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Growth performance and blood profile of broiler chickens as influenced by oral administration of lime fruit (*Citrus aurentifolia*)

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Abstract

The study was aimed at investigating the effect of oral administration of lime fruit on growth performance, haematology and serum biochemistry in broiler chicken. A total of 150, one day-old unsexed broiler chickens of Anak 2000 strain, were used in a completely randomized design (CRD). The birds were randomly allotted to five treatments with each group having 30 birds, replicated three times to give 10 birds per replicate. Treatment 1 was the control and had no additive; Treatment 2, had 5 mL of lime juice per litre of water every day; Treatment 3, had 7.5g of diced whole lime fruit per litre of water every day; treatment 4, had 5mL of lime juice per litre of water in alternate day while Treatment 5, had 7.5g of diced whole lime fruit per litre of water in alternate day. The experimental trial was for six (6) weeks after a brooding period of two weeks. Significant differences were observed in the final weight, feed intake, weight gain, and feed conversion ratio. Treatment 4 with 5mL of lime juice per litre of water in alternate day recorded better growth performance in the study, with weight gain and feed conversion ratio of 2963.35g/bird and 1.91, respectively. Results from haematological and serum biochemistry characteristics showed varied significant differences in some parameters. Haemoglobin values were between the range of 10.00g/dL in Treatment 3 and 11.80g/dL in Treatment 2. Total white blood cell (TWBC) was significantly highest in Treatment 2 (75.20 x10^3/μL), and least in Treatment 3 (66.20 x10^3/μL). Total plasma cholesterol was highest in the control treatment with 133mg/dL and the least value was obtained from Treatment 2 with 104mg/dL. The study supports the use of lime fruit (juice or whole fruit) to promote growth and blood characteristics of broiler chickens which ultimately contributes to the improvement of the bird's overall productivity.

Keywords: broiler chickens, lime fruit, *Citrus aurentifolia,* non-antibiotic growth promoter, growth performance, blood profile

Introduction

High level of production and efficient feed conversion are the need of modern poultry industry which to a certain extent can be achieved by the use of specific additives as growth promoters which when supplemented in poultry nutrition, stabilizes the microbial flora, and improve the health status and general performance of the animals (Windisch *et al.,* 2008). For this reason, antibiotic has been given at sub-therapeutic dosage to stabilize the intestinal microflora, improve the general performance and prevent some specific intestinal pathology, for about 50 years (Dibner 2004). But according to World Health Organization (WHO), resistance to antibiotics, which is the ability of bacteria to survive the inhibitory or the lethal effect of antimicrobial agent has grown as a result of previous exposure to sub-therapeutic levels of anti-microbial agents (Demir, 2005). The dangers posed by the development of resistance in poultry and human beings have received huge attention over the last decade and are well documented (Dibner, 2004; Paul *et al*., 2007; Agyare *et al.,* 2018). Ricke (2003)

showed that resistance to bacteria can be transferred from poultry products to the human population through consumption or handling of poultry products contaminated with the resistant pathogen. Attempts at resolving these challenges have increased research interest in finding suitable replacements. Alternatives to antibiotics are of great interest to the poultry industry as there is also an increased interest in the utilization of growth promoters of natural origin (Imasuen and Aikhomu, 2017). Most of these alternatives have effects on microflora directly or indirectly and one of such interest has given rise to the use of organic acids, spices and essential oils (Ndelekwute *et al.,* 2015). Organic acids such as citric, ascorbic, acetic butyric and formic acids occur naturally most especially in some fruits. However, the majority of information on organic acids is mostly on synthetic types, which most farmers may not have access to. Most of the previous studies have used a single organic acid as a dietary supplement but few studies have been conducted to determine the effect of a mixture of different organic acids and the capability of such mixtures to substitute antibiotics growth promoters in poultry production. The next practical alternative, therefore, is to use natural sources which contain more than one organic acid and which can be accessed easily. Lime fruit (*Citrus aurantifolia*), is a citrus spp., which abound in the tropical ecosystem and is a rich source of citric and malic acids. It also contains an appreciable amount of oxalic, tartaric, acetic and ascorbic acids (Pennistine *et al.,* 2008, NFH, 2013). This study was therefore undertaken to assess the growth performance and blood profile of broiler chickens as influenced by the oral administration of lime (C*itrus aurantifolia*) fruit.

