Dosage Effects of Aqueous Extract of Baobab Tree (*Adansonia digitata* Linn) Bark on Growth Performance, Blood Profile, Intestinal Morphology and Microflora of Cockerel Chickens

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RESEARCH ARTICLE

Abstract

This experiment investigated the dosage effects of the aqueous extract of Baobab Tree Bark (AEBTB) on growth performance, blood profile and intestinal micro-flora of cockerel chickens for 16 weeks. A total of 200 Isa brown day-old cockerel chicks were used in groups of AEBTB (0, 300, 325, 350 and 375 mg/ litre of water) for the experiment. Qualitative and quantitative (mg/100 g) phytochemical screening revealed that AEBTB contained flavonoid (36.33 mg), cardiac glycoside (31.46 mg), saponin (23.26 mg), alkaloid (24.86 mg), tannin (19.28 mg) and phenolic (17.06 mg). The most common components in GS-MS was 9-Octadecenoic acid (C₁₉H₃₆O₂; 296.0 g/mol). At the chick phase, significantly ($p < 0.05$) highest final weight (416.50 g/bird) and weight gain of 47.69 g/bird/day were recorded in birds on 375 mg/litre when compared with the control. Alkaline phosphatase, RBC, Hb, PCV and MCHC were significantly ($p < 0.05$) reduced by AEBTB in the birds when compared with the control and the lowest total bacterial count was in birds on 375 mg/litre of AEBTB. However, at the grower phase, birds on 300 mg/litre AEBTB had the best ($p < 0.05$) feed conversion ratio. The study concluded that AEBTB at 300 mg/litre improved health status and growth performance of meat-type chickens.

Keywords: *Adansonia digitata* bark extract; Performance; Phytochemicals; Gas Chromatography Mass Spectrometry; Intestinal microflora; Blood Profile.

INTRODUCTION

In the past, antibiotics have been used as feed additives to improve growth performance, egg production and to protect animals from pathogenic micro-organisms. The ban on the use of synthetic antibiotics as growth-promoting substance led to increase in research on organic acids and phytobiotics that are prebiotic and probiotic in nature (Patterson and Burkholder, 2003). These organic acids should be able to influence animal health and improve productivity. In spite of these promising medicinal values of *Adansonia digitata* bark, its full economic potentials have not been fully explored particularly in poultry production.

The Baobab fruit pulp shows interesting properties in the stimulation of the intestinal microflora growth. The hydrosoluble fraction of the fruit pulp has stimulating effects on the proliferation of bifido bacteria in *in-vitro* assays (Baobab Fruit Company, 2008). Soluble dietary fibres, as those contained in the pulp (about 25%), are known to have prebiotics effects stimulating the growth and/or the metabolic activity of beneficial organisms (BFCS, 2008). *Adansonia digitata* root-bark and leaf methanol extracts have shown high antiviral activity against Herpes simplex, Sindbis and Polio (Anani et al., 2000), together with viricidal (direct inactivation of virus particles) and also intracellular antiviral activity, which could indicate the presence of multiple antiviral compounds, or a single compound with multiple actions (Hudson et al., 2000).

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Extracts of baobab roots eliminate the motility in *Trypanosoma congolense* within 60 minutes and drastically reduce motility in *T. brucei* and *T. congolense* which are the causative agent of sleeping sickness in humans and related diseases in animals (Atawodi et al., 2003).

The aqueous extract of the tree bark also has growth-stimulating effect and antibacterial properties. It is often given to infants to promote weight gain (Lockett and Grivetti, 2000) and had been found to be high in fat, calcium, copper, iron, and zinc (Lockett and Grivetti, 2000). In addition, it contains betulinic acid, alkaloid 'adansonin', (Odetokun, 1996; Sidibe and Williams, 2002). Stem and root barks of baobab contain bioactive constituents which are responsible for antimicrobial activity of the crude aqueous and ethanolic extracts. This explains the scientific basis for the use of crude stem and/or root bark extracts in traditional medicine (Masola et al., 2009).

The rural small resource-poor poultry farmers engage more in the rearing of cockerel chicken than broiler chicken because of low cost of production and lower susceptibility to disease. It is the male (under one year) of the hen from the egg-type chicken. Nowadays, the rearing of cockerel chicken is been given prominence unlike when it was been disposed of with as hatchery waste together with unhatched incubator eggs due to its hardness and relatively high survival rate, though its production does not appear profitable due to its relatively long maturity period (Sogunle et al., 2013). Unlike in spent hens whose live weight decreases with increasing egg production and age, the live weight of a cockerel increases with age (Okosun, 1987). This study therefore investigated the potentials of aqueous extract of baobab tree bark on growth performance, blood profile, intestinal morphology and microflora of cockerel chickens.

