

Effect of feed supplementation of *Dacryodes edulis* parts' powder as prebiotic on the growth traits, ceca microbiota and blood parameters of local chickens

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ABSTRACT: This study aimed at testing the efficacy and safety of *Dacryodes edulis* plant parts in diets fed to chicken. The plant has potential for use as a natural prebiotic to substitute the conventionally used antibiotic growth promoters in poultry production. Phytochemical analyses of the plant leaves, stem, and bark combination (stembark) and seed powders from the *D. edulis* were carried out. The powder from the three *D. edulis* plant parts were used as supplement in formulating six experimental diets tested in this study. The diets were TL_{0Ed} (0.5% leaves powder), TL_{1Ed} (1.0% leaves powder), TB_{0Ed} (0.5% stembark powder), TB_{1Ed} (1.0% stembark powder), TS_{0Ed} (0.5% seeds powder), and TS_{1Ed} (1.0% seeds powder). Besides, a positive (T₊ positive control; 0.5-g oxytetracycline as recommended by the manufacturer) and a negative (T₋ negative control; having no commercial antibiotic and no plant supplement) diets were prepared for comparison purposes. The diets were fed to a total of 288 dual-purpose chicken for a period of 14 weeks. The chicken growth and body composition characteristics, blood chemistry, and microbiota count were collected and used as indicators of the plant parts efficacy and safety. The analysis of

the *D. edulis* plant parts significantly differed ($P \leq 0.05$) in their phytochemical contents. The initial body weight and feed conversion efficiency ratios were not significantly different ($P \geq 0.05$) between and among treatment groups. However, significant differences ($P \leq 0.05$) were detected in the feed intake and body weight gain at eighth week. Live weight at eighth week was significantly different ($P \leq 0.05$) with its values ranging between 503.32 and 614.93 g for treatments TL_{1Ed} and T_{Neg-}, respectively. The dietary treatment of *D. edulis* leaves, stembark, and seed powder at the two inclusion levels significantly ($P \leq 0.05$) decreased the colonies forming unit of *Escherichia coli* and *Salmonella* sp. as compared with negative control treatment in the eighth week phase. The level of glucose, total cholesterol, triglycerides, aspartate aminotransferase, alanine amino transferase, alkaline phosphatase, and the packed cell volume did not differ significantly ($P \geq 0.05$) between and among dietary *D. edulis* treatments. The findings from this research provide crucial information on the efficacy and safety of *D. edulis* plant parts. This is an important step in testing the potential of the plant in use as a prebiotic in chicken feeds production.

Key words: *Dacryodes edulis*, blood parameters, growth, microbiota, local chicken

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INTRODUCTION

For many decades, antibiotics have been used as feed additive to improve animal health and promote growth by inhibiting certain intestinal flora that produce toxins or compete with the host for available nutrients, resulting in better nutrient utilization and decreased feed conversion ratio (Sugiharto, 2016). However, the misuse of these antibiotics as growth promoters (AGP) has led to the emergence of antimicrobial resistance (AMR) (Mehdi et al., 2018) and accumulation in the human food chain. Antibiotic inclusion was not only increasing the cost of livestock feeds, but the growing concern of AMR as a public health issue initiated the ban of antibiotic in animal feed (Ragland et al., 2018). With that awareness of AMR risk and possibilities of safer alternatives, there is growing interest and pressure from consumers for poultry products without AGP (Fasanmi et al., 2014). Africa has traditionally relied on plant based therapies to treat human and animal diseases as well as a source of limiting nutrients. Many of these plants and their corresponding extracts have not been studied for efficacy and safety. This has hampered their increased adoption and possible commercialization.

Some few plants or their extracts have been confirmed to contain natural phytochemicals and have been screened for many bioactive components with potential to alter the abundance and/or pathogenicity of microorganisms (Nayim et al., 2018), while some may modulate the genes expression for growth profile, immunity, and reproduction. Furthermore, incorporation of some plants by-products as phytochemicals in animal feed have been shown to express positive effects on growth performance and different health parameters (Crhanova et al., 2011; Valenzuela-Grijalva et al., 2017). The *D. edulis* is one such tropical plant whose fruits are edible, while the bark, leaves, stems, and roots are used in locally to treat some human and animal diseases (Ogboru et al., 2015). For instant, different parts of the *D. edulis* are used in Nigeria, Cameroon, and in the Democratic Republic of Congo in the

treatment of dysentery, toothache, earache, anaemia, eradication of scars, and other skin diseases (Riwom et al., 2015).

Phytochemical screening of the *D. edulis* fruit, seed, stem bark, resin, and leaves has tested positive for alkaloids, tannins, saponins, cyanogen glycosides, flavonoids, and phytates (Yelwa et al., 2017). Besides, the plant's extracts and secondary metabolites have been found to exhibit some antimicrobial, antioxidant, anti-sickle-cell disease properties (drepanocytes) in treatment of parasitic skin diseases, cough, malaria, mouth infections, tonsillitis and eradication of jiggers (Ebana et al., 2016, 2017). Studies by Valenzuela-Grijalva et al. (2017) showed that the *D. edulis* seed, leaf, stem, and bark also showed antimicrobial properties when used in vitro against resistant strain of *Staphylococcus aureus*. Further, findings from a study by Oyetunji and Opeyemi (2017) indicate that various parts of the *D. edulis* have broad spectrum antibacterial activity. Decoctions from different parts of the plant are used to relieve certain digestive disorders caused by microbial infection (Ebana et al., 2016). The *D. edulis* fruit has traditionally been consumed (boiled or roasted) due to its nutritional and medicinal properties (Kadji et al., 2016), while the seeds, which are usually discarded, are known to contain high amounts of soluble carbohydrates and lipids (Bratte, 2010). The documented antimicrobial benefits to human health and nutrition informed the choice of the *D. edulis* plant for evaluation for its suitability in use as feed supplement and growth promoter in indigenous chicken in this study. The impact of its leaves, stem bark, and seed powder as feed additive on growth performance, carcass parameters, ceca microbiota count and serum biochemistry, and pack cell volume of indigenous chicken was evaluated.

MATERIALS AND METHODS

Ethical Considerations

All animal care and use procedures were carried out according to the guidelines provided

and supervised by Jomo Kenyatta University of Agriculture and Technology Animal Ethics Review Committee. An approval for use of animals in this research was sought after a thorough assessment of all procedures and resources and a compliance reference number JKU/2/4/896C was granted. There was constant monitoring of the adherence to the procedures and resources provision.

Experimental Site

The experiments and laboratory analyses were conducted at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), in Juja Sub-county, Kiambu County, Kenya. Juja Sub-county lies at a latitude of 1°11'0"S, 37°7'0"E with an altitude of 1,530 m above sea level and is in upper midland zone 4 which is semi-humid to semi-arid, and receives an annual rainfall range from 600 to 800 mm and mean annual temperature of 18.9 °C (Kaluli et al., 2011).

