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Enhancement of Bioactive Compounds in Lime Fruit Juice and its Dietary Effect on Growth Performance and Digestive Physiology of Chickens

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Abstract

This experiment was conducted to determine the bioactive substances in normal lime juice and mashed lime juice. The substances were citric acid, ascorbic acid, carotenoids, flavonoids and phenols. The effect of the mashed lime juice on growth performance and digestibility of broiler chickens was assessed. A total of one hundred and fifty (150) Anak strain day old chicks were used and were divided into five dietary groups of 30 birds each. The groups were further replicated with ten birds per replicate. The experiment was conducted in completely randomize design (CRD). A basal starter and finisher diets were formulated which formed the control (T1). Other dietary treatments (T2 - T5) were formed by adding 10, 15, 20 and 25 ml/kg diet of mashed lime fruit juice respectively. This was fed for seven weeks starting from day old. Feed and water were offered ad libitum throughout. Results indicated that the mashed lime juice contained higher bioactive substances than the normal juice. Final live weight, feed: gain ratio and protein efficiency ratio were significantly improved by 20ml/kg mashed lime fruit juice over the control at both the starter and finisher phases. Addition of 15, 20 and 25 ml/kg gave better protein, ether extract and ash digestibility than the control. Therefore, it is concluded that the incorporation of 20 ml/kg diet mashed lime fruit juice in broiler diet could be adopted.

Keywords: ***Bioactive substances; Broiler chickens; Digestibility; Growth performance; Mashed lime fruit juice***

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Introduction

For decades antibiotics have been used extensively in animal feeds and their drinking water all over the world to promote their productivity [1]. Antibiotics in animal production as growth enhancers have been in use since 1940s [2]. They are used mostly in pigs and poultry production at therapeutic and prophylactic doses to improve performance [3]. They are thought to stabilize the intestinal microbial flora and to prevent some specific intestinal pathogens [4,5]. This subsequently improved feed efficiency, growth rate, and reduced mortality rate in intensive poultry production [6]. Following the ban on the use of antibiotics as growth promoters in animal nutrition due to public health concern regarding antibiotic residues in animal products resulting to antibiotic resistance in human, attention has been shifted to searching for antibiotic growth promoting alternatives [7,8].

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Several alternatives to antibiotic growth promoters have been proposed such as organic acids, probiotics, herbs and herbal products, enzymes, spices and essential oils [8,9]. Among these, organic acids (citric, acetic, propionic, formic acids) and probiotics (for example lactic acid bacterium) are the most promising alternatives for poultry production [10]. Addition of organic acids in the diet of broilers enhanced nutrient utilization, growth and feed efficiency [11].

According to Afsharmanesh and Pourreza [12], Nezhad et al. [13] and Abdel-Fattah et al. [14] organic acids create an acid environment (pH 3.5 - pH 4.0) in the gut that favours the development of Lactobacilli and inhibits the replication of Escherichia Coli, Salmonella, and other gram-negative bacteria. Organic acids activate proteolytic enzymes, stimulate feed consumption, reduce production of ammonia and growth-depressing microbial metabolites, favor mineral absorption and lower the incidence of sub clinical infections [15,16] in broiler chicks. In addition, they improved immune responses by broilers [17].

Most available information on organic acids as feed additives is based on synthetic types. With recent emphasis on organic animal production, it is important to look inwards to discover natural sources of organic acids suitable as feed additives. Phytonutrients have suggested improving immune system of animals [18]. Natural types of organic acids are found in fruits especially citrus spp. (such as lime, lemon and grape). Citrus as a tree crop is abundant in the tropical ecosystem. Lime juice has been used to reduce microbial load of chicken feed, improve growth and gross margin [5,19]. It is a known fact that lime juice contains mostly water, ascorbic and citric acids. The positive result has been attributed to the organic acid content of lime juice [19]. However, a whole lime fruit contains other bioactive compounds such as essential oils in the seeds and peels, carotenoids, flavonoids and phenolic compounds in the peels. These bioactive compounds can be extracted and used to improve animal health as in human, but the process is expensive for farmers to bear. A better way could be to mash (grind) a whole lime fruit and the juice extracted which could contain these bioactive compounds. Therefore the objective of this research was to determine the effect of mashed lime fruit juice on growth and digestibility of broiler chickens.

Materials and Methods

Experimental Site

The experiment was conducted at the Animal Science Poultry Unit University of Uyo Annex Uyo, Akwa Ibom State, Nigeria, situated between Latitude 5-05° North and Longitude 80° East of the Equatorial rain Forest Belt, which is a Tropical Zone and a home to Vegetation of green foliage trees, shrubs and oil palm trees with annual rainfall of 800 mm.