MATERIALS AND METHODS

Fresh Boabab tree (*Adansonia digitata* L) bark was collected from the forest area of Abeokuta, Ogun State, Nigeria. The bark was chopped into smaller pieces and air-dried to reduce the moisture content to about 10%. The dried Baobab tree bark was further ground into powdery form.

A total of 100 g powdered baobab bark sample was dissolved in 750 mL water for 48 hours in order to extract the polar compounds (Elgorashi and Van Staden, 2004) and then filtered using muslin cloth. The extract was then oven dried to reduce the moisture at 80°C inside hot air oven. The dried extract (88 g) was then ground into powdery form and stored in an air-tight plastic at room temperature for further use.

Experiment 1 was carried out at the Biochemistry Department Laboratory, and Animal Research Laboratory, Department of Pharmacology, College of Medicine, University of Lagos, Lagos. The Gas Chromatography Mass Spectrometry (GCMS) was carried out at Shimadzu Training Centre for Analytical Instruments (STC), Federal Institute of Industrial Research (FIRO), Lagos State, Nigeria. Experiment II was carried out at the Poultry Unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on altitude of 127 m, latitude 7° 13' N and longitude 3° 26' E (Google Map, 2019).

Chemical tests were carried out on the aqueous extract of Baobab tree bark using standard procedures to identify the constituents as earlier described (Harborne, 1973; Sofowora, 1993; Madhu et al., 2016).

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites (saponins, alkaloids, phenolics content, tannins and flavonoids content) present in aqueous extract of baobab tree bark- AEBTB (Harborne, 1993; Trease and Evans, 1989; Madhu et al., 2016).

Gas Chromatography – Mass Spectrometry (GC-MS) technique was used to identify the phyto-components present in AEBTB. The GC-MS used was a Shimadzu QP2010PLUS system. All the conditions used in GC-MS method were recorded in (Table 1). Identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology library and by direct comparison with published data (Van Den Dool and Kratz, 1963).

Table 1: GC-MS Method

Model:	GCMS QP2010 SE Shimadzu
GC Condition:	
Column Oven temperature:	60°C
Injection port temperature:	250°C
Injection Mode:	Split (3:1)
Column Oven temperature program:	60°C by 15°C/min to 120°C (hold time 2min) by 15°C/min to 300°C (hold time 4min)
Column Type:	Optima 5MS 30m*0.25mm*0.25um
MS Condition:	
Ion Source Temperature:	200°C
Interface Temperature:	250°C
Detector Voltage:	Relative to tuning result
Acquisition Mode:	SCAN
Scan range:	45 – 700

The total phenolic content of AEBTB was estimated using Folin-Ciocalteu reagent by the method of Makkar et al. (2007). About 20 µg of AEBTB was taken and made up to 1 mL with distilled water. Then 500 µL of diluted Folin-phenol

reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na_2CO_3 (20%) was added. After incubation for 40 minutes in dark condition, the absorbance was measured at 750 nm. A calibration curve of gallic acid was constructed and linearity obtained in the range of 10-50 $\mu\text{g/mL}$. The total phenolic content of the AEBTB extracts was expressed as mg of gallic acid equivalent (mg GAE/100 g extract) by using the standard curve.

For flavonoids, a 5 mL sample of the extract dissolved in 3 mL of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 mL of Dragendroff's reagent were added to 1 mL of the filtrate and turbidity was observed. The filtrate of AEBTB was used for the test as follows: 5 mL of dilute ammonia solution was added to AEBTB aqueous filtrate followed by the addition of concentrated H_2SO_4 . A yellow colouration observed in the extract indicated the presence of flavonoids. The percentage of flavonoid was calculated as % Flavonoid = (Weight of flavonoid*100)/Weight of sample

The method described by Madhu et al. (2016) was used for steroids determination. Acetic anhydride (2 mL) was added to 0.5 g AEBTB extract and 2 mL H_2SO_4 . The colour changed from violet to blue or green in some samples indicated the presence of steroids. The absorbance was at 780 nm against the reagent blank.

