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Thermal stress causes oxidative stress and physiological changes in female rabbits

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ABSTRACT

The present study investigated the effects of heat stress on oxidative stress status and physiological changes using female New Zealand White rabbits. 24 sexually mature female rabbits weighing 1953.1–2375.4 g were divided into 4 groups of 6 animals each and subjected to ambient temperature (T0: 19–26 °C), 27–28 °C for T1, 31–32 °C for T2 and 35–36 °C for T3 using electrical heaters from 8:00 a.m. to 4:00 p.m. daily for 30 days. Feed intake and body weight gain were recorded daily. Behavioral alterations of anxiety, dizziness, aggression, withdrawal, impaired feed intake were observed. At the end of experimental period animals were sacrificed, blood samples and vital organs such as liver, kidney, heart, ovaries, uterus collected for appropriate analysis. Results revealed that animals of T2 and T3 had an 11% decrease in the final body weights and 62% body weight gain but increase in feed conversion ratio by 64.81%, 24.19% water intake, 3.64% in rectal and 2.42% in skin temperature compared to the control. Dizziness, withdrawal to a corner of the cage and reduced feed intake were observed. The live weight of lungs and kidneys increased by 37.71% and 33.78% while that of ovaries and uterus decreased in the same animals of T2 and T3. Animals from T2 and T3 showed significant decrease ($p < 0.05$) by 23.64% in hemoglobin concentration, 12.73% in red blood cells, 11.93% in packed cell volume, 12.02% in total protein while mean corpuscular volume, white blood cells, lymphocytes, creatinine, urea and aspartate transaminase increased respectively by 10.73%, 42.37%, 15.53%, 28.98%, 53.2% and 23.31% compared to the control. The kidney level of malondialdehyde was significantly increased in T2 and T3 animals by 74.29%, whereas protein, catalase, superoxide dismutase and glutathione peroxidase activity were significantly lower ($p < 0.05$) compared with control. It was concluded that long-term exposure of female rabbits to elevated ambient temperatures induces heat stress and accompanying oxidative stress that consequently impairs physiological function.

1. Introduction

Climate change, a long-term imbalance of customary weather conditions such as temperature, radiation, wind and rainfall characteristics of a particular region, is likely to be one of the main challenges of the present century for mankind (Ganaie et al., 2013). The earth's climate has warmed in the last century (0.74 ± 0.18 °C) with the 1990s and 2000s being the warmest on instrumental record (Intergovernmental Panel on Climate Change (IPCC, 2019)). Furthermore, the earth's climate has been predicted to change continuously at rates unprecedented in recent human history (IPCC , 2007). The variation in climatic variables like temperature, humidity and radiations have been recognized as the potential hazards in the growth performances and production of all domestic livestock species. High ambient temperature accompanied by high air humidity is reported to cause discomfort and enhance the stress levels which results in depression of the physiological and metabolic activities in animals (Ganaie et al., 2013).

In Africa, rabbits have been promoted as tool for poverty alleviation, food security management, reducing rural-urban migration, entrepreneurial skills, humanitarian services including recovery efforts from natural disasters and gender empowerment (Lukefahr, 2000; Mutwedu et al., 2015). These animals are highly preferred because of their body size, high rate of reproduction, adaptability to inexpensive housing and useful by-products (Mapara et al., 2012). However, African rabbit

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husbandry is facing several constraints such as lack of reproductive management, predation, uncontrolled cross-breeding practices, inbreeding, negative selection (Mapara et al., 2012; Mutwedu et al., 2015) and environmental stress (Kumar et al., 2011; Rafel et al., 2012; Sabah et al., 2016).

The term 'stress' here refers to a set of physiological and behavioral responses to a hostile environment (Merlot, 2004). According to physiologists, stress results from external forces that disrupt homeostasis. Several types of stress affecting animals include physical, nutritional, chemical, psychological and thermal factors (Ngoula et al., 2017a, 2017b). The latter arises when the environmental temperature exceeds the thermoneutrality zone of the animal, otherwise referred to as "thermal comfort zone" hence reducing its productive performances (Kumar et al., 2011). Rabbits ideal environmental temperature ranges between 16 °C and 21 °C (Marai et al., 1994; Sabah et al., 2016). Environmental temperatures above this range results into heat stress as a result of few sweat glands that aid in removal of excess body heat (Rafel et al., 2012). Their long exposure to thermal stress leads to the increase in free radicals which may induce oxidative stress (Kumar et al., 2011). Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the body's own natural antioxidant defense (Tremellen, 2008). In female, elevated oxidative stress increases not only the risk of spontaneous abortion (Barrington et al., 1996; Vural et al., 2000) but also other factors such as litter performance, the well-being and health status of animals including reduced milk production, reproductive performance and longevity (Agarwal et al., 2003; Jabbour et al., 2009; Zhao et al., 2011).

