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Thermoregulatory response and oxidative stress indices of rabbit bucks administered ascorbic acid and sodium bicarbonate in a humid tropical environment

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Abstract

Background: This study aimed to assess the effect of ascorbic acid and sodium bicarbonate on thermoregulatory response and oxidative stress markers of rabbit bucks during highest temperature-humidity index in a humid tropical environment as a mitigation strategy against impact of high ambient temperature on the animals. Twenty-eight exotic rabbit bucks of 9 months old weighing 2.54 ± 0.23 kg were randomly allotted to four treatments consisting of T1 (Control-2 ml of sterile water), T2 (2 ml of 300 ppm ascorbic acid), T3 (2 ml of 0.30% sodium bicarbonate) and T4 (2 ml mixture of 150 ppm ascorbic acid + 0.15% sodium bicarbonate, administered orally at 48 h interval for 12 weeks. After a month of administration, rectal temperature, ear temperature, respiratory rate and pulse rate were monitored twice daily (8:00 am and 4:00 pm). Semen samples were collected from all bucks weekly for seminal lipid peroxidation and total antioxidant activity assay. Blood samples were collected from all bucks and serum obtained using standard procedure to assay for lipid peroxidation and total antioxidant activity.

Results: The result revealed that the rabbits were exposed to very severe heat stress for greater part of the day. At week 5, bucks on T2, T3 and T4 had increased respiratory rate at 8 am than those on T1, similar trend was observed at week 4 and 6. At 4 pm, the pulse rate was significantly ($p < 0.05$) different among the treatments at weeks 11, bucks on T2, T3 and T4 had significantly lowered ($p < 0.05$) pulse rate than those on T1. The administration of ascorbic acid, sodium bicarbonate and its combination did not significantly ($p > 0.05$) influence the ear and rectal temperature of the bucks. At week 11, seminal total antioxidant activity of bucks on T1, T2 and T3 was significantly ($p < 0.05$) lower than bucks on control T4. Lipid peroxidation level was significantly ($p < 0.05$) lower in bucks on T2, T3 and T4 than those bucks on the control.

Conclusion: The administration of ascorbic acid, sodium bicarbonate and its combination had enhanced seminal total antioxidant and reduced lipid peroxidation in heat-stressed bucks.

Keywords: Heat stress, Antioxidant activity, Lipid peroxides, Sodium bicarbonate, Ascorbic acid

Background

Most part of Nigeria are characterised by extended periods of high ambient temperature and humidity and referred to as humid tropics (Jimoh & Ewuola, 2018a). Temperature-humidity index (THI), which is an indicator of thermal comfort level for animals in enclosure, has

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been discovered to peak from February to March, thus animals are exposed to varying severity of heat stress in Nigeria (Jimoh & Ewuola, 2016). Heat stress occurs when the core body temperature of a given specie exceeds its specified range for normal activity resulting from a total heat load (internal heat production and heat gained from environment), exceeding the capacity for heat dissipation. Thermal stress is often accompanied by oxidative stress in which ROS compounds are produced in greater amounts, while radical scavenging antioxidants are consumed by the increased free radicals (Jimoh et al., 2017).

Thermoregulation is a tool to maintain animal body temperature, via balance between heat gain and heat loss, associated with increased ambient temperature which leads to enhanced heat gain as compared to heat loss from the body and may cause heat stress in animals (Jimoh & Ewuola, 2016). Previous reports revealed that exotic rabbits exercise their thermoregulatory apparatus to cope with the prevailing heat stress conditions (Jimoh & Ewuola, 2018b). Heat stress inflicts suboptimal reproductive efficiency and heavy economic losses on rabbit farmers by limiting the breeding season of rabbits and attempts to improve semen quality of rabbit bucks under heat stress is linked or connected to enhancing the antioxidant activity and reducing lipid peroxidation of seminal fluid (Jimoh & Ewuola, 2018a). This is due to seminal oxidative stress in exotic rabbits resulting from increase in ROS production and decrease in antioxidants during heat stress.

In recent times, introduction of exotic rabbit to Nigeria, aimed at improving rabbit population, has been pelted with oxidative stress greatly compromised animal physiology, with high reactive oxygen species and its metabolites (ROS-M) accumulation in blood and seminal fluid of bucks during heat stress (Jimoh et al., 2017). Mitigating strategies to ameliorate the adverse effects of heat stress in exotic rabbits in humid tropics is important to unleash the potential of the animals, owing to the fact that heat stress perhaps poses detrimental effects on productivity and reproductive process of commercial flocks. $NaHCO_3$ in drinking water is useful for alleviating heat load, correcting acid–base balance disturbances under stress conditions (Majekodunmi et al., 2013). Inclusion of vitamin C in water during the hot season aid adaptation, reduce the generation of oxidants by reducing the blood glucose and preventing the lipid peroxidation (Majekodunmi et al., 2015). However, these previous studies are limited to poultry species as experimental subject used for the studies and little or none is known about the adoption of the mitigating agents in rabbit production. Therefore, the possibility of ascorbic acid and sodium bicarbonate to mitigate oxidative stress by thermoregulatory mechanism or increase antioxidant defence and/or its precursors in rabbit bucks exposed to heat stress was investigated in this study.

Methods

Experimental animals and management

Twenty-eight exotic rabbit bucks which include New Zealand White, Chinchilla, English spots and Fauve de Bourgogne, 9 months old weighing 2.54 ± 0.23 kg were used for the experiment conducted in Rabbitry Unit of the Teaching and Research Unit, University of Ibadan, Ibadan, Nigeria. The rabbits were randomly allotted to four treatments with seven rabbits/treatment and blocked for breed effect. The rabbits were housed in a 2-tier rabbit hutches measuring 50 cm × 50 cm × 30 cm. The hutches are made up of steel frame with wire gauze of 0.3 mm covering both sides of the hutch. The experimental rabbits were stabilized for two weeks before the commencement of the actual experiment. During this period, the rabbit bucks were given formulated diet as presented in Table 1, which includes crude protein of 17.78%, crude fibre of 7.55% and digestible energy of 3156.6 kcal/kg. The diets were not pelleted and animals were fed ad libitum and water was made available to the animals throughout the period of the experiment.

Between the hours of 7.00–9.00 am, there was oral gavage of ascorbic acid (vitamin C) and sodium bicarbonate at 48 h interval for 12 weeks. The experimental layout was:

Treatment 1 (Control): 2 ml of sterile water.