Experimental Procedure

In total, 288-d-old dual-purpose male chicks from a breeding stock described by Gikunju et al. (2018) were purchased from Kenya Agriculture and Livestock Research Organisation (KALRO) at Naivasha Centre, Kenya. The chicks were brooded for 3 wk during which the brooder temperatures were maintained between 30 °C in the first week with gradual adjustment to 24 °C in the last week. The chicks were intensively brooded in deep litter pens measuring 3 × 3.4 ft under the same experimental conditions. They were fed ad libitum on conventional starter diet formulated to supply 2,850 kcal/kg of metabolizable energy and 21% crude protein before being introduced to the experimental starter diet from day 8. The chicks received glucose solution the first day (as anti-stress factor), whereas vaccination against Newcastle and infectious bursal (Gumboro) diseases were administered on days 7, 14, 21, and 28 as recommended by the breeder. The chicks were subsequently transferred to cages and allowed to acclimatize for a period of 1 week before the start of the experiment. In total, 72 cages (experimental units) were used to accommodate eight treatment groups with nine replicates. Any chick that exhibited disease condition was quarantined for treatment and was not returned to the experiments.

The *D. edulis* plant parts were harvested from the North West region of Cameroon where the plant is commonly found in abundance. The leaves,

stem/bark combination (stembark), and seeds were prepared based on the methods described by Ebana et al. (2017) and Nkukwana (2012). Fresh, green, and undamaged leaves, stembark, and seeds were air-dried under shade in sunlight. The materials were constantly turned to avoid fungal growth. They were dried for 12 d before being finely ground into powder using a blender. The powder was then packed in airtight polythene plastic bags and stored in room temperature for use in formulation of the experimental diets. The dried samples of *D. edulis* were first analyzed for their phytochemical composition before being incorporated into various experimental diets. The experimental diets were formulated to meet the nutritional requirements of the chicks at different growth stages. Different raw materials were used in formulation of the diets to provide the necessary nutrients. Table 1 presents the proportions of different raw materials used in the formulation of the starter and finisher diets. An analysis of the diets was carried out to ascertain

Table 1. Basal diet formulae and nutrients composition

Composition, g/kg of basal diets	Starter (0–8 weeks)	Finisher (8–14 weeks)
Ingredients		
Maize	600	575
Soya meal	224	177
Wheat Bran	20	108
Fish meal	50	30
Limestone	40	40
Cotton seed meal	30	30
Fat	20	25
Dicalcium phosphate	6	6
Salt	3	3
Bone meal	2	2
Premix ¹	2	2
Methionine	2.3	1.8
Lysine	1.3	0.5
Total	1,000	1,000
Calculated bromatological composition		
Diet	Starter	Finisher
ME, kcal/kg	2836	2950
Crude protein, %	19	17
Crude fibre	2.4	3
Fat, %	1	2
Calcium, %	1	1
Phosphorus, %	0.2	0.2
Lysine, %	0.9	0.7
Methionine, %	0.4	0.3

¹an iron, zinc, manganese, magnesium, iodine, cobalt, molybdenum, selenium, vitamin A, vitamin D3, vitamin E, vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B12, biotin, folic acid, and vitamin K3 mix.

that the compounded basal diets met the nutritional requirements of the growing chicks (see Table 1).

Six experimental diets each for the starter and finisher were prepared by varying the three *D. edulis* plant parts inclusion into the respective basal diet. The diets were TL_{0Ed} (0.5% leaves powder), TL_{1Ed} (1.0% leaves powder), TB_{0Ed} (0.5% stembark powder), TB_{1Ed} (1.0% stembark powder), TS_{0Ed} (0.5% seeds powder), and TS_{1Ed} (1.0% seeds powder). Besides, a positive (T₊ positive control; 0.5-g oxytetracycline as recommended by the manufacturer) and a negative (T₋ negative control; having no commercial antibiotic and no plant supplement) diets were prepared. The basic diet without any additive was considered as negative control, whereas the one with oxytetracycline at the concentration recommended by the manufacturer was considered as positive control, against which the treatments were compared. The chicken had free access to feed and water. They were fed the experimental starter diet for 8 wk before being introduced to the finisher for a further 6 wk. Samples were collected at the end of the starter and finisher phase. Vitamins and trace elements were provided in drinking water.

DATA COLLECTION

Phytochemical Analysis of the D. edulis Parts and Proximate Analysis of the Dietary

The phytochemical analysis of the three *D. edulis* plant parts was determined qualitatively (Nwokonkwo, 2014; Oyetunji and Opeyemi, 2017) and quantitatively (Madhurima et al., 2014) using three replicate for each of the parts. While the proximate analysis was carried out for all experimental diets in which the dry Matter (DM) was determined by drying samples at 105 °C overnight. Ash content was determined by ashing samples in a muffle furnace at 550 °C for 6 h. Nitrogen (N) content was determined using Kjeldahl method (AOAC 1990). Crude protein (CP) was calculated as $N \times 6.25$. Crude fiber (CF) and ether extract (EE) were determined by the methods described (AOAC 2005) while the nitrogen-free extract was determined by difference obtained from $[100 - (CP + EE + CF + Ash)]$.

Growth Performance

Data collected for growth performance evaluation included the daily feed intake (dFI), daily weight gain (dWG), and feed conversion ratio (FCR). The dFI was obtained from difference

between the amount of feed provided to the birds and the remnant after a period of 24 h. The dWG was determined as

$$dWG = \frac{\text{Final bird weight (g)} - \text{Initial bird weight (g)}}{\text{Period (days)}}$$

The FCR was calculated per treatment group (Nkukwana, 2012). The conversion ratio was determined as follows:

$$FCR = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

Carcass Parameters

Carcass characteristics were obtained at slaughter at the 8th and 14th weeks. Two birds from each of the treatment groups were randomly selected and fasted for 24 h after which the live weight was measured. The birds were then stunned using concentrated CO₂ gas before being slaughtered by severing the carotid arteries and jugular veins using a sharp knife. The chicken was completely bled, scalded using hot water, feathers plucked, and washed before evisceration. The organs and carcass in each treatment groups were separated into different parts and weighed. The weights of the carcass, total visceral organs, spleen, heart, lungs, testicles, liver, small intestine, crop, kidney cecum and gizzard, the lengths of the small intestine, and cecum were measured and recorded.

Pack Cell Volume and Serum Biochemistry

A bird was sacrificed, and 5-mL blood sample obtained through cardiac punch. The blood was dispensed into two different eppendorf tubes; one containing ethylene di-amine tetra acetic acid (EDTA) anticoagulant and the second portion into a plain sterilized tube. The blood samples preserved in EDTA were mixed using a blood mixer with the capillary tube being sealed with plasticine and centrifuged in a microhematocrit centrifuge at 1,000 rpm for 5 min at 4 °C. The packed cell volume (PCV) was read using an HC 702 hematocrit scale as described by Audu et al. (2017). The blood samples in the plain tubes were left to coagulate and then centrifuged at 3,500 rpm for 15 min to obtain clear sera. The serum samples were stored in Eppendorf tubes at -20 °C until further processing for biochemical analysis. Total cholesterol, glucose, triglyceride, alanine amino transferase (ALT), alkaline phosphatase (ALP), Aspartate aminotransferase (AST) concentrations

were determined using commercial diagnostic kits protocol from Reflotron (Anonymous, 2016).

Ceca Microbiota Count

At the end of 8th and 14th weeks of ages, one bird from each replicate was randomly selected and euthanized. Then, 1 g of cecum contents was removed for culturing based on the methods described by Engberg et al. (2004), Ranjitkar et al. (2016), and Xavier and Umadevi (2014). Total viable count of *Escherichia coli* and *Salmonella* sp. were determined in Mac Conkey agar and xylose lysine deoxycholate agar media, respectively. Colonies were subjected to biochemical test for confirmation and identification (indole test, methyl red test, Simon citrate test, urease test, triple sugar ion, motility test, catalase test, and oxidase test).