Materials and methods

The experiment was conducted at the Poultry Unit of the Teaching and research farm, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The lime fruits were purchased from the local market in Ekpoma. The good fruits were washed with clean water and divided into two sets. The first set was immersed in hot water for about five minutes, to ease the juice extraction. They were thereafter cut into two halves with a sharp knife and the juice expelled manually by squeezing. The extracted juice containing the seeds was passed through a filter material to remove the seeds. The second set of fruits were diced whole and soaked in warm water overnight before use in the field the next morning. Completely randomized design (CRD) was employed. One hundred and fifty (150), one day-old unsexed broiler chicks of Anak 2000 strain were used for this study. The chicks were randomly divided into five treatments, with 30 birds per treatment group. Each treatment was replicated three times to give 10 chicks per replicate. Treatment 1 was the control containing neither lime juice nor diced whole lime fruit. Treatment 2 was offered 5mL of lime juice per liter of drinking water every day. Treatment 3 was offered 7.5g diced whole lime fruit per litre of water every day. Treatment 4 was offered 5mL lime juice per litre of water in an alternate day, while treatment 5 was offered 7.5g of diced whole fruit per litre of drinking water in an alternate day. The brooding pen and its environment were thoroughly cleaned and disinfected. The birds were brooded for 2 weeks after which they were moved to the experimental pen at the beginning of the third week. The birds were offered the treated water from the third week till the eighth week when the experiment was terminated. Feed and water were offered *ad libitum* throughout the experiment. The starter and finisher diets were formulated according to the recommendations of Olomu (2010), and are given in Table 1 below.

Table 1: composition of experimental broiler starter and finisher diets

Ingredients (%)	Starter diet	Finisher diet
Maize	55.20	54.00
Soybean meal	28.00	20.80
Palm kernel cake	9.00	12.40
Wheat bran	-	7.00
Fish meal	4.00	3.00
Bone meal	3.00	2.00
Common salt	0.30	0.30
Vitamin/mineral premix	0.50	0.50
Total	**100**	**100**
*Calculated composition		
Crude protein (%)	23.10	20.62
Metabolizable energy (Kcal/Kg)	3240.04	3088.15

*The calculated nutrient composition were derived using the following values; maize 9% crude protein and 3520 kcal/kg ME, soybean meal 48% crude protein and 3400 kcal/kg ME, palm kernel meal 21% crude protein and 2500 kcal/kg ME, fish meal with 70% crude protein and 3000 kcal/kg ME. Source: Olomo (2010).

Feed intake, weight gain, and feed conversion ratio were assessed weekly. Feed intake was determined by weighing the quantity of feed offered weekly and subtracting the leftover at the end of the week. Average feed intake and average daily feed intake were calculated from the recorded data. The feed conversion ratio was calculated as the ratio of feed intake to weight gain. At the end of the six weeks trial period, the birds were starved of feed for 12 hours and blood samples were collected from two birds per replicate for hematological and biochemical analysis. The blood samples were collected from each bird from the wing vein using sterilized disposable syringes and needles. Haematological parameters (total white blood cell (TWBC), lymphocytes, monocytes, neutrophils, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width, red blood cell, packed cell volume (PCV), mean cell volume (MCV), platelets and mean platelet volume (MPV)) were determined in the laboratory with the aid of an auto-analyzer while serum characteristics (cholesterol, total protein, albumin, and globulin) were determined with the help of an Epoll 20 photometer using original Alpha Diagnostics kits. All data collected were subjected to a one-way analysis of variance (ANOVA) by using the general linear model procedure of SAS (2012) and differences in means was compared by Duncan's multiple range tests as outlined by Steel and Torrie (1997). The level of statistical significance was preset at $p<0.05$.

Results and discussion

The growth performance of the experimental birds is as stated in Table 2 below. Significant differences ($P<0.05$) were recorded in all measured and estimated parameters except for average initial weight. Average final weight was highest and significantly different in treatment 4 (5mL of lime juice per litre of water in alternate days) with 3164.52g/bird while the control treatment had the least final weight of 2431.94g/bird.

Table 2: Growth performance of experimental birds

Performance characteristics	T1	T2	T3	T4	T5	SEM
Average initial weight (g)	203.17	203.00	198.83	201.17	201.33	5.51NS
Average final weight (g)	2431.94^{c}	2668.07^{b}	2770.65^{ab}	3164.52^{a}	2459.29^{bc}	22.76*
Average weight gain (g)	2228.77^{c}	2465.07^{b}	2571.82^{ab}	2963.35^{a}	2257.96^{bc}	34.13*
Average daily weight gain (g)	53.07^{c}	58.69^{b}	61.23^{b}	70.56^{a}	53.76^{c}	1.82*
Average feed intake (g)	5478.07^{b}	5496.31^{b}	5565.50^{a}	5676.10^{a}	5489.12^{b}	0.74*
Average daily feed intake (g)	130.43^{b}	130.87^{b}	132.51^{a}	135.15^{a}	130.69^{b}	0.60*
Feed conversion ratio (FCR)	2.46^{c}	2.23^{b}	2.16^{ab}	1.91^{a}	2.43^{c}	0.16*

NS = Not significant; * = significant difference; SEM = standard error of mean

a,b,c: means along the same row with different superscripts are significantly different

T1=Control; T2=5ml of lime juice per litre of water every day; T3=7.5g of diced whole lime fruit per litre of water every day; T4=5ml of lime juice per litre of water in an alternate day; T5=7.5g of diced whole lime fruit per litre of water in an alternate day.