Treatment 2: 300 ppm of ascorbic acid (dissolved in 2 ml of sterile water).

Treatment 3: 0.3% sodium bicarbonate (dissolved in 2 ml of sterile water).

Table 1 Gross composition of experimental diet for rabbit bucks

Ingredients (%)	Quantity
Maize	25.00
Palm kernel cake	12.00
Wheat offal	22.00
Corn bran	23.00
Soybean meal	15.00
Fish meal	0.50
Salt	0.25
Premix	0.25
Dicalcium phosphate	2.00
Total	100.00
Calculated nutrient analysis	
Crude protein (%)	17.78
Crude fibre (%)	7.55
Digestible energy (Kcal/kg)	3156.6

Treatment 4: 0.15% of sodium bicarbonate + 150 ppm of Aacorbic acid (dissolved in 2 ml of sterile water).

Rabbitry microclimate data

The rabbit pen was monitored throughout the 12 weeks experimental period, using a thermohygrometer (hydro thermograph). The temperature–humidity index (THI) computed using the formula established by Marai et al., (2002) for rabbits as following:

$$\mathrm{THI} = \mathrm{db}^{\circ}\mathrm{C} - \left\{(0.31 - 0.31\mathrm{RH})\left(\mathrm{db}^{\circ}\mathrm{C} - 14.4\right)\right\}$$

where db °C = dry bulb temperature in degrees Celsius and RH = relative humidity expressed in percentage.

The values of THI obtained for the temperate and subtropical region are classified as: < 27.8 °C = absence of heat stress, 27.8–28.9 °C = moderate heat stress, 28.9–30 °C = severe heat stress and above 30 °C = very severe heat stress (Marai et al., 2002).

Thermoregulatory assessment

After 4 weeks of treatment administration, rectal temperature, ear temperature, respiratory rate and pulse rate were taken twice daily (8:00am and 4:00pm). The ear temperature was taken by placing the digital thermometer in direct contact with the central area of the auricle. Rectal temperature was measured by inserting a digital thermometer (Hartman-UK) to a depth of approximately 4 cm into the rectum for 1 min. Respiratory rate was measured by visually counting the number of movements of the flanks of the rabbits in a resting position for 1 min with a stopwatch calculated as breaths/min, while heart/pulse rate was measured by placing the stethoscope on the chest of the rabbits for 1 min to determine the rhythmic beats of the heart which is calculated as beats/min as described by Jimoh and Ewuola (2016, 2018b).

Semen and blood sample collections

After a month of administration of treatments, semen was individually collected from the bucks. The semen was collected at weeks 6, 7 10 and 11 of administration by stimulation of the bucks using artificial vaginal developed by Ewuola et al., (2014). Semen samples per buck were centrifuged at 3000 rpm for 15 min to obtain seminal plasma. Seminal plasma obtained was used for oxidative status assay; lipid peroxidation and total antioxidant activity (Jimoh & Ewuola, 2019).

Blood samples were collected from the rabbits at the end of the trial via the jugular veins into plain sample bottles and allowed to clot as described by Ewuola and Egbunike (2008). The blood samples were centrifuged at 3000 rpm at 4 °C for 15 min and the clear supernatant was decanted as serum. The serum obtained was assayed for glucose, lipid peroxidation and total antioxidant activity (Jimoh & Ewuola, 2018a).

Glucose determination

Determination of glucose was carried out using Randox Glucose Assay kit with 4-amino phenazone as oxygen acceptor. 1.0 ml of reagent (GOD-PAP reagent made up of glucose oxidase (GOD), peroxide and 4-aminophenazone (POD)) was mixed with 0.1 ml of sample in a test tube; 0.1 ml of prepared standard glucose was also be mixed with 1.0 ml of reagent in another test tube while 1.0 ml of reagent was measured into the third test tube as blank. The mixtures were thoroughly mixed and incubated for 25 min at 25 °C. The absorbance of the standard and the samples were measured against the reagent blank at wavelength 500 nm. Glucose concentration (mg/dl) of samples was calculated by: Absorbance sample × standard concentration (mg/dl)/absorbance standard.

Lipid peroxidation assay

Lipid peroxidation in seminal plasma and serum was measured by reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) according to Yagi (1984). Content of MDA is measured spectrophotometrically using a spectrofluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of serum and seminal plasma was determined using various concentrations of tetraethoxypropane as standards. The results are expressed as nmol MDA/10×10^6 cells, nmol MDA/ml seminal plasma and nmol MDA/total seminal plasma.

Measurement of antioxidant activity

The antioxidant activity of the seminal plasma and serum was measured according to Koracevic et al. (2001). The reaction mixture containing 0.5 ml of a Na-Benzoate (10 mmol/l), 0.2 ml of H_2O_2 (10 mmol/l), 0.49 ml of phosphate buffer (100 mmol/l, pH = 7.4) (prepared by mixing 19.5 ml of KH_2PO_4 (100 mmol/l) with 80.5 ml of Na_2HPO_4 (100 mmol/l), then adjusted the pH to 7.4 and 0.2 ml of Fe-EDTA complex (2 mmol/l). (prepared freshly by mixing equal volumes of EDTA (2 mmol/l), and ferrous ammonium sulphate (2 mmol/l), then left at 25 °C for 60 min. Ten microliters of the blood serum were added to the latter reactive mixture and were incubated at 37 °C for 60 min. Finally, 1 ml glacial acetic acid (20 mmol/l) and 1 ml thiobarbituric acid (0.8% w/v in 100 ml of 50 mmol/l NaOH) were added, and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100 °C for 10 min. Total antioxidant capacity was calculated according to the following formula:

$$T\ A\ \text{capacity}(\text{mmol}/\text{l}) = (\text{CUA})(K - A)/(K - \text{UA})$$

where CUA (mmol/l); concentration of uric acid; K: absorbance of the control (K1 − K0); A: absorbance of the sample (A1 − A0); UA: absorbance of uric acid solution (UA1 − UA0).

Statistical analysis

Data obtained were subjected to descriptive analysis and analysis of variance using the General Linear Model Procedure while significant means were separated using Duncan multiple range test (SAS, 1999). $P<0.05$ was considered significant at 95% confidence interval.

Results

Temperature-humidity index

The temperature-humidity index of rabbitry microclimate is shown in Fig. 1. The temperature-humidity index range within the study period at 8am was between 24.87 and 26.53, at 12 pm values ranged between 30.39 and 31.47, while at 4 pm values was between 30.99 and 31.82. The average temperature-humidity index range within the study period was between 29.05 and 29.83, this is an indication of severe heat stress (28.9–30.0; Marai et al., 2002). The result revealed that the rabbits were exposed to very severe heat stress for greater part of the day (12 pm and 4 pm observation; 30.39–31.82).

