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Research Article
In vitro Antioxidant Potential and *In vivo* Effects of Diet Supplemented with *Balanites aegyptiaca* on Growth, Biochemical Parameters and Liveability of Heat-Stressed Broilers

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Abstract
Objective: This study was conducted to evaluate the effects of *Balanites aegyptiaca* leaves (BAL) and seeds (BAS) as a feed additive on growth, biochemical parameters and mortality of broilers during hot fattening periods. **Materials and Methods:** A total of 214 one-day old Cobb 500 broiler chicks (42±3.6 g) were acclimatized for 14 days. At fifteen days of age, 200 chicks (327.92±48.83 g) were randomly allocated to four different treatments with 5 replicates each (50 chicks/treatment). The four experimental groups were as follows: Group 1 (control) received basal diet and water without additives; Group 2 received basal diet and drinking water supplemented with ASPRO-C Plus (1g/L); Group 3 received basal diet supplemented with 1% of BAL, Group 4 received basal diet supplemented with 2% of BAL. **Results:** As compared to the seeds, the leaves showed higher content ($p<0.05$) of polyphenols but lower values ($p<0.05$) of saponins. The ferric-reducing antioxidant power (FRAP) activity of the BAL was higher ($p<0.05$) than that of the seeds. Broilers treated with BAL showed significantly higher ($p<0.05$) serum content of alpha-amylase and lower ($p<0.01$) mortality. The diet supplemented with 1% BAL significantly increased ($p<0.05$) the superoxide dismutase (SOD) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) activities in the liver. **Conclusion:** It can be concluded that diet supplemented with 1% *Balanites aegyptiaca* leaves improved the antioxidant status of the liver and liveability of broilers during hot fattening periods.

Key words: Antioxidant, *Balanites aegyptiaca* , broiler, growth, heat-stress, mortality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heat stress affects poultry and has been associated with various harmful impacts on livestock productivity, such as animal morbidity and growth reduction, resulting in dramatic losses to the livestock industry[1]. Heat exposure has been strongly associated with oxidative stress in poultry. In fact, when the body is exposed to high temperature, heat can break the balance of body redox and result in oxidative stress[2,3]. Oxidative stress is defined as the presence of reactive species in excess of the available antioxidant capacity of animal cells[4]. These reactive species can modify several biological cellular macromolecules, such as proteins, lipids and nucleic acids[5]. As a consequence of these phenomena, several metabolic dysfunctions develop, including cell death, altered expression of key enzymes in detoxification, antioxidant defence, inflammatory responses, nutrient absorption and metabolism[2].

Recent studies have suggested that enhancing the detoxifying capacity of reactive species in heat stressed broilers is the primary target[3,5]. Such results can be achieved by exogenous antioxidants. In poultry, herbal plants and their phytochemicals with antioxidant activity seem to offer a promising option for managing heat stress and improving performance[3,6]. In previous studies, plants such as *Forsythia suspense*[7], *Emblica officinalis*, *Terminalia chebula*[7,8], *Mentha longifolia*[9], *Curcuma xanthorrhiza* and *Oreganum compactum*[5] have shown positive effects in alleviating oxidative damage in response to heat stress. The beneficial effects of these plants have been associated with secondary metabolic compound, especially in polyphenols.

The beneficial effects of polyphenols compound is due to their antioxidant, anti-inflammatory and antimicrobial activities[2,5]. Polyphenols can act as radical scavengers depending on their chemical structures and eliminate the oxidative damage caused by heat stress. They may block the action of some enzymes that directly generate O^{-2}. Polyphenols may modulate cell signalling pathways to alleviate the impact of heat stress[2]. However, the efficacy and the potential mechanisms underlying the protective effects of polyphenols against heat stress are different[5]. It is therefore important to explore other natural phytochemicals locally available.

Balanites aegyptiaca is a sahelian plant rich in beneficial phytochemicals such as polyphenols, alkaloids and saponins[10]. The antioxidant activity of the *Balanites aegyptiaca* leaves has been reported. It prevents lipid peroxidation, neutralize the 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical and shiekling fibroblast cells from oxidative damage[11]. Moreover, the ethanolic extract of *Balanites aegyptiaca* leaves seem to have hepatoprotective effect[12]. However, studies related to the effect of *Balanites aegyptiaca* on heat-stressed chickens are rare. The double aim of this experiment was therefore to evaluate the *in-vitro* antioxidant potential of *Balanites aegyptiaca* seeds and leaves collected from Maroua; and the *in-vivo* effect of *Balanites aegyptiaca* leaves on the growth performance, blood parameters and antioxidant status of heat-stressed broilers.

MATERIALS AND METHODS

- **Experiment 1:**

In-vitro antioxidant potential of _Balanites aegyptiaca_ seeds and leaves:

Study area: The *in-vitro* study was carried out at the Laboratory of Bioscience, National Advanced School of Engineering, University of Maroua, Cameroon.

