Antibacterial action and dietary effect of lemon juice on nutrient digestibility and growth performance of broiler chickens

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Abstract

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A total of 150 one-day old unsexed Hubbard broiler chicks were used in a 49 days feeding trial to determine the bactericidal effect of lemon juice on feed bacterial load and its dietary effect on growth and apparent nutrient digestibility of broiler chickens. There were five dietary treatments (T1 – T5) containing lemon juice 0.0, 10, 15, 20 and 25mL/kg diet, respectively. Each treatment was replicated three times with 10 birds each, giving 30 birds per treatment. The experiment was arranged in completely randomized design (CRD). Starter diet was fed ad libitum for 3 weeks and finisher diet for 4 weeks. Water was given also ad libitum throughout the experimental period. Citric and ascorbic acids content of the lime and the pH were determined. At the end of the experiment, results showed that the juice contained ascorbic acid (2.76), citric acid (4.23) with low pH (3.87) and exhibited antibacterial action. Above 10mL/kg inclusion level, apparent nutrient digestibility of crude protein, ether extract, crude fibre, ash and energy utilization were improved. At the starter phase 20 and 25mL/kg improved final live weight and protein efficiency ratio. Final live weight was improved by 15, 20 and 25mL/kg at the finisher phase. In conclusion, addition of 15mL/kg lemon juice in broiler diets could be a good practice and is recommended.

Keywords: apparent nutrient digestibility, broiler chickens, growth, gut bacteria, lemon juice.

Introduction

Poultry nutrition has transcended from emphasis on quality of feed to adequacy of feed utilization. This is because feed could have high quality but may not be well utilized by birds due to some underlining factors such as the health condition of the gut which will process the feed (Ndelekwute *et al.*, 2016). Considering the fact that feed quality has been recognized, attention has been shifted to how to modulate the gut to improve its health status for better nutrient utilization efficiency. For decades, antibiotics have been efficaciously used for this purpose (Maynard *et al.*, 1981), but are recently regarded as unsafe for both the animals and consumers Islam *et al.*, 2012). Earlier, nutritional strategies using safe feed additives have been advocated for this purpose (Dibner, 2004). Later, reports indicated that use of natural ingredients as growth promoters such as organic acids,

spices, essential oils, carotenoids and flavonoids are key to achieving this task (Windisch *et al.*, 2008). To portray the importance of natural growth promoters, Ndelekwute *et al.* (2017) advocated the study of phyto-genecology in animal science. The authors defined phyto-genecology as the study of spices, plants parts, and plant extracts in relation to their application in farm animal nutrition and their use to mitigate nutrition-related health challenges. According to them it entails the study and use of extracted bioactive compounds in plants or plant parts in processed forms to solving nutrition and nutrition-related health challenges in monogastric animals. Before this, organic acids both in feed and drinking water have been viewed as effective feed additives to improve broiler productivity (Philipsen, 2009; Islam *et al.*, 2012; Ndelekwute *et al.*, 2014). Khan and Iqbal (2016) reported

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increase in the use of organic acids as substitutes for antibiotic growth promoters in broiler production. The dietary acidification of feeds and water for broilers is to inhibit intestinal bacteria that compete with the host for available nutrients, reduction of possible toxic metabolites which result in the improvement of nutrient digestibility, absorption and host immunity (Adil *et al.*, 2011).

On the other hand, 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 (Ndelekwute and Enyenihi, 2017). Since most organic acids currently in use are synthetic types which are expensive and most farmers do not have access to them, natural sources should be explored and exploited (Ndelekwute and Enyenihi, 2017). Fruits are major sources of organic acids especially fruits of citrus *spp* (lime, lemon, grape) which contain citric and ascorbic acids. Citrus fruits have been reported to have some health benefits due to the presence of bioactive compounds, such as ferulic acid, hydrocinnamic acid, cyanidin glucoside, hesperidine, carotenoid and naringin (Xu *et al.*, 2008). Citrus fruit extracts have antioxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (Abeyasinghe *et al.*, 2007).

However, information on their uses as dietary sources of organic acids and other bioactive compounds is scarce. Therefore, the objective of this study was to determine the citric and ascorbic acid content of lemon juice, its antibacterial and dietary effects on 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 75% and average ambient temperature was 30°C.

Processing of lemon juice

The lemon fruits used to process the juice were obtained from citrus farm of the Department of Crop Science, University of Uyo, Uyo, Nigeria. The lime fruits were washed and cut into two with a sharp knife. The juice was expelled manually by squeezing the cut halves with the hand. The juice was collected into a container. The juice containing the seeds and some pulp was filtered in order to have a clear juice. The juice was stored in the refrigerator at the temperature of 4°C to reduce oxidation and fermentation.