Treatment 4 also had the highest average weight gain of 2963.35g/bird and was also significantly different from the control treatment having the least value of 2228.77g/bird. Average daily weight gain followed the same pattern of significance as the average weight gain. Average feed intake and average daily feed intake were significantly higher in treatments 3 and 4. Increased feed intake in these two treatments was advantageous as it resulted in higher final weight and average weight gain in the experiment. Feed conversion ratio (FCR) was better in treatment 4 (1.91), though not significantly different from treatments 2 and 3 (2.23 and 2.16, respectively). The control and treatment 5 had the least significant feed conversion ratio of 2.46 and 2.43, respectively.

Ndelekwute and Enyenihi (2017), reported significant differences in the average final weight, average weight gain and average daily weight gain in a study with broiler chickens using lime as a feed additive. This observation was in agreement with the findings of this study. This could possibly be due to the composition of lime fruits which include essential oils, carotenoids, flavonoids and organic acids and synergy between them (Ndelekwute *et al*., 2015). This was also in line with Windisch *et al*. (2008) who stated that essential oils, improves nutrient utilization for better performance. Dibner (2004) and Paul *et al* (2007) also stressed the importance of organic acids such as citric acids in nutrient utilization which will result in better productivity. According to Ndelekwute *et al. (2015)*, feeding a diet containing 2.50% lime juice will result to an increase in protein intake and better protein efficiency ratio and eventually underscore its importance in broiler chicken production. This level according to these authors can supply the required amount of citric and ascorbic acids needed for better productivity. Paul *et al.* (2007) also reported that organic acids such as citric acid increases feed intake and subsequently the live weight of broiler chickens.

The result of the haematological analysis is shown in Table 3. Significant differences were observed in all measured parameters except red for blood cell and packed cell volume. Highest values were obtained in MCH (42.05pg) and MCHC (36.25g/dL) from Treatment 1 while the least values (40.10pg and 32.00g/dL) were obtained from Treatment 3 and Treatment 5, respectively. RDW and MPV values were highest in Treatment 3 with 4.80% and 5.90fl respectively, while the control had the least values in both instances with 3.35% and 4.65fl respectively. Significantly higher values were obtained in haemoglobin (11.80g/dL), MCV (127fl) and platelets ($33x10^{3}/\mu L$) from Treatment 2, Treatment 5 and Treatment 4

respectively, while Treatment 3 had the least value of 10.00g/dL of haemoglobin and Treatment 2 had the least values of 114.10 fl in MCV and 17 x10^3/µL in platelets.

Table 3: Haematological characteristics of experimental birds

Parameters	T1	T2	T3	T4	T5	SEM
RBC(x10^6/µl)	2.75	2.89	2.49	2.60	2.55	0.12NS
Haemoglobin (g/dl)	11.55^{ab}	11.80^{a}	10.00^{c}	10.85^{b}	10.40^{bc}	0.42*
PCV (%)	31.80	33.00	31.10	32.65	32.40	1.52NS
MCV (fl)	115.80^{c}	114.10^{c}	125.00^{b}	125.50^{b}	127.00^{a}	0.15*
MCH(pg)	42.05^{a}	40.80^{b}	40.10^{c}	41.65^{a}	40.80^{b}	0.11*
MCHC (g/dl)	36.25^{a}	35.70^{a}	32.10^{c}	33.20^{b}	32.00^{c}	0.11*
RDW (%)	3.35^{c}	4.50^{ab}	4.80^{a}	4.35^{b}	3.50^{c}	0.10*
Platelets(x10^3/µl)	20.00^{bc}	17.00^{c}	32.00^{ab}	33.00^{a}	21.50^{abc}	3.82*
MPV (fl)	4.65^{c}	4.70^{c}	5.90^{a}	4.75^{bc}	5.10^{b}	0.31*

NS = Not significant; * = significant difference; SEM = standard error of mean

a,b,c: means along the same row with different superscripts are significantly different

T1=Control; T2=5ml of lime juice per litre of water every day; T3=7.5g of diced whole lime fruit per litre of water every day; T4=5ml of lime juice per litre of water in an alternat e day; T5=7.5g of diced whole lime fruit per litre of water in alternate day

The results obtained from this research are in agreement with the work of Nobakht (2013), where dried lemon pulp was supplemented with broiler chicken diets and no significant effects were observed in red blood cell and packed cell volume. Haemoglobin value was also reported as not significantly influenced by the treatment. However, this is contrary to the findings of this work as significant differences between treatments was recorded in haemoglobin values. The MCV values which ranged between 114.10 – 127.00fl, are within the range recommended as optimum by Jain (1993) of 90 – 140fl. Mitruka and Rawnsley (1997), reported that MCV is an important trait which determines the cell size of the erythrocytes and thus an important factor in determining the ability of the birds to withstand oxygen starvation for a long time.

