# Growth performance, blood profile and gut microflora of broiler chickens on different dosages of Baobab tree (*Adansonia digitata* L.) bark extract

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**This study comprised two experiments; experiment I determined non-toxic doses of Baobab tree (*Adansonia digitata* L.) bark extract (BTBE) using white mice, while experiment II investigated the effects of Baobab tree bark extract on growth performance, carcass characteristics, blood profile, gut morphology and intestinal micro-flora of broiler chickens. Forty mice 6 - 8 weeks old, were used in experiment I and grouped to receive intra-peritoneal and oral administration, for 72 hours, of eight different BTBE preparations to determine lethal-dose, effective-dose and low-dose levels for use on broiler chickens in experiment II. Experiment II involved the use of 200 day-old Cobb 500 broiler chicks orally administered BTBE which lasted for 5 weeks. It consisted of five treatment groups (0 (control), 300, 325, 350 and 375 ppm) with 40 birds per treatment and four replicates of 10 birds each in 5 treatment groups in a completely randomized design. Haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, eosinophil, alkaline phosphatase and aspartate aminotransferase activity were significantly ($p < 0.05$) reduced in mice orally administered BTBE. Also, mean corpuscular volume, alkaline phosphatase and aspartate aminotransferase activity were significantly ($p < 0.05$) reduced in mice intra-peritoneally administered BTBE. Significantly ($p < 0.05$) higher feed intake (122.71 g/day) was recorded in broiler chickens on 350 ppm and, at that dosage, total protein, blood glucose and globulin of the birds were reduced ($p < 0.05$). The study concluded that BTBE can be used as alternative to conventional antibiotics. Therefore, BTBE of 300 ppm was recommended for improved health status and significant growth performance of broiler chickens.**

Keywords: Mice, broiler chickens, performance, intestinal microflora, *Adansonia digitata* bark extract, blood profile

The poultry industry supplies one of the cheapest and most complete sources of animal protein for human consumption. However, in most developing countries, the prevalence of disease is a major constraint to production. This has resulted in the failure of many poultry establishments and thereby led to reduced protein intake (FAO 2017). The diseases affect growth and muscle accretion in broilers, necessitating the use of antibiotics virtually throughout the life span of the birds with recourse only to the withdrawal period before slaughter (Lorençon et al. 2007).

In addition, the ban on the use of sub-therapeutic levels of antibiotics, coupled with increasing consumer demand for meat products free of antimicrobial residues and the concern related to the development of cross-bacterial resistance among humans, have led to increasing interest in the search for alternatives, including phytobiotics and/or phytogenics in poultry production. These alternatives are meant to eliminate antibiotic residues in poultry and meet consumer demand for meat without reduction in the levels of productivity achieved by the livestock or poultry farmers (Waldroup et al. 2003; Janardhana et al. 2009). Such alternatives include acidifiers (organic acids), prebiotics, probiotics, plant enzymes extracts, herbal products, microflora enhancers and immuno-modulators (Richards et al. 2005; Pirgozliev et al. 2008). It should be worthy of note that any replacement for antibiotic growth promoters would have to provide an improvement in feed efficiency that is economically viable.

These substances prevent the harmful effects of potentially pathogenic micro-

organisms and allow the host animals to derive increased benefits from the feed (Dibner and Buttin 2002; Al-Mansour et al. 2011). They do not leave residues in the animal products nor cause cross-resistance in humans as with antibiotics (Nepomuceno and Andreatti 2000). This is believed to improve the health of the host animal (White et al. 2002; Lemieux et al. 2003; Biggs et al. 2007). Some plant extracts such as that from the African Baobab (*Adansonia digitata* L.) tree, influence digestion, secretion of digestive enzymes and exhibit antibacterial, antiviral and antioxidant properties (Ertas et al. 2005; Cross et al. 2007).

The African Baobab (*A. digitata*) belongs to the family *Bombacaceae* and the genus *Adansonia.* The tree is found mainly across Africa and also outside Africa including Barbados, India, Indonesia, Jamaica, Malaysia, Netherlands Antilles, Philippines and the United States of America. *A. 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 (Anani et al. 2000). 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). However, the dearth of information regarding its utilization in poultry production necessitates this study.