Female rabbits are very sensitive to heat stress, which is considered as an important factor influencing their reproduction, fertility and physiological traits (Rafel et al., 2012; Sabah et al., 2016). Exposure to 41 °C to New Zealand rabbits, led to 18% decrease in red blood cells count, 20% decrease in hemoglobin content and 22% decrease in blood platelet count as well as 11.2% decrease in total protein, 24% of albumin, 21% of globulin (Sabah et al., 2016). Rabbits exposed to the temperature between 25 °C and 36 °C compared to those maintained between 14 °C and 20 °C during pregnancy and lactation, produced lower litter size (9.7 vs 11.4), lower litter weight (503.0 vs 630.5 g) and lower kit weight at birth (56.6 vs 61.4 g) as well as higher stillborn rate (25.4 vs 9.9%) (Marco-Jiménez et al., 2017). Marai et al. (2004) reported a decrease in milk yield at day 7 of suckling and milk intake per kit at 7 and 14 days of age in female rabbits reared in hot climate in Egypt. High temperature also affects oogenesis especially during telophase I and metaphase II of meiosis thereby affecting quality of oocytes produced (Hamam et al., 2001).

Rabbits, as a homoeothermic animal, can regulate the heat input and output of their bodies using physical, morphological, biochemical, and behavioral processes to maintain a fairly normal body temperature (Marai et al., 1994). Unfortunately, the link between the oxidative stress status, physiological parameters and heat stress in female rabbits under different ranges of temperature is not well documented in literature. This study aimed at evaluating the oxidative stress status and ensuing physiological and behavioral changes in female rabbits exposed to different ranges of temperature.

2. Materials and methods

2.1. Animals and housing

Twenty-four mature female New Zealand White rabbits, clinically healthy, aged 6 months and weighing between 1953.1 and 2375.4 g were used for the study. They were purchased from a local recognized and licensed breeder and transported to the animal house at the Department of Anatomy and Physiology, University of Nairobi, Kenya. Rabbit does were fed the basal commercial pelleted ration containing 18.18% crude protein, 13.43% crude fiber, 2656 MJ/kg diet digestible energy and 2.29% ether extract that met all nutritional requirements of rabbit does according to the National Research Council (NRC, 1977). Fresh water was available to the animals *ad libitum*. The rabbits were housed in wire cages (0.8 × 0.6 × 0.6 m) at room temperature of 22 ± 4 °C with animal house relative humidity of 68 ± 5% during the acclimatization period and kept under the same hygienic and managerial conditions. Fecal matter and urine were removed from the cages and floor every morning.

2.2. Experimental design

The experimental protocol was approved by Ethical Committee of the Faculty of Veterinary Medicine of the University of Nairobi (REF: FVM BAUEC/2019/244) and the experiments were performed in accordance with the internationally accepted standard ethical guidelines for Laboratory Animal Use and Care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986. Following 2 weeks of acclimatization, all the 24 female rabbits were randomly assigned to 4 groups (T0: ambient temperature (19–26 °C), T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C) of 6 animals each with comparable weight. The heat was induced in each rabbit cage, using electrical heaters (brand: ARMCO) from 08:00 h to 16:00 h followed by exposure to the normal air temperature as in the control group from 16.00 h to 08:00 h. During the experimental period, the relative humidity and ambient temperature were recorded twice daily using an automatic thermo-hygrometer. Animals were submitted during 30 consecutive days to the temperature, relative humidity and temperature humidity index (THI) as follow: T0: ambient temperature (19–26 °C), 58 ± 0.72%, 22.3 ± 1.84, T1: 27–28 °C, 65 ± 0.12%, 26.1 ± 0.6; T2: 31–32 °C, 62 ± 0.8%, 29.5 ± 0.6, T3: 35–36 °C, 63 ± 0.4%, 32.9 ± 0.6. The THI was calculated following the formula described by Marai et al. (2001): THI = db°C-[(0.31–0.31RH) (db°C-14.4) where RH = relative humidity/100, t = ambient temperature. The obtained values of THI for rabbit were classified as follows: < 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., 2001). In the present study, animals of T0 and T1 were exposed to no heat stress and moderate heat stress respectively while those of T2 and T3 were respectively exposed to severe heat stress and very severe heat stress. During the trial period, water consumption (W.C), feed intake (F.I.) and weight gain (W.G.) were measured daily. Daily body weight gain and feed conversion were calculated for each female rabbit according to the equations developed by Sabah et al. (2016): Daily body weight gain = final body weight - initial body weight/period (days); Feed conversion ratio = feed intake/body weight gain.

2.3. Behavioral assessment

Previous studies have shown that stress induces behavioral modifications in animals with the aim of coping with the stressor (Rafel et al., 2012; Nyongesa et al., 2014). It is on this basis that the present study assessed the behavioral changes following induction of heat stress. Anxiety was scored using behavioral indicators of pacing, scratch and self-directed behaviors; difficulty in standing in upright position, staying alert and moving were indicators of dizziness; aggression was assessed by animals showing ears held flat and turned back, nipping hands, shaking the cage wall, growling and thumping in presence of observer, biting and scratching at the slightest sign of danger; withdrawal was present when the animal appeared isolated to the corner of the cage) and impaired feed intake assessed by lag time in response to introduction of feed and water Shepers et al. (2009); Rafel et al. (2012); Nyongesa et al. (2014). These behaviors were evaluated during the last week of the experimentation.

