Response of broiler chickens to frequency of dietary inclusion of neem leaf meal

Egbeyale*, L.T., Adeleye, O. O., Ayo-Ajasa, O. Y., Shekoni, T. E., Ayoola, A. A., Sulaiman, O. M. and Shobukola, K.
Department of Animal Production and Health, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria.
*Corresponding author: egbeyalelt@funaab.edu.ng
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Abstract
A 5-week feeding trial was conducted to determine the growth performance, nutrient digestibility and carcass characteristics of 180 2-week old broiler chicks of Cobb strain fed diets containing neem (*Azardirachta indica*) leaf meal at different periods. The birds were allotted to 5 dietary treatments with each treatment consisting of 36 birds and were replicated 4 times with 9 birds per replicate. Feed and water were administered *ad libitum*. Birds in treatment 1 were fed control diet all through the period of experiment, birds in treatment 2 were fed diet containing 0.5 % neem leaf meal, birds in treatment 3 were fed diet containing 0.5% neem leaf meal for 3 d in a wk, birds in treatment 4 were fed diet containing 1% neem leaf meal, birds in treatment 5 were fed diet containing 1% neem leaf meal for 3 d in a wk. The best feed conversion ratio was in the birds in treatment 3 which was similar to the response of birds in treatment 5 at starter phase while only feed intake was significantly ($P<0.05$) influenced at finisher phase. Results on nutrient digestibility indicated that birds on 0.5% neem leaf meal for 3 d in a wk had better ($P<0.05$) nutrient utilization than their counterparts in other groups. Carcass characteristics was not significantly ($P>0.05$) influenced by the treatments. It was concluded that 0.5% neem leaf meal inclusion in the diet of broiler chickens for 3 d in a wk improves performance of broiler chickens.

Keywords: neem leaf meal, broiler chicken, performance, nutrient digestibility

Introduction

The use of antimicrobial agents as growth promoters is being discouraged due to human and animal health issues, mainly resulting from development of antimicrobial resistance (Castanon, 2007; Bonsu, 2012). The incidence of residual effect antibiotics in livestock products and the development of resistant strains of micro-organisms due to drug therapy have resulted in the search for natural substances that can effectively replace the functions of the drugs used in animal production (Iwuji and Herbert, 201 2). Due to the advocacy against the use of mo st anti-microbial feed additives in animal feed because of their residual effects on animals, plant extracts are becoming more popular (Singh *et al*., 2015). Among these plants is neem (*Azadirachta indica*) which has compounds of various constituents that play a role in disease management. Although more than 300 natural products have been isolated from different sections of this tree, with new compounds added to the list every year (Sharma *et al.*, 2015), studies at molecular level demonstrated that *A. indica* contained chemical constituents of alkaloids, terpenoids, tannins and flavonoids (Makkar *et al*., 2007) responsible to overcome microbial infection especially having antioxidant and antimicrobial biological activities (Manach *et al*., 2004). These chemicals might show the antibacterial activity having the ability to make a complex with the bacterial cell walls. Moreover, the

Gram-positive bacterial strains were found more sensitive than the Gram-negative ones (Sinaga *et al*., 2016).

The usual method of assessing feed quality is to conduct digestibility studies and growth performance trials with birds. Though the potential value of a feed for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the food to the animal can be arrived at only after making allowance for the inevitable losses that occur during digestion, absorption and metabolism (McDonald *et al.*, 1998b). Nworgu (2016) reported that carcass quality and organ weight of broilers declined with decreased feed quality.

Recent works by Ayoola *et al.* (2015) and Egbeyale *et al.* (2015) have shown that partial inclusion of neem (*Azadirachta indica)* leaf meal in the diets of broiler chickens without any administration of antibiotics produced encouraging results in terms of weight gain, feed efficiency ratio and survivability of the birds. Though there had been many studies on the inclusion of NLM in the diet of broiler chickens but there is scarce information on response of broiler chickens to frequency of inclusion of neem leaf meal in broiler diets. The frequency of administration is crucial and should be determined to avoid abuse of neem leaf meal usage knowing fully that antibiotics are not administered on daily basis but at interval. Therefore, the objectives of the study were to determine the best frequency of two dietary inclusion levels of neem leaf meal on the growth performance, carcass characteristics and nutrient digestibility of broiler chickens.