## Materials and methods

Site of the experiment

Experiment 1 was carried out at the Biochemistry Department Laboratory, and Animal Research Laboratory, Department of Pharmacology, College of Medicine, University of Lagos, Lagos, 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 at an altitude of 127 m, latitude $7^{\circ}$ 13' N and longitude $3^{\circ}$ 26' E (Google Earth 2015). The experiment to determine non-toxic dosages for mice was carried out between March and April, 2018 ($29^0$C and 66% average temperature and relative humidity, respectively) while the experiment on the broiler chickens was carried out between late July and August, 2018 (average temperature and relative humidity were $25^0$C and 85%, respectively).

Preparation of Baobab tree bark extract (BTBE)

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 powder.

A total of 100 g powdered Baobab bark sample (100 g/750 mL) was dissolved in water for 48 hours in order to extract the polar and non-polar compounds (Elgorashi and van Staden 2004) and then filtered through muslin cloth. The extract was oven dried for 48 hours at $80^0$C using a hot air oven. The dried extract was then ground into powder and stored in an air-tight plastic container at room temperature for future use.

Determination of zone of inhibition

Preparation of bacteria organisms: gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli* and *Pseudomonas fluoresce*) were inoculated into nutrient broth and incubated for 24 hours to obtain a viable broth for the antimicrobial assays.

Agar well diffusion method: Antimicrobial activity of the extract was determined using modified method of agar well diffusion (Chand et al. 1994). The broth culture of bacteria organisms was prepared and spread on nutrient agar, and allowed to air dry. Then 6 mm diameter wells of 4 mm depth were bored into the agar at 4 cm distance apart using a sterile cork borer. A 100 µl aliquot of each extract sample, diluted into 100%, 50%, 25% and 12.5%, was placed in each well while 100 µl sterile water served as negative control with 100 µl ciprofloxacin at 10 µg/ml as positive control. The nutrient agar plates were incubated at $37^0$C for 18 to 24 hours. The inhibition zones were measured using a graduated metre ruler.

Determination of the response of mice to different dosages of Baobab tree bark extract

The experiment for dose response was carried out using mice. A total of 40 mice (American white-footed mice, *Peromyscus leucopus*) about 6-8 weeks old, was used for the experiment. Eight different compositions of BTBE were prepared (four groups for intraperitoneal and four groups for oral administration). The mice were acclimatized for seven days before the administration of the treatments. The mice were monitored for 72 hours and feed was given an hour after the administration of the extracts.

Concentration of extract = 100 mg/ml
1 gram of BTBE dissolved in 10 ml of distilled water.

$$\text{Volume required / animal} = \frac{(\text{weight of mice} \div 1000) \times \text{dose}}{100}$$

$$\text{Volume required by control animal (distilled water)} = \frac{\text{weight of mice}}{1000} \times 10$$

At the 35th day of the experiment, blood samples were collected from all the remaining mice into EDTA bottles for haematological examination and into plain bottles for blood chemistry analysis. Toxic effects of the extract were determined by quantifying liver enzymes such as aspartate amino transferase (AST) and alanine amino transferase (ALT). Alkaline phosphatase (ALP) and total plasma protein (TP) values were also measured.

The most tolerable BTBE dosage, starting with the smallest dosage, was used in the experiments with broiler chicken.

Performance of broiler chicken on BTBE

Two hundred Cobb 500 strain, day-old broiler chicks were used for this experiment. The houses and the equipment were thoroughly washed, cleaned and disinfected before the arrival of the birds which were raised in cages. The birds were divided into five treatments of forty birds each. Each treatment was further divided into four replicates of ten birds each, brooded separately from day one. All routine management practices were adhered to during the experiment while the birds were managed intensively for five weeks. No vaccination and medication were administered on the Baobab groups during the period of the experiment (35 days). Formulated feeds (Table 1) were given *ad libitum* to the birds during the experiment. Aqueous extract, of BTBE were applied in drinking water at 0 ppm, 300 ppm, 325 ppm, 350 ppm and 375 ppm, three times a week for the period of the experiment.