A sample of 0.5 g AEBTB was boiled gently in 20 mL of water for 1 hour in a test tube and then filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 of distilled volumetric flask. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration which indicated the presence of tannin. Varying concentrations (0.2 – 1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 cm^3) and saturated Na_2CO_3 (10 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C. Optical density was ascertained at 700 nm. Optical density (absorbance) versus tannic acid concentration was plotted. The following formula was used in the calculation:

$$\text{Tannic acid (mg/100 g)} = (\text{C} \times \text{extract volume} \times 100) / (\text{aliquot volume} \times \text{weight of sample})$$

Where C is concentration of tannic acid read off the graph.

For saponin, AEBTB (2 mL) was boiled in 20 mL of distilled water in a water bath and filtered. About 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then the formation of emulsion was observed. The saponin content was calculated as a percentage: % Saponins = (Weight of saponins*100)/ weight of sample

AEBTB (0.5 mL) was boiled with 2 mL of 1% aqueous hydrochloric acid for 10 minutes. Deposition of a red precipitate indicates the presence of phlobatannin.

AEBTB (5 mL) was mixed with 50 mL of 95% ethanol for 24 hours. The filtrate was extracted with petroleum ether (60 to 80° C) and concentrated to dryness. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

For cardiac glycosides (Keller-Killani test), AEBTB (5 mL) was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution and cooled. After cooling, 2-3 drops of ferric chloride were added. This was underlain with 1 mL of concentrated sulphuric acid. A reddish brown ring of the interface indicated a deoxysugar characteristic of cardenoloides. A violet ring appeared below the reddish brown ring, while in the acetic acid layer, a greenish ring formed just gradually throughout thin layer.

To 1 mL AEBTB, 20 mL of chloroform was added. This was heated in steam bath for 5 minutes. The extract was filtered while hot and allowed to cool. An equal volume of 10% ammonia solution was added to the filtrate. This was shaken and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of Anthraquinones.

A total of two hundred Isa brown day-old cockerel chicks were used for the experiment. The birds were divided into five AEBTB treatment groups (0 mg/litre, 300 mg/litre, 325 mg/litre, 350 mg/litre and 375 mg/litre three times a week, respectively) of forty birds each. Each treatment was further divided into four replicates comprising of 10 birds each in a Completely Randomized Design and were brooded on replicate basis from day-old for two weeks. All routine management practices were adhered to during the experiment which lasted 8 weeks. No vaccination and medication were given to the birds on AEBTB in the course of the experiment. Chicks mash (Table 2) and water were given *ad libitum* to the birds for the period of the experiment. Performance indices including body weight gain, feed intake, mortality and feed efficiency per replicate were recorded on a weekly basis.

Blood samples (5 mL) were obtained from brachial vein of 2 birds per replicate via the wing vein and emptied into 2 sets of well-labelled bottle; one containing Ethylene Diamine Tetra Acetate (EDTA) as an anti-coagulant while the other contained no anti-coagulant. The samples were analysed in the laboratory for haematological parameters and biochemical measurements.

Faecal swap samples were taken from 2 birds per replicate for microbial load count and Identification according to Cowan (1974). Biochemical identification tests were done to further characterize the bacteria isolates according to World Health Organization (WHO, 1983) manual for laboratory investigation of acute enteric infections.

Table 2: Gross composition and proximate composition (%) of chick's mash and growers mash for cockerel chickens

Ingredients	Chick mash	Growers mash
Maize (Yellow maize)	45.00	45.75
Soyabean Meal	11.70	8.00
Wheat offals	18.00	26.60
Oyster shell	3.00	6.00
Bone meal	2.00	2.00
Groundnut cake	9.60	
Palm kernel cake	8.00	11.00
Fish meal	2.00	
Salt (NaCl)	0.25	0.25
Lysine	0.10	0.10
Methionine	0.10	0.10
Vitamin and Mineral Premix *	0.25	0.25
Total	100.00	100.00
Determined Proximate Analysis:		
Carbohydrate (%)	33.20	56.32
Protein (%)	21.10	19.61
Crude fat (%)	4.20	5.19
Moisture (%)	5.72	8.45
Ash (%)	12.10	3.71
Crude Fibre (%)	9.37	6.48
ME (Kcal/100g)	254.50	352.43

Vit. A 12,500,000 iu; Vit. D₃ 2, 5000,000 iu; Vit E 40,000mg; Vit. K 32,000mg; Vit. B₁ 3,000 mg; Vit. B₂ 5,500 mg; Niacin 55,000 mg; Calcium Pantothenate 11, 5000 mg; Vit. B₆ 5,000 mg; Vit. B₁₂ 25 mg; Choline Chloride 500,000 mg; Folic acid 1,000 mg; Biotin 80 mg; Manganese 120,000 mg; Iron 100,000 mg; Zinc 80,000 mg; Copper 8,5000 mg; Iodine 1,500 mg; Cobalt 300 mg; Selenium 120 mg; Anti-oxidant 120,000 mg.

