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Daniellia oliveri Leaf Extracts as an Alternative to Antibiotic Feed Additives in Broiler Chicken Diets: Meat Quality and Fatty Acid Composition

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ABSTRACT

The objective of the study was to examine the effect of feeding different levels of *Daniellia oliveri* leaf extract (DOFE) on the meat quality and fatty acid composition of broiler chicks. A total of 375 one –day old broiler chicks were randomly assigned into five dietary treatments of seventy-five birds per group; each group was further divided into 5 replicates consisting of 15 chicks each. The dietary treatments include a control diet-fed 1.25 g/litre Oxytetracycline (T1), T2, T3, T4 and T5 were fed DOFE at 20 ml, 40ml, 60ml and 80 ml/liter respectively. The basal diet was formulated to meet the nutritional requirements of birds according to NRC (1994), feed and water were given *ad libitum* and the experiment lasted for 56 days. Result obtained showed that *Daniellia oliveri* leaf contained Dry matter (89.11%), crude protein (18.95%), crude fiber (13.11%), ether extract (4.78%), ash (6.10%), neutral detergent fibre (28.10%), and acid detergent fiber (47.50%). Significant differences ($P<0.05$) were observed in the proximate composition of the breast meat. Total saturated fatty acid (TSFA), total unsaturated fatty acid (TUFA), and omega-6/omega -3 ratio (n-6: n-3) values were significantly influenced ($P<0.05$) by DOFE. Birds in T5 had the highest TUFA value of 77.87% followed by T4 (72.45%), T3 (66.43%), T2 (61.94%), and T1 (41.47%) respectively. While T1 (44.71%) had the highest value of TSFA ($P<0.05$) relative to other treatments. The atherogenic index was significantly ($P<0.05$) different among the treatments, the value increases as the level of DOFE increased. It was concluded that feeding DOFE to birds at 80 ml/liter highly influenced the composition of fatty acid and meat quality of animals without any negative effect on their general performance.

INTRODUCTION

The huge increase in poultry production to meet growing demand in the world has led to the rise in antibiotic use, leading to a worrying increase in cases of antibiotic resistance diagnosed in animals and humans via direct contact, environmental contamination, and feed consumption causing high cases in various ailments including cancer (Alagbe, 2020). Poultry meat is an excellent source of high protein, essential vitamins, minerals, and fatty acids especially polyunsaturated fatty acids (Muhammad *et al*., 2015; Akintayo and Alagbe, 2020; Oluwafemi *et al*., 2020). Consumption of antibiotics contaminated animal products is injurious to health while multiplications of antibiotic resistance pose a great threat and danger to the livestock industry, particularly poultry production and venture in Nigeria (Oluwafemi *et al*., 2020; Samarasinghe and Wenk, 2002; Ahsan et al., 2018). It thus becomes imperative to find cheap, readily available alternatives to antibiotics feed additives in poultry diets. Among the potential alternative is the use of medicinal plants.

Daniellia oliveri (Rolfe) is an evergreen uncultivated copiously available tree, particularly in the savannah zone of Nigeria (Olafadehan and Okunade, 2018; Olafadehan *et al*., 2020). The plant belongs to the family Caesalpiniaceae and the tree can grow up to 48 m with leaves deciduous to torch (Uzama and Bwai, 2012). Scientific studies have revealed that the roots, stems, and leaves demonstrated a considerable antimicrobial (Jimoh and Oladiji, 2005), anti-inflammatory (Oyegade *et al*., 1999; Muanda *et al*., 2011), cytotoxic (Topcu *et al*., 1993; Alagbe *et al*., 2018), antihyperglycaemic (Iwueke and Nwodo, 2008), antioxidant (Ahmadu *et al*., 2004), anti-diarrheal (Ahmadu *et al*., 2007), immunostimulatory (Lamy *et al*., 2010), hepatoprotective (Olatunji, 2000; Alagbe *et al*., 2017) and miracicidal activities (Arnao *et al*., 1998; Musa *et al*., 2020). The plant parts contain various phytochemicals (tannins, flavonoids, saponins, phenols, alakloids, terpenoids, glycosides), minerals, amino acids, and vitamins and they can be traditionally used for the treatment of malaria, typhoid, skin diseases, gastro intestinal infections, urinary infections and toothache (Alagbe *et al*., 2020; Onwukaeme *et al*., 1999; Musa *et al*., 2000).

In view of these abundant potentials, the use of *Daniellia oliveri* leaf extract will promote food safety. Therefore this study was carried out to examine the effects of *Daniellia oliveri* leaf extracts as an alternative to antibiotic feed additives in broiler chicken diets: meat quality and fatty acid composition.