Statistical Analysis

The data were analyzed using one-way analysis of variance in the General Linear Model using SPSS Version 21. Statistical significance was based on a probability of $P \leq 0.05$.

RESULTS

Phytochemical Variability in *D. edulis* Leaves, Stembark, and Seed Powder

The results for the phytochemical composition analyses of the *D. edulis* plant parts are presented in Table 2. There was significant difference in the quantities of the tannin, polyphenol, and flavonoids between and among the plant parts. All the parts contained saponin, terpenoids, steroids, and alkaloids although at varying levels. Anthraquinone and glycoside were absent in all the *D. edulis* plant parts. The leaves had the highest amounts of tannins,

polyphenol, and flavonoids followed by the seeds and least in the stembark. The amount of flavonoids was always higher than tannins and polyphenol in all the plant parts. Qualitatively, leaves had lower terpenoids, steroids, and alkaloids than the stembark and seeds.

Growth Performance and Carcass Traits

The growth performance and carcass traits' characteristics of the chicken-fed different treatment diets are summarized in Table 3. The assessment was carried out at the end the 8th and 14th weeks of the growth period. The initial chicken weight did not differ significantly. However, FI was statistically different between and among dietary treatment at the eighth week of the growth. The negative control diet recorded the least intake during the period. Results for the BWG at the eighth week of growth revealed a significant difference for different treatment diets. Conversely, no significant difference was detected in the BWG during at the 14th week assessment period. There were no significant differences between treatment diets for the FCR for the entire chicken growing period. However, there was a significant difference in live weight between and among treatment diet groups. The highest weight was obtained from chicken fed the negative control diet (614.93 ± 29.32 g), whereas the least (503.98 ± 17.53 g) was from TL_{0Ed} diet. There was no statistically significant difference between and among the carcass weights attributable to the different treatment diet groups.

The total weight of the viscera organs differed significantly for the period ending on the eighth week. It ranged between 84.43 g for chicken-fed TL_{1Ed} and 112.28 g for chicken fed on the negative control diet. There was no statistically significant difference in the weights of the spleen, heart, lungs, testicles, liver, small intestines, crop, kidney, and cecum between and among treatment diets. Similar

Table 2. Phytochemical contents of *D. edulis* plant parts, g/100 g

Phytochemical composition	Leaves	Stembark	Seed	P-value
Tannin	0.08 ^a ± 0.0	0.03 ^b ± 0.0	0.23 ^c ± 0.0	0.00 [*]
Polyphenol	2.32 ^a ± 0.3	0.21 ^b ± 0.1	0.75 ^c ± 0.3	0.00 [*]
Flavonoids	6.27 ^a ± 0.2	1.53 ^b ± 0.0	3.57 ^c ± 0.0	0.00 [*]
Saponin	++	+	+++	
Anthraquinone	–	–	–	
Terpenoids	+	+++	++	
Steroids	+	++	+++	
Alkaloids	+	+++	++	
Glycoside	–	–	–	

+, Low; ++, moderate; +++, high; and –, absent.

*P-value < 0.05 and there is significant difference.

^{a,b,c}Means with similar letter indices on the same row are not significantly different.

Table 3. Growth performance and carcass traits of indigenous chickens fed different experimental diets

Variable ²	Dietary treatment groups ¹							P-value	
	TL _{0fd}	TL _{1fd}	TB _{0fd}	TB _{1fd}	TS _{0fd}	TS _{1fd}	T+		T-
Initial BW, g	236.11 ± 4.66	239.11 ± 5.11	236.00 ± 3.58	239.22 ± 5.17	237.56 ± 6.32	239.89 ± 5.82	236.48 ± 0.08	237.06 ± 0.33	0.10
FI, g									
8th week	1299.70 ^b ± 19.3	1306.39 ^b ± 30.8	1276.14 ^b ± 14.5	1276.24 ^b ± 20.5	1282.87 ^b ± 23.8	1305.10 ^b ± 13.7	1347.16 ^a ± 10.7	1174.15 ^a ± 47.0	0.00*
14th week	2958.26 ± 144.6	3092.61 ± 155.9	2970.82 ± 151.1	3103.03 ± 122.9	3004.79 ± 124.4	3145.87 ± 81.0	2853.09 ± 187.0	2871.55 ± 70.7	0.74
BWG, g									
8th week	319.20 ^a ± 19.6	306.59 ^a ± 10.2	316.63 ^a ± 12.8	321.59 ^a ± 8.7	332.79 ^{ab} ± 15.0	309.33 ^a ± 13.7	338.49 ^{ab} ± 12.1	377.13 ^b ± 14.3	0.00*
14th week	1006.50 ± 58.0	898.89 ± 54.4	1010.26 ± 63.3	989.47 ± 36.6	892.95 ± 63.362	927.22 ± 54.6	1020.21 ± 64.7	1057.00 ± 72.5	0.42
FCR									
8th week	4.07 ± 0.0	4.26 ± 0.2	4.03 ± 0.1	3.97 ± 0.1	3.85 ± 0.1	4.21 ± 0.1	3.98 ± 0.1	3.91 ± 3.6	0.45
14th week	2.94 ± 0.3	3.44 ± 0.3	2.94 ± 0.3	3.14 ± 0.2	3.37 ± 0.2	3.39 ± 0.2	2.79 ± 0.3	3.09 ± 0.2	0.26
Live weight, g									
8th week	511.47 ^a ± 33.4	503.98 ^a ± 17.5	551.52 ^a ± 21.8	536.88 ^{ab} ± 22.8	559.12 ^{ab} ± 27.1	505.93 ^a ± 29.7	579.66 ^{ab} ± 19.2	614.93 ^{ab} ± 29.3	0.03*
14th week	1028.70 ± 44.4	978.28 ± 56.3	1123.55 ± 63.3	1070.23 ± 36.6	991.26 ± 60.6	1017.58 ± 45.4	1213.09 ± 66.0	1109.27 ± 71.8	0.23
Carcass, g									
8th week	436.96 ± 18.2	414.63 ± 17.6	437.73 ± 20.4	441.16 ± 22.1	466.54 ± 26.7	410.09 ± 27.7	470.64 ± 19.6	510.71 ± 25.6	0.06
14th week	855.13 ± 68.4	856.44 ± 56.8	947.27 ± 58.1	911.63 ± 33.7	845.52 ± 62.4	856.82 ± 51.7	1020.01 ± 54.3	984.04 ± 72.5	0.29
Total visceral organ, g									
8th week	104.27 ^{bc} ± 7.7	89.43 ^a ± 3.1	100.00 ^{abc} ± 3.3	97.41 ^{ab} ± 3.4	99.96 ^{abc} ± 3.8	94.45 ^{ab} ± 3.6	103.12 ^{abc} ± 2.9	112.28 ^c ± 7.3	0.04*
14th week	156.13 ± 6.1	156.44 ± 6.3	159.55 ± 6.8	158.17 ± 6.2	147.39 ± 8.9	169.58 ± 11.8	158.16 ± 5.4	140.41 ± 9.2	0.67
Spleen, g									
8th week	1.63 ± 0.2	1.68 ± 0.2	1.71 ± 0.2	1.74 ± 0.2	2.18 ± 0.3	2.24 ± 0.5	2.65 ± 0.7	2.04 ± 0.2	0.41
14th week	2.13 ± 0.3	1.76 ± 0.2	2.19 ± 0.4	2.19 ± 0.4	1.65 ± 0.2	1.85 ± 0.2	2.45 ± 0.3	2.53 ± 0.3	0.48
Heart, g									
8th week	4.83 ± 0.3	4.43 ± 0.2	5.13 ± 0.3	5.08 ± 0.2	4.91 ± 0.3	4.81 ± 0.3	5.08 ± 0.3	5.30 ± 0.4	0.61
14th week	7.52 ± 0.6	7.09 ± 0.5	6.64 ± 0.6	7.73 ± 0.5	6.78 ± 0.6	6.88 ± 0.6	7.32 ± 0.5	8.26 ± 0.8	0.57
Lung, g									
8th week	3.50 ± 0.2	3.72 ± 0.2	3.47 ± 0.3	3.91 ± 0.2	3.46 ± 0.2	3.36 ± 0.3	4.44 ± 0.5	3.40 ± 0.4	0.11
14th week	7.00 ± 0.6	6.81 ± 0.7	7.56 ± 0.4	7.47 ± 0.5	7.49 ± 0.9	7.43 ± 1.0	7.44 ± 0.6	7.00 ± 0.9	0.98
Testicles, g									
8th week	0.24 ± 0.0	0.28 ± 0.0	0.29 ± 0.1	0.25 ± 0.0	0.28 ± 0.1	0.23 ± 0.0	0.26 ± 0.0	0.28 ± 0.0	0.92
14th week	3.54 ± 1.3	3.34 ± 1.0	5.47 ± 1.6	2.23 ± 0.6	5.29 ± 1.9	4.39 ± 1.2	3.96 ± 0.8	3.46 ± 1.2	0.58
Liver, g									
8th week	19.64 ± 0.9	18.20 ± 0.9	20.99 ± 1.3	20.18 ± 1.1	20.52 ± 1.3	20.88 ± 1.5	21.25 ± 1.6	21.79 ± 1.8	0.66
14th week	26.23 ± 1.2	25.24 ± 1.1	25.98 ± 1.6	26.29 ± 0.9	26.11 ± 2.0	28.13 ± 1.4	28.86 ± 1.2	25.26 ± 2.2	0.40
Small intestine, g									
8th week	30.78 ± 2.1	27.60 ± 1.7	35.24 ± 5.6	26.67 ± 1.4	29.92 ± 1.4	26.89 ± 1.2	29.02 ± 1.5	30.15 ± 1.6	0.27
14th week	44.79 ± 2.7	55.24 ± 2.3	54.05 ± 6.7	48.62 ± 3.0	46.68 ± 3.2	49.92 ± 2.4	49.51 ± 3.6	50.71 ± 4.4	0.55

