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Lime Juice as a Source of Organic Acids for Growth and Apparent Nutrient Digestibility of Broiler Chickens

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Abstract

An experiment was conducted to determine the effect of lime juice (*Citrus aurentifolia*) on growth and nutrient digestibility of broilers. Three hundred day-old mix-sex broiler chicks of Anak strain were used. The lime juice was assessed for its citric and ascorbic acids content. Its antibacterial action on feed was determined. A basal diet was formulated as control and designated as treatment 1 (T1). Other treatments were formed by mixing the lime juice with the basal diet at different levels. The levels were 1.0, 1.50, 2.0 and 2.50% representing T2, T3, T4 and T5 respectively. Birds were randomly allotted to the 5 treatments and each treatment was replicated thrice. Each replicate had 20 birds. The experiment was arranged in completely randomized design (CRD). Feed and water were given *ad libitum.* Results showed that the juice contained 1.60 and 1.20% of citric and ascorbic acids respectively with pH value of 4.5. The bacteria population in the feed was reduced by the lime juice. At the starter phase, the lime juice improved feed intake and 2.50% significantly ($P<0.05$) improved final live weight and weight gain. At the finisher phase, final live weight was significantly ($P<0.05$) improved by 2.0 and 2.50%. Feed intake, weight gain, feed: gain ratio and protein efficiency ratio were not influenced ($P>0.05$). Digestibility of protein, fat and oil was improved by 1.50% and above. Therefore, lime juice could be used to sanitize poultry feed and addition of 2.0% lime juice in broiler diets is recommended to be adopted.

Keywords: Ascorbic acid; Broilers; Citric acid; Nutrient digestibility

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Introduction

Feed additives have numerous beneficial effects in meat animal production like control of pathogenic microorganisms and enhancement of growth of beneficial microorganisms [1]. High level of production, least cost feed and efficient feed conversion are the needs of the modern broiler production which to a certain extent could be achieved by the use of specific feed additives. Antibiotic growth promoters have been extensively used in animal feed especially in the poultry and pig industries [2]. They are thought to stabilize the intestinal microflora and to prevent some specific intestinal pathogens leading to better gain and feed conversion [1]. Antibiotics possess these beneficial effects, but their use in animal feeds has been intensely and extensively controversial because of the development of bacterial resistance and potential consequences on animal and human health [3].

Owing to antibiotic resistance and human health hazard, there is an increased pressure on livestock industry by consumers and regulatory agencies to phase out or reduce the use of antibiotic growth promoters. This has forced animal nutrionists to actively search for efficacious alternatives to antibiotic growth promoters. As alternative to antibiotics, organic acids (formic, citric, ascorbic, acetic and butyric acids etc.) among others have been reported to be efficacious by modifying intestinal pH, improving digestion and absorption of nutrients [4]. Organic acids currently in use are synthetic types which are expensive and most farmers do not have access to them. Also going by the campaign in favor of organic farming or use of natural feed additives in meat animal feeds, it is important to survey the natural ecosystem to discover and exploit some natural sources of organic acids. Lime juice is a good source of organic acids such as citric and ascorbic acids [5] and could serve this purpose. It was reported that lime juice also contained some bio-molecules such as flavonoids and

carotenoids [5]. This could be an added advantage to envisaged bioactive property of lime juice due to the organic acid content. Nevertheless, there is scarce information on the use of lime juice as a growth promoter in feeding of broiler chickens. Therefore, the objective of this work was to determine the antibacterial action of lime juice in the feed and its effect as natural source of organic acids on growth and nutrient digestibility of broiler chickens.

Materials and Methods

Site of the experiment

The experiment was conducted at the poultry unit of Teaching and Research Farm of the University of Uyo, Nigeria, located on latitude 5° 32' N and longitude 7° 54' E with average annual rainfall of 1500 mm. The average relative humidity during the experiment was 65% and average ambient temperature was 32°C.

Processing of test lime juice

The lime juice used as the source of organic acids was obtained from the market. The lime fruits were washed and cut into two transverse sections with a sharp knife. The juice was expelled manually by squeezing with the hand. The juice containing the seeds and some particles was filtered in order to have a clear juice.

Determination of pH, citric and ascorbic acids content of lime juice

The juice was tested for pH using a pH Meter (PHep, Hanna Instruments, Italy) by dipping the pH meter electrode into a glass tube containing 10 ml of the lime juice. The number at which the pointer was indicating was taken as the pH. The juice was also analyzed for citric and ascorbic acids content by titration method according to Ref. [5].