ME (kCal/100 g) = Metabolizable energy

ME = 4*(Carbohydrate + Protein) + 9*Crude fat (Source: Merrill and Watt, 1973).

All data in phase 1 of the experiment were also taken in the second phase. However, a total of ninety-six cockerels were randomly selected from the AEBTB treatment groups in phase 1 while 24 birds were selected from the control group and used in the grower phase. The birds were subjected treatment groups of AEBTB (as in phase 1) but containing 8 birds of 3 replicates each. All management practices were adhered to while the birds were managed intensively for the period of the experiment. Growers mash (Table 2) was given to the bird's *ad libitum*. Fowl pox vaccine was administered to the birds before the beginning of the 2nd phase of the experiment.

Data were subjected to One-way Analysis of Variance (ANOVA) in a Completely Randomized Design. Significantly (P<0.05) different means among variables were separated by Tukey test as contained in Minitab[®] version 17.1.0 (Minitab, 2013).

RESULTS AND DISCUSSIONS

Table 3 shows that saponin, alkaloids, phlobatanin, tannin, phenol, flavonoid and cardiac glycoside were phytochemical components of AEBTB while steroids, terpenoid and anthraquinone were absent in AEBTB.

Table 3: Phytochemical components of Baobab Tree Bark Extract

Compounds	Availability
Saponin	+
Alkaloids	+
Phlobatanin	+
Tannin	+
Phenol	+
Steroids	-
Flavonoids	+
Cardiac glycoside	+
Terpenoid	-
Anthraquinone	-

+ Present; - Absent

The quantification of phytochemical composition of AEBTB (mg/100 g) is presented in Table 4. AEBTB extract has higher concentration of flavonoid (36.33 mg) and cardiac glycosides (31.46 mg). It also contained 23.26 mg saponin, 24.86 mg alkaloid, 19.28 mg tannin and 17.06 mg phenol.

Table 4: Quantitative estimation of the phytochemical constituents of Baobab Tree Bark Extract

Compounds	Quantitative (mg/100g)
Saponin	23.26
Alkaloids	24.86
Phlobatanin	ND
Tannin	19.28
Phenol	17.06
Steroids	-
Flavonoids	36.33
Cardiac glycoside	31.45
Terpenoid	-
Anthraquinone	-

ND = Not determined

- Absent

The result of the gas chromatography-mass spectroscopy (GC-MS) analysis of the AEBTB identified the various compounds. These results are in line with the findings of Shukla et al. (2001) who reported that varieties of chemicals have been isolated and characterized from *A. digitata* which includes flavonoids, alkaloids, saponin and phlobatannin. The result however contradicted the reports by Abiona et al. (2015) that aqueous baobab tree (*Adansonia digitata*) leave extract contained steroids, terpenoids and anthraquinones. This could be due to the difference in geographical location and the parts of the baobab tree. Unlike the present study, Sugandha et al. (2014) reported reduced number of metabolites in aqueous AEBTB. These metabolites according to Narenda et al. (2012) and Vishnu et al. (2013) were reported to have many therapeutic properties.

In Table 5, the active compounds, area of peaks concentration percentage, retention time, molecular weight and formulae are presented. The most common components and highest peak in the ethyl acetate fraction as identified by GC-MS was 9-Octadecenoic acid. The GC-MS spectrum gives the structure of the compound, molecular formula ($C_{19}H_{36}O_2$) and molecular weight of 296 g. This phenolic hydroxyl group has a wide range of cellular activities (Geeta and Padma, 2013).