Three observers, standing at strategic positions in full view of focal subjects, made the behavioral scoring. Following heat-induction, all behaviors were observed seven consecutive days before the end of the trial from 10.00 h to 12.00 h of each observation day. Focal subjects in

individual cages were observed for their interest with empty toys and/or toys enriched with food. A video camera connected to a video camera recorder was placed at a strategic position within the animal house and three different observers that had been habituated to the animals scored individual behavioral scores. Inter-rator reliability use was 90%; it defined number of times behavior was scored divided by the number of times each of the observers scored the behavior. Following the method developed by Ngoula et al. (2017a), each behavioral alteration was scored from 0 to +4 (0 = none, +1 = very weak, +2 = weak, +3 = moderately and +4 = severely) depending on the severity and frequency.

2.4. Blood and organ collections

At the end of the experimental period (30 days), all animals were fasted for 24 h and humanely sacrificed by euthanizing. For hematological and biochemical analysis, 10 ml of the blood was collected directly by cardiac puncture before euthanasia. After sacrifice, the ovaries, uterus, lung, heart, liver and kidney were collected by dissection, freed of adipose tissue, washed using saline solution and blot-dried for weight evaluation. The relative weights of the organs were expressed as percentage of slaughter weights.

2.5. Oxidative stress biomarkers

After harvesting and weighing, the kidney was quickly homogenised. The homogenate was then centrifuged at 4800 rpm for 60 min at 4 °C and the supernatant analyzed for oxidative stress biomarkers. Protein content in the supernatant was determined using bovine serum albumin as standard according to method described by Lowry et al. (1951). The kidney enzymatic activities of catalase (CAT) and reduced glutathione (GSH) as well as the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were assessed in kidney homogenates using a spectrophotometer (GENESYS 20.0) according to the methods described respectively by Habbu et al. (2008), Dimo et al. (2006), Kodjo et al. (2016) and Sajeeth et al. (2011).

2.6. Hematological and biochemical analysis

Blood for hematological analysis was collected in a test tube with K3 EDTA anticoagulant and hematological parameters of white blood cell count (WBCs), red blood count (RBCs), haemoglobin content (Hb), lymphocytes (LYM), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV) and Platelet count (PLT) were analyzed immediately after collection using the Coulter Counter System (Beckman Coulter®, Thermo Fisher, UK) (Sabah et al., 2016; Jensen, 2009). Meanwhile, 5 ml of blood for biochemical analysis collected in tube free from anticoagulant was centrifuged at 3000 rpm for 15 min and supernatant separated as serum and preserved at −20 °C for the evaluation of serum content of total cholesterol, albumin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), urea, creatinine, protein and glucose using commercial assay kits. Analysis were done according to manufacturer's instructions (Ngoula et al., 2017b).

2.7. Statistical analysis

All data were submitted to analysis of variance using XL STAT for Windows 10 Software. Results are expressed as mean ± SD, and treatment effects among experimental groups alongside controls assessed using one-way ANOVA. The differences in mean values were compared using the Tukey HSD post hoc test at 5% significance level. The Effect Rate (ER) was calculated as the ratio between the value of the control group and the value of the group with the most relevant impact.

3. Results

3.1. Effect of heat stress on growth performances

There was a significant decrease ($P < 0.05$) in final body weight and body weight gain, respectively by 11% and 62%, in animals from exposed to 31–32 °C and 35–36 °C compared to those from the control (19–26 °C). However, there was apparent opposite trend in feed conversion ratio (64.81%) and water intake (24.19%) while no significant effect ($P > 0.05$) was observed on the average feed intake in all treatment groups compared to controls (Table 1).

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C; ER: Effect Rate. *, *, **: significant at $P = 0.05$; 0.01 and 0.001, respectively.

3.2. Effect of heat stress on behavioral scores

The behavioral scores recorded on heat stressed female rabbits are presented in Table 2. No behavioral changes were observed in animals of the control group (19–26 °C) as well as those exposed to 27–28 °C. Dizziness was observed in animals exposed to 31–32 °C and 35–36 °C. All animals exposed to 31–32 °C and 35–36 °C showed a very weak response to feed introduction (score 1) but severely withdrawn to a corner of the cage (score 4). On the other hand, animals exposed to 19–26 °C and 27–28 °C animals had severe response to feed introduction (score 4).

T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C; 0, +1, +2, +3, +4 represent respectively none, very weak, weak, moderately and severely behavioral scores.

