory cells (slender arrow), the submucosal layer appear infiltrated by inflammatory cells and also show focal area of lymphocytes aggregate (blue arrow). Muscularis layers appear normal (red arrow).

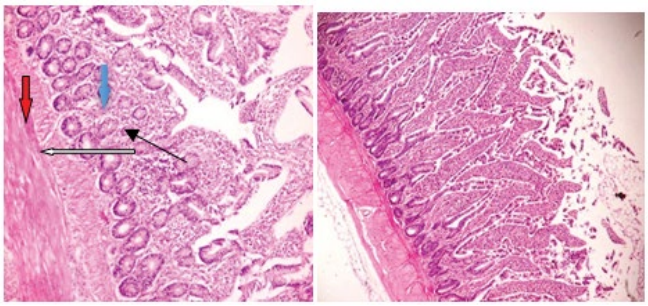

Figure 2: Plate 2- Birds fed on control diet +0.05% Antibiotics (positive control). Photomicrograph shows the submucosal layer appear infiltrated by inflammatory cells and also show focal area of lymphocytes aggregate (blue arrow). Muscularis layers appear normal (red arrow).

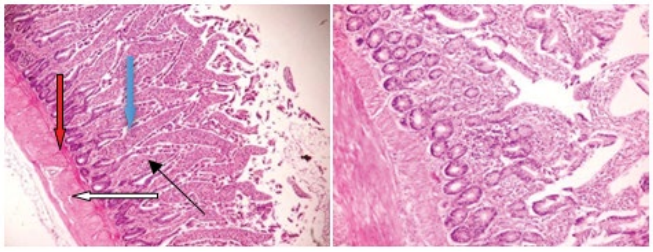

Figure 3: Plate 3- Birds fed on control diet + 3% *Carica papaya*. Photomicrograph shows mucosa layer with moderately sloughed/ eroded villi (white arrow), the glands and lamina propria show moderate infiltration of inflammatory cells (slender arrow), the submucosal layer appear mildly infiltrated by inflammatory cells (blue arrow). Muscularis layers appear normal (red arrow).

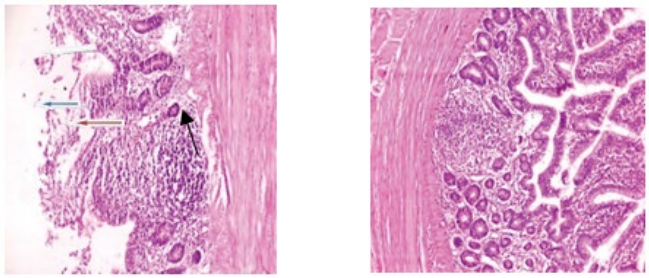

Figure 4: X100 Plate 4- Birds fed on control diet + 3% *Chromolaena odorata*. Photomicrograph shows moderately normal mucosa layer, the glands and lamina propria show moderate infiltration of inflammatory cells (slender arrow).

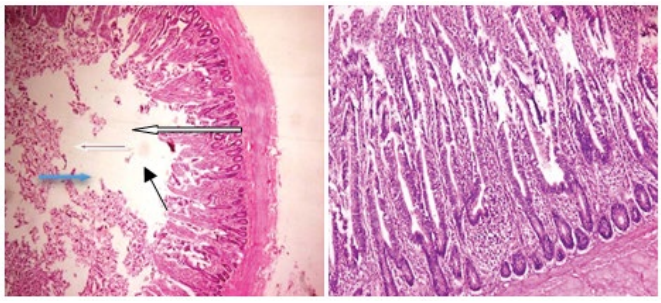

Figure 5: X100 Plate 5- Birds fed on control diet + 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meals. Photomicrograph shows normal mucosa layer, the glands and lamina propria show severe to chronic infiltration of inflammatory cells (slender arrow).

within the dietary treatments fell within the normal physiological range of 22.0-35.0 % and 7.0- 13.0 g/dL reported by Jain [26], respectively. This is in agreement with the normal range of PCV and Hb reported by Jiwuba *et al.* [21] in the study of utilisation of *Chromolaena odorata* leaf meal by broilers. The red blood cell (RBC) value of birds on combination of 1.5% *Carica papaya* + 1.5% *Chromolaena odorata* leaf meals was outside the range and this could

Table 8: Haematological indices of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets.