METHODS

The experiment was carried out at the University of Abuja Teaching and Research Farm, Gwagwalada, Nigeria. Mature leaves of *Daniellia oliveri* leaves were collected from different plants within the University premises; it was authenticated at the herbarium of the Department of Crop Science, University of Abuja, Nigeria, with a voucher number CS – 012 D. The leaves were washed and allowed to dry under shade until a constant weight was obtained and made into a powder using a Panasonic electric blender Model (AA-7301A) and stored in a well-labeled air-tight container. 200 g of the sample was soaked in 1000 ml of ethyl alcohol (80 % BDH), the mixture was agitated using an electric blender, poured in a container, and then kept in the refrigerator at 4^{o}C for 48 hours, sieved with What Man No 1 filter paper to obtain *Daniellia oliveri* leaf extracts (DOFE).

Animals and Their Management

Three hundred and seventy-five one-day-old (Ross 308) broiler chicks of mixed sex were used for the experiment. The birds were purchased from a commercial hatchery in Ibadan, Oyo State, Nigeria, and weighed on arrival on the farm to obtain their initial body weight and thereafter weekly. A deep litter housing system was used, it was fumigated two weeks before the commencement of the study, and the surrounding environment was also cleaned daily to ensure proper hygiene. Birds were divided into five treatments with five replicates of fifteen (15) birds in a completely randomized design. Electric brooders were used and wood shavings serve as the litter material. Vaccines were administered according to the prevailing disease condition in the environment and all other management practices were strictly adhered to throughout the experiment which lasted for 56 days.

Ration Formulation

Three (3) basal diets were formulated at different stages of production to meet up the requirements of birds according to NRC (1994). Broiler starter's mash (0-21 days), growers mash (22-35 days), and finishers mash (36-56 days).

Treatment 1: Basal diet + Oxytetracycline 2.5 g/litre
Treatment 2: Basal diet + 20 ml/liter DOFE /liter of water
Treatment 3: Basal diet + 40 ml/liter DOFE /liter of water
Treatment 4: Basal diet + 60 ml/liter DOFE /liter of water
Treatment 5: Basal diet + 80 ml/liter DOFE /liter of water

Measurements

Proximate compositions of experiment diet and meat (breast and thigh) were determined by using the official method of analysis by AOAC (2000).

Weight gain (g) = final weight (FW) – initial weight (IW)

Feed intake (g) = Amount of feed consumed – remaining feed

Fatty Acid Analysis

At the end of the experiment, five birds were randomly selected from each treatment for fatty acid analysis (FA). Meat lipids (breast) from freeze-dried; grounded samples were extracted with chloroform-methanol (2:1 v/v; Folch *et al*. (1957) with slight modification as described by Elshater *et al*. (2009). After extraction, FAs in the residual fat were esterified, using acid and base-catalyzed methods as described by Elshater *et al*. (2009). Fatty acid methyl esters (FAMEs) analysis was performed by gas chromatography-mass spectrometry (GC–MS; Mussek-QM- 2010 plus, China) equipped with an electron impact (EI) detector. Separations of Fas were carried out on capillary column Model 7009 A, Punjab Technologies, India (30m × 0.32 mm × 0.25 µm) using helium as carrier gas. The column temperature was held at 50°C for 1 min, and then the temperature was raised up to 150°C at the rate of 15°C per min. The temperature was later increased to 175°C at the rate of 2.50°C and hold for 5 min and finally increased to 220°C at the rate of 2.50°C per min and kept for 5 min. The identification of the peaks was made by comparison of the equivalent chain length with those of authentic fatty acid methyl esters. Peak areas were determined automatically using the Agilent gas chromatography chemstation software. The fatty acid concentrations were expressed in percentage of the sum of total identified peaks measured in each sample.

Statistical Analysis

All data were subjected to one–way analysis of variance (ANOVA) using SPSS (18.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

Table 1. Chemical Composition of Experimental Diets

Materials	Starter (1-21 days)	Grower (22-35 days)	Finisher (36-56 days)
Maize	50.00	56.00	60.50
Wheat offal	8.00	7.00	8.05
Soya meal	28.55	22.00	21.00
Groundnut cake	10.00	11.55	6.05
Fish meal	2.00	2.00	2.00
Bone meal	0.35	0.40	0.40
Limestone	0.20	0.20	0.20
Lysine	0.15	0.15	0.15
Methionine	0.20	0.20	0.20
Premix	0.25	0.25	0.25
Salt	0.30	0.30	0.30
TOTAL	100.0	100.0	100.0
Calculated analysis			
Crude protein	23.08	20.11	19.33
Ether extract	5.03	4.87	4.28
Crude fibre	3.06	3.95	3.42
Calcium	0.98	1.00	1.10
Phosphorus	0.47	0.40	0.51
Lysine	1.17	1.29	1.60
Meth +Cyst	0.87	0.82	0.51
ME (Kcal/kg)	2936	3000.8	3100.2

*Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg.