Table 3. Continued

Variable ^c	Dietary treatment groups ¹								P-value	
	TL _{0Fd}	TL _{1Fd}	TB _{0Fd}	TB _{1Fd}	TS _{0Fd}	TS _{1Fd}	T+	T-		
Crop, g										
8th week	4.27 ± 0.3	3.65 ± 0.3	4.26 ± 0.4	4.07 ± 0.3	4.04 ± 0.2	3.15 ± 0.2	4.47 ± 0.3	4.44 ± 0.5	0.07	
14th week	7.09 ± 0.8	5.57 ± 0.5	5.48 ± 0.4	6.86 ± 0.9	5.65 ± 0.7	5.87 ± 0.4	5.87 ± 0.3	6.49 ± 0.7	0.41	
Kidney, g										
8th week										
14th week	7.29 ± 0.7	6.77 ± 0.6	7.16 ± 0.7	6.94 ± 0.6	6.64 ± 0.7	6.60 ± 0.6	7.63 ± 0.6	7.08 ± 1.1	0.96	
Gizzard, g										
8th week	20.70 ^a ± 1.1	20.52a ± 1.1	23.76 ^{ab} ± 1.3	23.07 ^{ab} ± 1.4	22.34 ^{ab} ± 1.3	20.65 ^a ± 1.0	23.93 ^{ab} ± 1.0	25.46 ^b ± 1.1	0.02*	
14th week	39.36 ± 2.1	35.94 ± 2.2	36.45 ± 2.5	34.91 ± 1.9	34.07 ± 2.2	36.47 ± 1.8	36.25 ± 2.4	40.15 ± 3.4	0.64	
Cecum, g										
8th week	6.50 ± 0.6	5.60 ± 0.5	6.86 ± 0.6	6.28 ± 0.6	6.28 ± 0.4	6.55 ± 1.1	5.865 ± 0.4	6.02 ± 0.5	0.88	
14th week	9.10 ± 0.7	10.96 ± 0.6	11.21 ± 0.9	10.31 ± 0.8	9.76 ± 1.0	9.58 ± 0.4	11.68 ± 1.2	11.36 ± 1.1	0.23	
Small intestine, cm										
8th week	127.44 ± 3.8	123.77 ± 4.3	117.79 ± 6.4	122.07 ± 4.8	124.66 ± 5.6	117.73 ± 6.9	128.45 ± 4.3	115.55 ± 3.6	0.62	
14th week	148.63 ± 5.4	142.37 ± 4.9	147.23 ± 3.7	143.76 ± 3.6	152.88 ± 3.8	142.78 ± 4.7	134.69 ± 7.5	132.14 ± 4.4	0.09	
Cecum, cm										
8th week	14.62 ± 0.6	20.18 ± 6.8	13.47 ± 0.9	13.92 ± 0.8	14.32 ± 0.9	14.82 ± 0.6	14.27 ± 0.6	13.11 ± 0.5	0.60	
14th week	18.72 ± 0.9	18.98 ± 1.2	17.82 ± 0.8	19.57 ± 0.9	16.44 ± 0.7	17.47 ± 1.1	17.73 ± 0.1	17.30 ± 1.1	0.23	

*P-value < 0.05 and there is significant difference.

^{a,b,c}Means with similar letter indices on the same row are not significantly different.¹See text for description of the dietary treatments.²See text for description of the parameters.

results were obtained for the lengths of the small intestines and cecum. Gizzard was the only visceral organ whose weight differed significantly between and among treatment groups. The gizzard weights ranged between 20.52 and 25.46 g for TL_{1Ed} and negative control diet, respectively.

Ceca Microbiota Count

Results for the ceca microbial count are presented in Table 4. There was significant decrease in viable colonies forming unit of *E. coli* and *Salmonella sp* between and among treatment groups in the first 8 weeks of the growing period. The negative control diet had higher colonies forming units in all cases. In all cases, an increase in the level of inclusion of the *D. edulis* resulted in a decrease in the colonies counts irrespective of the chicken growth phase.

Serum Biochemistry

The results for the serum biochemistry of chicken fed on the experimental diets are summarized in Table 5. The glucose, total cholesterol, triglycerides, ALT, AST, and ALP were not significantly altered by feeding the chicken on the experimental diets. However, the negative control diet resulted in insignificantly higher values for all the test parameters. Besides, the parameter values tended to reduce with an increase in level of *D. edulis* plant parts powder.

Packed Cell Volume

The results on the PCV are shown in Figure 1. There was no significant difference in PCV values obtained from chicken fed on different dietary treatments in the two growing phases.