Table 5: Components in ethyl acetate fraction of Baobab Tree Bark Extract by GC-MS analysis

Peak	Retention Time	Area (%)	Chemical formula	Molecular weight (g/mol)	Name of Compound
1	4.832	0.20	$C_6H_{12}S$	116	Cyclohexanethiol
2	5.784	0.15	$C_8H_{12}O_4$	172	Diethyl maleate
3	7.618	0.21	$C_7H_{12}O_2$	128	Butyl acrylate
4	8.553	0.07	$C_{16}H_{28}O_2$	252	Hydnocarpic acid
5	9.145	0.10	$C_9H_{16}O_4$	188	Azelaic acid
6	11.027	0.25	$C_{10}H_{18}O_2$	170	1-Octen-3-yl acetate
7	11.216	0.30	$C_9H_{12}O_4$	184	3-Methoxy-4-hydroxyphenylglycol
8	11.716	0.37	$C_{22}H_{32}O_2$	328	Docosahexaenoic acid
9	12.066	0.21	$C_{18}H_{34}O_2$	282	Oleic acid
10	12.529	0.21	$C_{13}H_{26}O$	198	1-Methylcyclododecanol
11	13.618	0.37	$C_{13}H_{26}O$	198	Tridecan-2-one
12	14.107	0.88	$C_{18}H_{32}O_2$	280	Linoleic acid
13	14.778	1.62	$C_{19}H_{36}O_2$	296	Methyl oleate
14	15.303	1.10	$C_{18}H_{35}NO$	281	9-Octadecenamide
15	15.660	1.45	$C_{35}H_{68}O_5$	568	2-(Decanoyloxy)-3-hydroxypropyl docosanoate
16	15.938	4.18	$C_{15}H_{30}O_3$	258	Methyl 3-hydroxytetradecanoate
17	16.349	4.19	$C_{18}H_{35}NO$	281	9-Octadecenamide
18	16.469	1.66	$C_{18}H_{35}NO$	281	9-Octadecenamide
19	16.668	6.49	$C_{18}H_{33}ClO$	300	13-Octadecenoic acid chloride
20	16.791	2.40	$C_{21}H_{42}O_4$	358	2,3-Dihydroxypropyl octadecanoate
21	16.995	16.88	$C_{18}H_{32}O_2$	280	Octadecadienoic acid
22	17.057	6.13	$C_{15}H_{30}O_3$	258	Methyl 3-hydroxytetradecanoate
23	17.125	9.36	$C_{19}H_{38}O_4$	330	Propylene glycol monopalmitate
24	17.625	2.65	$C_{35}H_{68}O_5$	568	Glyceryl 1,3-dipalmitate
25	17.855	4.22	$C_{15}H_{34}OSi$	258	1-Triethylsilyloxynonane
26	18.090	20.12	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid
27	18.177	6.00	$C_{21}H_{42}O_4$	358	Glyceryl monostearate
28	18.544	6.53	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid
29	18.656	1.62	$C_{11}H_{16}O_4$	212	3,9-Diethylidene-2,4,8,10-tetraoxaspiro (5.5) undecane
30	20.800	0.12	$C_{35}H_{52}$	472	2-Tert-butyl-10-nonyl-9-octylanthracene

These compounds show a wide spectrum of action which includes anti-tumour, antiviral, antibacterial and cardio-protective (Rice-Evans et al., 1996; Chen et al., 1998; Wang et al., 2008). Ultee et al. (2002) also reported that the presence of hydroxyl group plays an important role in antimicrobial activity. This attest to the fact that AEBTB may be used as an alternative to antibiotics.

Table 6 shows the effect of AEBTB on growth performance of cockered chicks. At the chicks' phase, the dosage of administration significantly ($P<0.05$) influenced final weight, daily weight gain and mortality of the cockerels. The weight gain ranged from 34.16 g/bird/day to 47.69 g/bird/day and it followed the same trend in the final weight gain. The mortality ratio was highest in the birds on 375 mg/L and lowest in birds on the control. However, at the growing phase, the dosage of administration significantly ($P<0.05$) affected the feed conversion ratio. The poorest feed conversion ratio feed conversion ratio was obtained in birds on 300 mg/L while the best was obtained in birds on 350 mg/L dosage administered. The improvement in the body weight gain may be as a result of the positive effect of the extract on the beneficial microorganism which helps in digestion. The result of this present study regarding weight gain and final weight coincides with the findings of Gunal (2006) and Owens et al. (2008) who reported that supplementation of organic acid in broiler chickens improved the body weight gain when compared with conventional antibiotics group. The higher mortality in AEBTB treatment compared to the control group resulted from disease (Salmonellosis) in the flock between weeks two and three of the experiment. In the growing phase, AEBTB improved feed conversion ratio when compared with the control treatment. The best feed conversion ratio was from birds on 350 mg/L of AEBTB. The improvement could result from improved feed utilization by AEBTB dosage administered on beneficial microorganism in the GIT. This observation supported the earlier findings of Hooze (2003) and Murshed and Abudabus (2015) who noted that supplementation of prebiotics improved feed conversion ratio.