Experimental design

Completely randomized design (CRD) was employed. The experiment was conducted with 300 day old unsexed broiler chicks of Anak strain. The chicks were randomly divided into five dietary groups (T1, T2, T3, T4 and T5) each having 60 chicks. Each treatment was replicated three times with 20 chicks each. Starter and finisher basal diets **(Table 1)** were formulated to form the control (T1). 10 ml, 15 ml, 20 ml and 25 ml of the lime juice /kg feed were respectively added to the basal diet to form T2, T3, T4 and T5. This represented 1.00, 1.50, 2.00 and 2.50% of the diet respectively.

Starter premixes supplied per kg diet: Vitamin A 15,000/10,000 IU, vitamin D_3 13000/12,000 IU, thiamin 2/2.0 mg, riboflavin 6/3.0 mg, pyridoxine 4/4.0 mg, niacin 40/20 mg, cobalamine 0.05/0.05 g, Biotin 0.08/0.08 mg, choline chloride 0.05/0.2 g, manganese 0.096/0.006 g, zinc 0.06/0.03 g, iron 0.024/0.02 g, copper 0.006/0.006 g, iodine 0.014/0.001 g, selenium 0.24/0.24 mg, cobalt 0.024/0.25 mg, antioxidant 0.125/0.125 g.

Finisher premix supplied per kg diet: Vitamin A 10, 000 IU, vitamin D_3 12,000 IU, vitamin E 20 IU, vitamin K 2.5 mg, thiamine 2.0 mg, riboflavin 3.0 mg, pyridoxine 4.0 mg, niacin 20 mg, cobalamin 0.05 mg, pantothenic acid 5.0 mg, Folic acid 0.5 mg, Biotin 0.08 mg, choline chloride 0.2 mg, manganese 0.006 g, Zinc 0.03 g, copper 0.006 g, iodine 0.0014 g, selenium 0.24 g, cobalt 0.25 g and antioxidant 0.125 g.

Table 1A: Ingredient composition of the basal diets.

Ingredients (%)	Starter	Finisher
Maize	53	54
Soybean meal	30	27
Fish meal	3	2
Palm kernel cake	6.2	9.3
Wheat offal	4	6
Bone meal	3	3
Lime juice	0	0
Salt	0.25	0.25
Lysine	0.2	0.1
Methionine	0.1	0.1
Premix	0.25	0.25
Total	100	100

Table 1B: Nutrient composition of the basal diets.

Nutrient Composition (%)	Starter	Finisher
Crude Protein	22.35	20.45
Crude Fibre	4.13	4.13
Ether Extract	4.1	4.1
Total Ash	8	8
Calcium	1.08	1.08
Phosphorus	1.01	1.01
Lysine	1.1	1.1
Methionine	0.5	0.5
Energy (KcalME/kg)	2875	2901

Management of experimental birds

The experiment was conducted according to the rules and regulations of the University Research Ethics Committee regarding animal welfare. The birds on arrival to farm the chicks were given glucose solution after they were allotted randomly to the various treatments. Heat was provided using kerosene stove for three weeks. All necessary vaccinations against Newcastle and infectious bursal (gumboro) diseases were done under the supervision of a veterinary officer. The birds were raised in an open sided deep litter house with pens measuring 2 m × 2 m each. Each pen formed a replicate which contained twenty birds. Feed and water were provided *ad libitum*.

Determination of apparent nutrient digestibility

At the end of the experiment, total collection method was used to determine the apparent nutrient digestibility. One bird from each of the replicates was used for the digestibility trial. There were housed in metabolism cages that were thoroughly washed and disinfected. The birds were acclimatized for four days during which their respective feeds were given to them. Thereafter, they were fed *ad libitum*, a known quantity of their respective diets for another four days during which faecal collection was carried out daily. Plastic trays were placed under the cages to collect the

faeces. Collected samples were quickly taken to the laboratory and dried to a constant weight in an oven. The proximate composition was determined according to the methods of Ref. [6]. The apparent nutrient digestibility was calculated thus, according to Ref. [7].

$$Nutrient\ digestibility\ (\%) = \frac{Nutrientin\ feed - nutrient\ in\ faeces}{Nutrient\ in\ feed} \times \frac{100}{1}$$

Determination of antibacterial action of lime juice in feed

A kilogram of the basal diet was measured into 5 places labeled A, B, C, D and E representing the treatments. Thereafter 0.0, 10, 15, 20 and 25 ml of the lime juice was added to the feed samples respectively. Five (5 g) was collected from each of the acidified sample feeds and the one without lime juice which served as the control. Each of the samples was left for 24 hours. At the end of the 24 hours they were incubated at 37°C for 48 hours. At the end of the incubation period serial dilution method according to Ref. [8] was used to determine the bacteria load of each sample.

Collection of data and statistical analysis

Live weight was measured weekly and feed intake daily. The live weight and feed intake were used to calculate the feed: gain ratio. All data collected were subjected to one way analysis of variance (ANOVA). Significant means were separated using Duncan New Multiple Range Test according to Ref. [9]. Total bacteria load was represented by bar chart.