3.3. Effect of heat stress on rectal and skin temperature

Results obtained in Fig. 1 show that severe and very severe heat stress caused a significant increase ($P < 0.05$) of both rectal and skin temperatures. Rectal temperature increased significantly by 3.64% with the increase in temperature range while the skin temperature has increased by 2.42% in rabbits submitted to the highest temperature compared to controls.

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C.

3.4. Effect of heat stress on relative weight of some internal organs

The relative weights of heart and liver was not significantly affected ($P > 0.05$) by the temperature levels considered in this study (Table 3). However, the relative weight of the lung and kidney were significantly increased ($P < 0.05$), respectively by 37.72% and 19.5%, in animals exposed to 31–32 °C and 35–36 °C while that of ovaries and uterus decreased significantly ($P < 0.05$) by 30.4% and 18.1% respectively at the same temperatures when compared to those submitted to the control group (19–26 °C) and 27–28 °C.

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C; ER: Effect Rate. *, *, **: significant at $P = 0.05$; 0.01 and 0.001, respectively.

3.5. Effect of heat stress on hematological parameters

There was a significant decrease ($P < 0.05$) in Hb by 23.64% and 12.73% of RBC contents in animals exposed to 31–32 °C and 35–36 °C but total WBC, MCV and LYM counts increased in the same animals respectively by 42.37%, 10.73% and 15.53% when compared to control. The PCV decreased only in animals exposed to 35–36 °C when compared to control and other treated groups. The other blood parameters of MCH,

Table 1
Growth performances for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C	ER (%)	P-value
Initial body weight (g)	2178.30 ± 167.10	2110.10 ± 110.75	2143.5 ± 91.88	2156.30 ± 161.75	–	0.852
Final body weight (g)	2608.20 ± 163.65^{a}	2556.40 ± 129.03^{a}	2359.1 ± 65.70^{b}	2319.60 ± 75.10^{b}	−11	<0.001***
Body weight gain (g)	429.90 ± 194.53^{a}	446.28 ± 72.41^{a}	215.60 ± 84.44^{b}	163.28 ± 107.68^{b}	−62	0.009**
Feed conversion ratio (g)	2.19 ± 0.93^{b}	1.81 ± 0.32^{b}	3.95 ± 0.49^{b}	3.61 ± 0.61^{ab}	64.81	0.006**
Average feed intake (g)	750.37 ± 130.95	756.42 ± 136.03	781.02 ± 178.62	776.82 ± 116.12	3.56	0.8149
Average water intake (ml)	1102.30 ± 149.98^{ab}	985.00 ± 161.43^{b}	1196.70 ± 256.69^{ab}	1368.80 ± 219.10^{a}	24.19	<0.001***

Table 2
Behavioral alterations for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C
Anxiety	0	0	0	0
Dizziness	0	0	+2	+2
Aggression	0	0	0	+1
Withdrawal	0	+1	+4	+4
Response to feed introduction	+4	+4	+1	+1

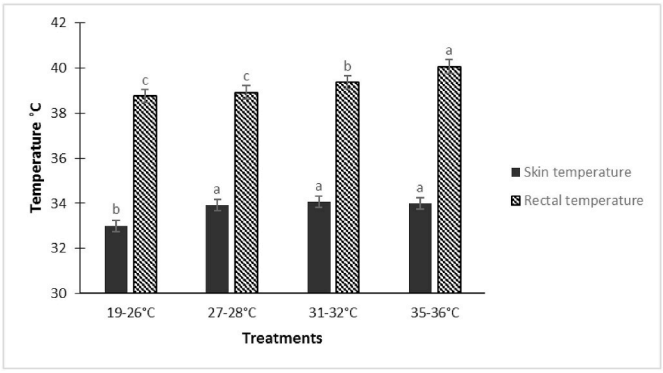

Fig. 1. Skin and rectal temperatures for female rabbits, as affected by different ranges of temperature.

MCHC and PLT were not significantly affected by the heat stress (Table 4).

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C. Hb: hemoglobin; PCV: packed cell volume; RBC: Red blood Cell; MCV: mean cell volume; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; WBC: white blood cells; PLT: Platelet count; LYM: lymphocytes; ER: Effect Rate. *, *, **: significant at $P = 0.05$; 0.01 and 0.001, respectively.

3.6. Effect of heat stress on biochemical parameters

Creatinine, urea and ASAT were significantly increased ($P < 0.05$) respectively by 28.98%, 53.20% and 23.31% in 31–32 °C and 35–36 °C while total protein decreased by 12.02% in animals in the same groups compared to those of the control group (19–26 °C) and 27–28 °C. There was no significant difference on cholesterol, ALAT, glucose and total albumin in treated groups compared to controls (Table 5).

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3: 35–36 °C. ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase, ER: Effect Rate. *, *, **: significant at $P = 0.05$; 0.01 and 0.001, respectively.