Determination of pH, citric and ascorbic acids content of lemon 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 lemon juice (Ndelekwute and Enyenihi (2017)). 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 (Novella, 2014).

Determination of antibacterial action of lemon juice in feed

This was determined by the methods described by Ndelekwute and Enyenihi (2017). A kilogram of the basal diet was measured into 5 plastic containers labeled A, B, C, D and E representing the treatments. Thereafter 0, 10, 15, 20 and 25 mL of the lemon juice was added to the feed samples respectively and thoroughly mixed. Five grams (5 g) was collected from each of the acidified sample feeds and the one without lemon 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 according to Aibuni *et al.*, 2007). At the end of the incubation period serial dilution method according to Fawole and Oso (1988) was used to determine the bacteria load of each sample.

Experimental design

Completely randomized design (CRD) was employed. The experiment was conducted with 150 one-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 30 chicks. Each treatment was replicated three times with 10 chicks each. Starter and finisher basal diets (Table 1) were formulated to form the control (T₁). To the basal diet 0, 10, 15, 20 and 25 mL of the lime juice /kg feed were respectively added to form T₁, T₂, T₃, T₄ and T₅. This represented 0.0, 1.00, 1.50, 2.00 and 2.50% of the diet, respectively.

Table 1: Ingredient and Nutrient Composition of Experimental Diets

Ingredients	Starter	Finisher
Maize	51.0	50.0
Soybean meal	30.0	28.0
Fish meal	4.00	2.00
Palm kernel cake	10.20	15.3
Bone ash	4;00	4.00
Salt	0.25	0.25
Lysine	0.20	0.10
Methionine	0.10	0.10
*Premix	0.25	0.25
Total	100	100
Nutrient composition (%)		
Crude protein	23.05	20.10
Crude fibre	4.38	4.96
Ether extracts	4.56	3.65
Lysine	1.20	1.00
Methionine	0.47	0.47
Calcium	1.25	1.10
Phosphorus	1.00	0.85
Energy (KcalME/kg)	2 880	2 901

***premix supplied per kg starter diet:** vitamin A 15,000 i.u., vitamin D₃ 13000 i.u, thiamine 2mg, riboflavin 6mg, pyridoxine 4mg, niacin 40mg, cobalamine 0.05g, biotin 0.08mg, choline chloride 0.05g, manganese 0.096g, Zinc 0.06g, iron 0.024g, copper 0.006g, iodine 0.014g, selenium 0.24mg, cobalt 0.024mg and antioxidant 0.125g ***premix supplied per kg finisher diet:** vitamin A 10, 000 i.u., vitamin D₃ 12,000 i.u. vitamin E 20 i.u., vitamin K2.5mg, thiamine 2.0mg, riboflavin 3.0mg, pyridoxine 4.0mg, niacin 20mg, cobalamin 0.05mg, pantothenic acid 5.0mg, Folic acid 0.5mg, Biotin 0.08mg, choline chloride 0.2mg, manganese 0.006g, Zinc 0.03g, copper 0.006g, iodine 0.0014g, selenium 0.24g, cobalt 0.25g and antioxidant 0.125g.

Management of experimental birds

The experiment was approved and conducted according to the rules and regulations of the University Research Ethics Committee regarding experimental animals' welfare. The birds on arrival to farm 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

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the apparent nutrient digestibility (Ojewale *et al.*; Dourado *et al.*, 2010; Ndelekwute and Enyenihi, 2017). 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 AOAC. (2000). The apparent nutrient digestibility was calculated according to Maynard *et al.* (1981).

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's New Multiple Range Test according to Steel and Torrie (1980). Total bacteria load was represented by a bar

chart. The statistical model used was;

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where;

Y_{ij} is the response variable recorded by the broiler chickens.

μ is the overall mean.

T is the treatment (lemon juice) effect on the broilers and,

ϵ_{ij} is the random or residual effect.

Results and discussion

The lemon juice contained 2.76% and 4.23% of ascorbic and citric acids respectively and a pH of 3.87. The ascorbic and citric acid content, and the pH value is an indication that the tested lemon was acidic in nature. This could be linked to its ability to reduce the bacterial load of the feed as shown in Fig. 1. Several reports are in consonant with this report in terms of the acidic nature of the lemon but differ in the amount of ascorbic and citric acids content. Penniston *et al.* (2009) reported 3.89% while Jamil *et al.* (2015) reported 3.30%. Tareen *et al.* (2015) reported that lemon contained 1.27% of ascorbic acid and Nour *et al.* (2010) reported higher value of citric acid. This variation could be due to differences in some factors such as soil type, stage of ripening and season of harvest of the fruits.