Source of Lime Fruits and Production of Juice from Mashed Fruits

The lime juice fruits used were purchased from the market. They were cut into pieces and mashed (grinded) using a grinding machine. The mashed lime juice in paste form was put in a cheese cloth and pressed with the hands to extract the juice. The juice was collected into a plastic container and stored in a refrigerator at temperature of 4°C. This was to reduce oxidation of bioactive compounds in the juice.

Determination of Bioactive Substances

Bioactive substances determined were ascorbate (vitamin C), carotenoids, flavonoids and phenols. The ascorbic acid, citric acids, carotenoids, flavonoids and phenol content of the mashed and normal lime fruit juice were. The ascorbic and citric acids content were determined using titration method according to [20]. The Folin-Ciocalteu assay was used for the determination of total phenolic compounds present in the mashed lime juice [21]. Colorimetric aluminum chloride method was used for

flavonoids determination according to the methods described by [22] with some modifications as described by Ahmed et al. [23].

Experimental Design

The experiment was conducted on a Completely Randomized Design (CRD). One hundred and fifty (150) Anak strain of birds were used. There were five dietary treatments (T1, T2, T3, T4, and T5) each having 30 chicks. Each treatment was replicated three times, each having 10 birds. The birds were fed diets containing 0, 10, 15, 20 and 25 ml/kg mashed lime fruit juice only at the starter phase for four weeks.

Experimental Diet

A basal starter diet was formulated and the required quantity of mashed lime fruit juice was added according to the treatments. At the finisher phase the juice was withdrawn from the fifth week. A single finisher diet was formulated and fed to all the birds (Table 1).

Ingredients (%)	**Starter**	**Finisher**
Maize	51.00	50.00
Soybean meal	30.00	28.00
Palm kernel cake	10.00	15.30
Fish meal	4.00	2.00
Bone ash	4.00	4.00
Salt	0.25	0.25
Lysine	0.20	0.10
Methionine	0.20	0.10
Premix	0.35	0.25
Total	100	100.00
Nutrient composition (%)		
Crude protein	22.00	20.20
Ether extract	3.98	4.68
Crude fibre	4.63	5.06
Total ash	4.40	7.17
Lysine	1.25	1.01
Methionine	0.43	0.38
Calcium	1.25	1.34
Phosphorus	1.07	1.03
Energy (KcalME/kg)	2866	2824

Table 1: Ingredient and nutrient composition of basal diets.

Note: *__premix supplied per kg starter diet:__ Vitamin A 15,000 i.u., Vitamin D_3 13,000 i.u, Thiamine 2 mg, Riboflavin 6 mg, Pyridoxine 4 mg, Niacin 40 mg, Cobalamine 0.05 g, Biotin 0.08 mg, Choline chloride 0.05 g, Manganese 0.096 g, Zinc 0.06 g, Iron 0.024 g, Copper 0.006 g, Iodine 0.014 g, Selenium 0.24 mg, Cobalt 0.024 mg and Antioxidant 0.125 g. *__premix supplied per kg finisher diet:__ Vitamin A 10,000 i.u., Vitamin D_3 12,000 i.u. Vitamin E 20 i.u., 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.

Management of Experimental Birds

The management of the birds was according to the ethics that govern Animal welfare as approved by the research and ethics Committee of the University. The birds on arrival to the farm were given glucose solution after which they were allotted randomly to the various treatments. Heat was provided using kerosene stove for three weeks. All necessary vaccinations against

Newcastle and Gumboro diseases were carried out. The birds were raised in an open sided deep litter house. 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 washed metabolism cages and acclimatized for four days during which their respective feeds were given to them. Thereafter, they were fed a known quantity of their respective diets for another four days. During this time faecal collection was carried out daily. Plastic trays were placed under the cages where the faeces were collected. Faecal collection was made for four days and on each day sample collected was quickly taken to the laboratory and dried to a constant weight in an oven. Thereafter, the proximate analysis according to AOAC [24] was conducted and the digestibility calculated according to Maynard et al. [2].

Statistical Analysis

All data collected were subjected to one way analysis of variance (ANOVA). Significant means were separated using Duncan New Multiple Range Test according to Steel and Torrie [25].

Results

Bioactive Substances in Mashed Lime Fruit Juice

The result of the citric and ascorbic acid content of the mashed fruit juice indicated that it contained ascorbic acid (1.65%) and citric acid (2.90%) while the normal juice contained 1.25% and 2.03% respectively. The quantity of carotenoids, flavonoids and phenolic compounds were 87 mg/ml, 202 mg QE/100 ml and 788 mg garlic acid/100 ml respectively for the mashed lime juice. The levels in the normal juice were insignificant.