Table 1: Gross composition and proximate composition (g/kg) of experimental diets

| **Ingredient** | **Pre-starter (0 –7 days)** | **Starter (8-21 days)** | **Finisher (22 – 35 days)** |
|---|---|---|---|
| Maize (Yellow maize) | 568.10 | 582.00 | 612.60 |
| Soybean Meal | 370.00 | 363.50 | 317.70 |
| Fat and oil (Palm oil) | 22.00 | 18.00 | 24.00 |
| Limestone | 10.00 | 10.00 | 11.00 |
| Bone meal | 17.50 | 17.50 | 16.00 |
| Salt (NaCl) | 3.50 | 3.50 | 13.50 |
| Lysine | 4.00 | 1.00 | 1.20 |
| Methionine | 2.40 | 2.00 | 1.50 |
| Vitamin and Mineral Premix * | 2.50 | 2.50 | 2.50 |
| Total | 1000 | 1000 | 1000 |
| **Determined Proximate Analysis** | | | |
| Carbohydrate (g/kg) | 561.80 | 488.00 | 530.80 |
| Protein (g/kg) | 233.10 | 233.90 | 226.70 |
| Crude fat (g/kg) | 22.90 | 32.50 | 39.00 |
| Moisture (g/kg) | 67.80 | 66.00 | 75.60 |
| Ash (g/kg) | 32.10 | 30.20 | 60.90 |
| Crude Fibre (g/kg) | 81.00 | 88.00 | 65.90 |
| ME (kCal/100g) | 338.52 | 353.49 | 338.55 |

**Vit. A 12,500,000 iu; Vit. $D_3$ 2, 5000,000 iu; Vit E 40,000mg; Vit. K 32,000mg; Vit. $B_1$ 3,000 mg; Vit. $B_2$ 5,500 mg; Niacin 55,000 mg; Calcium Pentothenate 11, 5000 mg; Vit. $B_6$ 5,000 mg; Vit. $B_{12}$ 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*

Data Collection

*Growth parameters*

Weekly body weight gains were determined as the difference in the body weight of two consecutive weighing for each replicate group. Feed intake was determined weekly through deduction of leftover feed from initial feed supplied. Percentage mortality per replicate was recorded on a weekly basis (as the quotient of number of dead birds/number of birds stocked x 100). Feed conversion ratio as the ratio of feed intake to weight gain was also determined weekly.

*Blood sample analysis*

On day 35 of the study, five ml of blood were collected from the brachial vein of two birds per replicate into two sets of well-labelled bottles; one containing ethylene diamine tetra acetate (EDTA) as anticoagulant, while the other contained no anti-coagulant. All samples were collected in the morning before feeding (between 07:00 am to 09:00 am). Blood samples collected were kept in cool containers and transported to the laboratory within two hours of blood withdrawal.

Haematological parameters measured were analyzed according to the procedures described by Sood (2016). Packed cell volume (PCV) was determined using microhaematocrit capillaries. Haemoglobin concentration (Hb) was determined using cyanmethaemoglobin method which involves mixing five ml of Drabkin's solution (1000 ml of deionised water was mixed with 400 mg of potassium ferricyanide, 280 mg of potassium dihydrogen phosphate, 100 mg of potassium cyanide and one ml of non-ionic detergent) with 20 µl of blood sample. The mixture was read in a photocolorimeter at 540 nm (green filter). Blood counts were determined using the improved Neubauer's chamber (area of 9 sq/mm and depth of 0.1 mm).

Serum biochemical parameters (total protein, albumin, globulin, creatinine, alanine transaminase (ALT) and aspartate transaminase (AST), uric acid, cholesterol, triglycerides) were analysed using commercially available test kits by Randox laboratories, United Kingdom (Model BT294QY).

*Gut microbial count and identification*

A section of about two cm from the ileum of the small intestine, from two birds per replicate, was incised and dropped into sterile sample bottles for microbial load count and identification according to Cowan and Steel (1974) method for bacteria identification. All the samples were homogenized in sterile water, cultured on nutrient agar using a sterile wire loop and incubated at 37°C for 18-24 hours. The isolates obtained were sub-cultured on nutrient agar to produce a pure colony. Each bacterial isolate was examined for its colonial appearance. The following colonial characteristics were used to identify each colony: size, shape, consistency, colour, elevation and opacity. Biochemical identification tests were performed to further characterize the bacterial isolates according to World Health Organization (WHO) manual for laboratory investigation of acute enteric infections (WHO 1983).