Thermoregulatory indices

Respiratory rate

The respiratory rate of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and their combination are shown in Table 2. Significant ($p<0.05$) differences were observed among the treatments at 8am in weeks 4, 5, 6, 8 and 9. At week 5, bucks on T2, T3 and T4 (93.55, 92.71 and 87.50 breath/min, respectively) had increased respiratory rate than those on T1 (77.48 breath/min), similar trend was observed at weeks 4 and 6. At 4 pm, significant ($p<0.05$) differences were observed in the respiratory rate of the rabbits among the treatments in weeks 4, 5, 6, 8, 9, 10 and 11. Rabbit bucks on T2, T3 and T4 (121.11, 121.41 and 121.77 breath/min, respectively)

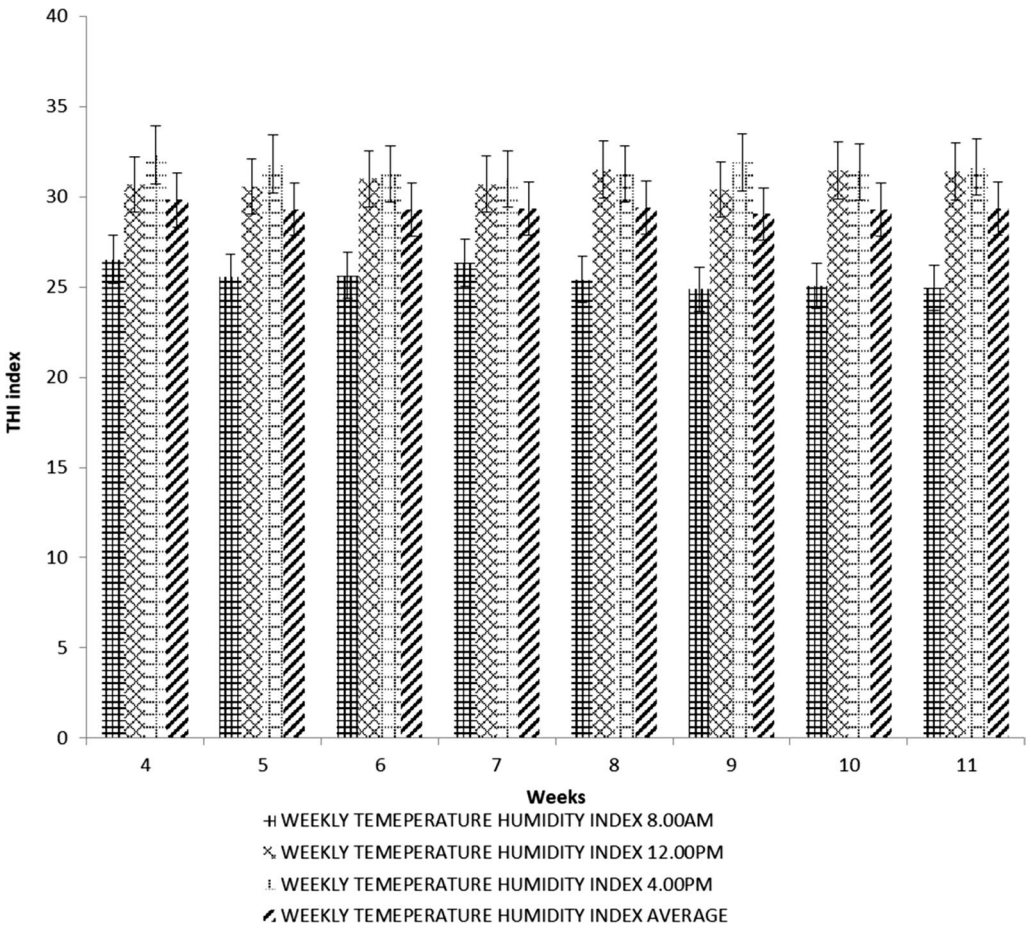


Fig. 1 Temperature-humidity index (THI) patterns during the weeks of study

Table 2 Respiratory rate (breath/min) of heat-stressed bucks administered ascorbic acid and sodium bicarbonate and their combination

Weeks	Time	T1	T2	T3	T4
4	8am	91.78 ± 7.91^{b}	96.37 ± 7.62^{a}	96.43 ± 9.12^{a}	96.50 ± 12.61^{a}
	4pm	105.96 ± 8.05^{b}	112.56 ± 9.18^{a}	108.45 ± 14.75^{b}	111.30 ± 13.02^{a}
	Daily	98.87 ± 7.53^{b}	104.46 ± 8.05^{a}	100.94 ± 11.36^{a}	108.90 ± 11.50^{a}
5	8am	77.48 ± 11.22^{b}	93.55 ± 7.36^{a}	92.71 ± 7.38^{a}	92.50 ± 8.80^{a}
	4pm	96.68 ± 8.32^{b}	111.53 ± 9.79^{a}	116.56 ± 6.91^{a}	117.31 ± 11.12^{a}
	Daily	87.08 ± 3.85^{c}	102.54 ± 5.70^{b}	116.56 ± 6.91^{a}	102.40 ± 5.23^{b}
6	8am	99.20 ± 7.31^{b}	105.98 ± 5.89^{a}	109.75 ± 6.54^{a}	105.24 ± 6.99^{a}
	4pm	113.96 ± 6.72^{b}	119.26 ± 5.38^{a}	123.90 ± 7.61^{a}	121.61 ± 4.87^{a}
	Daily	106.58 ± 6.17^{b}	112.62 ± 4.45^{a}	116.82 ± 6.73^{a}	113.42 ± 4.62^{a}
7	8am	104.46 ± 2.29	106.22 ± 5.97	110.80 ± 6.82	108.02 ± 7.49
	4pm	125.26 ± 5.07	126.73 ± 7.33	125.05 ± 8.46	127.05 ± 5.83
	Daily	114.86 ± 2.83	116.47 ± 4.80	117.92 ± 3.35	117.54 ± 5.66
8	8am	102.20 ± 5.34^{a}	106.37 ± 8.89^{a}	96.30 ± 40.36^{b}	108.62 ± 9.94^{a}
	4pm	120.42 ± 5.15^{b}	143.73 ± 16.77^{a}	123.66 ± 9.69^{b}	122.11 ± 7.81^{b}
	Daily	111.31 ± 5.03^{b}	125.05 ± 12.34^{a}	109.98 ± 17.92^{b}	115.37 ± 6.19^{b}
9	8am	103.90 ± 6.32^{b}	105.05 ± 8.82^{b}	112.76 ± 12.65^{a}	99.52 ± 6.24^{c}
	4pm	120.04 ± 5.37^{a}	119.96 ± 10.74^{b}	112.76 ± 12.65^{b}	122.70 ± 6.36^{a}
	Daily	111.97 ± 3.45	112.50 ± 9.09	106.99 ± 6.99	111.11 ± 4.09
10	8am	108.88 ± 12.15	111.26 ± 7.66	106.15 ± 8.40	108.68 ± 5.10
	4pm	125.18 ± 7.59^{a}	121.11 ± 4.04^{b}	121.41 ± 4.74^{b}	121.77 ± 4.76^{b}
	Daily	125.03 ± 8.70^{a}	116.19 ± 7.16^{b}	114.28 ± 5.58^{b}	116.22 ± 5.41^{b}
11	8am	98.22 ± 7.24	104.73 ± 5.93	106.60 ± 4.37	108.74 ± 4.86
	4pm	113.26 ± 8.09^{b}	120.16 ± 6.53^{a}	122.70 ± 6.55^{a}	127.40 ± 9.26^{a}
	Daily	105.74 ± 7.49^{b}	112.45 ± 5.98^{a}	114.65 ± 4.13^{a}	118.07 ± 6.52^{a}