Materials and Methods

Experimental site

The research work was carried out at the poultry unit, Teaching and Research Farms of the Federal University of Agriculture, Abeokuta (FUNAAB) Ogun State, Nigeria. The farm is located in the rain forest vegetation zone of south western Nigeria altitude of 127m, latitude 7^0 13^0 N and longitude 3^0 to 26' E (Google Earth, 2017). The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature is 34.7^o C and relative humidity of 82%.

Preparation of the test ingredient

Mature neem leaves (*Azadirachta indica*) were collected from fully grown (more than 15 yrs) neem trees at the University farm, air-dried at ambient temperature and ground using 1 mm sieve into fine powder using an electric blender. It was stored in a well labelled air-tight container at room temperature as neem leaf meal (NLM) for later use.

Experimental diet

Three experimental diets were made by incorporating NLM at 3 levels of 0, 0.5 and 1.0 g/kg. The gross composition of the experimental diets at starter and finisher phases are presented in Table 1.

Table 1: Composition (%) of starter and finisher diets

Ingredients	Starter	Finisher
Maize	47.00	53.50
Soya bean meal	18.50	16.50
Fish meal	2.00	0.40
Groundnut cake	17.50	13.80
Wheat offal	10.00	10.80
Bone meal	3.00	3.00
Oyster shell	1.00	1.00
Vitamin/Min Premix	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Salt	0.25	0.25
Total	100.00	100.00
Calculated analysis		
Crude protein	23.00	20.01
Crude fibre	3.61	3.51
Ether extract	4.04	3.88
Metabolisable energy(KJ/kg)	11.47	11.62

Experimental birds and design

One hundred and eighty Cobb day old broiler chicks were purchased from Zartech breed farm, Ibadan, Oyo State. The birds were brooded together for 2 wk after which they were distributed into 5 treatment groups of 36 birds per group. Each group was subdivided into 4 replicates of 9 birds per replicate. The treatment groups were arranged in a completely randomized design. The groups were defined as below:

T_1: Control diet (commercial feed)
T_2: Diet containing 0.5% NLM
T_3: Diet containing 0.5% NLM for 3 d and 4 d on control diet/week.
T_4: Diet containing 1.0% NLM
T_5: Diet containing 1.0% NLM for 3 d and 4 d on control diet/week.

Growth performance

The initial weights of the birds were taken at the beginning of the study and subsequent live weight measurements were carried out on a weekly basis with the use of a weighing scale. Feed intake was determined on weekly basis as the difference between the feed supplied and feed leftover. Feed conversion ratio was calculated as the ratio of feed to body weight gain.

Nutrient digestibility

Nutrient digestibility was carried out on the second and fifth week of the study. Two birds per replicate were transferred and individually housed in a steel metabolic cage; 4 d of acclimatization of birds to the cage was allowed and 3 d for collection of excreta after the acclimatization period, a known quantity of feed was offered to each replicate of the treatments represented while the leftover was weighed to determine the feed intake during the metabolic trial. The daily excreta output was dried in an oven at a temperature of 80^{o}C for 3 d and then weighed and milled (1 mm serve). Samples of the excreta and diets were taken for chemical and

proximate analyses using AOAC (2005) methods.

$$\% \text{ Nutrient digestibility} = \frac{\text{Nutrient in feed intake(g)} - \text{Nutrient in excreta output (g)}}{\text{Nutrient in feed intake(g)}} \text{ X } 100$$

where

Nutrient in feed intake
= % nutrient in feed
× quantity of feed intake (g)
Nutrient in excreta output
= % nutrient in excreta
× quantity of faecal sample (g)

Carcass characteristics

At the 35th day of the experiment, 4 birds of mean weight of the treatment were picked and subjected to carcass evaluation. The feed was withheld from the birds for 12 h before slaughter to ensure emptying of the digestive tract. The birds were slaughtered, defeathered after immersing them in boiled water and eviscerated. After evisceration, the weight was taken and recorded. The cut-up parts (head, neck, breast, back, thigh, drumstick, shank), organs (gizzard, proventiculus, heart, liver, kidney, and lung) and abdominal fat were cut and weighed and expressed in percentage of live weight.