Parameters	T1	T2	T3	T4	T5	SEM	P Values
Packed cell volume (%)	27.80	27.00	28.6	27.20	30.00	0.58	0.49
Haemoglobin (g/dL)	9.26	8.86	9.44	9.00	9.68	0.19	0.66
Red blood cell (x10^6 uL)	3.12^{ab}	2.67^{b}	3.28^{ab}	3.04^{ab}	4.64^{a}	0.26	0.018
White blood cell (x 10^7 uL)	1.91	1.73	1.59	1.90	1.64	682	0.48
Platelets (x 10^5 uL)	2.44	1.88	2.10	2.00	1.72	1.02	0.26
Lymphocytes (%)	65.00	63.40	62.20	64.40	66.60	0.85	0.56
Heterophils (%)	28.80	29.80	30.60	28.40	27.00	0.95	0.79
Heterophil/Lymphocytes	0.45	0.48	0.49	0.45	0.41	0.02	0.75
Monocytes (%)	3.60	3.60	3.80	3.20	3.20	0.27	0.94
Eosinophil (%)	2.20	4.00	2.60	4.00	3.00	0.39	0.51
Basophil (%)	0.20	0.20	0.40	0.00	0.20	0.085	0.70

[ab] Means on the same row with different superscript are significantly (p<0.05) different, SEM- Standard Error of Mean, T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

possibly suggest polychytemic condition. The WBC concentration of birds on 3% pawpaw leaf agreed with the findings of Agboola *et al.* [27] who postulated that sundried pawpaw leaf meal contains some secondary metabolites that enhance the immune system. The lymphocyte, heterophil, Monocyte, eosinophil, basophil percentages and heterophil to lymphocyte (HET/LYM) ratio were within the physiological normal range as reported by Jain [26].

Table 9 shows the results of the analysis of some serum metabolites of birds fed experimental diets. The diets had no significant effect on total protein, albumin, creatinine and urea except globulin and Alb/Glo ratio. Globulin concentration of birds fed 3% *Chromolaena odorata* leaf meal diet was similar to other diets. The alb/glo ratio of birds on control diet was significantly (p<0.05) higher (1.22) than those on other dietary treatment. The chemistry of serum is normally used for the diagnosis of organ diseases in farm animals and detection of the amount of available protein in the diets [28]. There were no appreciable differences observed in total protein and albumin of birds which contrast the significant differences reported by Jiwuba *et al.* [21]. The serum urea and creatinine values were within the normal physiological range as reported by Mitruka and Ranwsley [29].

Conclusion

The inclusion of either *Carica papaya* or *Chromolaena odorata* leaf meal in broiler diet did not enhance performance, gut morphology and histopathology. The combination of 1.5% *Carica papaya* and 1.5% *Chromolaena odorata* in broiler diet had positive effect on the performance indices. The combination could be considered as suitable natural alternatives to synthetic antibiotics in broiler diet.

Recommendation

Carica papaya and *Chromolaena odorata* at inclusion level of 1.5% + 1.5% is recommended in broiler starter and finisher diets for improved proliferation of beneficial microorganisms. Further studies should be carried out on probably higher inclusion levels of *Carica papaya* and *Chromolaena odorata* in broiler diet and also in characterising the beneficial microorganisms.

Table 9: Serum biochemical parameters of broiler chickens fed *Carica papaya* and *Chromolaena odorata* leaf meal supplemented diets.

Parameters	T1	T2	T3	T4	T5	SEM	P Values
Total protein (g/dL)	3.42	4.18	4.04	3.96	4.18	0.12	0.29
Albumin (g/dL)	1.87	1.78	1.77	1.89	1.94	0.55	0.84
Globulin (g/dL)	1.55^{b}	2.40^{a}	2.28^{a}	2.08^{ab}	2.24^{a}	0.087	0.047
ALB/GLO	1.22^{a}	0.76^{b}	0.79^{b}	0.93^{b}	0.90^{b}	0.035	0.0033
Creatinine (mg/dL)	0.50	0.68	0.56	0.60	0.52	0.045	0.73
Urea (mg/dL)	5.31	5.38	6.37	6.29	6.06	0.22	0.39

[ab] Means on the same row with different superscript are significantly (p<0.05) different, SEM- Standard Error of Mean, T1- Basal (Control) diet; T2- basal diet + 0.05% antibiotics; T3- Basal diet + 3% Carica papaya leaf meal; T4- Basal diet + 3% Chromolaena odorata leaf meal and T5- Basal diet + 1.5% Carica papaya + 1.5% Chromolaena odorata leaf meals

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