Statistical analysis

Data generated from the study were subjected to one-way analysis of variance (ANOVA) in a completely randomized design arrangement. Significant ($p < 0.05$) differences among treatment means were separated by Tukey's test as contained in Minitab® version 17.1.0 (Minitab 2013).

## Results

Expt 1- Effect of BTBE on mice

The responses of mice to oral administration of different BTBE dosages are given in Table 2. There was no mortality recorded after 72 hours of exposure**.** Increasing doses of BTBE administered intra-peritoneally resulted in increased mortality in mice. The highest mortality was recorded in mice administered 1500 ppm of BTBE intra-peritoneally while none was recorded in mice on 375 ppm of BTBE after 72 hours of exposure. The activity of BTBE on four selected bacteria is shown in Table 3. The zone of inhibition was measured in mm and it ranged from 0 to 7 for *Staphylococcus aureus*. The inhibition zone for *Bacillus subtilis* ranged from 7 to 15. That of *Escherichia coli* and *Pseudomonas aeruginosa* ranged from 2 to 9 and 0 to 10, respectively. The blood parameters and liver enzymes of mice orally administered BTBE are

presented in Table 4. Haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, alkaline phosphatase and aspartate aminotransferase were significantly ($p < 0.05$) affected by dosage of administration. The haemoglobin ranged from 6.65 g/dl in mice on 5000 ppm to 13.20 g/dl in mice on 1250 ppm. Mice orally administered 1250 ppm BTBE had the highest mean corpuscular haemoglobin while mice orally administered 5000 ppm BTBE had the lowest mean corpuscular haemoglobin. Also, mice on 1250 ppm dose of administration of BTBE had the highest mean corpuscular haemoglobin concentration value of 31.60 while the lowest value, 12.90, was obtained on mice with 5000 ppm oral administration of BTBE. The eosinophil did not follow any pattern but ranged from 0.45% in mice on 1250 ppm to 2.00% in mice on 2500 ppm. Mice on 0 ppm BTBE had the highest values of 342.00 g/dl and 10.00 g/dl for alkaline phosphatase and aspartate aminotransferase, respectively, and the lowest values of 209.00 g/dl and 7.00 g/dl for alkaline phosphate and aspartate amino transferase, respectively, were recorded in mice on 2500 ppm BTBE.

Table 2: Response of mice to oral and intra-peritoneal administration of different dosages of Baobab tree bark extract

| **Parameter** | **Dosage of Administration (ppm)** | | | |
|---|---|---|---|---|
| **Oral administration** | **0** | **1250** | **2500** | **5000** |
| Number of mice | 5 | 5 | 5 | 5 |
| Mortality after 24 hours | 0 | 0 | 0 | 0 |
| Mortality after 48 hours | 0 | 0 | 0 | 0 |
| Mortality after 72 hours | 0 | 0 | 0 | 0 |
| **Intra-peritoneal administration** | **0** | **375** | **750** | **1500** |
| Number of mice | 5 | 5 | 5 | 5 |
| Mortality after 24 hours | 0 | 0 | 2 | 3 |
| Mortality after 48 hours | 0 | 0 | 0 | 1 |
| Mortality after 72 hours | 0 | 0 | 0 | 0 |

Table 3: Antibacterial activity of Baobab tree bark extract (BTBE) and antibiotic (Ciprofloxacin) on selected microorganisms

| | Zone of activity (mm) | | | |
|---|---|---|---|---|
| **Micro-organisms** | BTBE 100 % | BTBE 50 % | BTBE 25 % | Ciprofloxacin |
| *Staphylococcus aureus* | 7 | 5 | 0 | 30 |
| *Escherichia coli* | 9 | 4 | 2 | 34 |
| *Pseudomonas aeruginosa* | 10 | 4 | 0 | 42 |
| *Bacillus subtilis* | 15 | 9 | 7 | 34 |