Statistical analysis

Data collected were subjected to one way analysis of variance using SAS 9.1 version (2010) while the Duncan's Multiple Range Test was used to separate means that were significant at $P<0.05$.

Results and Discussion

The growth response of broiler chickens to the frequency of administration of NLM in the diet is presented in Table 2. At the starter phase, the results showed that daily feed intake and feed conversion ratio were the indices influenced ($P<0.05$) by the treatments. The feed intake was highest in birds fed diet containing 1.0% NLM throughout the experimental period while the other groups had similar values. The feed conversion ratio was poorest in the birds fed diet containing 1.0% NLM throughout the period while their counterparts on 0.5% NLM for 3 d in a wk had the best result which was also similar to the response of birds fed 1.0% NLM for 3 d in a wk. The only parameter among all the growth indices that was significantly ($P<0.05$) influenced by the treatment was daily feed intake at the finisher phase.

Table 2: Effect of frequency of NLM inclusion in the diet of broiler chickens at starter phase on growth performance

Parameter /Treatments	T1	T2	T3	T4	T5
Starter phase					
Initial weight (g)	452.78±3.59	450.69±3.65	450.00±3.93	451.39±1.39	450.00±2.27
Final weight (g)	831.60±23.35	838.88±29.22	872.23±11.57	833.35±23.57	850.05±18.97
Weight gain (g)	378.82±25.45	388.18±31.99	422.23±14.88	381.96±24.47	400.05±18.56
Daily weight gain (g)	27.06±1.82	27.73±2.29	30.16±1.06	27.28±1.75	28.58±1.33
Daily Feed intake (g)	63.79±3.07^{b}	67.86±0.63^{b}	63.39±1.43^{b}	73.19±0.43^{a}	65.08±0.81^{b}
Feed conversion ratio	2.37±0.10^{ab}	2.49±0.18^{ab}	2.11±0.05^{b}	2.71±0.17^{a}	2.29±0.10^{b}
Finisher phase					
Final weight (g)	1882.99±52.68	1814.76±25.32	1900.52±76.08	1968.06±82.07	1975.18±36.80
Weight gain (g)	1051.39±66.09	975.88±35.70	1028.30±66.72	1134.71±74.79	1125.13±27.84
Daily weight gain (g)	50.07±3.15	46.47±1.70	48.97±3.18	54.03±3.56	53.58±1.33
Daily feed intake (g)	180.60±3.90^{c}	189.39±4.51^{bc}	209.77±5.27^{a}	191.06±2.82^{bc}	203.81±6.42^{ab}
Feed conversion ratio	3.66±0.30	4.09±0.17	4.36±0.37	3.59±0.30	3.81±0.11

^{ab}Means with different superscripts along the same row were different significantly (P<0.05)

The nutrient digestibility of broiler chickens fed with diet containing varying levels of neem leaf meal at the starter phase is presented in Table 3. The result showed significant (P<0.05) difference in all parameters except crude fibre. Birds fed 0.5% NLM diet for 3 d in a wk had the highest (P<0.05) value of 76.09% for dry matter digestibility, while the least value (69.89%) was recorded by birds fed 1.0% NLM diet for 3 d in a wk. Crude protein digestibility followed the trend of dry matter digestibility and the values ranged from 78.33 to 83.34%. The highest ash and carbohydrate digestibility values were recorded by birds fed 0.5%NLM diet for 3 d in a wk while their counterparts fed 1.0% NLM diet throughout the experimental period had the highest fat digestibility.