3.7. Effect of heat stress on oxidative stress biomarkers

In female rabbits exposed to 31–32 °C and 35–36 °C, the protein levels in the kidney significantly decreased by 3.55% as compared to those exposed to 19–26 °C and 27–28 °C. The opposite trend was recorded for MDA concentration (74.29%) (Table 6). The activities of CAT, SOD and GSH were significantly lower ($P < 0.05$) in animals exposed to 35–36 °C respectively by 32.82%, 24.28% and 34.34% compared to other temperature ranges.

The superscripts a, b, c: means the mean values are significantly different at $P < 0.05$; T0 control group, T1: 27–28 °C, T2: 31–32 °C, T3:

Table 3
Relative organs weight for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C	ER (%)	p-value
Heart	0.51 ± 0.08	0.54 ± 0.11	0.55 ± 0.06	0.56 ± 0.13	9.83	0.101
Lung	1.14 ± 0.32^{b}	1.19 ± 0.22^{b}	1.28 ± 0.14^{b}	1.57 ± 0.06^{a}	37.71	0.027*
Liver	4.18 ± 1.09	4.21 ± 1.52	5.36 ± 1.47	5.59 ± 1.40	33.78	0.258
Kidney	0.82 ± 0.04^{b}	0.81 ± 0.08^{b}	0.94 ± 0.04^{a}	0.98 ± 0.07^{a}	19.55	0.023*
Ovaries	0.23 ± 0.02^{a}	0.22 ± 0.03^{a}	0.19 ± 0.06^{ab}	0.16 ± 0.02^{b}	−30.43	<0.001***
Uterus	0.44 ± 0.01^{a}	0.46 ± 0.06^{a}	0.35 ± 0.01^{b}	0.36 ± 0.02^{b}	−18.11	<0.001***

Table 4
Hematological parameters for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C	ER (%)	p-value
Hb (g/dl)	14.17 ± 1.12^{a}	14.02 ± 1.26^{a}	11.33 ± 1.04^{b}	10.82 ± 1.54^{b}	−23.64	0.017*
PCV (%)	41.21 ± 1.76^{a}	43.72 ± 2.16^{a}	40.10 ± 0.65^{a}	36.29 ± 0.76^{b}	−11.93	0.032*
RBC (x10^{12}/l)	5.89 ± 0.22^{a}	5.81 ± 0.25^{a}	5.36 ± 0.16^{b}	5.14 ± 0.21^{b}	−12.73	0.018*
MCV (fl)	69.90 ± 0.81^{b}	71.33 ± 2.21^{b}	75.33 ± 1.46^{a}	77.40 ± 1.83^{a}	10.73	<0.001***
MCH (pg)	25.73 ± 0.85	26.22 ± 0.13	25.41 ± 0.77	26.85 ± 0.91	4.35	0.246
MCHC (g/dl)	33.36 ± 1.82	32.44 ± 2.87	33.06 ± 2.13	34.87 ± 1.48	4.52	0.083
WBC (x10^{9}/l)	11.61 ± 2.73^{b}	11.72 ± 1.89^{b}	12.62 ± 1.34^{b}	16.53 ± 1.12^{a}	42.37	<0.001***
PLT (× 10^{3}/μl)	185.33 ± 5.60	190.50 ± 6.93	192.67 ± 8.12	188.09 ± 6.74	3.96	0.061
LYM (%)	56.57 ± 3.67^{b}	58.06 ± 2.51^{b}	63.36 ± 1.49^{a}	65.36 ± 2.33^{a}	15.53	0.024*

Table 5
Serum biochemical parameters for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C	ER (%)	p-value
Cholesterol (mg/dl)	122.12 ± 6.08	123.40 ± 9.13	111.93 ± 9.11	108.42 ± 10.21	−11.21	0.241
Creatinine (mg/dl)	0.69 ± 0.06^{b}	0.69 ± 0.04^{b}	0.84 ± 0.05^{a}	0.89 ± 0.04^{a}	28.98	0.004**
Urea (mg/dl)	92.12 ± 11.15^{b}	89.30 ± 17.42^{b}	136.93 ± 13.81^{a}	141.13 ± 12.87^{a}	53.20	0.003**
ALAT (U/L)	49.22 ± 3.70	46.29 ± 6.02	51.93 ± 5.15	53.01 ± 7.19	7.70	0.072
ASAT (U/L)	23.90 ± 1.19^{c}	23.64 ± 1.22^{c}	25.91 ± 0.08^{b}	29.47 ± 1.28^{a}	23.31	0.036*
Glucose (mmol/L)	7.26 ± 0.08	6.55 ± 0.16	6.61 ± 0.12	5.81 ± 0.39	−19.97	0.241
Total protein (g/L)	73.87 ± 2.04^{a}	72.12 ± 1.41^{a}	67.80 ± 1.08^{b}	64.99 ± 2.25^{b}	−12.02	0.014*
Total albumin (g/dl)	4.51 ± 0.23	4.60 ± 0.19	4.48 ± 0.31	4.55 ± 0.33	0.88	0.131

Table 6
Oxidative stress biomarkers for female rabbits, as affected by different ranges of temperature.