Growth Performance of Starter Chicks

The effect of mashed lime fruit juice on growth performance of starter broiler chicks is shown on (Table 2). The initial weight, daily gain, feed intake and daily protein intake were not significantly (P>0.05) influenced by the juice. However, it was observed that the juice had significant (P<0.05) effect on final live weight, feed; gain ratio and protein efficiency ratio.

Parameters	T_1(0)	T_2(10)	T_3(15)	T_4(20)	T_5(25)	SEM
Initial weight (g)	37.62	38.00	37.90	37.50	38.05	3.76
Final live weight (g)	1004^{b}	1015^{ab}	1020^{ab}	1056^{a}	1000^{b}	51.08
Daily weight gain (g)	34.51	34.89	35.08	36.38	34.36	3.41
Total feed intake (g)	1678	1624	1651	1670	1691	53.16
Daily feed intake (g)	59.93	58.00	58.96	59.64	60.39	3.66
Feed: gain ratio (g)	1.74^{a}	1.66^{b}	1.68^{b}	1.64^{b}	1.71^{ab}	0.05
Daily protein intake (g)	13.18	12.75	12.19	13.13	13.29	1.40
Protein efficiency ratio	2.61^{b}	2.73^{a}	2.87^{a}	2.77^{a}	2.58^{b}	0.22

Table 2: Effect of mashed lime fruit juice (ml/kg diet) on performance of starter broilers.

Note: *ab: Means along the same row with different superscripts are significantly (P<0.05) different.

Addition of 20 ml/kg diet of juice produced larger final live weight in comparing it with control and 25 ml/kg level which did not differ (P>0.05). Its value was similar to those of 10ml and 15ml/kg which in turn were the same with those of control and 25 ml/kg. There was improvement in feed: gain ratio by 10, 15 and 20 ml/kg juice, while there was no difference between

control and 25 ml/kg. Within the groups that consumed mashed lime fruit juice, the feed: gain ratio was the same. Protein utilization was significantly (P<0.05) higher in treated groups except 25 ml/kg that was similar to control.

Growth Performance of Finisher Broilers

The effect of mashed lime fruit juice on the growth performance of finisher broilers is shown on (Table 3). Diet containing mashed lime fruit juice did not significantly (p>0.05) influence initial weight (i.e. final live weight at starter phase), total feed intake, daily feed intake and daily protein intake. However, significant difference (p<0.05) was observed on final live weight, feed gain ratio and protein efficiency ratio. Nevertheless, the initial weight showed a marginal improvement at dietary inclusion level of 20 ml/kg.

Parameters	T_1(0)	T_2(10)	T_3(15)	T_4(20)	T_5(25)	SEM
Initial weight (g)	1005	1015	1020	1056	1000	30.24
Final weight (g)	1803^{b}	1842^{ab}	1911^{ab}	2039^{a}	1778^{b}	60.39
Daily weight gain (g)	38.02^{b}	39.38^{b}	42.43^{a}	46.81^{a}	37.05^{b}	2.16
Total feed intake (g)	2749	2748	2813	2917	2846	105.57
Daily feed intake (g)	130.89	129.17	133.62	138.90	135.53	4.97
Feed: gain ratio (g)	3.44^{ab}	3.28^{abc}	3.17^{abc}	3.00^{c}	3.66^{a}	0.45
Daily protein intake (g)	26.18	25.83	26.73	27.78	27.11	0.99
Protein efficiency ratio	1.46ab	1.53^{ab}	1.59^{ab}	1.69^{a}	1.37^{b}	0.08

Table 3: Effect of Mashed Lime Fruit Juice (ml/kg Diet) on Growth Performance of Finisher Broilers

Note: *ab: means along the same row with different superscripts are significantly (p<0.05) different.
*SEM: Standard error of means.

But at 25 ml/kg, the initial live weight was marginally lower than that of control and other inclusion levels which showed sign of detrimental effect at that higher level. The final live weight was higher in birds on 20 ml/kg compared to control and 25 ml/kg. Within the lime juice groups there was no difference between the final live weight of 20 ml/kg, 15 ml/kg and 10ml/kg. Daily weight gain was improved by 15 and 20 ml/kg compared to control, 10 ml/kg and 25 ml/kg which were similar. Addition of 20 ml/kg improved feed: gain ratio over the control and 25 ml/kg. However, there was no difference (p>0.05) in feed: gain ratio between control, 10 ml/kg, 15 ml/kg and 25 ml/kg groups. Protein efficiency ratio was significantly (p<0.05) different showing higher value at 20 ml/kg. All dietary levels were similar to control in terms of protein efficiency ratio.

Apparent Nutrient Digestibility

Effect of mashed lime juice is presented on (Table 4). There was no significant difference (P>0.05) in dry matter and fibre digestibility.