DISCUSSION

Use of antibiotics in animal feed production and their subsequent accumulation in human food chain and the environment have resulted in negative impact to human and animal health by promoting an accelerated AMR to various diseases. This complicates the diseases management in the humans and animal populations. The antibiotics have been used in poultry feeds production to promote growth (AGP) as well as inhibiting production of toxins by intestinal flora. Besides, some of the microorganisms compete with the chicken for the available nutrients, thus diverting them from

Table 4. Ceca microbial count for different experimental dietary treatment groups

Mean Viable count, Log ₁₀ /CFU	Dietary treatment groups ¹							P-value	
	TL0Ed	TL1Ed	TB0Ed	TB1Ed	TS0Ed	TS1Ed	T+		T-
<i>Escherichia coli</i>									
8th week	3.35 ± 0.4 ^{bc}	2.41 ± 0.3 ^a	2.82 ± 0.7 ^{ab}	1.93 ± 0.4 ^b	2.76 ± 0.7 ^{ab}	2.19 ± 0.2 ^a	2.64 ± 0.7 ^{ab}	4.07 ± 0.8 ^c	0.00*
14th week	2.79 ± 0.4	2.33 ± 0.4	2.72 ± 0.4	2.58 ± 0.7	2.56 ± 0.6	2.39 ± 0.4	2.38 ± 0.5	3.12 ± 0.8	0.29
<i>Salmonella</i>									
8th week	3.22 ± 0.6 ^{bcd}	2.77 ± 0.3 ^{abc}	2.82 ± 0.5 ^{abc}	2.53 ± 0.7 ^{ab}	2.57 ± 0.7 ^{ab}	2.38 ± 0.1 ^a	3.52 ± 0.0 ^{cd}	3.69 ± 0.0 ^d	0.01*
14th week	3.35 ± 0.5	2.82 ± 0.5	2.98 ± 0.2	2.75 ± 0.2	3.40 ± 0.7	2.89 ± 0.8	3.00 ± 0.7	3.61 ± 0.4	0.39

* P-value < 0.05 and there is significant difference.

^{a,b,c,d} Means with similar letter indices on the same row are not significantly different.

¹ See text for description of the dietary treatments Log = logarithm, CFU = Colonies forming unit

Table 5. Serum biochemistry chicken fed on different experimental diets

Variables ²	Dietary treatment groups ¹										p-value	
	TL0Ed	TL1Ed	TB0Ed	TB1Ed	TS0Ed	TS1Ed	T+	T-				
Glucose, mg/dL												
8th week	250.6 ± 70.4	220.80 ± 30.6	236.40 ± 90.6	216.00 ± 14.0	309.00 ± 6.8	244.80 ± 6.5	239.52 ± 5.4	271.80 ± 2.0	0.89			
14th week	273.60 ± 80.8	231.60 ± 92.1	275.40 ± 46.9	216.60 ± 22.8	211.62 ± 76.2	190.20 ± 75.2	234.72 ± 16.8	251.40 ± 19.1	0.65			
Total cholesterol, mg/dL												
8th week	139.21 ± 21.5	131.67 ± 39.4	190.26 ± 2.7	164.47 ± 48.4	166.15 ± 8.9	141.92 ± 49.6	116.53 ± 16.2	181.88 ± 46.8	0.18			
14th week	169.50 ± 52.9	147.46 ± 4.0	183.03 ± 31.7	167.83 ± 48.0	172.85 ± 18.1	151.19 ± 6.6	133.02 ± 2.0	187.03 ± 7.2	0.33			
Triglycerides, mg/dL												
8th week	153.52 ± 6.8	163.56 ± 12.6	180.98 ± 28.6	161.19 ± 17.4	174.78 ± 5.7	165.92 ± 13.3	164.44 ± 19.7	192.19 ± 17.0	0.13			
14th week	168.87 ± 1.0	153.52 ± 10.9	179.79 ± 21.8	160.61 ± 16.0	173.59 ± 0.9	157.95 ± 8.4	163.26 ± 11.0	190.43 ± 35.0	0.21			
AST, U/L												
8th week	131.00 ± 18.0	138.93 ± 58.3	145.28 ± 31.0	125.33 ± 53.1	140.33 ± 52.7	147.17 ± 38.7	133.83 ± 15.1	148.55 ± 35.0	0.99			
14th week	162.17 ± 14.5	144.67 ± 6.1	139.17 ± 6.8	147.83 ± 29.1	132.50 ± 9.0	148.17 ± 34.6	149.17 ± 28.1	171.00 ± 32.9	0.57			
ALT, U/L												
8th week	34.67 ± 0.5	33.00 ± 1.0	34.92 ± 0.9	31.67 ± 1.8	34.67 ± 1.5	32.0000 ± 2.7	32.33 ± 1.5	34.73 ± 0.9	0.52			
14th week	34.34 ± 2.1	32.28 ± 2.9	34.35 ± 2.5	33.35 ± 1.5	34.68 ± 0.8	33.87 ± 0.6	33.87 ± 1.2	36.30 ± 0.4	0.31			
ALP, U/L												
8th week	55.87 ± 5.4	66.88 ± 37.1	50.18 ± 17.2	62.90 ± 13.4	51.87 ± 17.3	60.33 ± 10.1	54.20 ± 9.6	69.17 ± 4.7	0.19			
14th week	47.07 ± 7.5	51.30 ± 13.9	58.90 ± 3.5	44.80 ± 3.6	50.23 ± 6.5	46.47 ± 6.5	54.00 ± 0.0	59.03 ± 8.1	0.09			

¹See text for the description of the dietary treatment groups.²See text for the description of the variables.

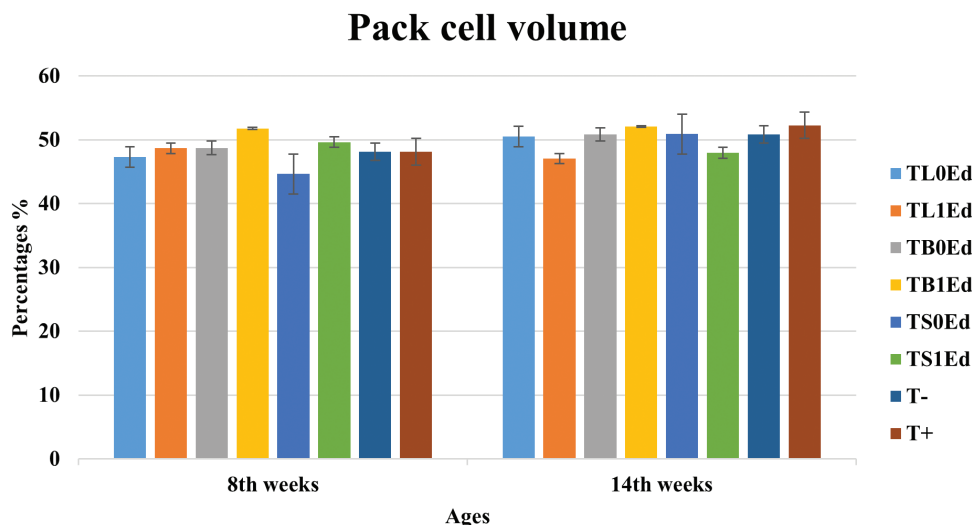


Figure 1. Pack cell volume of the experimental birds at 8th and 14th weeks.

production and reproduction (Sugiharto, 2016). The use of the antibiotics has, thus, been justifiable; however, prolonged use as well as failure to use them as directed has resulted in health, nutritional, and environmental problems (Mehdi et al., 2018). For instance, there has been a notable increase in the cost of the antibiotics resulting in increased cost of livestock feeds. The growing concerns of AMR and the decreasing cost benefits of using antibiotics has led many countries to ban their use in animal feeds (Ragland et al., 2018). The ban has resulted in an increase in search for safer alternatives (Fasanmi et al., 2014).