a, *b*, *c* means within a row with different superscript are significantly different ($p < 0.05$)

T1 control, *T2* Ascorbic acid, *T3* sodium bicarbonate, *T4* ascorbic acid + sodium bicarbonate

had lowered respiratory rate than those on T1 (125.18 breath/min) at week 10. At average daily respiratory rate, significant ($p < 0.05$) differences were observed among the treatments in weeks 4, 5, 6, 8, 10 and 11. Rabbit bucks on T2, T3 and T4 (116.19, 114.28, 116.22 breath/min, respectively) had lowered respiratory rate compared to those on T1 (125.03 breath/min) at week 10.

Pulse rate

The pulse rate of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and their combination are shown in Table 3. The pulse rate was significantly ($p < 0.05$) different among the treatments at 8am in weeks 4, 6, 7 and 11. At week 7, bucks on T2, T3 and T4 (144.38, 140.83 and 139.88 beat/min, respectively) had increased pulse rate than those on T1 (128.64 beat/min), similar trend was also observed at week 4. At 4 pm, the pulse rate was significantly ($p < 0.05$) different among the treatments at weeks 11, bucks on T2, T3 and T4 (157.03, 154.03 and 153.39 beat/min, respectively) had significantly lowered ($p < 0.05$) pulse rate than those on T1 (163.40 beat/min). The average daily pulse rate at weeks 4, 5 and 11 was significantly ($p < 0.05$) higher in the control bucks (T1) than other treatments.

Ear temperature

The ear temperature of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and their combination are shown in Table 4. At 8 am, 4 pm and average daily ear temperature values obtained were not significantly different among the treatments and the control throughout the study.

Rectal temperature

The rectal temperature of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combination are shown in Table 5. At 8 am, 4 pm and average daily rectal temperature values obtained in all the weeks were statistically ($p > 0.05$) similar. Indicating that the administration of ascorbic acid, sodium bicarbonate and its combination did not significantly influence the rectal temperature of heat-stressed bucks.

Serum biochemical indices

The serum glucose of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combination are shown in Fig. 2. The glucose of bucks on control (95.5 ± 0.22 mg/dL) was significantly ($p < 0.05$) higher than bucks on T4 (71.83 ± 0.12 mg/dL).

Table 3 Pulse rate (beat/min) of heat-stressed bucks administered ascorbic acid and sodium bicarbonate and their combination

Weeks	Time	T1	T2	T3	T4
4	8am	109.66 ± 6.20[b]	128.46 ± 8.15[a]	121.36 ± 2.43[a]	126.30 ± 1.86[a]
	4pm	169.78 ± 6.72	168.60 ± 5.22	161.98 ± 4.37	168.54 ± 3.99
	Daily	155.22 ± 52.50[a]	148.53 ± 29.02[b]	140.67 ± 20.82[b]	145.92 ± 23.98[b]
5	8am	120.64 ± 12.08	113.90 ± 9.68	130.51 ± 4.00	124.27 ± 4.71
	4pm	225.76 ± 32.57	186.76 ± 33.65	155.96 ± 13.52	147.08 ± 13.85
	Daily	173.20 ± 12.40[a]	150.33 ± 19.56[b]	143.24 ± 7.39[b]	135.67 ± 7.75[b]
6	8am	127.08 ± 3.08[c]	137.85 ± 15.57[c]	155.60 ± 16.48[b]	173.00 ± 17.84[a]
	4pm	163.42 ± 7.83	160.71 ± 13.70	168.31 ± 10.09	143.85 ± 19.00
	Daily	145.25 ± 2.95	149.28 ± 12.89	161.95 ± 12.91	178.35 ± 17.72
7	8am	128.64 ± 7.90[b]	144.38 ± 16.15[a]	140.83 ± 2.13[a]	139.88 ± 16.81[a]
	4pm	159.26 ± 15.47	163.25 ± 8.97	160.61 ± 15.37	169.90 ± 7.30
	Daily	143.95 ± 11.27	153.81 ± 10.50	150.72 ± 9.90	150.39 ± 10.50
8	8am	145.22 ± 16.47	155.65 ± 13.40	121.71 ± 11.18	120.05 ± 7.96
	4pm	162.22 ± 7.80	164.46 ± 7.40	186.65 ± 42.50	136.02 ± 14.87
	Daily	153.72 ± 11.88	160.05 ± 6.68	154.18 ± 21.97	128.04 ± 10.30
9	8am	121.66 ± 11.93	121.13 ± 8.74	123.78 ± 8.08	122.27 ± 5.83
	4pm	150.54 ± 20.26	156.50 ± 14.84	159.13 ± 13.74	154.10 ± 16.52
	Daily	136.10 ± 14.23	138.81 ± 11.10	141.45 ± 8.82	138.18 ± 8.88
10	8am	144.88 ± 13.93	141.28 ± 14.36	146.88 ± 13.30	143.85 ± 15.22
	4pm	169.32 ± 8.35	161.25 ± 10.31	167.95 ± 9.62	162.92 ± 11.43
	Daily	157.10 ± 9.42	151.26 ± 11.90	157.41 ± 11.37	153.39 ± 12.98
11	8am	148.54 ± 15.10[a]	146.61 ± 10.27[a]	135.98 ± 7.51[b]	132.94 ± 4.73[b]
	4pm	163.40 ± 13.49[a]	157.03 ± 11.61[b]	154.03 ± 7.31[b]	154.17 ± 4.71[b]
	Daily	155.97 ± 13.19[a]	141.82 ± 10.89[b]	145.00 ± 7.14[b]	143.55 ± 3.34[b]