Experimental design: Fresh leaves and seeds of *Balanites aegyptiaca* were collected from the town of Maroua in February 2023. The taxonomic identity of the plant was confirmed at the Department of Biological Sciences, Faculty of Science, University of Maroua, Maroua City, Cameroon. The purchased leaves and seeds were separately washed in clean water several times to remove soil, dust or dirt. The samples were cut into small pieces and air-dried to crispiness in the laboratory (prevailing room temperature of 33±4°C) for a week. The dried materials were reduced to fine particles using a pestle and mortar and sieved with 0.5 mm sieve into fine powder before storing in airtight plastic containers. The obtained powders of leaves and seeds were subsequently used for analyses.

Data collection

Phytochemistry: The phytochemical screening of *Balanites aegyptiaca* leaves and seeds were performed at the laboratory of Bioscience of the University of Maroua. Each dried powder (10 g) of leaves and seeds was extracted separately with 100 mL of 80% methanol. Using a funnel choked with non-absorbent cotton, extracts were filtered into a conical flask and diluted 10 fold before analysis[13-15].

Qualitative screening: The qualitative analyses were carried out to highlight the presence or absence of secondary metabolites, including phenols, alkaloids, flavonoids, quinones, saponins, glycosides or tannins.

Test for phenols: Exactly 2 mL of Ferric Chloride ($FeCl_3$) was added to each of the 2 mL extracts of root, stem bark, leaf and fruit. The resultant mixture turns deep bluish green indicating the presence of phenols.

Test for alkaloids: In order to test for alkaloids, 2 mL of leaf and seeds extracts were measured in a 5 mL measuring cylinder and poured into a test tube. Few drops of Dragendorff's reagent were added using a dropper. Formation of orange colour indicates the presence of alkaloids.

Test for flavonoids: Exactly 2 mL of the leaf and seeds extracts were poured into a test tube and few drops of 5% lead acetate was added using a dropper, cream light yellow colour formation indicates the presence of flavonoids.

Test for saponins: Using a 5 mL measuring cylinder and a dropper, 1 mL of each leaf and seeds extract was measured and poured into a tube. A vigorous shake was performed after adding 4ml of distilled water. Formation of honey comb froth indicates the presence of saponins.

Test for tannins: Few drops of 10% Ferric chloride ($FeCl_3$) were added to 2 mL of the leaf and seed extracts using a dropper. A deep bluish or greenish Colour indicates the presence of tannins.

Test for glycosides: In a test tube, 5 mL of Fehling's solution chloroform was added to 5 mL each of the leaf and seed extracts. The mixture was placed in a water bath at 70°C for 3 min. A reddish-brown colour formation indicates the presence of glycosides.

Test for quinones: In a test tube, 2.5 mL of ammoniac solution (NH_4OH) (20 %) was added to 5 mL each of the leaf and seed extracts. Formation of reddish-brown colour indicates the presence of free anthraquinones.

Quantitative determination: Quantitative phytochemistry was carried out to quantify the *Balanites aegyptiaca* leaves and seeds content of polyphenols[16], flavonoids[17], saponins[14], or tannins[18]. The quantitative analyses were performed using colorimetric methods.

Total polyphenol content: Only 0.5 mL of leaf and seed extracts and gallic acid was introduced into tubes and mixed with 1 mL of a tenfold diluted Folin-Ciocalteu reagent and 1 mL of 7.5% sodium carbonate. The tubes were covered with aluminium foil and allowed to stand for 30 min at room temperature before the absorbance was read at 750 nm using UV/V spectrophotometer. For each analysis, samples were prepared in triplicate and the mean absorbance was determined. Gallic acid was used as a standard and the total polyphenols were expressed as mg/g gallic acid equivalents (GAE). For standard curve 0, 25, 50, 75, 100 and 125 µg/mL of gallic acid were used.

Total flavonoids content: Exactly 1 mL of extracts or standard solution was added to 1 mL of 10% aluminium chloride in a test tube. The mixture was thoroughly mixed and two drops of acetic acid were added. The absorbance of the mixture was determined at 430 nm versus a blank. Total flavonoids content of the extract was expressed as mg/g of sample (mg/g). Quercetin was used as a standard and the total flavonoids were expressed as mg/g quercetin equivalents. For standard curve 0, 25, 50, 75 and 100 µg/mL of quercetin were used.

Total saponins content: A volume of 1mL of the extract samples or standard was transferred into a capped test tube with 1 mL of ADNS reagent. The mixture was heated for 5 min in a water bath at 60°C. The absorbance measurement was performed against a blank at 5330 nm. The total saponins content was determined by using a standard calibration curve with galactose as standard.

Total tannins content: Exactly 0.2 mL of the leaf and seed extracts or standard solution was added to 2 mL of vanillin hydrochloride reagent in a test tube. The mixture was thoroughly mixed and incubated at 30°C for 5 mn. The absorbance of the mixture was determined at 500 nm versus a blank. Total tannins content of the extract was expressed as mg/g of sample. Catechin was used as a standard and the total tannins were expressed as mg/g Catechin equivalents. For standard curve 0, 10, 20, 30, 40 and 50 µg/mL of catechin were used.