Table 4: Effect of oral administration of Baobab tree bark extract on blood parameters and liver enzymes of mice

| | Dosage of Administration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| **Blood Parameters** | **0** | **1250** | **2500** | **5000** | **SEM** | **P-value** |
| Total Protein (g/dl) | 31.70 | 62.00 | 46.30 | 24.60 | 12.70 | 0.302 |
| Red Blood Cell ($x10^{12}$/l) | 8.26 | 9.16 | 9.60 | 10.72 | 0.637 | 0.190 |
| White Blood Cell ($x10^3$/µl) | 3.10 | 4.22 | 3.92 | 5.32 | 1.36 | 0.727 |
| Haemoglobin (g/dl) | 11.40[a] | 13.20[a] | 11.30[a] | 6.65[b] | 0.822 | 0.013 |
| Packed Cell Volume (%) | 36.50 | 41.75 | 43.05 | 48.45 | 2.47 | 0.109 |
| Mean Corpuscular Haemoglobin (g/dl) | 13.80[a] | 14.40[a] | 14.35[a] | 6.00[b] | 0.506 | 0.001 |
| Mean Corpuscular Haemoglobin Concentration (g/dl) | 31.20[a] | 31.60[a] | 30.95[a] | 12.90[b] | 0.470 | 0.000 |
| Mean Corpuscular Volume (g/dl) | 44.20 | 45.55 | 46.35 | 45.00 | 2.11 | 0.902 |
| Neutrophil ($x10^3$/µl) | 2.32 | 1.62 | 2.15 | 1.69 | 0.437 | 0.643 |
| Lymphocyte ($x10^3$/µl) | 1.21 | 1.48 | 1.43 | 1.71 | 0.329 | 0.764 |
| Eosinophils ($x10^3$/µl) | 0.02 | 0.01 | 0.11 | 0.03 | 0.035 | 0.309 |
| Monocytes ($x10^3$/µl) | 0.21 | 0.22 | 0.20 | 1.26 | 0.481 | 0.420 |
| Basophils ($x10^3$/µl) | 0.02 | 0.15 | 0.15 | 0.15 | 0.008 | 0.961 |
| **Liver enzyme** | | | | | | |
| Alkaline Phosphatase (IU/L) | 342.00[a] | 257.50[ab] | 209.5[b] | 214.00[b] | 20.90 | 0.032 |
| Aspartate Amino Transferase (IU/L) | 10.00[a] | 7.00[b] | 7.00[b] | 9.50[a] | 0.25 | 0.002 |
| Alanine Amino Transferase (IU/L) | 9.00 | 8.75 | 9.25 | 8.50 | 0.31 | 0.443 |

[ab]: Means on the same row with different superscripts differs significantly (P<0.05)

Table 5: Effect of intra-peritoneal administration of Baobab tree bark extract on blood parameters and liver enzyme of mice

| | Dosage of Administration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **0** | **375** | **750** | **1500** | **SEM** | **P-value** |
| Total Protein (g/dl) | 67.00 | 63.35 | 73.15 | 29.60 | 8.29 | 0.082 |
| Red Blood Cell($x10^{12}$/l) | 8.67 | 9.94 | 9.30 | 7.75 | 0.707 | 0.435 |
| White Blood Cell ($x10^3$/µl) | 4.32 | 5.26 | 7.86 | 3.20 | 1.50 | 0.388 |
| Haemoglobin (g/dl) | 13.4 | 13.6 | 12.9 | 15.6 | 0.404 | 0.106 |
| Packed Cell Volume (%) | 42.35 | 43.20 | 37.80 | 47.40 | 1.33 | 0.088 |
| Mean Corpuscular Haemoglobin (g/dl) | 15.20 | 13.70 | 25.10 | 16.00 | 0.677 | 0.672 |
| Mean Corpuscular Haemoglobin Concentration (g/dl) | 31.20 | 31.50 | 32.45 | 32.90 | 0.688 | 0.487 |
| Mean Corpuscular Volume (g/dl) | 48.30[a] | 43.40[b] | 40.60[b] | 48.60[a] | 0.911 | 0.025 |
| Neutrophil ($x10^3$/µl) | 1.79 | 1.17 | 1.38 | 0.03 | 0.336 | 0.076 |
| Lymphocyte ($x10^3$/µl) | 1.72 | 3.33 | 1.72 | 3.03 | 1.06 | 0.597 |
| Eosinophils ($x10^3$/µl) | 0.00 | 0.02 | 0.84 | 0.02 | 0.388 | 0.432 |
| Monocytes ($x10^3$/µl) | 0.25 | 0.28 | 0.24 | 0.12 | 0.047 | 0.156 |
| Basophils ($x10^3$/µl) | 0.03[a] | 0.01[b] | 0.02[a] | 0.00[b] | 0.002 | 0.003 |
| **Liver Enzyme** | | | | | | |
| Alkaline Phosphate(IU/L) | 273.00[ab] | 227.50[ab] | 303.30[a] | 151.60[b] | 23.50 | 0.037 |
| Aspartate Amino Transferase (IU/L) | 10.00[a] | 9.00[ab] | 7.00[b] | 7.00[b] | 0.50 | 0.030 |
| Alanine Amino Transferase (IU/L) | 8.00 | 9.25 | 8.00 | 10.00 | 0.38 | 0.046 |