Results and Discussion

Results indicated that the lime juice contained 1.60% of citric and 1.20% of ascorbic acid. The pH was also recorded to be 4.50. The low level of pH of the lime juice was an indication of acidity and thus confirming the presence of organic acids in the lime juice as confirmed by the presence of citric and ascorbic acids. According to Ref. [10] lime juice contained citric and ascorbic acids.

Antibacterial action of lime Juice in feed

The antibacterial action of test lime juice is shown in **Figure 1.** It was observed that 2.50% level reduced bacteria load more than any other level. It was also observed that as the level of lime juice was increased the bacteria load was reduced. The ability of the lime juice to reduce the bacterial load of the diet could be as a result of the presence of the organic acids (citric and ascorbic acids) in the lime juice. These acids have been reported to be anti-bacterial in nature [1,3,10-13].

Growth performance of starter broilers

Effect of lime juice on performance of starter broiler chicks is shown on **Table 2.** Diets containing lime juice did not significantly (P>0.05) influence feed: gain ratio. The lime juice however, influenced live weight, weight gain, feed intake, and protein intake and protein efficiency ratio. The final live weight, daily weight gain, protein intake and protein efficiency ratio were higher (P<0.05) in birds that fed diet containing 2.50% lime juice compared to control and other lime juice levels. There were no significant differences (P>0.05) between the control, 1.0, 1.50

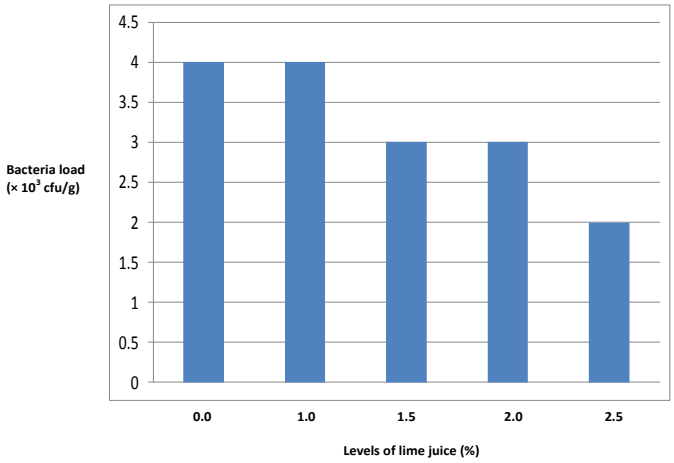

Figure 1 Effect of lime juice on bacterial load of experimental diet.

and 2.0% levels of lime juice. Both final and daily feed intakes were higher (P<0.05) in all the levels of lime juice. It was also observed that birds on 2.50% lime juice diet consumed more feed compared to other levels of lime juice.

The result of performance at the starter phase as indicated showed the capacity of lime juice to act as growth promoter in broiler chickens. Improvement in feed intake was advantageous as it led to higher live weight. Also, the feeding of diet containing 2.50% lime juice which resulted to increase in protein intake and better protein efficiency ratio underscores its importance at this phase of production. That level could have supplied the required amount of citric and ascorbic acids needed for better productivity. Organic acids such as citric acid have been reported to increase feed intake and subsequently the live weight of broiler chickens [2]. It could also be affirmed that the positive result at this phase was as a result of better utilization of protein and oil as indicated by the digestibility result in which protein and ether extract were better digested using lime juice. Also actions of other bioactive compounds such as flavonoids, carotenoids and synergism between them and the organic acids may not be ruled out according to Ref. [11].

Growth performance of finisher broilers

The effect of lime juice on performance of broilers during the finisher phase was shown in **Table 3.** Except the live weight, lime juice did not influence the parameters. Inclusion of 2.0 and 2.50% levels of lime juice posted better live weight than the control. The difference in weight between 1.50%, 2.0% and 2.50% was not significant (P>0.05). It was observed that 2.0% lime juice produced higher live weight than the control as against the result at the starter phase where non significant difference existed between them.

Going by the results obtained at the finisher phase it was clear that the lime juice was better utilized at the starter phase as indices which were significant and positively influenced by 2.50% juice at the starter phase became non-significant at the finisher phase. This was clearly expressed in final live weight where 2.50% lime juice gave the highest value than the other levels at starter

phase, but significantly posted similar values as those of 1.50 and 2.0% levels at the finisher phase. This is an indication that at certain level of the lime juice and for long dietary exposure, performance of birds could be hampered. According to Ref. [11], at 2.50% level, lime juice significantly reduced abdominal fat of broilers. This could be linked to the reduction in live weight at the finisher phase by 2.50% compared to other levels. Lime juice has been recommended by human nutritionists to be used to reduce human body weight [14-16]. In rat citrus products were reported to reduce both body weight, serum lipid and liver weight [17]. It could be opined therefore that lime juice may not be necessarily be added to diets during the finisher phase but during the starter phase. It has been reported that inclusion of 0.25% organic acids [acetic, butyric, citric and formic acids] in broiler diets at the finisher phase did not result to better growth performance [18].