Antioxidant power: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay was carried out as recommended by Sun *et al.*[19], meanwhile, the ferric reducing antioxidant power (FRAP) antioxidant activity was performed as described by Benzie and Strain[20].

DPPH antioxidant assay: To perform the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, 0.2 mL solvent extract of the samples or trolox solution was introduced in a test tube and 2 mL of 0.1 mM DPPH was added. The mixture was shaken well and

incubated at room temperature in the dark for 5 min, the decrease in absorbance of the resulting solution was then read at 517 nm using UV/Vis spectrophotometer. IC50 value, the concentration of sample required to scavenge 50% of DPPH free radical, was calculated from the plotted graph of radical scavenging activity against the concentration of extracts. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Trolox was used as a standard and the DPPH activity were expressed as mg/g trolox equivalents (TroloxEq). For standard curve 0, 25, 50, 75, 100 and 125 µg/mL of trolox were used.

FRAP antioxidant activity: To perform FRAP test, fresh FRAP reagent was prepared by mixing 300 mm acetate buffer (pH 3.6), 10 mm TPTZ solution and 20 mm $FeCl_3.6H_2O$ solution at the ratio of 10 :1 :1. A volume of 0.1 mL each of the leaf and seed extracts or standard solution was added to 1 mL of FRAP reagent. The mixture was thoroughly mixed for 5 min. The absorbance of the mixture was determined at 593 nm versus a blank. Vitamin C was used as a standard and the FRAP activity were expressed as mg/g vitamin C equivalents. For standard curve 0, 25, 50, 75, 100 and 125 µg/mL of vitamin C were used.

- **Experiment 2:**

***In-vivo* effect of *Balanites aegyptiaca* leaves on the growth performance, blood parameters and antioxidant status of heat-stressed broilers:**

Study area: The present study was carried out at the application and research farm of the National Zootechnical and Veterinary Training Center of Maroua, located in the Far-North region of Cameroon at latitude: 10E60'N, longitude: 14E30' and altitude 384 m. During the experimental period (8th March - 20th April 2023), the mean ambient temperature registered at the study area was over 32°C, while the relative humidity was around 18%.

Experimental animals and their management: A total of 214 one-day old Cobb 500 broiler chicks (42±3.6 g) were acclimatized for 14 days. At fifteen days of age, 200 chicks (327.92±48.83 g) were randomly allocated to four different treatments with 5 replicates each (50 chicks/treatment). All birds were reared on the floor pens with wood chips as a litter material, at the density of 8 birds/m^2. Chicks were kept under similar environmental and managerial conditions during the experiment. Throughout the experiment, between 10 a.m. and 4 p.m., broilers were daily exposed to maximum temperatures (31-44°C). Minimum temperature in the room (23-34°C) were recorded between 10 p.m. and 6 a.m. Feed and water were given *ad libitum* in adapted equipment. Standard health and vaccination programs against Newcastle and Gumboro diseases were administered. Chicks were observed and checked daily for any syndromes, during the entire experiment. Throughout the experiment, all birds were fed the standard diet (Table 1). Animal manipulation carried out in this study followed the protocol approved by the Cameroonian Bioethics Committee (Reg N° FWA-IRB00001945) and following HIN-care and use of laboratory animals manual (8th Edition).

Experimental design: Chicks were assigned randomly to 4 treatment groups. Each group was divided into 5 replicates of 10 chicks. Group 1 received basal diet and water without

Table 1: Ingredients and nutrient composition of experimental diet

Ingredients (%)	Starter diet	Grower diet	Finisher diet
Maize	56.2	59	61
Rice bran	3	4	5
Peanut meal	10	8	7
Soybean meal	17.5	15	12
*Premix 10%	10	10	10
Bone meal	3	3.8	4.8
Salt	0.2	0.2	0.2
Antitoxin	0.1	-	-
Total	100	100	100
Nutrient values			
Metabolizable energy (kcal/kg)	2950.23	3050.93	3100.26
Crude protein (%)	21.7	20	18.55
Phosphorus (%)	0.8	0.9	1
Calcium (%)	2	2.3	2.6
Lysine (%)	1	0.9	0.8
Methionine (%)	0.4	0.4	0.5

*Premix 10%: Crude protein: 40%, Lysine: min. 3.00%, Methionine: min. 1.70%, Calcium: min. 7.00%, Phosphorus: min. 1.80% and EM: 2100 kcal/kg

additives (control); Group 2 received basal diet and drinking water supplemented with ASPRO-C Plus (1 g/L); Group 3 received basal diet supplemented with 1% of *Balanites aegyptiaca* leaves; Group 4 received basal diet supplemented with 2% of *Balanites aegyptiaca* leaves.

Balanites aegyptiaca leaves and seeds were collected from the town of Maroua near the study area. They were washed, dried in the shade for a week and then finely ground to obtain powder which was subsequently used for supplementation.