Table 6: Effect of Aqueous extract of Baobab Tree Bark on growth performance of cockerel chicken

Dosage of administration (mg/l)	Initial weight (g)	Final weight (g/bird)	Weight gain (g/bird/day)	Feed intake (g/bird/day)	Feed conversion ratio	Mortality (%)
Chicks Phase						
0	35.00	396.80 ^a	45.22 ^a	224.10	5.00	15.00 ^b
300	35.00	380.00 ^{ab}	43.13 ^{ab}	244.30	5.67	60.00 ^a
325	35.00	416.50 ^a	47.69 ^a	249.40	5.27	20.00 ^b
350	35.00	394.50 ^a	44.94 ^a	252.80	5.65	32.00 ^{ab}
375	35.00	308.30 ^b	34.16 ^b	227.80	6.86	70.00 ^a
SEM	0.91	17.10	2.17	13.30	0.57	8.92
P-value	1.000	0.005	0.005	0.465	0.242	0.002
Grower Phase						
0	480.00	1147.50	11.92	70.89	5.98a	8.34
300	475.00	1155.00	12.14	74.91	6.14a	8.33
325	475.00	1252.00	13.88	77.19	5.60ab	16.67
350	520.00	1305.00	14.01	68.88	4.91b	6.25
375	502.50	1230.00	12.99	70.45	5.41ab	12.50
SEM	28.90	51.00	0.79	0.47	9.22	6.25
P-value	0.750	0.199	0.262	0.713	0.009	0.775

ab: Means on the same column by phase of growth with different superscripts differ significantly ($P<0.05$)

The effect of AEBTB on haematology, serum biochemistry and liver enzyme of cockerel chicks is presented in Table 7. The dosage of administration significantly ($P<0.05$) influenced red blood cell, haemoglobin concentration, packed cell volume and mean corpuscular haemoglobin concentration. Red blood cell and PCV followed the same trend with a numerically highest and lowest values from birds on 325 mg/l and 375 mg/l dosage of administration, respectively. Birds on 300 mg/l had the highest haemoglobin concentration of 10.90 and the lowest value 5.10 was obtained in birds on 375 mg/l dosage administered. The effect of AEBTB on liver enzymes and serum biochemistry of cockerel chicks revealed that only alkaline phosphate was significantly ($P<0.05$) affected by dosage administered. The values ranged from 138.00 μ mL to 165.60 μ mL with the highest value from birds in 300 mg/l and 350 mg/litre groups while the lowest was observed in birds administered 375 mg/litre.

Table 7: Effect of Baobab Tree Bark Extract on blood parameters and liver enzymes of cockerel chicks

Parameters	Dosage of Administration (mg/l)					SEM	P-value
	0	300	325	350	375		
Red Blood Cell ($\times 10^{12}/l$)	2.20 ^{ab}	2.20 ^{ab}	2.25 ^a	1.17 ^{ab}	0.86 ^b	0.244	0.024
White Blood Cell ($\times 10^3/\mu l$)	159.10	159.30	159.10	122.30	87.70	15.8	0.076
Haemoglobin (g/dl)	10.30 ^a	10.90 ^a	10.05 ^a	7.00 ^b	5.10 ^b	0.478	0.001
Packed Cell Volume (%)	32.20 ^a	32.20 ^a	32.60 ^a	19.00 ^b	15.00 ^b	0.910	0.00
Mean Corpuscular Haemoglobin (Pg)	11.30	13.50	10.40	9.90	9.70	0.738	0.068
Mean Corpuscular Haemoglobin Concentration (g/dl)	33.20 ^{ab}	39.60 ^a	27.30 ^{ab}	22.60 ^b	25.80 ^b	2.26	0.017
Mean Corpuscular Volume (fl)	146.50	150.10	146.30	162.90	173.60	13.10	0.556
Lymphocyte (%)	2.20	2.10	3.15	9.60	4.20	3.76	0.6333
Total Protein (g/dL)	52.50	54.80	53.50	52.50	51.40	1.60	0.657
Albumin (g/dL)	48.60	47.40	46.10	44.50	43.70	3.26	0.814
Globulin ($\times 10^3/\mu l$)	3.90	7.40	7.40	8.00	7.70	2.63	0.992
Blood Glucose (mg/dL)	94.60	96.40	96.40	98.20	107.30	4.44	0.386
Liver Enzyme							
Alkaline Phosphate (IU/L)	163.60 ^a	165.60 ^a	142.50 ^{ab}	165.60 ^a	138.00 ^b	4.24	0.012
Aspartate Amino Transferase (IU/L)	12.00	11.00	12.50	10.00	13.00	0.700	0.133
Alanine Amino Transferase (IU/L)	14.00	14.50	16.00	13.20	16.00	1.23	0.481