T1 control, *T2* ascorbic acid, *T3* sodium bicarbonate, *T4* ascorbic acid + sodium bicarbonate

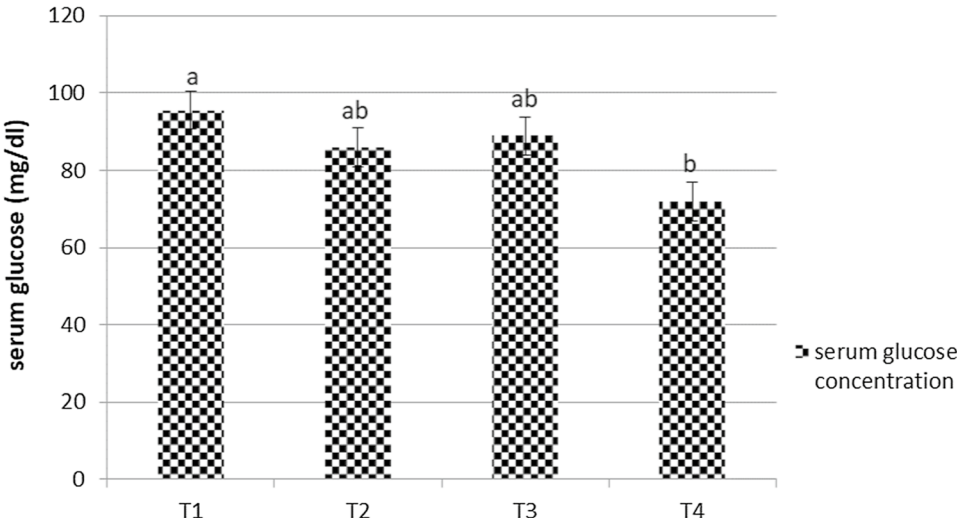
a, *b*, *c* means within a row with different superscript are significantly different ($p < 0.05$)

Table 4 Ear temperature (°C) of heat-stressed bucks administered ascorbic acid and sodium bicarbonate and their combination

Weeks	Time	T1	T2	T3	T4
4	8am	37.16 ± 0.62	37.01 ± 1.12	36.68 ± 0.97	37.28 ± 0.51
	4pm	38.56 ± 0.45	38.83 ± 0.57	38.66 ± 0.78	38.78 ± 0.29
	Daily	37.86 ± 0.44	37.92 ± 0.77	37.67 ± 0.66	38.03 ± 0.36
5	8am	37.44 ± 1.00	37.41 ± 0.83	37.71 ± 0.96	38.01 ± 0.71
	4pm	38.70 ± 0.47	38.55 ± 0.32	38.53 ± 0.45	38.51 ± 0.58
	Daily	38.07 ± 0.69	37.98 ± 0.51	38.12 ± 0.70	38.26 ± 0.52
6	8am	37.22 ± 0.56	37.06 ± 0.18	37.85 ± 0.84	37.24 ± 0.53
	4pm	38.12 ± 0.22	38.48 ± 0.30	38.63 ± 0.54	38.70 ± 0.33
	Daily	37.67 ± 0.36	37.77 ± 0.21	38.24 ± 0.61	37.97 ± 0.30
7	8am	36.78 ± 0.29	37.53 ± 0.31	37.50 ± 0.82	37.90 ± 0.36
	4pm	38.20 ± 0.23	38.30 ± 0.31	38.15 ± 0.37	38.38 ± 0.29
	Daily	37.49 ± 0.26	37.91 ± 0.29	37.82 ± 0.58	38.14 ± 0.30
8	8am	37.40 ± 0.71	37.75 ± 1.04	36.85 ± 1.13	37.78 ± 0.87
	4pm	38.66 ± 0.30	38.68 ± 0.30	38.41 ± 0.43	38.90 ± 0.23
	Daily	38.03 ± 0.44	38.21 ± 0.64	37.63 ± 0.71	38.34 ± 0.46
9	8am	38.36 ± 0.41	38.20 ± 0.51	38.56 ± 0.25	37.60 ± 0.58
	4pm	38.74 ± 0.28	38.50 ± 0.40	38.66 ± 0.32	38.84 ± 0.44
	Daily	38.55 ± 0.32	38.35 ± 0.38	38.61 ± 0.26	38.22 ± 0.46
10	8am	37.30 ± 0.43	37.48 ± 0.33	37.21 ± 0.58	37.61 ± 0.56
	4pm	38.20 ± 0.10	38.08 ± 0.21	37.96 ± 0.15	38.50 ± 0.36
	Daily	37.75 ± 0.25	37.78 ± 0.25	37.59 ± 0.36	38.05 ± 0.37
11	8am	37.56 ± 0.48	37.68 ± 0.31	37.56 ± 0.22	37.68 ± 0.29
	4pm	38.22 ± 0.16	38.53 ± 0.13	38.21 ± 0.21	38.54 ± 0.25
	Daily	37.89 ± 0.29	38.10 ± 0.18	37.89 ± 0.20	38.11 ± 0.19

Table 5 Rectal temperature (°C) of rabbit bucks administered ascorbic acid and sodium bicarbonate