Table 3: Apparent nutrient digestibility coefficient of broiler chickens fed with neem leaf meal diet at starter phase

Parameter (%)/ Treatments	T1	T2	T3	T4	T5	SEM
Starter phase						
Dry matter	71.29^{ab}	70.39^{b}	76.09^{a}	70.94^{ab}	69.89^{b}	0.86
Crude protein	80.79^{ab}	80.39^{ab}	83.34^{a}	81.95^{ab}	78.33^{b}	0.67
Crude fibre	68.98	67.23	70.66	66.60	67.85	0.82
Ash	57.69^{a}	46.34^{b}	69.87^{a}	47.66^{b}	45.33^{b}	1.62
Fat	76.31^{c}	82.31^{a}	81.76^{ab}	85.23^{a}	78.58^{bc}	0.85
Carbohydrate	76.13^{ab}	71.51^{b}	79.11^{a}	78.81^{a}	72.64^{ab}	1.12
Finisher phase						
Dry matter	75.37^{ab}	77.63^{ab}	78.76^{a}	7 4 . 4 8 ^{b}	76.95^{ab}	0.57
Crude protein	81.38^{b}	84.49^{a}	85.54^{a}	86.19^{a}	84.04^{ab}	0.53
Crude fibre	70.91^{c}	76.08^{a}	76.60^{a}	73.01^{bc}	74.74^{b}	0.61
Ash	58.67^{ab}	61.50^{b}	62.67^{a}	58.42^{bc}	56.32^{c}	0.68
Fat	77.81^{d}	80.12^{c}	90.50^{a}	64.27^{b}	81.46^{c}	1.04
Carbohydrate	80.65^{ab}	79.89^{ab}	82.72^{a}	79.67^{ab}	77.49^{b}	0.59

$^{abc;}$Means in same row with varying superscripts differ significantly (P<0.05)
T1 = Control diets
T2=Diet containing 0.5% NLM
T3=Diet containing 0.5% NLM for 3 d in a wk
T4=Diet containing 1.0% NLM
T5=Diet containing 1.0% NLM for 3 d in a wk

The nutrient digestibility at the finisher phase was significantly (P<0.05) different in all values of the measured parameters (Table 3). The results revealed that birds on diets containing 0.5% NLM for 3 d in a wk had better (P<0.05) nutrient digestibility among all the treatment groups. The value of ash digestibility was lower when compared with the values of other nutrient digestibility. The birds on the control diet had the least values for crude protein, crude fibre and fat digestibility while their counterparts fed diet containing 1.0%NLM for 3 d in a wk recorded the least value of ash and carbohydrate.

The result on the effect of different levels of inclusion of neem leaf meal on dressing percentage and cut up parts of broiler chickens at week 7 is presented in Table 4. Among the parameters measured, head, neck, breast and intestine were influenced (P<0.05) by the treatments. The birds fed diet containing 1.0% NLM for 3 d in a wk had highest value (2.81%) of head percentage which was similar to the values obtained for the birds fed diet containing 1.0% NLM throughout the period. Birds on control diet had the least value of 2.30%, while those on 0.5% NLM throughout and 3 d in a wk had similar values of 2.56 and 2.47%, respectively. The value of neck ranged from 3.87 to 4.85%. The highest (P<0.5) value was recorded in birds on 0.5% NLM for 3 d in a wk while birds on 1.0% NLM throughout the period had the lowest value. The values for breast weight percentage was highest (P<0.05) in birds fed diet containing 1.0% NLM throughout the period which was also similar with birds on 0.5% NLM for 3 d in a wk (19.41%) while their counterparts on 0.5% NLM throughout the period recorded least value of 15.36%. The birds on control

diet and 0.5% NLM fed 3 d in a wk recorded values of 17.78 and 18.45%, respectively. The intestine weight percentage ranged from 5.40 to 7.34% with the highest value obtained in birds on diet containing 0.5% NLM throughout the period was similar to the values obtained for the birds on control diet. Birds on 0.5% NLM for 3 d in a wk had the lowest value birds on 1.0% NLM throughout and 3 d in a wk had values of 6.03% and 5.77%, respectively.