Plant extracts have been shown to have antimicrobial characteristics implying that they potential to serve as growth promoters in animal feed production. Furthermore, they have traditionally been used to treat human and animal diseases in many parts of the world.

Africa has traditionally relied on plant based therapies to treat human and animal diseases besides supplying some limiting nutrients. Many of these plants and their corresponding extracts have not been studied for efficacy and safety. This has hampered their increased adoption in mainstream human and health diseases management and possible commercialization. The main problem is further complicated by the fact that information on the use of the plants use is not documented (Abdullahi, 2011). In most African communities, the information was maintained within families and passed on from one generation to the next through trusted family members who would safeguard the information on the plant and the ailment it treated from getting out of the family (Abdullahi, 2011). With an increase in use of conventional human and animal

health medicine, the indigenous medicine knowledge has become less important and the need to keep the information within families has reduced. This has led to an increased sharing of the information and has attracted researchers from human and animal health sciences (Zeng et al., 2015; Bwana et al., 2016).

The African Safou plant which is scientifically known as *D. edulis* plant has attracted the attention of the researchers in Africa due to its traditional use in treating many human ailments as described by Ebana et al. (2016) and its availability in many West African countries (Nwokonkwo, 2014). There exists little information on the plant use in managing animal diseases and correcting nutritional deficiencies in Africa and its use in treating human ailments (Ebana et al., 2016) and its reported nutritional characteristics (Vidanarachchi et al., 2005) informed its choice in this study. Determination of product efficacy and safety is the first step in considering its use in animal feed or human food chain (Okolo et al., 2016). A phytochemical analysis is a good starting point in testing the efficacy and safety. This is because the presence of specific phytochemical contents can explain the product action or reaction. An analysis of the *D. edulis* plant parts was carried and the results are as shown Table 2. Clearly, there were differences in the phytochemical composition in different *D. edulis* plant parts implying that its inclusion in animal feeds would dictate that the part with the highest microbial activity be harvested. Generally, all parts contained tannins, polyphenols, flavonoids, saponin, terpenoids, steroids, and alkaloids, but tested negative for anthraquinone and glycoside. These findings concur with those obtained by Aristimunha et al. (2016)

and Ogbe and Affiku (2012). Proportionately, the amounts determined from different plant parts in this study (see Table 2) compare well with those reported in other studies evaluating *D. edulis* phytochemical composition (Ogboru, 2015; Ebana et al., 2016; Okolo et al., 2016; Oyetunji and Opeyemi, 2017). These compounds have been noted to possess bactericidal, virucidal, fungicidal, and antiparasitical properties which can be exploited in pharmaceutical medicine or animal feed additives. Despite confirming the presence of these beneficial compounds in the *D. edulis* plant parts, their inclusion in chicken feeds requires further study on their impact on growth and body composition characteristics, blood chemistry, and microbiota count of the chicken fed on diets supplemented with the plant parts.

Starter and finisher diets were formulated to supply growing dual-purpose chicken with sufficient nutrients for growth and other body functions (see Table 1). For each of the two formulations, six experimental diets were prepared by either adding a 0.5% or 1% *D. edulis*' leaves, stem bark, and seed powder. Besides, negative and positive diets were made for comparison purposes. The diets were fed to a total of 288 dual-purpose chicken and their growth and body composition characteristics (see Table 3), blood chemistry (see Table 4), and microbiota count (see Table 5) determined. No parameter deviated unexpectedly, and in most cases, the values or trends obtained from this study matched those reported in other studies that tested the potential of different plants parts for use as source of animal growth promoter. For instance, Arif et al. (2019) showed that diets supplemented with humic acid and black cumin seeds did not significantly affect the length or weight of the intestine, liver, gizzard, heart, and spleen.

There were significant differences in FI, BWG, live weight, total weight viscera organs, and gizzard weight. In all cases, the differences were detected in the first 8 weeks of the chicken growth. The differences can be attributed to the increase in the FI as the extra nutrients would result in gains across different body components. The increase in FI can be explained by the fact that phytochemical feed additives are often claimed to improve the flavor, texture, and palatability of feed, thus increasing voluntary feed intake which could result in improved weight gain (Zeng et al., 2015). The corresponding insignificant change in FCR implies that the extra FI is beneficial to the chicken growth and body composition. Besides, the extra nutrients would be mobilized to counteract the negative impact related to

the excess of some anti-nutrients elements within the plant (Tannins, saponins and phytates). These antinutrients factors could influence feed intake by negatively impacting the bioavailability of minerals and protein digestibility (Ogbe and Affiku, 2012). Tannins, which have traditionally been considered as antinutritional factor, are widely present in medicinal plants especially when present in monogastric animal diets. Qianqian et al. (2018) noted that high concentrations tannins interfere with feed intake, nutrient digestibility because of their strong affinities with protein nutrients and others nutrients digestion, through over protecting protein nutrients, which lead to the decrease of intestinal microbial activity and inhibition of GIT digestive enzyme activities which decrease microflora activity and inhibit endogenous digestive enzyme activities thereby negatively impacting growth performance. Different levels of tannins were detected in the *D. edulis* in this study as presented in Table 2.

Khaksar et al. (2012) and Landy et al. (2011) noted that phytochemical feed additive have the potential to reduce pathogenic intestinal bacteria that produce toxins and/or compete with the host for available nutrients, resulting in better nutrient utilization and decreased feed conversion ratio. This was evidenced from the findings obtained from this study that indicated a significant decrease in colonies forming unit of *E. coli* and *Salmonella* sp. mainly during the first growth phase when the chicken would be more susceptible to microbial diseases. Similar results were obtained by Arif et al. (2018) from quail-fed diets supplemented with humic acid and black cumin seeds powder. Ebru et al. (2008) hypothesised that the reduction in *E. coli* and *Salmonella* sp. could emanate from production of bactericidal secretions and decreased intestinal pH resulting from inclusion of phytochemical additive in animal feeds. Besides, plant secondary metabolites in contact with pathogenic bacteria cell membrane strongly increase their hydrophobicity which modulates virulence properties of the microbe thus exposing them to plant secondary metabolite (Vidanarachchi et al., 2005).

The serum biochemistry is good indicator of metabolite diseases that might arise from feeding chicken diets contaminated with chemical substances (Okolo et al., 2016). Findings from this study presented indicated that inclusion of *D. edulis*' plant parts in the dietary treatments fed to the dual-purpose did not induce significant changes in glucose, total cholesterol, triglycerides, AST, ALT, and ALP. These findings are in agreement with those obtained by Arif et al. (2018), whereas

studying the effects of supplementing broiler feed with eucalyptus extracts on glucose, cholesterol, and triglycerides level. It is noteworthy that there were slight decreases in all the blood serum parameters tested in this study on increasing each of the plant part from 0.5% to 1% in all cases. The decrease in glucose, cholesterol, and triglycerides absorption could be attributed to phytochemicals (tannins, flavonoids, and phenolic) ability to inhibit carbohydrate digestive enzymes, enhanced insulin secretion, improved sensitivity of insulin, decreased intracellular pH, and scavenging of free radical (Ononamadu et al., 2019). A decrease in ALT, AST, and ALP serum levels in birds fed with diets incorporating *D. edulis* plant parts is a good attribute as high the parameters' high levels in chicken indicates liver failure as well as bile disorders Biasato et al. (2017).