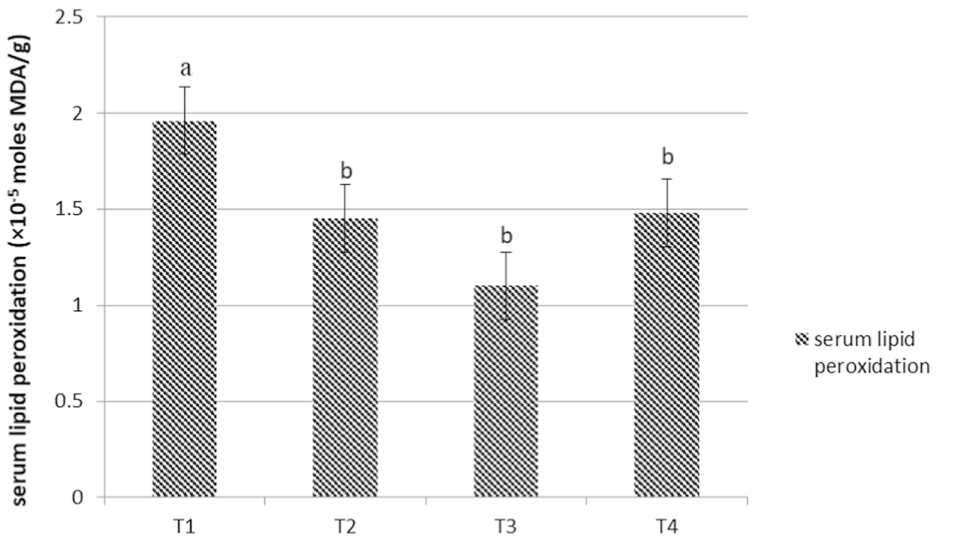
Weeks	Time	T1	T2	T3	T4
4	8am	38.28 ± 0.69	37.95 ± 1.01	38.53 ± 0.64	38.37 ± 0.76
	4pm	38.90 ± 0.50	39.08 ± 0.20	39.45 ± 0.55	39.78 ± 0.51
	Daily	38.59 ± 0.58	38.51 ± 0.53	38.99 ± 0.45	39.26 ± 0.22
5	8am	38.90 ± 0.18	38.66 ± 0.73	39.33 ± 0.30	39.01 ± 0.26
	4pm	39.36 ± 0.20	39.78 ± 0.43	39.80 ± 0.34	39.51 ± 0.42
	Daily	39.13 ± 0.18	39.22 ± 0.51	39.56 ± 0.28	39.53 ± 0.25
6	8am	38.74 ± 0.23	38.51 ± 0.17	39.05 ± 0.56	39.21 ± 0.45
	4pm	39.10 ± 0.23	39.38 ± 0.44	39.66 ± 0.37	39.85 ± 0.26
	Daily	38.92 ± 0.19	38.95 ± 0.25	39.35 ± 0.36	39.25 ± 0.26
7	8am	38.58 ± 0.23	38.61 ± 0.33	38.73 ± 0.18	38.75 ± 0.23
	4pm	38.20 ± 0.23	39.35 ± 0.62	39.45 ± 0.37	39.75 ± 0.32
	Daily	38.81 ± 0.06	38.98 ± 0.27	39.09 ± 0.20	39.42 ± 0.49
8	8am	38.64 ± 0.63	38.21 ± 0.64	38.21 ± 0.70	39.15 ± 0.56
	4pm	38.64 ± 0.63	39.75 ± 0.38	39.10 ± 0.35	39.70 ± 0.43
	Daily	38.98 ± 0.33	39.43 ± 0.41	38.65 ± 0.48	39.64 ± 0.15
9	8am	39.18 ± 0.37	38.35 ± 0.38	39.20 ± 0.54	39.30 ± 0.17
	4pm	39.18 ± 0.37	39.48 ± 0.42	39.78 ± 0.24	39.98 ± 0.18
	Daily	39.48 ± 0.32	39.07 ± 0.35	39.49 ± 0.37	39.07 ± 0.54
10	8am	38.10 ± 0.14	37.78 ± 0.25	38.21 ± 0.24	38.51 ± 0.24
	4pm	38.10 ± 0.14	39.41 ± 0.31	39.50 ± 0.23	39.74 ± 0.41
	Daily	38.57 ± 0.09	38.87 ± 0.14	38.85 ± 0.25	39.12 ± 0.30
11	8am	38.50 ± 0.29	38.81 ± 0.44	38.23 ± 0.15	38.32 ± 0.18
	4pm	39.14 ± 0.15	39.46 ± 0.27	39.21 ± 0.16	39.31 ± 0.13
	Daily	38.82 ± 0.20	39.14 ± 0.28	38.14 ± 0.30	38.82 ± 0.14



T1- Control, T2- Ascorbic acid, T3- Sodium bicarbonate, T4- Ascorbic acid + Sodium bicarbonate.

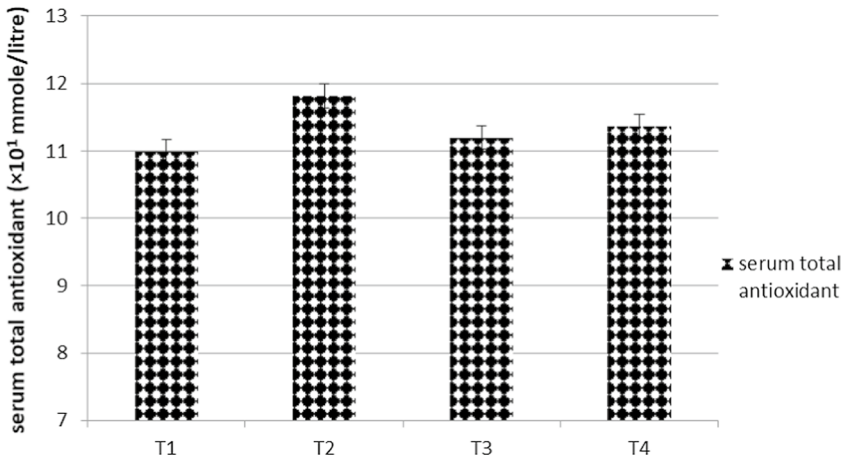
Fig. 2 Serum glucose level of the rabbit bucks administered ascorbic acid and sodium bicarbonate. T1-control, T2-ascorbic acid, T3-sodium bicarbonate, T4-ascorbic acid + Sodium bicarbonate

Heat-stressed bucks on T2, T3 and T4 (1.45 ± 0.18, 1.10 ± 0.21 and $1.48 \pm 0.20 \times 10^{-5}$ mol MDA/g, respectively) had significantly ($p < 0.05$) lowered serum lipid peroxidation values compared to those on T1 ($1.96 \pm 0.31 \times 10^{-5}$ mol MDA/g) as shown in Fig. 3. The serum total antioxidant profile of heat-stressed



T1- Control, T2- Ascorbic acid, T3- Sodium bicarbonate, T4- Ascorbic acid + Sodium bicarbonate.