ASPRO-C Plus was purchased from a veterinary pharmacy in Maroua (CAPHAVET) and used as recommended by the manufacturer (LCM) on the packaging, (1 g/L of drinking water in poultry). ASPRO-C Plus is constitute of Acetylsalicylic acid (20 g); betaine (25 g); sorbitol (37.4 g); sodium ascorbate (10 g); calcium chloride dihydrate (4 g); sodium chloride (2 g); potassium chloride (0.02 g); magnesium chloride hexahydrate (0.4 g); excipients qsp (100 g).

Data collection: Before experiment, the *Balanites aegyptiaca* leaves and seeds were submitted for phytochemical analyses. During the experiment, feed intake and live body weight were recorded weekly. At 42 days of age, two birds per pen replicate i.e., ten per treatment were randomly selected and fasted for 12 hrs, weighed and slaughtered and blood samples were collected for biochemical analyses. The liver was also collected for the determination of the oxidative stress parameters.

Animal manipulation carried out in this study was in accordance with recommendations of institutional guidelines for the care and use of laboratory animals. Chicks were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Heat index (HI): Every day, the room temperature and the relative humidity were recorded at 1pm, using a Taylor brand (FCC ID: WEC-1502) temperature and humidity sensor, placed at the level of chicken back in the middle of the room. The heat index was determined by the formula described by Kendall and Webster[21].

$$HI = (1.8\ T+32)-[(0.55-0.0055\ H)\times(1.8\ T-26)]$$

Where, T represents the ambient temperature (°C) and H is the relative humidity (%).

Growth parameters: After measuring the feed intake and live body weight with a scale of 5 kg and 1g precision, the body weight gain and feed conversion ratio were calculated in accordance with the following formulas:

$$\text{Daily Feed Intake} = \text{Feed quantity served one morning} - \text{Feed quantity remaining the following morning}$$

$$\text{Weekly Body weight gain} = \text{Live body weight of week}_{(n+1)} - \text{Live body weight of week}_{(n)}$$

$$\text{Daily Body weight gain} = \frac{\text{Weekly body weight gain}}{7}$$

$$\text{Feed conversion ratio} = \frac{\text{Weekly feed intake (g)}}{\text{Weekly body weight gain (g)}}$$

Biochemical analysis: Blood samples of each slaughtered bird were collected from the jugular vein into dry tubes. After clotting, the serum was separated by centrifugation at 3000 rpm for 15 min and the aliquots were stored at -20°C for biochemical analysis. Serum contents of total protein, albumin, total cholesterol and alpha-amylase were measured using a spectrophotometer (URIT-810) and according to the protocol described by the manufacturer's kits (HUMAN commercial kit). Globulins were determined as follows:

$$\text{Globulins} = \text{Total protein} - \text{Albumin}$$

Oxidative stress parameters: The levels of total protein[22] and the oxidative status in liver samples were measured. The activity of superoxide dismutase was evaluated by the colorimetric method of Misra and Fridovish[23]. The level of reduced glutathione was determined as described by Ellman[24]. The homogenate content of malondialdehyde was evaluated according to Wilbur *et al.*[25]. The DPPH antioxidant assay was carried out as recommended by Sun *et al.*[19], meanwhile, the FRAP antioxidant activity was performed as described by Benzie and Strain[20].

Data analyses: Data were analysed using one way ANOVA (type of supplement) and expressed as Mean ± SD followed by Tukey multiple tests for comparison among means using the SPSS 21.0 Statistical Software Program (SPSS, Inc., IBM, Chicago, Illinois, USA).

RESULTS

- **Experiment 1:**

Phytochemistry of *Balanites aegyptiaca* leaves and seeds

Qualitative phytochemistry: The qualitative composition of *Balanites aegyptiaca* leaves and seeds are presented in Table 2. Except the quinone which was not found in the seeds, the leaves and seeds contained many secondary metabolites such as alkaloids, phenols, quinones, flavonoids, saponins, tannins or glycosides[26]. However, the phenols, flavonoids and tannins are more represented in the leave and seeds.

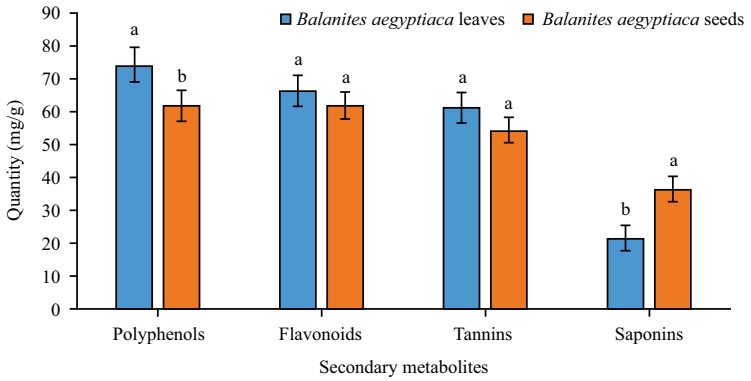

Fig. 1: Quantitative content in some secondary metabolites of *Balanites aegyptiaca* leaves and seeds