[ab]: Means in the same row with different superscripts differ significantly (P<0.05)

Table 6: Effect of Baobab tree bark extract on growth performance, blood parameters and liver enzymes of broiler chickens

| | Dosage of Administration (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Blood Parameters** | **0** | **300** | **325** | **350** | **375** | **SEM** | **P-value** |
| **Growth Performance** | | | | | | | |
| Initial Weight (g) | 37.50 | 38.75 | 36.26 | 38.75 | 37.50 | 1.33 | 0.657 |
| Final Weight (g/bird) | 1616.30 | 1556.30 | 1523.80 | 1578.50 | 1689.50 | 56.5 | 0.326 |
| Weight Gain (g/bird/day) | 45.11 | 43.36 | 42.50 | 43.99 | 47.20 | 1.62 | 0.330 |
| Water Intake (ml/bird/day) | 209.54 | 208.26 | 200.59 | 214.99 | 210.29 | 3.49 | 0.114 |
| Feed Intake (g/bird/day) | 115.23$^{b}$ | 118.43$^{ab}$ | 116.70$^{b}$ | 122.71$^{a}$ | 117.77$^{ab}$ | 1.30 | 0.012 |
| Feed Conversion Ratio | 2.56 | 2.74 | 2.74 | 2.80 | 2.50 | 0.97 | 0.182 |
| Mortality (%) | 0.00 | 0.00 | 2.50 | 5.00 | 0.00 | 1.71 | 0.199 |
| Water : Feed Ratio | 1.82 | 1.76 | 1.72 | 1.75 | 1.79 | 0.013 | 0.525 |
| **Blood parameters** | | | | | | | |
| Red Blood Cell ($x10^{12}$/l) | 2.08 | 2.09 | 2.40 | 2.26 | 2.28 | 0.340 | 0.296 |
| White Blood Cell ($x10^{3}$/µl) | 151.80 | 149.70 | 155.60 | 152.90 | 84.40 | 27.80 | 0.411 |
| Haemoglobin (g/dl) | 11.40 | 11.20 | 12.35 | 11.75 | 6.90 | 1.96 | 0.401 |
| Packed Cell Volume (%) | 30.05 | 29.70 | 32.40 | 31.85 | 18.30 | 4.98 | 0.367 |
| Mean Corpuscular Haemoglobin (g/dl) | 54.60 | 53.40 | 51.35 | 51.85 | 52.10 | 1.52 | 0.593 |
| Mean Corpuscular Haemoglobin Concentration (g/dl) | 37.85 | 37.65 | 38.00 | 36.85 | 37.20 | 0.943 | 0.895 |
| Mean Corpuscular Volume (g/dl) | 145.20 | 141.90 | 135.65 | 140.90 | 140.45 | 3.53 | 0.507 |
| Lymphocyte (%) | 12.30 | 9.50 | 4.80 | 10.85 | 2.85 | 1.70 | 0.062 |
| Total Protein (g/dl) | 83.90$^{a}$ | 60.20$^{b}$ | 56.85$^{b}$ | 58.30$^{b}$ | 59.20$^{b}$ | 3.23 | 0.008 |
| Albumin ($x10^{3}$/µl) | 42.45 | 40.50 | 42.90 | 40.50 | 42.90 | 0.530 | 0.044 |
| Blood Glucose (mg/dl) | 107.30$^{a}$ | 100.50$^{ab}$ | 98.20$^{ab}$ | 94.60$^{b}$ | 96.40$^{b}$ | 1.85 | 0.027 |
| Globulin ($x10^{3}$/µl) | 41.45$^{a}$ | 19.70$^{b}$ | 13.93$^{b}$ | 17.80$^{b}$ | 16.30$^{b}$ | 3.44 | 0.012 |
| **Liver enzymes** | | | | | | | |
| Alkaline Phosphate (IU/L) | 128.10 | 106.90 | 110.50 | 96.80 | 138.80 | 11.80 | 0.227 |
| Aspartate Amino Transferase (IU/L) | 39.50 | 40.55 | 31.00 | 36.00 | 31.00 | 7.90 | 0.845 |
| Alanine Amino Transferase (IU/L) | 48.40 | 41.70 | 41.70 | 48.40 | 41.25 | 7.93 | 0.905 |