Fig. 3 Serum lipid peroxidation profile of rabbit bucks administered ascorbic acid and sodium bicarbonate. T1-control, T2- ascorbic acid, T3-sodium bicarbonate, T4-ascorbic acid + sodium bicarbonate



T1- Control, T2- Ascorbic acid, T3- Sodium bicarbonate, T4- Ascorbic acid + Sodium bicarbonate.

Fig. 4 Serum total antioxidant profile of rabbit bucks after the administration of ascorbic acid and sodium bicarbonate. T1-control, T2-ascorbic acid, T3-sodium bicarbonate, T4-ascorbic acid + sodium bicarbonate

bucks presented in Fig. 4 was not significantly different across the treatments.

Seminal plasma lipid peroxidation

The seminal lipid peroxidation in heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combination are shown in Fig. 5.

At weeks 6, 7 and 10, administration of ascorbic acid, sodium bicarbonate and its combination to bucks had no significant ($p > 0.05$) difference on seminal lipid peroxidation, but apparently seminal lipid peroxidation was lower in bucks on T2-T4 over same period. However, at week 11, lipid peroxidation level was significantly ($p < 0.05$) lower in bucks on T2, T3 and T4 (1.78 ± 0.46, 1.70 ± 0.36, $1.47 \pm 0.22 \times 10^{-5}$ mol MDA/g, respectively) than those bucks on the control treatment ($2.75 \pm 0.14 \times 10^{-5}$ mol MDA/g).

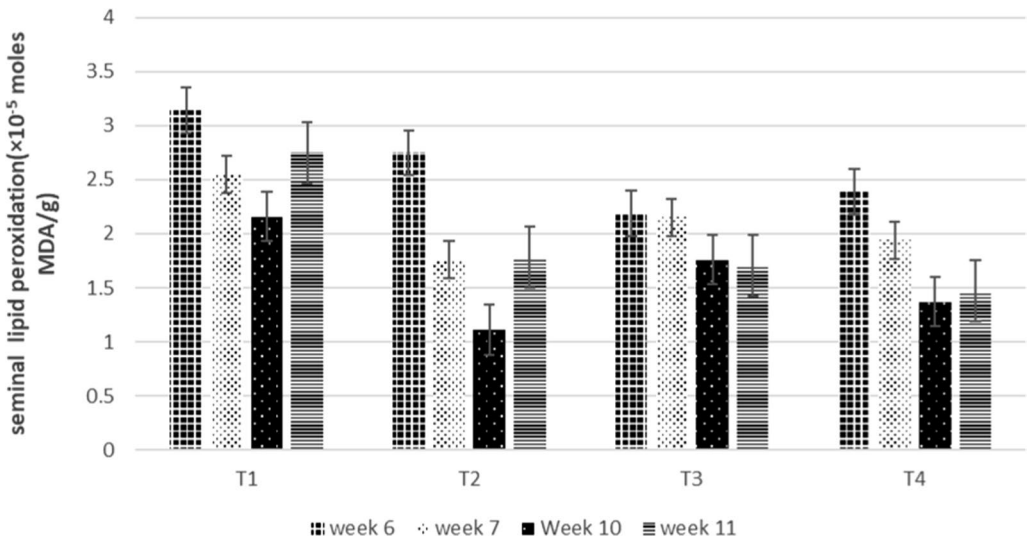


Fig. 5 Seminal lipid peroxidation in heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combinations

Seminal plasma total antioxidant

The seminal total antioxidant activity of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combination are shown in Fig. 6. At weeks 6, 7 and 10, the seminal total antioxidant heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combinations were apparently ($p > 0.05$) higher than bucks on control. However, at week 11, seminal total antioxidant activity of bucks on T1, T2 and T3 (0.88 ± 0.34, 1.06 ± 0.81 and $1.26 \pm 0.67 \times 10^1$ mmol/l, respectively) was significantly ($p < 0.05$) lower than bucks on control T4 ($1.66 \pm 0.72 \times 10^1$ mmol/l).

Discussion

Heat stress increases the demand of antioxidants; vitamin C important in cellular antioxidant defence not only by reacting with all oxygen species through formation of dehydroascorbyl (inert radical) but also by transferring radical equivalents from lipid phases to aqueous compartments (Abidin & Khatoon, 2013; Ciftci et al., 2005).

The range of afternoon/evening temperature-humidity index recoded in this study at 30.39–31.82 demonstrates that the animals were exposed to very severe heat stress for greater part of the day. The values obtained were lower than 30.66–35.02 recorded in the evening in the same study site in February and March 2014 for exotic breeds of rabbit (Jimoh & Ewuola, 2016). This reveals that the severity of heat stress could vary depending on the prevailing effects of global warming yearly. This justifies higher range of pulse and respiratory rates and similar range of ear and rectal temperature recorded in heat stress exotic rabbits (Jimoh & Ewuola, 2016) as compared to the values obtained in this study. In hot environments, animals attempt to maintain heat balance by increasing their respiratory activity, thereby losing more heat by evaporation from the respiratory tract than under normal circumstances. This explains the increase respiratory rate observed in this study compared to exotic rabbits at least temperature-humidity index reported by Jimoh and Ewuola (2018b).

Heat-stressed rabbits tends to have high rectal temperature due to its poor functional sweat glands to lose excess heat. The administration of ascorbic acid, sodium bicarbonate and its combination did not influence the rectal temperature of heat-stressed bucks in this study. This is at variance to report of El-Desoky et al., (2017) that bucks supplemented with moringa oleifera leaf extract had lower rectal temperature. Also, Al-Zafry and Medan (2012) indicated that supplementation of vitamin E and selenium complex decreased both rectal and skin temperatures in heat-stressed rabbits. This could suggest that the supplements could not lower the rectal temperature beyond the values and/or exert other mechanism to ameliorate its effects. Similar reports of vitamin C supplementation and providing cool water were found to be effective in alleviating the heat load of rabbits (Al-Shanty, 2003). Vitamin C has been reported to reduce panting and body temperature in heat-stressed birds (Abidin & Khatoon, 2013).