Table 4 shows the values of the differential white blood cell counts in total and percentages. All assessed parameters were significantly influenced by the treatments. Total white blood cell count was highest (75.20x 10^3/µL) in treatment 2, though not significantly different from the control with 74.90 x10^3/µL. However, Treatment 3 was least and significantly different from other treatments with 66.20 x10^3/µL. Lymphocyte count was highest in treatment 3 (86.00%) and least in treatment 4 (79.80%). Monocytes and neutrophils were highest in treatment 4 (9.80% and 10.40% respectively) which was also significantly different from the control. Lowest values in monocytes and neutrophils were obtained from treatment 5 (7.75%) and treatment 3 (6.20%) respectively. The values obtained from this research are contrary to the report of Nobakht (2013), where white blood cell and lymphocyte values were reportedly not influenced in their work with dried lemon pulp and broiler chickens. Kadam *et al.* (2009) also reported no significant differences between treatments in lymphocytes and monocytes in their work with lemon juice and broiler chickens, which is contrary to the results obtained from this study.

Serum biochemistry values obtained from the study are stated in Table 5. Total cholesterol ranged from the least in Treatment 2 with a value of 104mg/dL to 133mg/dL obtained from Treatment 1. Total protein was highest in Treatment 4 with 4.80g/dL and least in Treatment 1 with 4.15g/dL. Albumin values were also least

Table 4: Differential white blood cell count

Parameters	T1	T2	T3	T4	T5	SEM
TWBC (x10^3/µL)	74.90^a	75.20^a	66.20^d	72.70^b	70.50^c	0.40*
Lymphocytes (%)	83.65^{ab}	85.20^a	86.00^a	79.80^b	84.80^{ab}	0.79*
Monocytes (%)	8.15^b	8.20^b	7.80^{bc}	9.80^a	7.75^c	0.47*
Neutrophil (%)	8.20^b	6.60^c	6.20^c	10.40^a	7.50^{bc}	0.13*

* = significant difference; SEM = standard error of mean

a,b,c: means along the same row with different superscripts are significantly different

T1=Control; T2=5ml of lime juice per litre of water every day; T3=7.5g of diced whole lime fruit per litre of water every day; T4=5ml of lime juice per litre of water in an alternate day; T5=7.5g of diced whole lime fruit per litre of water in alternate day

in Treatment 1with 1.75g/dL. However, the highest albumin level was obtained from Treatment 5 with 2.11g/dL. Globulin value of 2.76g/dL was the highest obtained from Treatment 4, while the least value of 2.15g/dL was obtained from Treatment 3. According to Nobakht (2013), *Citrus aurantifulia* did not significantly influence the cholesterol values of broiler chickens. Values however ranged between 99.59mg/dL to 127.80mg/dL in the study. This was contrary to the findings of this study, where significant differences were observed among treatments with a range of 104mg/dL to 133mg/dL. The reported cholesterol values from this study are however considerably lower than the recommended range of 155mg/dL – 194mg/dL for table birds reported by Wikivet (2013).

Table 5: Serum biochemistry of experimental birds

Parameters	T1	T2	T3	T4	T5	SEM
Total cholesterol (mg/dL)	133.00^c	104.00^a	122.00^{bc}	108.00^a	115.00^{ab}	3.82*
Total protein (g/dL)	4.15^c	4.55^b	4.10^c	4.80^a	4.68^{ab}	0.07*
Albumin (g/dL)	1.75^c	1.82^c	1.95^b	2.04^{ab}	2.11^a	0.11*
Globulin (g/dL)	2.40^b	2.73^a	2.15^c	2.76^a	2.57^{ab}	0.06*

* = significant difference; SEM = standard error of mean

a,b,c: means along the same row with different superscripts are significantly different

T1=Control; T2=5ml of lime juice per litre of water every day; T3=7.5g of diced whole lime fruit per litre of water every day; T4=5ml of lime juice per litre of water in an alternate day; T5=7.5g of diced whole lime fruit per litre of water in alternate day

Conclusion

The results presented in this study has shown that the oral administration of lime juice and diced whole lime either every day or in an alternate day, generally improved growth performance and some blood parameters. It can be concluded that as a result of the significant positive effect on broiler performance in comparison to the control, lime juice or diced whole lime fruit administered orally holds great promise and potential as a growth promoter in broiler production.

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