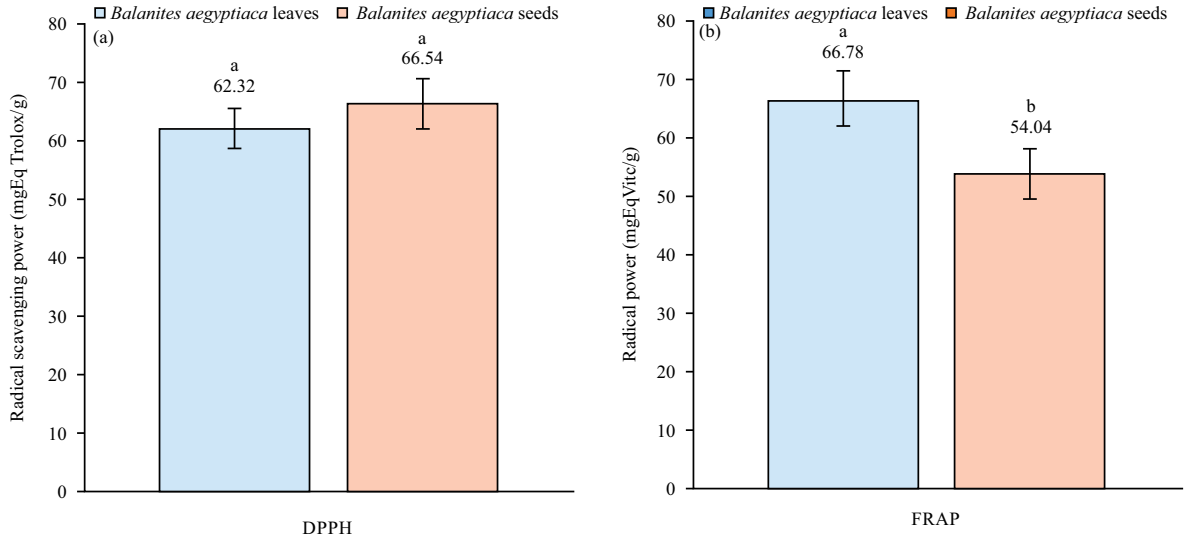

Fig. 2: *In vitro* Reducing power at FRAP (a) and DPPH radical scavenging activity (b) of the *Balanites aegyptiaca* leaves and seeds

Table 2: Qualitative composition of *Balanites aegyptiaca* leaves and seeds

Metabolites	Alkaloids	Phenols	Quinone	Flavonoïds	Saponins	Tannins	Glycosides
Balanites aegyptiaca leaves	+	+++	+	+++	++	+++	++
Balanites aegyptiaca seed	+	+++	-	+++	++	+++	++

+++/++ High presence, +: Moderate presence and –: Absence

Quantitative phytochemistry: As illustrated in Fig. 1, irrespective of the *Balanites aegyptiaca* part, the polyphenols compounds constitute the highest proportion of the secondary metabolites and the saponins the lowest. As compared to the seeds, the leaves showed higher content ($p<0.05$) of polyphenols and lower values ($p<0.05$) of saponins; meanwhile the flavonoids and tannins contents were statistically similar ($p>0.05$).

Total antioxidant potential of the *Balanites aegyptiaca* leaves and seeds: The total antioxidant potential of the *Balanites aegyptiaca* leaves and seeds are presented in Fig. 2a and b. The DPPH radical scavenging activity of the *Balanites aegyptiaca* leaves and seeds were high and statistically similar (Fig. 2a). As for DPPH, total antioxidant activity measured by the FRAP showed high reducing ability of the *Balanites aegyptiaca* leaves and seeds (Fig. 2b). However, this ability was significantly higher ($p<0.05$) with the leaves.

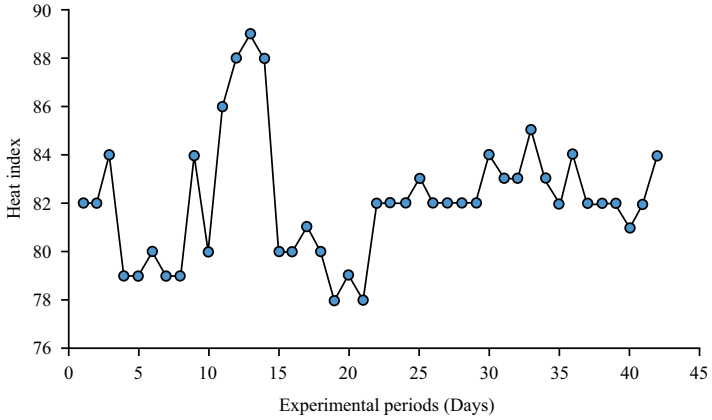

Fig. 3: Evolution of the heat index of the room during experiment

Table 3: Growth parameters of broilers fed on a diet supplemented with *Balanites aegyptiaca* leaves and Aspro-C