$^{ab}$: Means in the same row with different superscripts differ significantly (P<0.05)

Table 7: Effect of Baobab tree bark extract on total bacterial count and identification

| Dosage of Administration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **0** | **300** | **325** | **350** | **375** | **SEM** | **P-value** |
| Total Bacterial Count (cfu/mL) | 2.80 | 2.85 | 2.60 | 2.50 | 2.35 | 0.555 | 0.774 |
| *Escherichia coli* | ++ | ++ | + | ++ | ++ | | |
| *Staphylococcus saprophyticus* | ++ | + | + | ++ | -- | | |
| *Klebsiella oxytoca* | -- | + | + | + | + | | |
| *Pseudomonas fluoresce* | ++ | + | + | ++ | + | | |
| *Pseudomonas aeruginosa* | + | + | ++ | -- | ++ | | |
| *Citrobacter* species | + | ++ | + | ++ | + | | |
| *Bacillus subtilis* | ++ | -- | + | + | ++ | | |

(++) Highly positive
(+) Moderately positive
(--) Negative

The effects of intra-peritoneal administration of BTBE on blood parameters and liver enzymes of mice are presented in Table 5. Significant ($p < 0.05$) differences were observed in the mean corpuscular volume, basophil, alkaline phosphatase and aspartate aminotransferase of the mice. Mice on 1500 ppm had the highest (48.60 g/dl) mean corpuscular volume while mice on 750 ppm recorded the lowest value of 40.60 g/dl. The highest value 0.03 for basophil was recorded from mice on 0 ppm while the lowest value 0.00 was recorded from mice on 5000 ppm dosage of BTBE. Mice on 750 ppm had the highest (303.30 µ/mL) alkaline phosphatase level while the lowest (151.60 µ/mL) was found in mice on 1500 ppm. Aspartate aminotransferase decreased as the dosage of administration increased.

EXPT 2 - BTBE effect on broilers

The effect of BTBE on growth performance, blood parameters and liver enzymes of broiler chickens is presented in Table 6. It was observed that dosage of administration significantly ($p < 0.05$) affected feed intake. The highest feed intake (122.71 g/day) was recorded in birds on 350 ppm while the lowest values (115.23 g/day and 116.70 g/day) were recorded for birds on 0 ppm and 325 ppm dosages, respectively. The dosage administered significantly ($p < 0.05$) influenced serum total protein, blood glucose and globulin. Birds on 0 ppm had the highest value (83.90 g/dl) for total protein while the lowest values were recorded for treated birds. The highest blood glucose level (107.30 mg/dl) was in birds on 0 ppm while birds on doses of BTBE recorded lower blood glucose levels. Birds on 0 ppm had the highest globulin levels while the lowest globulin levels were obtained in birds on 325 ppm dosage of BTBE. Administration of BTBE had no significant ($P>0.05$) effect on total bacterial count (Table 7). However, *Staphylococcus saprophyticus, Pseudomonas aeruginosa* and *Bacillus subtilis* were absent in groups administered dosages of BTBE at 375, 350 and 300 ppm, respectively.

## Discussion

BTBE effect on mice

The objective of dose response analysis was to

estimate a bench mark dose (BMD), which is a dose or concentration that produces a predetermined change in the response rate of an adverse effect (Davis et al. 2011 and Filipsson et al. 2003). In this study, no lethal effect was seen in mice on oral route of administration after 72 hours of exposure to BTBE at different doses. On the other hand, more than 40% mortality was recorded for levels of 750 ppm and 1500 ppm after 72 hours of exposure to BTBE through the intra-peritoneal route. This may be as a result of faster systemic absorption. This confirmed the finding of Howmed (2015) that systemic absorption is more rapid and predictable than oral administration.