Apparent nutrient digestibility of broiler birds

The result of the effect of lime juice on apparent nutrient digestibility is shown on **Table 4.** It indicates that only protein and ether extract were improved (P<0.05) by the lime juice at 1.50, 2.0 and 2.50%. There were no significant differences (P>0.05) in both digestibility of protein and ether extract at the three different levels (1.50, 2.0 and 2.50%) of lime juice that improved digestibility. It was observed that digestibility of protein and ether extract followed similar trend. Dry matter, crude fiber and ash were not significantly affected (P>0.05) by lime juice.

The positive influence of lime juice on digestibility of protein and ether extract was in line with what has been reported about lime juice in human nutrition. Lime juice has been reported to improve digestion in human [5,16]. This result could be linked with the result of better performance in live weight observed in treated birds especially those fed 2.0% lime juice. The organic acids in the lime juice could have played this important role as they have been reported to improve digestibility [3,15,19].

Conclusion

Going by the results obtained on the antibacterial effect, growth performance and digestibility, 2.0% level of lime juice could be used in broiler diets for optimum productivity.

Acknowledgements

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Table 2 Effect of lime juice (%) on growth performance of starter broiler chicks.

Parameters	T1 (0.00)	T2 (1.0)	T3 (1.5)	T4 (2.0)	T5 (2.5)	SEM
Initial live weight (g)	40.1	40.8	40	40.75	40.5	10.71
Final live weight (g)	950^{b}	963^{b}	977^{b}	982^{b}	1133^{a}	80.5
Daily weight gain (g)	32.50^{b}	32.94^{b}	33.46^{b}	33.62^{b}	39.02^{a}	4.16
Total feed intake (g)	1426^{c}	1540^{b}	1555^{b}	1558^{b}	1736^{a}	95.66
Daily feed intake (g)	50.93^{c}	55.00^{b}	55.54^{b}	55.60^{b}	62.00^{a}	3
Feed gain ratio	1.57	1.67	1.64	1.65	1.59	0.11
Daily protein intake (g)	11.72^{b}	12.66^{b}	12.78^{b}	12.80^{b}	14.27^{a}	2.05
Protein efficiency ratio	2.61^{b}	2.60^{b}	2.62^{b}	2.63^{b}	2.73^{a}	0.09

ab means along the same row with different superscripts are significantly different (P<0.05); SEM=Standard error of the mean

Table 3 Effect of lime juice (%) on growth performance of broilers at the finisher phase.

Parameters	T1 (0.00)	T2 (1.0)	T3 (1.5)	T4 (2.0)	T5 (2.5)	SEM
Initial live weight (g)	950.00^{b}	963.00^{b}	977.00^{b}	982.00^{b}	1133.00^{a}	80.5
Final live weight (g)	2755.00^{c}	2806.00^{bc}	2838.00^{abc}	2888.00^{ab}	2927.00^{a}	118.75
Daily weight gain (g)	64.46	65.82	66.25	68.07	64.07	6.05
Total feed intake (g)	4320	4335	4350	4343	4320	145.06
Daily feed intake (g)	154.29	154.82	155.36	155.11	154.29	25.76
Feed:Gain ratio	2.39	2.35	2.35	2.29	2.41	0.25
Daily protein intake(g)	30.94	31.04	31.15	31.1	30.94	3.05
Protein efficiency ratio	2.08	2.12	2.13	2.19	2.07	0.13

ab means along the same row with different superscripts are significantly different (P<0.05); SEM=standard error of the mean

Table 4 Effect of lime juice (%) on apparent nutrient digestibility of broilers.

Parameters	T1 (0.00)	T2 (1.0)	T3 (1.50)	T4 (2.0)	T5 (2.50)	SEM
Dry matter (%)	75.05	75.77	76.45	76.75	77.05	9.56
Protein (%)	65.23^{c}	71.01^{bc}	74.21^{ab}	78.30^{ab}	78.87^{ab}	10.05
Ether extract (%)	74.03^{c}	76.10^{bc}	88.00^{ab}	88.23^{ab}	88.10^{ab}	12.05
Crude fibre (%)	45.05	45.33	46.03	45.98	45.65	5.01
Ash (%)	68.02	67.99	69.02	68.99	67.89	6.34

abc means along the same row with different superscripts are significantly different (P<0.05); SEM=Standard error of the mean

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