The results for the PCV in the present study fell within the range reported by Ogbe and Affiku (2012) and Oleforuh et al. (2015). Generally, the PCV values from chicken fed with diets fortified with different parts and levels of *D. edulis* did not differ significantly which is desirable. Ogbe and Affiku (2012) observed that it was normal for chicken PCV to vary over a wide range the phenomenon being attributed to age and physiological status of the bird.

CONCLUSION

The findings from this study indicate that *D. edulis* contains tannins, polyphenols, flavonoids, saponin, terpenoids, steroids, alkaloids, but lacks anthraquinone, and glycoside. The presence and concentrations of the chemicals depends on the plant part. This implies that the part of the plant to be used as an additive in animal feeds would rely on the targeted chemical compound. The chemicals in *D. edulis* positively impacted on the FI, BWG, live weight, total weight viscera organs, and gizzard weight in the early age of the dual-purpose male chicken. Increased in FI is desirable in fast growing animals as it accelerates biomass accumulation resulting in a quicker investment turnover. An analysis of the serum indicated that the chemical compounds found in *D. edulis* do not significantly alter the acceptable biochemical ranges which would have negative impact on growth, health, and general welfare of the chicken-fed diets fortified with the plant parts. Further, findings from this study provide an evidence that inclusion of *D. edulis* plant parts in chicken diets resulted in significant decrease in colonies forming unit of *E. coli* and

Salmonella sp. mainly during the first growth phase when the chicken would be more susceptible to microbial diseases. Diseases have been identified as a major constraint to increased poultry production and profitability and any sustainable and cheaper approach to eradicating or reducing this negative impact would be plausible. Considering all the findings obtained from this study on using *D. edulis* in poultry feed formulations point to the plants potential for use as an additive in chicken diets but further confirmatory studies are desirable.

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LITERATURE CITED

- Abdullahi, A. 2011. Trends and challenges of traditional medicine in Africa. Afr. J. Tradit. Complement. Altern. Med. 8:115–123. doi:10.4314/ajtcam.v8i5S.5.
- Anonymous. 2016. Reflotron® Plus Information Booklet. Stockport (Cheshire): Bio-Stat Healthcare Group; p. 1–28.
- AOAC. 1990. AOAC official methods of analysis, 15th ed. (vol. 1). Washington (DC): Association of Official Agricultural Chemists. p. 136–138.
- AOAC. 2005. Official Methods of Analysis. 18th Edn, Washington DC: Association of Officiating Analytical Chemists.
- Arif, M., Z. Hayat, M. E. A. El-hack, M. Saeed, H. M. Imran, and A. N. Alowaimer. 2019. Impacts of supplementing broiler diets with a powder mixture of black cumin *Moringa* Chicory seeds. S. Afr. J. Anim. Sci. 49(3):564–572. doi:10.4314/sajas.v49i3.17
- Arif, M., A. Rehman, M. E. A. El-hack, M. Saeed, F. Khan, M. Akhtar, and A. N. Alowaimer. 2018. Growth, carcass traits, cecal microbial counts, and blood chemistry of meat-type quail fed diets supplemented with humic acid and black cumin seeds. Asian-Australas. J. Anim. Sci. 31(12):1930–1938. doi:10.5713/ajas.18.0148.
- Aristimunha, P. C., A. P. Rosa, L. S. Boemo, D. C. Garcez, D. P. Rosa, A. Londero, and J. Forgiarini. 2016. A blend of benzoic acid and essential oil compounds as an alternative to antibiotic growth promoters in broiler diets. J. App. Poultry Res. 25(4):455–463. doi:10.3382/japr/pfw015
- Audu I. S., S. T. Ubwa, O. G. Igbum, and S. A. N. Hati. 2017. Analytical comparison between microhematocrit and automated methods for packed cell volume (PCV) determination. Int. J. Hematol. Blo. Dis. 2(1):1–4. doi:10.15226/2639-7986/2/1/00105
- Biasato, I., L. Gasco, M. De Marco, M. Renna, L. Rotolo, S. Dabbou, and A. Schiavone. 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology Effects of yellow mealworm