^{ab}: Means on the same row with different superscripts differs significantly (P<0.05)

Reports have shown that administration of medicinal compounds or drugs can alter the normal range of haematological parameters which could either be positive or negative (Adeneye, 2008). Either way, it could be used to explain blood relating function of chemical compound and plant extract (Yakubu et al., 2007) because it shows a physiological, nutritional and pathological state of an animal (Muhammed et al., 2000). The reduction in the RBC, haemoglobin, PCV and mean corpuscular concentration in the treatment indicated that the oxygen carrying capacity of the birds' blood would be reduced. This may be as a result of period of ill health caused by salmonella infection experienced by the birds in weeks 2 and 3 of the experiment. The result of this study supports the findings of Moss (1999) that reduction in the haemoglobin may be accompanied by fall in red blood cells and PCV. Muhammed et al. (2000) also opined that very low reading of RBC, haemoglobin can indicate anaemia. TP, ALT and AST were not affected by AEBTB extract when compared with the control at the chick phase. However, the ALP reduces as the dosage of AEBTB administered increases. This may be as a result of lower deamination done by liver when given AEBTB as compared to conventional antibiotics.

The result of the effect of AEBTB on microbial count and identification in cockerel chicks is presented in Table 8. It revealed that AEBTB significantly (P<0.05) affected the Total bacterial count. The highest was from 0 mg/l while numerically lowest values were observed from AEBTB dosed groups. The identification of bacteria shows that *Klebsiella oxytoca* and *Pseudomonas aeruginosa* were absent in 350 mg/l and 375 mg/l doses, respectively. Significantly reduced total bacterial count obtained in the dosed when compared the control may be as result of the phenolic compounds in the AEBTB to effect antibacterial properties which also increased as the dosage increased. The result of this present study is in agreement with the findings of Gunal et al. (2006) that organic acid significantly reduced the total bacterial count in broiler chickens. In addition, all the microorganism identified in the control were missing in two of out of AEBTB treatments. This could be a pointer that AEBTB had antibacterial effect on them especially during their ill health period. Unlike this present study, Mookiah et al. (2014) found reduced caeca *E. Coli* with increased probiotic usage in broiler chickens.

Table 8: Effect of Baobab Tree Bark Extract on bacterial count and identification of cockerel chicks

Parameter	Dosage of Administration (mg/l)					SEM	P-value
	0	300	325	350	375		
Total bacterial count (cfu/ML)	2.80 ^a	1.90 ^b	1.30 ^b	2.00 ^b	1.30 ^b	0.127	0.003
<i>Escherichia coli</i>	+	+	+	+	+		
<i>Staphylococcus saprophyticus</i>	+	-	-	+	+		
<i>Klebsiella oxytoca</i>	+	+	+	-	-		
<i>Pseudomonas fluoresce</i>	+	-	-	+	+		
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-		
<i>Citrobacter species</i>	-	-	+	-	+		
<i>Bacillus subtilis</i>	+	-	-	+	+		

(+) Positive; (-) Negative

The effect of AEBTB on blood parameters and liver enzymes of growing cockerels is presented in Table 9.