Table 2. Vaccination Schedule for Birds

Vaccine	Day/week	Route of administration
1st ND Lasota	Day 5	Drinking water
1st IBD (Gumboro)	Day 8	Drinking water
Immucox vaccine (Coccidial vaccine)	Day 10	Drinking water
2nd ND Lasota	Day 15	Drinking water
2nd IBD (Gumboro)	Day 21	Drinking water
3rd ND Lasota	Day 28	Drinking water
3rd IBD (Gumboro)	Day 33	Drinking water

Table 3. Proximate Composition of *Daniellia oliveri* Leaf Meal

Parameters	% Composition
Dry matter	89.11
Crude protein	18.95
Crude fibre	13.11
Ether extract	4.78
Ash	6.10
Neutral detergent fibre (NDF)	28.10
Acid detergent fibre (ADF)	47.50
Nitrogen free extract (NFE)	46.17

Table 4. Proximate Composition of Breast Meat

Parameters (%)	T1	T2	T3	T4	T5	SEM
Breast meat						
Moisture	71.63^{b}	72.00^{b}	74.18^{a}	75.20^{a}	76.08^{a}	2.06
Crude protein	19.93^{c}	22.24^{b}	23.00^{a}	23.47^{a}	23.86^{a}	1.10
Fat	1.15^{b}	1.83^{b}	2.00^{a}	2.21^{a}	2.44^{a}	0.06
Ash	1.29^{b}	1.42^{b}	1.94^{b}	2.00^{a}	2.02^{a}	0.10

Table 5. Effect of *Daniellia oliveri* Leaf Extract on the Fatty Acid Profile of Broiler Chicks (Breast) Meat

Fatty acids	T1	T2	T3	T4	T5	SEM
C12:0	2.91^{a}	1.98^{b}	1.65^{b}	1.61^{b}	1.52^{b}	0.07
C14:0	3.18^{a}	2.48^{b}	2.42^{b}	2.37^{b}	2.21^{c}	0.08
C16:0	22.9^{a}	18.0^{b}	17.2^{b}	16.3^{c}	15.0^{c}	0.51
C18:0	11.2^{a}	8.71^{b}	7.62^{b}	6.00^{c}	5.73^{c}	0.30
C20:0	4.21^{a}	3.93^{a}	2.81^{b}	2.40^{b}	1.88^{c}	0.73
C22:0	0.31^{a}	0.21^{b}	0.27^{b}	0.20^{b}	0.24^{b}	0.01
C14:1c	1.81^{c}	2.88^{a}	2.73^{a}	2.91^{a}	2.97^{a}	0.08
C16:1c	2.01^{b}	2.21^{b}	2.93^{b}	3.18^{a}	3.51^{a}	0.04
C18:1c	13.4^{c}	18.7^{b}	19.5^{b}	21.0^{a}	21.7^{a}	0.26
C18:1n9t	1.20^{c}	1.51^{b}	1.40^{b}	1.44^{b}	1.86^{a}	0.06
C18:1n9c	0.82	0.86	0.80	0.83	0.87	0.02
C:22:1	0.13^{b}	0.47^{a}	0.51^{a}	0.53^{a}	0.66^{a}	0.04
C18:2n6	15.4^{c}	19.8^{b}	22.0^{a}	22.8^{a}	23.4^{a}	0.23
C20:5n3	0.88^{c}	1.05^{b}	1.10^{b}	1.14^{b}	1.51^{a}	0.18
C18:3n3	3.04^{c}	8.08^{b}	10.3^{b}	13.1^{a}	14.3^{a}	0.22
C20:4n6	2.08^{b}	2.21^{b}	2.59^{b}	2.87^{b}	3.81^{a}	0.05
C20:3n6	0.92^{b}	1.08^{a}	1.17^{a}	1.21^{a}	1.28^{a}	0.01
C22:6n3	0.05^{c}	1.35^{b}	1.40^{b}	1.44^{b}	2.00^{a}	0.61
TSFA[1]	44.71^{a}	35.31^{b}	31.97^{b}	28.88^{c}	26.58^{c}	0.06
TUFA[2]	41.47^{c}	61.94^{b}	66.43^{b}	72.45^{a}	77.87^{a}	0.02
MUFA[3]	19.37^{c}	26.63^{b}	27.87^{b}	29.89^{b}	31.57^{a}	0.14
PUFA[4]	22.37^{c}	33.57^{b}	38.56^{b}	42.56^{a}	46.30^{a}	0.47
n-6:n-3[5]	4.67^{a}	2.20^{b}	2.01^{b}	1.71^{c}	1.75^{c}	0.03
Ant. Index[6]	0.93^{a}	0.48^{b}	0.43^{b}	0.38^{c}	0.33^{c}	0.05