	Control	ASPRO-C	1%Ba	2%Ba	p-value
DFI (g)	109.5±18.60^{a}	108.7±11.71^{a}	106.9±8.088^{a}	106.7±6.647^{a}	0.9981
DBWG (g)	47.64±7.521^{a}	48.29±6.484^{a}	47.75±7.565^{a}	47.46±8.270^{a}	0.9999
FCR	2.347±0.592^{a}	2.275±0.357^{a}	2.273±0.346^{a}	2.303±0.290^{a}	0.9964
Mortality (%)	31.48±11.28^{a}	20.60±5.675^{ab}	9.922±6.725^{b}	10.01±2.676^{b}	0.0006

Values in the same line sharing a common superscript are statistically similar (p>0.05). DFI: Daily feed intake, DBWG: Daily body weight gain; FCR: Feed conversion ratio

Table 4: Blood Biochemical parameters of broilers fed on a diet supplemented with *Balanites aegyptiaca* leaves and Aspro-C

	Control	ASPRO-C	1%Ba	2%Ba	p-value
Total Proteins(g/l)	93.44±3.167	94.67±3.606	94.89±4.961	93.22±4.324	0.7635
Albumin (g/l)	43.44±3.909^{ab}	43.22±3.492^{ab}	41.11±2.028^{b}	46.00±3.279^{a}	0.0292
Globulins (g/l)	52.33±3.571^{a}	52.44±4.216^{a}	52.67±5.148^{a}	45.00±3.905^{b}	0.0010
α-Amylase (U/L)	58.33±9.394^{a}	60.44±9.567^{a}	74.67±5.196^{b}	72.67±8.000^{b}	0.0002
Cholesterol (g/l)	1.56±0.193^{a}	1.57±0.171^{a}	1.91±0.306^{b}	1.71±0.208^{ab}	0.0093

Values in the same line not sharing a common superscript differ significantly (p<0.05)

- **Experiment 2:**

In-vivo effect of _Balanites aegyptiaca_ leaves on the growth performance, blood parameters and antioxidant status of heat-stressed broilers

Heat stress index: The evolution of the heat index of the room during the experiment is presented in Fig. 3. Throughout the experiment, the lowest values of heat index was 78 and the highest was 89.

Growth parameters: As shown in Table 3, the feed intake, the body weight gain and the feed conversion ratio were not statistically affected by diet supplemented with *Balanites aegyptiaca* leaves or water supplemented with Aspro-C. As compared to control, the mortality rate of broilers fed a diet supplemented with *Balanites aegyptiaca* leaves was significantly lower (p<0.05). However, the values obtained with *Balanites aegyptiaca* were statistically similar (p>0.05) to that registered with Aspro-C Plus.

Biochemical parameters: The diet supplemented with *Balanites aegyptiaca* leaves or water supplemented with Aspro-C Plus did not affect the serum levels of total protein (Table 4). However, A significant reduction in serum globulin content was observed in broilers fed diet supplemented with 2% *Balanites aegyptiaca* (p<0.05) as compared to other treatments. Broilers fed diet supplemented with *Balanites aegyptiaca* leaves showed significantly higher (p<0.05) content of alpha-amylase. Table 4 also indicates that the total cholesterol level was increased with *Balanites aegyptiaca* leaves but the increase was significant only at 1% (p<0.05).

Antioxidant enzyme activities and oxidative status indicators: The effects of diet supplemented with *Balanites aegyptiaca* leaves or water supplemented with Aspro-C Plus on the antioxidant capacity, the antioxidant enzyme activity and oxidative status indicators in the liver of broilers are presented in Table 5. As compared to the non-supplemented group, the total protein concentration in the liver and the

Table 5: Oxidative stress parameters of the liver of broilers fed on a diet supplemented with *Balanites aegyptiaca* leaves and Aspro-C

	Control	ASPRO-C	1%Ba	2%Ba	p-value
TP (mg/100 mL hom.)	41.9000±5.828^{a}	36.0100±9.040^{ab}	40.1200±3.696^{ab}	32.9900±3.290^{b}	0.0137
FRAP(mgET/100 mL)	66.5800±6.449^{a}	40.9200±7.257^{c}	68.4500±6.175^{a}	56.2900±2.996^{b}	<0.0001
DPPH (mgET/100 mL)	37.0300±8.246^{c}	66.4200±4.460^{a}	52.6700±10.88^{b}	43.4900±6.275^{bc}	<0.0001
SOD (U/mg protein)	45.4100±3.906^{b}	44.5900±5.008^{b}	56.1600±5.374^{a}	42.7400±4.939^{b}	<0.0001
MDA (µmole/g of organ)	0.0274±0.004^{b}	0.0375±0.012^{ab}	0.0395±0.013^{ab}	0.0467±0.008^{a}	0.0046
GSH (µmole/mg Protein)	43.6200±6.903^{a}	34.2300±4.588^{b}	36.0000±6.508^{ab}	37.3500±7.756^{ab}	0.0263