Fig. 1 Effect of lemon juice on bacterial load (x 10⁴ cfu/g) of experimental diets

The antibacterial activity of the lemon juice in the feed is shown in Fig. 1. The antibacterial effect was observed at supplementation beyond 10mL/kg diet. Antibacterial effect of the present lemon juice corroborated earlier reports which indicated lemon to be antibacterial due to its organic acids content. Pisoschi *et al.* (2011) and Bahboudi *et al.* (2016) stressed the need to sanitize the drinking water and feeds of broiler chickens with lemon juice because of its antibacterial activity. Feed used to

produce birds is regarded as a potential source of bacterial infection and need to be sanitized to reduce the bacteria load (Diner, 2014).

The effect of lemon juice on performance of starter broiler chicks is shown in Table 2. Addition of 20 and 25mL/kg diet significantly ($P<0.05$) improved final live weight, daily gain and protein efficiency ratio compared to the control. Feed intake, protein intake and feed: gain ratio were not significantly ($P>0.05$) influenced.

Table 2: Effect of lemon juice (mL/kg diet) on growth of starter broilers

Parameters	T1 (0)	T2 (10)	T3(15)	T4 (20)	T5 (25)	SEM
Initial live weight (g)	42.05	41.85	42.0	41.01	41.90	5.75
Final live weight (g)	700.0 ^c	720.05 ^{bc}	760.23 ^{abc}	780.11 ^{ab}	800.50 ^a	59.05
Daily gain (g)	23.50 ^c	24.22 ^{bc}	25.65 ^{abc}	26.40 ^{ab}	27.09 ^a	4.04
Total feed intake (g)	1350	1360	1378	1375	1425	145.05
Daily feed intake (g)	48.21	48.57	49.21	49.11	50.89	6.14
Feed: gain ratio	1.89	2.01	1.92	1.86	1.88	0.08
Daily protein intake (g)	11.11	11.20	11.34	11.32	11.73	3.01
Protein efficiency ratio	2.12 ^b	2.16 ^b	2.26 ^{ab}	2.33 ^a	2.31 ^a	0.13

abc means along the same row with different superscripts are significantly ($P<0.05$) different.

Within the lemon juice groups, the final live weights produced by 15, (760.23g), 20 (780.11) and 25mL/kg (800.50g) were similar ($P>0.05$) but higher ($P>0.05$) than that produced by 10mL/kg (720.05g). The same parameter was similar ($P>0.05$) in control, 10 and 15mL/kg. The protein efficiency ratio followed similar trend as the final live weight. The improved growth performance of the starter chicks fed lemon juice above 15mL/kg revealed the ability of lemon juice to support broiler productivity. This fit has been linked to its antibacterial action both in the feed and in the gut, and other endogenous activities of lemon juice due to presence of bioactive substances such as organic acids (ascorbic and citric acids) and flavonoids (Bahboudi *et al.*, 2016). Young chicks require acidic medium to be able to effectively digest protein, but

the level produced by young chicks is inadequate to digest protein properly (Dibner, 2004). Also Dibner (2004) advocated for dietary application of organic acids to reduce the pH of the *proventriculus* for adequate protein digestion. This was shown in better performance of inclusion level of 20 and 25ml/kg at the starter phase where protein was better utilized resulting to better final live weight, even when there was no difference in feed intake. Studies have shown that administration of ascorbic acid alleviated the deleterious effects of heat stress on performance and metabolism of broiler chickens (Sahin *et al.*, 2002; Gursu *et al.*, 2004). McDowell (1989) reported that ascorbic acid is the first line of defense against reactive oxygen species, and this could improve the immunity and health status of the birds and thus higher productivity.

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Table 3: Effect of Lemon Juice (mL/kg diet) on Growth of Finisher Broilers

Parameters	T1 (0)	T2 (10)	T3(15)	T4 (20)	T5 (25)	SEM
Initial live weight (g)	700.0 ^c	720.05 ^{bc}	760.23 ^{ab}	780.11 ^{ab}	800.50 ^a	59.05
Final live weight (g)	2200 ^b	2240 ^b	2350 ^a	2360 ^a	2405 ^a	160.89
Daily gain (g)	71.43	72.38	75.70	75.23	76.40	8.45
Total feed intake (g)	3052 ^d	3073 ^{cd}	3162 ^{bcd}	3229 ^{bc}	3380 ^a	115.23
Daily feed intake (g)	145.33	146.33	150.57	153.76	160.95	25.56
Feed: gain ratio	2.03	2.02	1.99	2.04	2.11	0.18
Daily protein intake (g)	29.21	29.41	30.26	30.91	32.35	3.98
Protein efficiency ratio	2.44	2.46	2.50	2.43	2.36	0.17

abc means along the same row with different superscripts are significantly ($P<0.05$) different.