Parameters	19–26 °C	27–28 °C	31–32 °C	35–36 °C	ER (%)	p-value
Protein (mg/ml)	10.65 ± 0.55^{a}	10.50 ± 0.67^{a}	9.30 ± 0.84^{b}	6.62 ± 0.43^{c}	−3.55	<0.001***
MDA (nmol/mg tissues)	19.88 ± 0.99^{a}	20.63 ± 1.64^{b}	30.75 ± 1.65^{c}	34.65 ± 1.29^{c}	74.29	<0.001***
CAT (UI/mg tissues)	9.75 ± 1.03^{a}	9.65 ± 1.39^{a}	8.40 ± 0.60^{a}	6.55 ± 0.84^{b}	−32.82	<0.001***
SOD (UI/mg tissues)	6.30 ± 0.52^{a}	6.73 ± 0.42^{a}	5.02 ± 0.41^{b}	4.77 ± 0.69^{b}	−24.28	<0.001***
GSH (nmol/mg of tissue wet)	9.55 ± 0.57^{a}	9.55 ± 0.39^{a}	6.38 ± 0.60^{b}	6.27 ± 0.75^{b}	−34.34	<0.001***

35–36 °C. CAT: catalase, GSH: reduced glutathione, SOD: superoxide dismutase, MDA: lipid peroxidation; ER: Effect Rate. *, *, **: significant at $P = 0.05$; 0.01 and 0.001, respectively.

4. Discussion

Heat stress causes several drastic changes in the biological function which are responsible for damages in both production and reproduction performances in rabbits (Abdel –Samee et al., 2005). Results of the present study showed effect of elevated temperatures on some parameters defining growth performance where final body weight and body weight gain decreased while feed conversion ratio (FCR) increased in animals exposed to 31–32 °C and 35–36 °C as compared to the controls. In earlier studies where New Zealand White rabbits exposed to hot ambient temperature ranges from 33 to 38 °C (Sabah et al., 2016) and 36 ± 3 °C (Ondruska et al., 2011) for one month, similar findings were recorded. The decrease in final body weight and body weight gain observed in the present study can be ascribed to the increase in the FCR, which might have led to less protein biosynthesis and less fat deposition (Marai et al., 2001, 2004; Ogunjimi et al., 2008). In fact, increase in FCR with the increasing temperature indicates a poor digestibility of feed and absorption of the resultant nutrients in heat stressed rabbits. Poor digestibility, in this context, has been explained in earlier findings where elevated temperature suppressed enzymatic activities at the brush border and pancreatic secretions on duodenum, which led to a weakened digestion and nutrients assimilation (Jinap and Hajeb, 2010). It has also been associated with increase in the energy loss used in synthesis of macromolecules (Garlick, 2005). Studies have shown that extreme temperatures can disrupt stability of alpha diversity within gut microbiota while favoring beta diversity (relative abundance of specific bacteria) microbiota within host organisms and consequently influence beneficial and deleterious effects that play key role on host phenotypes and fitness (Kohl and Yahn, 2016). Each host species displays a distinct microbial response to thermal stress but some gut bacterial taxa (Firmicutes and proteobacteria) show shift with temperatures that appear to be reproducible across host species. It is however, not clear whether changes in gut microbiota in response to high ambient temperature are responsible for reduced host energy acquisition from diet. Thermal stress also interferes with redistribution of blood from internal organs such as intestines thereby interfering with digestion and absorption. Thermoregulatory mechanisms here favor cutaneous heat dissipation while internal tissues experience hypoxia (Pearce et al., 2013) hence impairment of secretion of digestive enzymes and absorption. In addition, high ambient temperatures have been shown to impact on the composition of animal gut microbiota thus altering their function and consequently influencing host phenotypes and general performance (Sepulveda and Moeller, 2020). The observed increase in FCR in the present study shows the influence of high temperature on food assimilation by gut microbiota. This is supported by earlier studies in salamanders that reported high temperature different from their preferred body temperature led to specific changes in microbiome composition hence decrease in energy assimilation, food intake, and digestive efficiency. Energy assimilation, in this case, was associated with the relative abundances of *Sphingopyxis*, *Roseococcus* and *Stenotrophomonas*, which contains lineages capable of digesting cellulose polymers (Dantur et al., 2015). Similar studies in *Bos taurus* reared at 20 °C, 28 °C and 30 °C showed decrease in relative abundance of *Firmicutes* within gut microbiota (Tajima et al., 2007). From the foregoing, understanding how changes in ambient temperature impacts on gut microbiota of animals may help predict future responses of animal genotypes and phenotypes to climate change.