Values in the same line not sharing a common superscript differ significantly (p<0.05). GSH: Reduced glutathione, MDA : Malondialdehyde, SOD: Superoxide dismutase, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power, TP: Total protein, mgET/100 mL: mg Equivalent Trolox/100mL of homogenate, hom.: Homogenate

FRAP antioxidant activity were significantly lower (p<0.05), meanwhile the malondialdehyde content was significantly increased (p<0.05) with 2% *Balanites aegyptiaca* leaves. Statistical analyses also showed that 1% Balanites aegyptiaca leaves increased (p<0.05) the DPPH antioxidant capacity and the superoxide dismutase (SOD) activity compare to negative control. When compared to positive control (ASPRO-C Plus), the FRAP antioxidant activity was significantly increased with the diet supplemented with *Balanites aegyptiaca* leaves and the SOD activity was significantly higher (p<0.05) with 1% *Balanites aegyptiaca*. However, the DPPH antioxidant capacity was significantly increased (p<0.05) with ASPRO-C Plus. The level of reduced glutathione (GSH) in the liver of broilers treated with ASPRO-C Plus was reduced but the decrease was statistically different (p<0.05) when compared to negative control.

DISCUSSION

The phytochemical screening of *Balanites aegyptiaca* leaves revealed the presence of alkaloids, phenols, quinones, flavonoids, saponins or glycosides. Our findings are in line with those of Murthy *et al.*[10] who pointed out the presence of these secondary metabolites in the different part of *Balanites aegyptiaca* in India. The quantitative analyses confirmed that polyphenols, flavonoids and tannins are the major secondary metabolites in the *Balanites aegyptiaca* leaves and seeds. These results are in accordance with those of previous authors who reported important quantity of polyphenols in the different part of *Balanites aegyptiaca*[27,28]. Phenolic compounds have been reported to exert various pharmacological or biological activities such as antioxidant, antidiabetic or anticancer[28]. The total equivalent antioxidant capacities of the *Balanites aegyptiaca* leaves and seeds showed high values for DPPH (62.32±3.4 mgEq Trolox/g DM and 66.54±4.3 mgEq Trolox/g DM for leaves and seeds respectively) or FRAP (66.78±4.9 mgEq Vit C/g DM and 54.04±4.2 mgEq Vit C/g DM for leaves and seeds respectively). These results suggest that the *Balanites aegyptiaca* leaves and seeds have stronger antioxidant properties. Sedky *et al.*[28] also reported high antioxidant and scavenging activity of the aqueous extract of *Balanites aegyptiaca* fruit at 1.5 mg/mL of concentration. The capacity to scavenging free radicals were similar between leaves and seeds but the capacity in reducing ferric ion to ferrous ion was higher with the leaves. Previous authors reported that some plants might exhibit higher capacity in reducing ferric ion to ferrous ion and weaker scavenging free radicals and inversely for others[29,30]. Our results are in agreement with several studies that reported higher correlation between the amount of total phenols and the antioxidant capacity obtained from FRAP and DPPH assays[30,31]. DPPH and FRAP assays have been reported to be reliable in vitro methodologies used to measure the antioxidant capacity of synthetic and natural substances before addition to a food matrix[30].

Based on its *in vitro* antioxidant potential, the effects of diet supplemented with *Balanites aegyptiaca* leaves on performance of broilers under heat temperature were evaluated. The diet supplemented with *Balanites aegyptiaca* leaves did not affect feed intake. Our findings are in line with those of Musa *et al.*[32] who reported that the aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark didn't affect the feed intake of broiler chicks. Other authors stated that, despite feed intake reduction, phytogenic feed additives may enhance feed efficiency through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut[33,34]. Our result showed that the body weight gain was not increased by the diet supplemented with *Balanites aegyptiaca* leaves, suggesting low feed efficiency capacity of *Balanites aegyptiaca* leaves. These results are different from those of several authors who reported that plants rich in polyphenols can improve the growth performance of birds under heat stress[3,6]. In fact, polyphenols can act as radical scavengers and eliminate the oxidative damage caused by heat stress, or may modulate cell signalling pathways to alleviate the impact of heat stress[2]. These results may be explained by the fact that during the first 14 days of experiment, the diet of broilers

was not supplemented with *Balanites aegyptiaca* leaves; meanwhile, broilers were under heat stress conditions. During the first 14 days of experiment, the heat stress index of the experimental room showed higher values (79-89), which had been reported to be associated with heat stress conditions for chickens[35]. Heat stress decreases the gastrointestinal motility and prolongs gastric emptying, which in turn reduced the feed intake[2]. Our result showed that irrespective of the treatment, the feed intake was 28.7% lower than expected value and the body weight of the chicken at 15 and 42 days of age were 20% lower than the expected value. The inefficacy of *Balanites aegyptiaca* leaves may be attributed to its inability to reverse some adverse effects of heat stress occur during the first 14 days. In fact, broilers under heat stress display less crypt depth, mucous area and villus height of small intestine, leading to negative impact on nutrient absorption and weight gain[36]. Moreover, chronic heat stress results in excessive ROS generation that further results in oxidative injury[37]. Several antioxidant enzymes help in protecting the cells from the adverse effects of ROS. However, the protection provided is limited. According to Sahin *et al.*[37], the levels of these antioxidant enzymes may increase initially to protect the cells during the first few hours and then become constant or reduced chronically.