Parameters	T1 (0)	T2 (10)	T3 (15)	T4 (20)	T5 (25)	SEM
Dry matter (%)	72.02	72.67	74.08	74.15	75.00	11.50
Protein (%)	65.23^{c}	71.01^{bc}	74.21^{ab}	78.30^{a}	78.87^{a}	7.05
Ether extract (%)	74.03^{b}	76.10^{b}	88.00^{a}	88.23^{a}	88.10^{a}	10.05
Crude fibre (%)	44.01	45.00	44.87	45.08	45.15	4.51
Ash (%)	60.08^{c}	62.99^{bc}	65.42^{ab}	68.99^{a}	69.00^{a}	6.00

Table 4: Effect of mashed lime juice (ml/kg diet) on apparent nutrient digestibility of broilers.

Note: abc: Means along the same row with different superscripts are significantly different (P< 0.05).

The digestibility of protein, ether extract and ash were significantly (P<0.05) influenced. Addition of 15, 20 and 25 ml/kg mashed lime juice to the feed improved protein digestibility over the control. Inclusion of 10 ml/kg did not give better protein

digestibility compared to the control. In like manner, above 10 ml/kg mashed lime juice led to better digestibility of ether extract and minerals.

Discussion

Citric acid and ascorbic acid content of the mashed juice were higher than that reported by [19] who reported 1.60 and 1.20% respectively for citric and ascorbic acids of ordinary lime juice. The higher values could have resulted from the seeds and peels of the fruits which were mashed together. The presence of carotenoids, flavonoids and phenolic compounds in mashed lime fruit juice could have emanated from the peel. This acidic nature of the mashed fruit juice could have been responsible for the improved feed: gain ratio and protein utilization. Positive effect of citric and ascorbic acids on feed utilization by mono gastric animals has been reported [19].

Ndelekwute et al. [19] using 10, 15, 20 and 25 ml/kg pure lime juice discovered that 25 ml/kg performed better in terms of final live weight. In this current work 20 ml/kg was observed to be better than the control. Improvement of lower level compared to 25 ml/kg reported by [19] underscores the fact that other bioactive substances in the mashed lime fruit juice (carotenoids, flavonoids essential oils and phenolic compounds) could have played a major role. According to Nyak et al. [26] these substances have antibacterial, antioxidant and anti-carcinogenic properties which are important in health management. They further stressed that anti-oxidants reduce the negative effect of free radicals on internal organs such as the liver and kidney thereby improving the productivity of animals.

Improvement in the final live weight and the daily weight gain at the finisher phase could not have been as a result of feed intake. This is because feed intake was not significantly influenced. Olomu [27] and Oyeyemi and Roberts [28] reported that feed intake and body weight have positive relationship i.e. feed intake influenced growth performance positively. This shows that the weight of the birds was not as a result of the quantity of feed taken by the birds but could be ascribed to better utilization of the feed. Also the feeding of diet containing 20 ml/kg juice which resulted in better feed: gain ratio proved that mashed lime juice at 20 ml/kg encouraged feed utilization which resulted in improvement of final live weight. The protein efficiency ratio has higher value in birds that consumed diet that contained 20 ml/kg juice than those that were fed 25 ml/kg. Further observation indicated that protein utilization was the same ($p>0.05$) in control, 10 ml/kg, 15 ml/kg and 25 ml/kg, hence protein efficiency ratio took similar trend as the feed gain ratio. This goes a long way to indicate that the quantity of feed consumed was not a sole determinant of growth performance. Many authors have suggested that quantity and quality of feed though important may not lead to better feed utilization, but the interplay of the gut function [29,9].

Juice from lime has been reported to improve digestion in human [30,31]. This was attributed to the presence of organic acids (citric and ascorbic acids) in lime juice [31]. Other bioactive compounds such as carotenoids and flavonoids in citrus fruits could improve secretion of endogenous fluids such as bile [30]. Bile is important for digestion of fats and oils. The bitter taste of citrus peels which in this case was mashed could stimulate the wall of the intestine to secret mucous resulting to better nutrient digestibility. Report elsewhere indicated that phyto-nutrients could be used to improve nutrient digestibility [32]. Considering dry matter, crude protein and ether extract, this result agreed with the report of Ndelekwute et al. [33] on lime juice but at variance with the result of ash.

Conclusion

Citrus peels are known to contain carotenoids, flavonoids and phenols [23]. Going by the result of this work mashing of the lime fruits brought about increase in bioactive substances in the lime juice like the vitamin and citric acid content. Also the normal lime juice was enriched with carotenoids, flavonoids and phenolic compounds from the peel. Nutritionally, the juice from the mashed lime fruits improved digestibility and growth. Therefore 20 ml/kg diet could be added to diets for broiler chickens and is recommended.

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