[1]Total saturated fatty acid= C12:0 + C14:0 + C16:0 + C18:0 + C20:0 +C22:0

[2]Unsaturated fatty acid = (3 + 4)

[3]Mono unsaturated fatty acid= C14:1C + $C16:1_C$ + $C18:1_C$ + C18:1n9t + C18:1n9c + C22:1
[4]Polyunsaturated fatty acid = C18:2 n6 + C20:5 n3 + C18:3n3 + C20:4n6 + C20:3n6 + C: 22:6n3
[5]n-6: n-3 = (C18:2 n6 + C20:4n 6 + C20:3n 6 / (C20:5n 3 + C18:3n 3 + C: 22 6n 3), [6]Antherogenic index = (C12:0+ 4×C14:0+ C16)/ε of UFA

RESULTS AND DISCUSSION

The Proximate Composition of Experimental Diet and *Daniellia oliveri* Leaf Meal

The proximate composition of experimental diet (Table 1) revealed that it contains crude protein, crude fibre, ether extract, calcium, phosphorus and metabolizable energy which ranged between 19.33 – 23.08 %, 3.06 – 3.95 %, 4.28 – 5.03 %, 0.98 – 1.10 %, 0.40 – 0.51 % and 2936 – 3100. 2 Kcal/kg respectively. The experimental diet was in three (3) phases with starter mash fed between 0 – 21 days, growers mash (22 – 35 days), and finishers mash (36 – 56 days). The crude protein and ether extract values obtained in this study conform with the values obtained by Musa *et al*. (2020); Alabi *et al*. (2017) and Ahsan *et al*. (2018) when phytogenic feed additives were supplemented in the diet of broiler chicks. The crude fiber (4.28 – 5.03 %) falls within the recommended ranges by Olafadehan *et al*. (2020); Alagbe *et al*. (2020); Oluwafemi et al. (2020) and Wati *et al*. (2015). The calcium and phosphorus values are in close agreement with the findings of Vukic *et al*. (2013) who examined the effect of phytogenic additives on the performance and caecal microflora of broiler chickens. The energy contents (2936 – 3100.2 kcal/kg) are in conformity with the values obtained by Toghyani *et al*. (2010) but lower than those obtained by Fascina *et al*. (2012) and Hong *et al*. (2012) who examined the effects of supplemental essential oil on the performance and carcass traits in broilers. However, all values in the experimental diets were within a nutritional requirement for broilers according to NRC (1994).

Proximate analysis of *Daniellia oliveri* leaf meal

Proximate analysis of *Daniellia oliveri* leaf meal is presented in Table 2. The sample contained dry matter, crude protein, crude fibre, ether extract, ash, neutral detergent fibre, acid detergent fibre and nitrogen-free extract at 89.11 %, 18.95 %, 13.11 %, 4.78 %, 6.10 %, 28.10 %, 47.50 % and 47.50 % respectively. The crude protein (18.95 %) value in *Daniellia oliveri* leaf meal is in agreement with those reported for *Delonix regia* leaves (18.77 %) by Alagbe (2020), but contrary to those reported for *Piliostigma thonningii* stem and root (4.22 % and 7.40%), *Indigofera tinctoria* leaves (30.53 %) reported by Alagbe (2020). The result suggests that the sample cannot supply an adequate amount of protein in the diets of animals (NRC, 1994; Alagbe, 2016). The crude fiber obtained is higher than the values for *Sida acuta* leaves (6.24 %) by Shittu and Alagbe (2020). Dietary inclusion of fiber encourages proper digestion, reduces serum cholesterol levels and the risk of cardiovascular diseases (Fasola *et al*., 2011; Oluwafemi *et al*., 2020). The result showed that the sample contains ether extract (4.78%) and ash (6.10%). These values are lower than the values for *Pentadiplandra brazzeana* (5.70% and 12.11%) respectively reported by Alagbe *et al*. (2020). All values obtained in this study were in agreement with the findings of Olafadehan *et al*. (2020).