The high water consumption in heat stressed rabbits observed in the present study can be explained by the normal homeostatic mechanisms governing thermoregulation. Rabbits are almost dependent on pulmonary ventilation for regulation of body temperature in hot conditions and so the increase in water consumption may help them to increase heat loss through pulmonary ventilation mechanisms (Marai et al., 2001; Badr, 2015). Results of the present study showed high skin and rectal temperatures in rabbits exposed to heat, with significant effects at 31–32 °C and 35–36 °C compared to the group exposed to moderate temperatures as well as for controls. These results are similar to the findings of Sabah et al. (2016) in New Zealand White rabbits subjected to hot ambient temperature in the range 33 °C–38 °C. The high skin temperature observed in the present study may be due to the insulating effect of the hair coat (Marai et al., 2008). On the other hand, the increase in rectal temperature in animals at high temperatures may be the result of increasing metabolic rate and ultimately hyperthermia as a result of impaired thermoregulation (Shafie et al., 1982). In separate studies, Marai and Rashwan (2004) reported that at temperatures above 30 °C, rabbits stretch out in an attempt to lose as much heat as possible by radiation and convection with significant rises in rectal temperature. This finding is linked to the behavioral manifestations reported in animals exposed to 31–32 °C and 35–36 °C in the present study. Rafel et al. (2012) in their study indicated that stress induces reactions in rabbits including behavioral modifications in attempt to cope with the stressor. The appearance of dizziness in heat stressed animals reported in the present study may be due to the inhibition of acetyl cholinesterase enzymes (AchE) by the heat thus resulting to accumulation of

acetylcholine in cholinergic synapses (Sarkar et al., 2000). The withdrawal near the window of the cage observed in heat stressed animals indicates an attempt to cooling effect with cooler ambient temperatures outside of the animal house. The heat stressed animals were not excited with food introduction in cages. McManus et al. (2009) reported that heat stressed animals are not very attracted to feed as they need to slow down their basal metabolism causing hypo-function of thyroid gland in order to prevent the additional metabolic heat production.

Results of this study showed that the relative weight of lung and kidney were significantly increased in heat stressed animals. The increase in weight of these organs has been previously reported in studies of Badawi and El-Aasar (2018) in New-Zealand White and Baladi Black rabbits exposed to Egyptian hot conditions for 35 consecutive days. On the contrary, the weights of uterus and ovaries decreased as compared to the control. The reduction in uterine and ovary size has been previously associated with the fetal growth restraint (IbaÂnÄez et al., 2003). The increased weight of lung in heat stressed rabbits reported in the present study may be due to the larger air volumes required by these animals to release heat to the environment through the vapor released in the breath (Marai et al., 2007). The increase in weight of the kidney could be due to the intensive activity of detoxification carried out by this organ due probably to the increased insensible loss of body water and salt. This leads to substantial fluid deficit which, if not replaced, may result in vasoconstriction resulting in renal injury driven by effects of hyperosmolarity (via activation of the polyol pathway) (Lloyd, 1994; Glaser et al., 2016). Urea and creatinine are biochemical markers usually used in the exploration of the renal function. It was observed from this study that the urea and creatinine concentrations significantly increased in animals at 31–32 °C and 35–36 °C. This is in agreement with findings of Okab et al. (2008) in New-Zealand White rabbit males submitted to 26.5°C-32.2 °C corresponding to summer conditions in Egypt. The level of increase of creatinine and urea in the study is probably indicative of damage to the kidney functional units hence compromised glomerular filtration (Walmsley and White, 1994). Further, the increase in urea and creatinine could also be a result of increase in protein catabolism owing to high stimulation of synthesis of the enzyme arginase by elevated heat, which intervenes in the production of urea (Yanardag and Sacan, 2007). The latter argument is complimented by results in the same animals where a decrease in serum protein level was noticed in rabbits exposed to 31–32 °C and 35–36 °C and similar to earlier reports in New Zealand White rabbits exposed to 33–38 °C (Sabah et al., 2016) and 36 ± 3 °C (Ondruska et al., 2011) for one month. The decrease in total protein observed in the present study was probably a consequence of dilution of plasma proteins caused by the increase in water consumption or could have issued from enhanced protein utilization and amino acid transamination in the rabbits exposed to heat (Ayyat et al., 2002).

It has been reported that the liver functional transaminases (ASAT and ALAT) enzyme activities are indicators of liver diseases such as infectious hepatitis, alcoholic cirrhosis, biliary obstruction, toxic hepatitis and liver cancer (Abdel-Wahab et al., 2007). In the present study, ALAT concentration was not significantly affected by the heat. This contradicts findings of Okab et al. (2008) in New-Zealand White male rabbits exposed to 26.5°C-32.2 °C where a significant decrease in liver ALAT levels was recorded. On the contrary, ASAT levels significantly increased in rabbits exposed to 31–32 °C and 35–36 °C in the present study. This finding points to the fact that ASAT is dependent on the amino acid groups of alanine and glutamine taken up by the liver and reflect the changes in the liver metabolism associated with glucose synthesis (El-Maghawry et al., 2000). The decrease in serum glucose content in the heat stressed rabbits by the rise in glucose utilization during muscular movements required for high respiratory activity or due to increase in corticosteroid concentration (Habeeb et al., 1997).