Table 4: Effect of frequency of administration of varying inclusion levels of neem leaf meal on dressing percentage and cut up parts of broiler chickens

	Inclusion level (%) of NLM				
Parameters	T1	T2	T3	T4	T5
Live weight (g)	1969.25±59.12	1744.50±69.34	1981.25±164.40	1853.25±57.86	1882.00±66.57
Eviscerated weight (%)	83.34±1.52	82.69±1.46	84.90±0.44	83.86±1.27	84.79±2.20
Dressed weight (%)	65.83±1.00	64.99±2.43	66.37±0.80	66.87±1.32	66.72±1.30
Heart	0.47±0.06	0.44±0.08	0.47±0.11	0.42±0.05	0.51±0.05
Liver	1.68±0.33	2.14±0.29	1.69±0.09	2.01±0.06	2.01±0.06
Lungs	0.59±0.04	0.57±0.04	0.59±0.09	0.54±0.04	0.54±0.03
Gizzard	2.10±0.19	2.28±0.11	2.40±0.30	2.37±0.28	2.22±0.09
Heart	0.47±0.06	0.44±0.08	0.47±0.11	0.42±0.05	0.51±0.05
Fat	2.01±0.34	1.10±0.17	1.14±0.23	1.10±0.36	1.47±0.61

[abc] Means in same row with varying superscripts differ significantly (P<0.05)
T1 = Control diets
T2=Diet containing 0.5% NLM
T3=Diet containing 0.5% NLM for 3 d in a wk
T4=Diet containing 1.0% NLM
T5=Diet containing 1.0% NLM for 3 d in a wk

The *Azadirachta indica* leaf meal fed to broilers in this study did not have any significant difference on the live weight which opposes the findings of Bonsu *et al.* (2012) who reported significant influence of neem leaf meal on weight gain and final weight of broiler chickens fed diet containing neem leaf meal at different levels (0, 1.5, 2.0 and 2.5%). The difference in the observations might be attributed to levels of inclusion of neem leaf meal which was lower in this study. The leaf meal was used as an additive and not administered throughout the experiment across the treatments in this study. Similarly, Kale *et al*. (2003), Bishnu *et al*. (2009) and Sarker *et al.* (2014) also reported that birds supplemented with neem leaf extract had higher body weight and weekly gain in weight. The variation could be attributed to the difference in the form of the test ingredient (extract vs leaf meal) and the means of administration.

The significant increase in the digestibility of crude protein and ether extract in birds fed 0.5% NLM inclusion level for 3 d in a wk showed that the crude protein and ether extracts in the neem leaf meal was properly absorbed and utilized. This also agrees with the finding of Kamel (2001) that the use of herbs, spices and various plant extracts have appetite and digestion stimulating properties and

antimicrobial effects. The results on crude protein digestibility, crude fibre and ether extract (fat) agree with the findings of Edeh (2013) who recorded that birds fed neem leaf extract had higher value for crude protein, crude fibre and ether extract digestibility.

At the finisher phase, the present result agrees with the report of McDonald *et al.* (1998) that digestibility of feed was influenced not only by its composition but also by the composition of other feed ingredients. The nutrient digestibility values obtained at both starter and finisher phases in this experiment were higher than the findings of Hejdysz *et al*. (2017) who included pea (*Pisum sativum*) in the diet of broiler chickens. The non-significant variation in the organ values was also similar to the findings of Bonsu *et al*. (2012) who reported that organ weights and dressing percentage were not significantly influenced by inclusion of NLM in the diet of broiler chickens at finisher phase. However, the authors observed that higher inclusion of NLM reduced fat deposition which negates the result in the present study. The variation might be attributed to the expression of the values. Bonsu *et al.* (2012) reported raw value while the results in this study were expressed as percentage of live weight of the birds. It is also note worthy that the values decreased numerically in birds fed diets containing NLM when compared with their counterparts in the control diet. Therefore, NLM has the potential to reduce fat deposition in broiler chickens. The similar values recorded for liver and lungs were an indication that air-drying reduced anti-nutritional elements in neem leaves and confirmed their safety. This observation was also reported by Nodu *et al.* (2016) who reported that neem leaf extract prevented the enlargement of liver and lungs in broiler chickens and Omar *et al*. (2016) who worked on natural herb extracts as supplements for broiler chickens.

Conclusion

Based on this experiment it can be recommended that diet containing 0.5% NLM level of inclusion for 3 d in a wk in the production of broiler chickens could be adopted as an alternative to the use of antibiotics since it produces similar results with the control group.

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