Proximate Composition of Breast Meat

The proximate composition of breast meat is presented in Table 4. The moisture, crude protein, lipid and ash ranged between 71.63 – 76.08 %, 19.23 – 23.86 %, 1.15 – 2.44 % and 1.29 – 2.02 % respectively. The values were highest in T4 and T5, intermediate in T2, T3 and lowest in T1 ($P < 0.05$). This is a clear indication that feeding *Daniellia oliveri* leaf extract (DOFE) is capable of modifying the meat composition of birds; it could also be attributed to the presence of phytochemicals in DOFE (Oluwafemi *et al*., 2019, 2020; Omokore and Alagbe, 2019). Concentrations of phytochemicals or bioactive chemicals in plants are determined by variety and environmental growth conditions, harvesting time, stage of maturity, method and duration of conservation and storing, extraction methods as well as possible synergistic or antagonistic effects and anti-nutritional factors in plants (Norton, 1994; Wenk, 2002; Alagbe *et al*., 2020, 2017; Hyun et al., 2018). The result obtained in this study is in agreement with the findings of Alagbe and Soares (2018); Teodora *et al*. (2020) who examined the effect of black soldier fly (*Hermetia illucens*) meals on the meat quality in broilers.

Fatty Acid Composition of Breast Meat of Broiler Chicks Fed Different Levels of DOFE

The effects of *Daniellia oliveri* leaf extract on the fatty acid profile of broiler chicks (breast) meat are presented in Table 5. C12:0 (Lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C20:0 (arachidic acid) and C22:0 (behenic acid) ranged between 1.52 – 2.91 %, 2.21 – 3.18 %, 15.0 – 22.9 %, 5.73 – 11.20 %, 1.88 – 4.21 % and 0.24 – 0.31 % was lowest (P<0.05) for T5 relative to other treatments. C22:1 (myristoleic acid), C14:1c (palmitoleic acid), C16:1c (linoleic acid), C18:1c (oleic acid), C18:1n9t (elaidic acid), C18:1n9c (linolelaidic acid), C:22:1 (erucic acid), C20:5n3 (eicosapentenoic acid), C18:3n3 (α – linolenic acid), C20:4n6 (arachidonic acid), C20:3n6 (dihomogammalinolenic acid) and C22:6n3 (docosahexenoic acid) ranged between 0.10 – 0.65 %, 1.81 – 2.97 %, 2.01 – 3.51 %, 13.4 – 21.7 %, 1.2 – 1.86 %, 0.82 – 0.87 %, 0.13 – 0.66 %, 15.4 – 23.4 %, 0.88 – 1.51 %, 3.04 – 14.3 %, 2.08 – 3.81 %, 0.92 – 1.28 %, 0.05 – 2.00 % respectively. Values were highest in T5, intermediate in T3, and lowest in T1 (P<0.05). Total saturated fatty acid (26.58 – 44.71 %) and total unsaturated fatty acid (41.47 – 77.87) were affected (P<0.05) by the oral administration of *Daniellia oliveri* leaf extract. TSFA were highest and lowest for T1 and T5 respectively (P<0.05) while TUFA was lowest (P<0.05) in T1 relative to other treatments. TUFA and TSFA obtained in this study conform with the values obtained by Alagbe and Omolere (2020); Suriya *et al.* (2014) and Li et al. (2012). Birds in T5 had the highest concentration of polyunsaturated fatty acid (PUFA), which is in agreement with the reports of Kamran *et al.* (2018). According to Bourre (2005), meat from birds is low in lipids or fats, but high in PUFA. Atherogenic index (A.I) values range between 0.33 – 0.93 %, the values significantly reduced as the level of DOFE increased (P<0.05). Antheriogenicity is an index used to ascertain the safety of the meat and prevent the incidence of cardiovascular diseases (Suriya *et al.*, 2014; Alagbe and Omolere, 2020). According to Katalin and Loana (2017), omega – 3 and omega -6 polyunsaturated fatty acids perform multiple biological roles such as influencing the inflammatory cascade, reducing oxidative stress, presenting neuro-protection, and cardiovascular protection.

CONCLUSION

It was concluded that DOFE contains several bioactive chemicals or phytochemicals which are capable of modification of meat fatty acid composition of animals, these chemicals are safe, effective, cheap, and can also influence the secretion of digestive fluids and total feed intake in animals, thus promoting food safety. Feeding birds' DOFE at 80 ml/liter is safe and does not have any deleterious effect on the general performance of the animal.

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