Previous authors stated that heat stress is a major source of systemic oxidative stress since it causes a redox imbalance between the pro- and anti-oxidants in favor of prooxidants[4]. Heat stress may induce reactive oxygen species (ROS) and cause anti-oxidant system disorders, which affect nutrient absorption and metabolism[2]. In our study, the diet supplemented with 2% *Balanites aegyptiaca* significantly increased the MDA content in the liver of broiler suggesting an increment of lipid peroxidation in the liver. The increase of MDA may be attributed to the imbalance antioxidant response or can reflect the degree of oxidative damage in the liver[2,3]. Our results showed that the FRAP antioxidant activity was significantly lower ($p<0.05$) with 2% *Balanites aegyptiaca* as compared to the non-supplemented group. Even though the MDA content in the liver of broiler fed on the diet supplemented with 1% *Balanites aegyptiaca* leaves and that of the non-supplemented broiler were similar, the diet supplemented with 1% *Balanites aegyptiaca* leaves increased significantly the DPPH and the SOD activities, suggesting an antioxidant activity of *Balanites aegyptiaca* leaves at 1%. Our findings are consistent with that of Annan and Dickson[11] who pointed out that *Balanites aegyptiaca* has powerful antioxidant activity by preventing lipid peroxidation, neutralizing the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical and shielding fibroblast cells from oxidative damage. Moreover, Suky *et al.*[38] reported hepatoprotective effect and antioxidant potential of *Balanites Aegyptiaca* (L.) Del against CCl_4 induced hepatotoxicity in rats. As for the MDA content in the liver, the diet supplemented with 2% *Balanites aegyptiaca* significantly decreased the globulins content in the blood of broilers. Globulins are a heterogeneous group of large serum proteins other than albumin. These include clotting proteins, complement, many acute phase proteins, immunoglobulins and lipoproteins. The decrease in total globulins may be attributed to decreased production, resulting from liver insufficiency[39]. Our findings revealed that the total protein concentration in the liver was significantly reduced ($p<0.05$) with 2% *Balanites aegyptiaca* leaves. Such findings suggest that at 2%, *Balanites aegyptiaca* leaves may alter nutrient metabolism in liver.

The alpha-amylase content in the serum of broilers receiving *Balanites aegyptiaca* leaves were significantly increased ($p<0.05$) as compared to other treatments. These results indicated that *Balanites aegyptiaca* increased the synthesis of alpha-amylase, an enzyme produced in the gastrointestinal tract especially in the pancreas. It is a responsible enzyme for starch digestion in birds. This result suggested a positive effect of *Balanites aegyptiaca* on the gastrointestinal tract of broilers. By increasing the production of digestive enzyme like alpha-amylase, *Balanites aegyptiaca* leaves may enhance feed digestibility in the animal gut[34]. The total cholesterol level in the serum was increased with *Balanites aegyptiaca* leaves but the increase was significant only at 1%. *Balanites aegyptiaca* could have improved fat digestion and absorption of lipids, leading to an increase in serum cholesterol. In fact, as a result of limited lipogenesis in birds, most of the absorbed fatty acids will be converted into cholesterol, phospholipids and nonpolar lipids through the monoglyceride pathway[40]. The probable beneficial effect of *Balanites aegyptiaca* was supported by the low mortality rate of broilers receiving diet supplemented with *Balanites aegyptiaca* leaves at 1 and 2%. Even though their mortality rate (9.92 and 10.01% respectively) was higher than 5% expected in broiler production, it was three-fold lower than that registered in non-supplemented group. *Balanites aegyptiaca* may have protected broilers from heat stroke which, under acute heat stress, increases drastically the body temperature and lead to death. The mortality rate in the non-supplemented group (31.7%) is neat to the 37% reported by Brossi *et al.*[41] in broilers under acute heat stress. Result of the present study is in accordance with the results of Srankova *et al.*[42], who pointed out that mortality occurred mostly during the last week of fattening.

CONCLUS1ION

Balanites aegyptiaca leaves and seeds are rich in polyphenol compounds and have good antioxidant potential. Irrespective of the incorporation rate (1 or 2%), *Balanites aegyptiaca* leaves did not affect growth performance but improved alpha-amylase synthesis and liveability of broilers during hot fattening periods. However, 2% *Balanites aegyptiaca* showed deleterious effects on liver metabolism and oxidative status. The diet supplemented with 1% of *Balanites aegyptiaca* leaves could be recommend to improve the antioxidant status and reduce mortality of broilers during hot fattening periods. It will be interesting to evaluate the in vivo effect of *Balanites aegyptiaca* leaves on broilers during the same period but from the age of one day.

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