During the finisher phase, different trends were observed (Table 3). Final live weights of birds on 15 (2350g), 20 (2360) and 25mL/kg (2405g) were better ($P<0.05$) compared to the control (2200g) and 10mL/kg (2240g). There were no differences ($P>0.05$) between the final live weights of chickens on 15, 20 and 25mL/kg.

Total feed intake was higher in chickens on 20 and 25mL/kg compared to control, while it was the same in control, 10 and 15mL/kg. Within the lemon juice 25mL/kg group consumed more feed in terms of total feed intake compared to others. Significant ($P>0.05$) differences were not observed in daily gain, daily feed intake, feed: gain ratio, protein intake and protein efficiency ratio at this phase. During the finisher phase, more feed consumed by chickens on 20 and 25mL/kg lemon juice was an advantage as it resulted to higher final live weight compared to control. The protein efficiency ratio which was not significantly different shows that the reason of addition of lemon juice could better realized at the starter phase. Ndelekwute *et al.* (2016) added 0.25% of different organic acids to broiler feeds at the finisher phase and reported that the acids did not improve growth at that phase of production and suggested increasing the level of the acids or to apply the organic acids during the starter phase of production. The present

report which showed that below 15% the tested lemon juice did not improve growth confirmed earlier report by Islam *et al.* (2012) that 2ml/kg lemon juice did not improve growth of broilers. Also, Ndelekwute and Enyenihi (2017) observed that at 15% and bellow, lime juice did not improve growth of both starter and finisher broiler chickens. This present report is in line with the report of Behboudi *et al.* (2016).

Table 4 indicates the effect of lemon juice on apparent nutrients digestibility of the broilers. There was significant ($P<0.05$) improvement in some of the parameters measured.

Above 10ml/kg protein, crude fibre, and ash were better digested and energy utilization was improved. Dry matter and ether extract were not influenced significantly ($P>0.05$) by dietary inclusion of lemon juice. Improved nutrient digestibility of broilers fed lemon juice above 10ml/kg manifested in the live weight especially observed in birds fed 20 and 25ml/kg. This shows that the lemon juice not only aided in digestion but probably facilitated and improved nutrient absorption and utilization. Steen (2015) suggested that daily intake of lemon juice is necessary for better digestion and normal function of the digestive system. Lemon juice could achieve this because of presence of bioactive compounds like organic acids and pectin which could modulate the gut.

Table 4: Effect of Lemon Juice (mL/kg diet) on Apparent Nutrient Utilization of Broilers

Parameters	T1 (0)	T2 (10)	T3(15)	T4 (20)	T5 (25)	SEM
Dry matter (g)	78	76	78	80	81	6.07
Crude protein (%)	65.02 ^b	68.00 ^{ab}	70.32 ^a	71.11 ^a	72.12 ^a	4.01
Ether extract (%)	80.05	82.21	85.96	86.01	86.76	4.98
Crude fibre (%)	40.07 ^b	41.98 ^b	45.88 ^a	46.04 ^a	46.88 ^a	3.09
Ash (%)	58.01 ^b	59.03 ^b	66.12 ^a	65.99 ^a	66.01 ^a	5.32
Energy utilization (%)	68.01 ^b	67.89 ^b	75.09 ^a	78.44 ^a	74.76 ^a	5.66

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

According to Steen (2015) and Thompson (2017) lemons are high in a fibre called pectin, which is a prebiotic that assists good gut health and removal of harmful chemical from the body. This report agreed with the report of Islam *et al.* (2012) that lemon juice aided protein and ether extract digestibility. Also similar report has been reported on lime juice according to Ndelekwute *et al.* (2017).

Conclusion and recommendation

The lemon juice used in this study was acidic with presence of ascorbic and citric acids. It showed antibacterial property on feed bacterial load which is a good property which could be used to improve the quality of the feed and modulate the gut. At 20 and 25mL/kg in the diet it improved growth. The apparent nutrient digestibility was improved above 10mL/kg of inclusion. Therefore, it is recommended to include 20mL/kg diet of lemon juice in diets for broiler chickens for optimum productivity.

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