- larvae (*Tenebrio molitor*) inclusion in diets for female broiler chicken. *Anim. Feed Sci. Technol.* 234:253–263. doi:10.1016/j.anifeedsci.2017.09.014
- Bratte, L. 2010. Effect of partial replacement of dietary maize with African Pear (*Dacrydium edulis*) seed meal on performance, nutrient digestibility and retention of broiler chickens in the humid tropics. *Asian J. Anim. Sci.* 5(2):127–135. doi:10.3923/ajas.2011.127.135
- Bwana, M. O., L. W. Njagi, P. N. Nyaga, P. G. Mbuthia, L. C. Bebor, M. W. Wahome, W. U. Mutinda, and P. M. Kitale. 2016. Growth promotive effects of neem and stinging nettle in indigenous chickens in Kenya. In: Fifth African Higher Education Week and RUFORUM Biennial Conference 2016, “Linking agricultural universities with civil society, the private sector, governments and other stakeholders in support of agricultural development in Africa, Cape Town”; October 17 to 21, 2016; South Africa.
- Crhanova, M., H. Hradecka, M. Faldynova, M. Matulova, H. Havlickova, F. Sisak, and I. Rychlik. 2011. Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar enteritidis infection. *Infect. Immun.* 79(7):2755–63. doi:10.1128/IAI.01375-10.
- Ebana, R., U. Edet, U. Ekanemesang, G. Ikon, J. Ekpenyong, N. Ntukidem, and N. Brown. 2016. Comparison of antimicrobial activity and phytochemical screening of seeds and testas of *Dacryodes edulis* and *garcinia kola*. *J. Adv. Microbiol.* 1(3):1–7. doi:10.9734/JAMB/2016/31173.
- Ebana, R., U. Edet, U. Ekanemesang, G. Ikon, E. Umoren, N. Ntukidem, and N. Brown. 2017. Proximate composition and nutritional analysis of seeds and testas of *dacryodes edulis* and *garcinia kola*. *Asian J. Biol.* 2(1):1–8. doi:10.9734/AJOB/2017/31159
- Ebru, U., U. Burak, S. Yusuf, B. Reyhan, K. Arif, T. H. Faruk, and E. Kemal. 2008. Cardioprotective effects of nigella sativa oil on cyclosporine a-induced cardiotoxicity in rats. *Basic Clin. Pharmacol. Toxicol.* 574–580. doi:10.1111/j.1742-7843.2008.00313.x
- Engberg, R. M., M. S. Hedemann, S. Steinfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poult. Sci.* 83:925–938. doi:10.1093/ps/83.6.925.
- Fasanmi, O. G., I. O. Akanbi, I. F. Adekunle, G. E. O. Makinde, and F. A. Fagbohun. 2014. Comparing the phytobiotic effects of feed-grade and liquid Oregostim® on performance characteristics, haematology and serum biochemistry of finisher broilers. *Sch. J. Agric. Vet. Sci.* 1(3):137–142.
- Gikunju, M., L. Kabuage, A. Wachira, G. Oliech, and M. Gicheha. 2018. Evaluation of pure breeds, cross-breeds and indigenous chicken egg quality traits in Kenya. *Livestock Res. Rural Develop.* 30(170). <http://www.lrrd.org/lrrd30/10/giche30170.html>
- Kadji, B., R. Léonce, K. Fankroma, M. Thierry, and S. A. Emmanuelle. 2016. Physico-chemical properties of Safou (*Dacryodes edulis*) fruits grown in Côte d’Ivoire. *J. Appl. Biosci.* 10103–10110.
- KALRO. 2018. Kenya agriculture and livestock research organisation. Kenya:Non-ruminant Research Institute Naivasha Centre. p. 4–5.
- Kaluli, J. W., C. Githuku, P. Home, and B. M. Mwangi. 2011. Towards a national policy on waste water re-use in Kenya. *J. Agric. Sci. Technol.* 13(1): 116–125.
- Khaksar, V., M. V. Krimpen, H. Hashemipour, and M. Pilevar. 2012. Effects of Thyme essential oil on performance, some blood parameters and ileal microflora of Japanese quail. *J. Poultry Sci* 49(2):106–110. doi:10.2141/jpsa.011089.
- Landy, N., G. Ghalamkari, and M. Toghyani. 2011. Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. *Livestock Sci.* 142(1–3):305–309. doi:10.1016/j.livsci.2011.08.017
- Madhurima, D., R. Sarmistha, S. D. Shahid Jamal, and. 2014. Study of phytochemical constituents and antibacterial activity of *calotropis gigantea*. *Int. Sci. J.* 4(4):15–28.
- Mehdi, Y., M. M. P. Letourneau, M. L. Gaucher, Y. Chorfi, G. Suresh, T. Rouissi, S. K. Brar, C. Cote, and S. G. Antonio Avalos Ramirez. 2018. Use of antibiotics in broiler production : global impacts and alternatives. *Anim. Nutri.* 4:170–178. doi:10.1016/j.aninu.2018.03.002.
- Nayim, P., A. T. Mbaveng, B. E. N. Wamba, A. G. Fankam, J. K. Dzutam, and V. Kuete. 2018. Antibacterial and antibiotic-potentiating activities of thirteen cameroonian edible plants against gram-negative resistant phenotypes. *ScientificWorldJournal.* 2018:4020294. doi:10.1155/2018/4020294
- Nkukwana, T. T. 2012. The effect of *Moringa oleifera* leaf meal on growth performance, gut integrity, bone strength, quality and oxidative stability of meat from broiler chickens. *S. Afr. J. Anim. Sci.* 44(3):228–239. doi:10.4314/sajas.v44i3.4.
- Nwokonkwo, D. C. 2014. The phytochemical study and antibacterial activities of the seed extract of *Dacryodes edulis* (African native pear). *Am. J. Sci. Indus. Res.* 5:7–12. doi:10.5251/ajsir.2014.5.1.7.12
- Ogbe, A. O., and J. P. Affiku. 2012. Effect of polyherbal aqueous extracts (*Moringa oleifera*, gum arabic and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chickens. *Res. J. Recent Sci.* 1(7):10–18.
- Ogboru RO, O. P. and A. I. 2015. Phytochemical screening and medicinal potentials of the bark of *Dacryodes edulis* (G. Don) HJ Lam. *J. Environ. Anal. Chem.* 02(05):2–4. doi:10.4172/2380–2391.1000158.
- Okolo, C. A., V. C. Ejere, C. O. Chukwuka, I. I. Ezeigbo, D. Daniel, A. N. Okorie, and E. State. 2016. Hexane extract of *Dacryodes edulis* fruits possesses anti-diabetic and hypolipidaemic. *Afr. J. Tradit Complement Altern. Med.* 13:132–144. doi:10.21010/ajtcam.v13i4.18
- Oleforuh-Okoleh, V. U., H. M. Ndofor-Foleng, S. O. Olorunleke, and J. O. Uguru. 2015. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. *J. Agric. Sci.* 7(4):167. doi:10.5539/jas.v7n4p167
- Ononamadu, C. J., A. J. Alhassan, A. Ibrahim, A. A. Imam, G. O. Ihegboro, T. A. Owolarafe, and M. S. Sule. 2019. Methanol-extract / fractions of *dacryodes edulis* leaves ameliorate hyperglycemia and associated oxidative stress in streptozotocin-induced diabetic wistar rats. *J. Evid. Based Integr. Med.* 24:1–12. doi:10.1177/2515690X19843832.
- Oyetunji, O., and A. Opeyemi. 2017. Antibacterial and antioxidant activities of *Dacryodes edulis* methanolic leaf

- extract. *J. Adv. Med. Pharm. Sci.* 14(1):1–11. doi:[10.9734/JAMPS/2017/33831](https://doi.org/10.9734/JAMPS/2017/33831).
- Qianqian, H., X. Liu, G. Zhao, T. Hu, and Y. Wang. 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Anim. Nutr.* 4(2):137–150. doi:[10.1016/j.aninu.2017.09.004](https://doi.org/10.1016/j.aninu.2017.09.004)
- Ragland, W. L., M.P. Zlatko, and I. K. Franciosini. 2018. Antibiotic growth promoters in poultry, and their potential alternatives. *Zagreb Biotek d.o.o. Frane Petrinovica 1, 21400 Supetar, Croatia. 21400 Supe(1): 1–8.*
- Ranjitkar, S., B. Lawley, G. Tannock, and R. M. Engberg. 2016. Bacterial succession in the broiler gastrointestinal tract. *Appl. Environ. Microbiol.* 82:2399–2410. doi:[10.1128/AEM.02549-15](https://doi.org/10.1128/AEM.02549-15).
- Riwom, S., F. Foe, M. Nyegue, R. Wanki, S. Ologu, and F. X. Etoa. 2015. Chemical composition and in vitro antibacterial activity of the essential oils of the leaves, resin and stem-barks of *Dacryodes edulis* (G. Don) H. J Lam growing in cameroon on diarrhea associated strains. *J. Appl. Pharm. Sci.* 5(10):006–011. doi:[10.7324/JAPS.2015.501002](https://doi.org/10.7324/JAPS.2015.501002).
- Sugiharto, S. 2016. Role of nutraceuticals in gut health and growth performance of poultry. *J. Saudi Soc. Agric. Sci.* 15(2):99–111. doi:[10.1016/j.jssas.2014.06.001](https://doi.org/10.1016/j.jssas.2014.06.001).
- Valenzuela-Grijalva, N. V., A. Pinelli-Saavedra, A. Muhlia-Almazan, D. Domínguez-Díaz, and H. González-Ríos. 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. *J. Anim. Sci. Technol.* 59:8. doi:[10.1186/s40781-017-0133-9](https://doi.org/10.1186/s40781-017-0133-9)
- Vidanarachchi, J. K., L. L. Mikkelsen, I. Sims, P. A. Iji, and M. Choct. 2005. Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds. *Recent Adv. Anim. Nutr. Austr.* 15(Kamel 2001): 131–144.
- Xavier, S. M., and D. Umadevi. 2014. Hepatoprotective effect of gotu kola (*Centella asiatica* Linn.) on carbon tetrachloride induced liver injury in rats. *Int. Res. J. Pharm.* 5(12):929–931. doi:[10.7897/2230-8407.0512189](https://doi.org/10.7897/2230-8407.0512189).
- Yelwa, A. S., H. E. Mshelia, O. Cyril, I. Lawal, H. B. Bature, and A. Shamsiya. 2017. Phytochemical screening, acute toxicity study and evaluation of *in vitro* antimicrobial activities of the fractions of *Dacryodes edulis* against selected clinical bacterial isolates. *J. Pharmacogn. Phytochem.* 6(4):1910–1915.
- Zeng, Z., S. Zhang, H. Wang, and X. Piao. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *J. Anim. Sci. Technol.* 6(1):7. doi:[10.1186/s40104-015-0004-5](https://doi.org/10.1186/s40104-015-0004-5)