Prostaglandin output turnover increases during stress and has a direct effect on the hypothalamic thermoregulatory zone. Vitamin C have an ameliorating effect upon heat-stressed animals by decreasing prostaglandin output (Al-Zafry & Medan, 2012). Similarly, Anwar and Aslam (2013) reported that supplementing a combination of dietary vitamin C and sodium bicarbonate reduce heat stress-related decreases in broiler Japanese quails. Ascorbic acid levels in the blood decrease as environmental

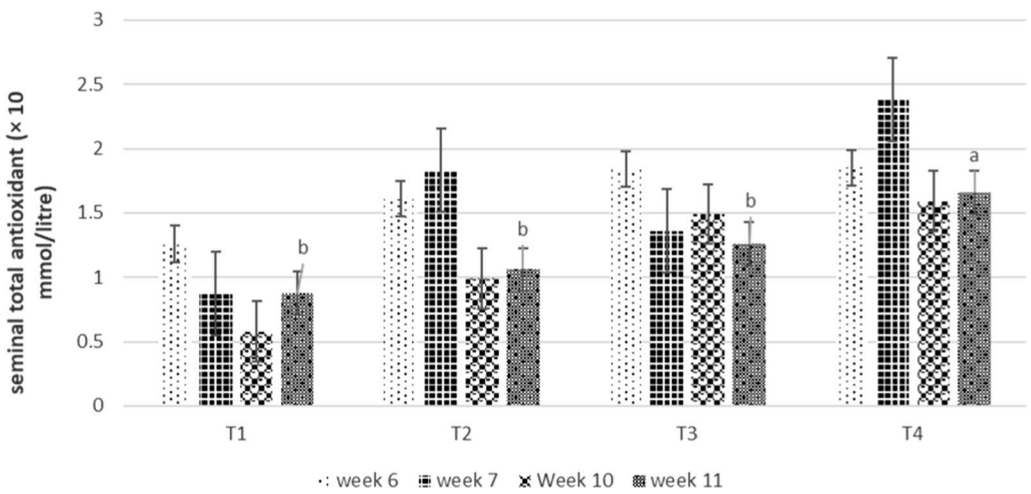


Fig. 6 Seminal total antioxidant activity of heat-stressed bucks administered ascorbic acid, sodium bicarbonate and its combinations

temperature increases, hence exogenous ascorbic acid will be beneficial for proper metabolism of amino acids and minerals as well as for the synthesis and production of hormones involved in resistance to stress (Abidin & Khatoon, 2013).

Higher glucose in control rabbits could be due to increase in glucose probably led to stress induced activation of cortisol secretion and the consequent stimulation of gluconeogenesis (Marai et al., 2007). The range of serum glucose obtained in this study is higher than values of 60–75.65 in exotic rabbits (Jimoh, 2019) and 38.04–46.73 in heat-stressed exotic rabbits (Jimoh et al., 2017). This suggests a possible stimulation of gluconeogenesis as a result of heat stress.

The result of this study shows that the supplement lowers serum lipid peroxidation. This is in accordance with claims that antioxidant vitamins have a scavenging effect on reducing oxidative stress by the elimination of excessive ROS, have been demonstrated in various species (Jang et al., 2014). The values of serum total antioxidant activity of heat-stressed bucks supplemented with ascorbic acid, sodium bicarbonate and its combination were higher than values of 0.8–1 mmol/l in heat-stressed exotic bucks (Jimoh et al., 2017), and 0.62–0.76 mmol/l in exotic bucks at thermal comfort (Jimoh, 2019). Apparently, this coupled with lower values in control groups of this study, which indicates that antioxidant systems of bucks could be enhanced in serum of heat-stressed bucks. Jang et al. (2014) reported similar trend in heat-stressed broilers administered vitamin C had enhanced hepatic and serum total antioxidant status and reduced lipid peroxidation.

The ameliorative effect of $NaHCO_3$ could be attributed to the acid-base balance due to increased metabolic acidosis (Voiculeț et al. 2016; Abdel-Latif et al., 2018). Yin et al. (2018) reported that supplementation with vitamin C or vitamin C-sodium bicarbonate greatly enhanced exogenous antioxidants, which decreased the need for endogenous antioxidants. Ascorbic acid being an essential nutrient in maintenance of homeostasis, actively transported in tissues and increase in its utilisation and demand during hot weather. Similarly, Yassein et al., (2008) reported that vitamin C alleviates heat stress in rabbit does, and Jang et al. (2014) revealed that vitamin C is more effective in alleviating heat stress than vitamin E in birds, despite both antioxidant vitamins having beneficial effect in maintaining immunity and antioxidant status.

The results of seminal oxidative status show the beneficial effect of the supplement to enhance oxidative stability of spermatozoa and improve its fertility. Similarly, addition of ascorbic acid to the drinking water of rabbits does relieved the adverse effect of heat stress on reproductive parameters (Zeweil et al., 2009). Similarly, Jimoh and Ewuola (2018a, 2018b) reported that oxidative stress due to hyperthermia reduces seminal antioxidant activity and result in impairment of sperm membrane integrity. The range of seminal total antioxidant values obtained in heat-stressed bucks supplemented with ascorbic acid, sodium bicarbonate and its combination in this study is higher than values recorded by Jimoh (2019) in exotic breeds of rabbit at thermal comfort (0.60–0.82 mmol/l) and Jimoh et al., (2017) in heat-stressed exotic rabbit bucks (0.19–0.60 mmol/l). This signifies the antioxidant enhancement in seminal fluids of heat-stressed bucks by the supplementation of ascorbic acid and sodium bicarbonate. Abdel-Latif et al., (2018) reported that vitamin C, and $NaHCO_3$ can ameliorate heat stress-induced symptoms in rabbit.

Conclusions

This study reveals that administration of ascorbic acid at 300 ppm, sodium bicarbonate at 0.3% or its combination at 150 ppm ascorbic and 0.15% $NaHCO_3$ to heat-stressed bucks enhanced antioxidant status and prevented seminal and serum oxidative stress by reducing the lipid peroxidation. Therefore, when ambient temperature is high to the extent of inducing heat stress in rabbits, any of ascorbic acid, sodium bicarbonate or their combination could be used by farmers to improve performance and overall productivity of the animals.

Abbreviations

AA: Ascorbic acid; EDTA: Ethylene dimethyl tetra acetic acid; MDA: Malondialdehyde; RH: Relative Humidity; ROS: Reactive oxygen species; SB: Sodium bicarbonate; THI: Temperature-humidity index.

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Author contributions

EOE designed the study, approved the study layout and supervised the research. SRO, OT and AT carried out the procurement of experimental materials, field work and statistical analysis as well as wrote the first manuscript. JOA jointly approved the study lay out with EOE, read and corrected the first manuscript. All authors read and approve the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

In Line with NIH guide for the care and use of laboratory animals, the study was approved by the institutional committee on the care and use of animals for the experiment. The number was not applicable and not available.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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