The result of the examination of antibacterial properties showed that BTBE was active against the tested organisms. The zone of inhibition increased as the dosage increased. The inhibitory effect was more pronounced in *Bacilus subtilus* and less pronounced in *Staphylococcus auresus* and *Escherichia coli.* Similar results were observed for aqueous Baobab leaf extract by Abiona et al. (2015). This may be as a result of the presence of the phenolic hydroxyl group. It could be concluded that BTBE contains an active ingredient against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa.*

After 72 hours of oral administration of BTBE on mice, haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were reduced as the dosage increased. The elevated values of white blood cell, lymphocyte and monocyte count for mice on 5000 ppm could be an indication that the mice were reacting to factors in the feed or environment which could be pathogenic. The RBC decreased as the dosage of BTBE increased. BTBE may contain a substance that inhibits the production of RBC which increased as the dosage increased. However, the values obtained for haematology in mice fall within the recommended value for healthy mice reported by Laurie et al. (2003) and Mouse Haematology (2017).

ALP and AST were lower for mice on both oral and intra-peritoneal routes of administration when compared to the control which may be due to reduction in damage to the liver. This observation is in line with the finding of Sahu 2016 who reported reduced liver enzymes for mice taking leaf extract of *Hibiscus rosa-sinensis.* However, ALP values were higher than the values of 119- 83 and 43-171, respectively, presented by Laurie et al. (2003). The administration of BTBE significantly reduced these liver enzyme levels.

BTBE effect on Broilers

It was observed that the birds were in good health and condition during the experimental period of five weeks and the mortality was minimal. This may be as a result of the positive effect of BTBE on performance and health status of the birds. The present findings were in accordance with reports of Valancony et al. (2001) that prebiotics have positive effects on performance and health status of chickens by reducing disease incidence, inhibiting gut lining colonization and preventing pathogenic bacteria from proliferating, thereby reducing toxin production. The dosage of administration had no significant effect on performance of broiler chickens except in feed intake (FI) when compared with the control group. This result disagrees with the findings of Hernandez et al. (2006) who reported no difference in the cumulative feed consumption between the group fed organic acid and control group. The final weight and weight gain recorded for birds on 375 ppm compared to the control may be as a result of the growth stimulating effect of BTBE which is linked to decreased pathogenic bacterial activity, improved feed intake and digestion (Kabir 2009). Also, feed conversion ratio from birds on 375 ppm conforms to the findings of Podmaniczky et al. 2006 and Zakeri

2006 who reported that supplementation with prebiotics was shown to improve growth and feed conversion ratio of broilers.

Blood parameters are good indicators of the physiological and health status of birds and are predictive of physiological changes caused by various stress factors (Kilgas et al. 2006). It was noted that blood glucose was significantly lower when compared with the control but reasons for this were not identified. The lower WBC and lymphocytes in birds on 375 ppm of BTBE gave an indication of little or no pathogenic challenge as pointed out in the findings of Oduguwa (2006). It was also noted that blood glucose significantly decreased in broiler chickens on 350 and 375 ppm BTBE compared to the control and this negates the earlier finding of Al-Saad et al. (2014) that organic acids supplementation had no effect on blood metabolites. However, the variation in values observed for PCV, Hb and red blood cells across the treatment groups fall within the range for normal avian species as reported by Pellet and Young (1980) and Cynthia (2005).

Total bacterial count values were reduced in broiler chickens as the dosage of BTBE increased during the treatment when compared with the control. This may be as a result of increased bactericidal effect with increased dosage as opined by Gunal et al. (2006) in the use of organic acid for broiler chickens' production. According to Bedford (2000), most alternatives to antibiotics target gram positive bacteria and suggest that this group of bacteria is an important cause of impaired performance. In this study, BTBE had antibacterial effect on *S. saprophyticus, P. fluoresce* and *B. subtilus* but not on *E. coli* compared to the control group.

## Conclusion and recommendation

1. BTBE can be used as an alternative to conventional antibiotics.
2. BTBE at 300 ppm is recommended for improved health status and significant growth performance of broiler chickens.

## Conflict of interest statement

There is absolutely no conflict of interest with any individual or organisation regarding the materials discussed in the manuscript.

**Ethical approval:** All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

**Informed Consent:** Consent of every individual included in this study was obtained.

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