Table 9: Effect of Baobab Tree Bark Extract on blood parameters and liver enzymes of growing cockerel chickens

Parameters	Dosage of Administration (mg/l)						P-value
	0	300	325	350	375	SEM	
Red Blood Cell ($\times 10^{12}/l$)	1.57	0.97	1.42	1.27	1.33	0.234	0.563
White Blood Cell ($\times 10^3/\mu l$)	134.70	78.90	122.00	118.40	118.20	22.00	0.524
Haemoglobin (g/dl)	10.00	5.80	8.60	8.10	9.45	1.09	0.204
Packed Cell Volume (%)	24.30	14.85	23.25	20.65	21.40	4.09	0.570
Mean Corpuscular Haemoglobin (Pg)	63.45	59.35	60.95	65.05	63.70	3.08	0.704
Mean Corpuscular Haemoglobin Concentration (g/dl)	41.10	38.95	37.15	40.15	39.90	1.96	0.691
Mean Corpuscular Volume (fl)	154.45	152.55	164.20	162.55	160.22	2.30	0.056
Lymphocyte (%)	1.85	0.55	1.40	3.50	1.10	174	0.791
Total Protein (g/dl)	59.60	60.40	63.60	62.30	63.35	2.50	0.734
Albumin ($\times 10^3/\mu l$)	42.10	41.53	42.10	42.35	41.25	0.641	0.111
Blood Glucose (mg/dL)	74.25	87.70	108.55	89.25	108.55	8.14	0.111
Globulin ($\times 10^3/\mu l$)	17.50	18.85	21.50	19.95	22.10	2.89	0.786
Liver Enzyme							
Alkaline Phosphate (IU/L)	84.00	92.40	75.60	84.00	120.00	11.90	0.220
Aspartate Amino Transferase (IU/L)	45.05	46.15	45.05	49.10	50.20	2.65	0.565
Alanine Amino Transferase (IU/L)	53.05	48.65	53.05	48.65	57.50	6.57	0.856

The dosage of administration had no significant ($P>0.05$) effect on all the parameters measured. None of the parameters considered on liver enzymes was significantly ($P>0.05$) affected by dosage administered. The present finding on the effect of AEBTB on haematology of growing cockerels is in accordance with earlier findings of Sheikh et al. (2010) that organic acid supplementation had no effect on the blood metabolites of the broiler chickens. However, the haematological values obtained in this study falls within the normal range for chicken reported by Mitruka and Rawnsley (1997). The liver enzymes concentration of growing cockerels among the treatments shows no significant variation when compared with control. This is in agreement with the findings of Abdel Fattah et al. (2008) that liver functions as measured by AST, ALT and TP were not affected by acetic and lactic acid. Birds on 375 mg/L had the numerically lowest Total bacterial count when compared with the control. This may be as a result of the antibacterial effect of the metabolites present in AEBTB at higher dosage. This result negates the findings of Gunal et al. (2006) that organic acid or mixture decrease the total bacteria and gram negative bacteria count. However, *B. subtilis* was absent in ileum at 375 mg/L dosage. This may be as a result of antibacterial properties of AEBTB.

The effect of dosage of AEBTB on gut microbial count and identification on growing cockerel chicken is shown in Table 10. It shows that the dosage of AEBTB did not significantly ($P>0.05$) affect the gut microbial count of the birds up to 375 mg/L.

Table 10: Effect of Baobab Tree Bark Extract on bacterial count and identification of growing cockerel chickens

Parameter	Dosage of Administration(mg/L)					SEM	P-value
	0	300	325	350	375		
Total bacterial count (unit)	2.05	1.75	1.80	1.90	1.35	0.417	0.807
<i>Escherichia coli</i>	++	++	+	++	++		
<i>Klebsiella oxytoca</i>	+	++	++	+	+		
<i>Citrobacter freundii</i>	+	+	+	+	++		
<i>Pseudomonas aeruginosa</i>	+	+	++	+	++		
<i>Pseudomonas fluoresces</i>	+	+	++	+	++		
<i>Bacillus subtilis</i>	+	++	++	++	+		
<i>Bacillus mycoides</i>	+	+	+	+	--		
<i>Proteus mirabilis</i>	++	+	+	+	+		
<i>Staphylococcus Saprophyticus</i>	++	++	++	+	+		
(++) Highly positive (+) Moderately positive (- -) Negative							

CONCLUSION

The result of the analysis revealed that AEBTB contained secondary metabolites and phenolic compounds which could make it appropriate as alternative to synthetic antibiotics.

AEBTB significantly reduced the total bacterial count in the ileum of cockerel chickens.

AEBTB can be used in cockerel chicken production from 300 mg/L and above without any deleterious effect.

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Conflict of Interest

The authors hereby assert that there is absolutely no conflict of interest with any individual or organization regarding the materials used and discussed in the manuscript.

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