The results of this study showed a decrease in Hb, PCV and RBC counts but an increase in MCV, WBC, LYM in female rabbits exposed to 31–32 °C and 35–36 °C. These results are similar to the findings in New-Zealand White male rabbits exposed to summer conditions in Egypt (Okab et al., 2008) and in New Zealand White rabbits exposed to 33–38 °C (Sabah et al., 2016). Hemoglobin of erythrocytes plays a vital role of carrying approximately 98% of oxygen throughout the animal body system while the PCV is a measure of the proportion of blood that is made up of cells (Jensen, 2009). In the present study, we reported suppression of appetitive behavior in terms of increased latency of feed intake with high temperature exposure to animals. The reduced feed intake may have been a strategy to minimize metabolic heat production, which may explain, in part, the physiological and biochemical and behavioral adaptations that were observed in female does in the present study. Studies in piglets showed that heat stress increase skin blood flow circulation that promote heat dissipation (Collin et al., 2001) while most internal organs such as intestinal epithelium may have less blood supply that leads to tissue hypoxia (Pearce et al., 2013). Thermoregulatory mechanisms in this case favor increased erythropoiesis hence more red blood cells to allow oxygen redistribution to curb hypoxia in internal tissues. The decrease in hemoglobin concentration in the present study is surprising since increased erythropoiesis means increase in hemoglobin content in red blood cells. Nonetheless, it is possible there was increased attack by free radicals on red blood cell membrane, which is rich in lipid content thereby leading to hemolysis. Measurements on the RBC deformability were not considered in the present study. Earlier studies showed involvement of increased insulin concentration during thermal stress (Pearce et al., 2013) in synthesis of nitric oxide by red blood cells, which causes deformability (Grau et al., 2013). The increase in RBC deformability during heat exposure has been shown to slow down peripheral resistance hence accelerating tissue oxygenation and blood supply to heat exchange surfaces (Zhou et al., 1999). Although these studies were mainly done on pigs, the mechanisms of homeothermic thermoregulation favor extrapolation of these results to rabbit physiology among other terrestrial homeotherms. In fact, studies in sheep exposed to high ambient temperatures for 8 h daily for 45 days showed significant effect on increased RBC, hemoglobin and PCV (Al-Haidary, 2004; Rana et al., 2014). The increase in PCV in their study was ascribed to adaptation mechanism to provide water necessary for evaporative cooling process. The same scenario could hold true for rabbits in the present study since rabbits have few functional sweat glands (Naqvi et al., 1995). Moreover, heat stress has been reported to decrease adrenocorticotropic hormone (ACTH) levels, which in turn decreases RBC, Hb and PCV contents (Okab et al., 2008). Lymphocytes and WBC are responsible for both humoral and cellular immunity. The increase in their contents in heat stressed rabbits reported in the present study may be attributed to stress due to pathogens during high temperature exposure, which may increase blood viscosity and produce allergic effects that induce WBC increase (Okab et al., 2008). The increase in MCV is a consequence of the decrease in salt concentration in blood plasma of rabbits exposed to 31–32 °C and 35–36 °C (Badawi and El-Aasar, 2018).

In the present study, the level of oxidative stress biomarkers such as kidney protein, MDA, CAT, SOD, GSH were significantly affected ($p < 0.001$) by the increase in temperature. Similar results have been reported in exotic breeds of rabbits during peak of heat stress in Nigeria during 7 consecutive weeks (Jimoh et al., 2019) and in guinea pigs exposed to heat stressed temperatures for 60 days (Ngoula et al., 2017b). Oxidative stress has been reported to impair both productive and reproductive functions in animals (Spears and Weiss, 2008; Celi, 2011; Ngoula et al., 2017a). An increase of the activity of MDA as well as a decrease in activity of SOD, CAT and GSH reported in the present study may have been caused by their intensive utilization in protection against oxidative tissue damage (Seven et al., 2001). In fact, the increased amount of MDA levels indicates the lipid peroxidation process in tissues wherever the fatty acids in the cell membrane lose hydrogen molecules (Celi, 2011). Serum GSH activity has a major role in the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (Halliwell and Chirico, 1993). Superoxide dismutase catalyses the dismutation of superoxide to hydrogen peroxide (H_2O_2) and this is considered the first line of defense against pro-oxidants while CAT is

known for its facile ability to convert hydrogen peroxide into water and oxygen thereby reducing H_2O_2 concentration in animal cells (Halliwell and Chirico, 1993).

5. Conclusion

Results of this study indicated that exposure of female rabbits to 31–32 °C and 35–36 °C for 30 consecutive days impaired their growth performances, relative organ weights and caused some behavioral abnormalities. Moreover, heat stress increased rectal and skin temperature, impaired hemato-biochemical parameters, resulted in increase in the level of MDA while reducing the levels of enzymatic antioxidant biomarkers. The deleterious effects of heat stress observed in the present study may be attributed to the oxidative stress. However, further studies are needed for alternative ways of alleviating the effects of heat stress on oxidative stress and physiological damages in animals.

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