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Research Application Summary

Effect of different ranges of temperature exposition on oxidative stress and biochemical parameters in nulliparous rabbit does

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Abstract

Heat stress negatively impact rabbit production and reproduction performances as they do not have enough sweat glands which can remove the body heat excess. The objective of this study was to investigate the effects of heat stress on oxidative stress status and biochemical parameters. For this purpose, 24 nulliparous female rabbits aged 6 months and weighing between 1953.1 and 2375.4 g were divided into four groups of six animals each and subjected for 30 consecutive days to following temperatures: ambient temperature (19–26 °C) for the control group (T0), 27–28°C for group 2 (T1), 31–32°C for group 3 (T2) and 35–36°C for group 4 (T3) using electrical heaters from 8:00 am to 4:00 pm. At the end of experimental period all animals were humanely sacrificed and blood samples and kidney were collected for analysis of respectively biochemical parameters and oxidative stress biomarkers. Data were submitted to analysis of variance and mean values were compared at 5% significance level. Results revealed that animals submitted to 31-32°C and 35-36°C had significantly decreased total protein content while the content in creatinine, urea and Aspartate Amino Transferase (ASAT) increased. The level of lipid peroxidation (MDA) was significantly increased in animals exposed to 31-32°C and 35-36°C, whereas the level of kidney protein, catalase, superoxide dismutase and reduced glutathione were significantly lower in exposed animals as compared with controls. It was concluded that exposure of female rabbits to 31-32°C and 35-36°C for 30 days induce heat stress that causes oxidative stress and physiological disorders. Alternative strategies are needed for heat stress alleviation.

Keywords: Climate change, female rabbit, heat, oxidative stress

Résumé

Le stress thermique a un impact négatif sur les performances de production et de reproduction des lapins car ils n'ont pas suffisamment de glandes sudoripares qui peuvent éliminer l'excès de chaleur corporelle. L'objectif de cette étude était d'étudier les effets du stress thermique sur l'état de stress oxydatif et les paramètres biochimiques. A cet effet, 24 lapines nullipares âgées de 6

mois et pesant entre 1953,1 et 2375,4 g ont été réparties en quatre groupes de six animaux chacun et soumises pendant 30 jours consécutifs aux températures suivantes : température ambiante (19–26 C) pour le groupe témoin (T0), 27–28 C pour le groupe 2 (T1), 31–32 C pour le groupe 3 (T2) et 35–36 C pour le groupe 4 (T3) en utilisant des radiateurs électriques de 8h00 à 4h : 00 h. À la fin de la période expérimentale, tous les animaux ont été sacrifiés sans cruauté et des échantillons de sang et de rein ont été prélevés pour analyser respectivement les paramètres biochimiques et les biomarqueurs du stress oxydatif. Les données ont été soumises à une analyse de variance et les valeurs moyennes ont été comparées à un seuil de signification de 5 %. Les résultats ont révélé que les animaux soumis à 31-32 C et 35-36 C avaient une teneur totale en protéines significativement réduite tandis que la teneur en créatinine, urée et aspartate amino transférase (ASAT) augmentait. Le niveau de peroxydation lipidique (MDA) a été significativement augmenté chez les animaux exposés à 31-32 C et 35-36 C, tandis que le niveau de protéines rénales, de catalase, de superoxyde dismutase et de glutathion réduit était significativement plus faible chez les animaux exposés par rapport à les contrôles. Il a été conclu que l'exposition des lapines à 31-32 C et 35-36 C pendant 30 jours induit un stress thermique qui provoque un stress oxydatif et des troubles physiologiques. Des stratégies alternatives sont nécessaires pour atténuer le stress thermique.

Mots-clés : Changement climatique, lapin femelle, chaleur, stress oxydatif

Introduction

Climate change, defined as the 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 for mankind during the present century (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), 2007). The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. High ambient temperature accompanied by high air humidity is reported to cause discomfort and enhance the stress level which in turn result 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 (Kaplan-Pasternak, 2011; Mutwedu *et al*., 2015). It is highly preferred because of its body size, high rate of reproduction, adaptability to inexpensive housing and useful by-products. However, African rabbit husbandry is facing several constraints such as the lack of reproductive management, predation, uncontrolled crossings, inbreeding and negative selection (Mutwedu *et al*., 2015), and environmental stress (Kumar *et al*., 2011).

Stress results from external forces that disrupt homeostasis. Animals are affected by several types of stress, including physical, nutritional, chemical, psychological and thermal (Ngoula *et al*., 2017). The latter occurs when the environmental temperature exceeds the thermoneutrality zone of the animal (Kumar *et al*., 2011).

Rabbits ideal environmental temperature ranges between 16 and 21ºC (Marai *et al*., 1994). Above

this range, they are subject to heat stress because they are very sensitive to the heat as they do not have enough sweat glands which can remove the body heat excess. Their long exposure to the thermal stress leads to the increase of free radicals which may induce the 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) and increases not only risk of spontaneous abortion but also other factors such as litter performance, the well-being and health status of animals including impaired milk production, reproductive performance, and longevity (Zhao *et al*., 2011).

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 constant body temperature (Marai *et al*., 1994). Assessment of whether female rabbits are able to maintain a homeostatic condition in spite of heat stressed environment would be very useful in considering the health status of female rabbits (Pasquini *et al*.. 2008). Unfortunately, the link between the oxidative stress status, biochemical parameters and heat stress in female rabbits under different ranges of African temperature is not well defined in literature. This study hypothesized that biomarkers, blood parameters and biochemical parameters of female rabbits are not affected by the change of the environment temperature. Hence this work evaluated the oxidative stress status and physiological changes in female rabbits submitted to different levels of temperature.

Materials and Methods

Animal husbandry. Twenty four mature female New Zealand rabbits, clinically healthy, aged 6 months and weighing between 1953.1 and 2375.4g were used for the study. They were purchased from a local provider and kept in the animal house of the Anatomy and Physiology Department, University of Nairobi, Kenya. Rabbit does were fed ad libitum the basal commercial pelleted ration containing 18.18% crude protein, 13.43% crude fibre, 2656 Kcal/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) and housed in wire cages (0.8 × 0.6 × 0.6 m). Fresh water was made available to the animals ad libitum. After two weeks of acclimatization, animals were randomly assigned to four groups of six animals each with comparable weight for 30 consecutive days. The heat was induced, in each rabbit cage, using electrical heaters from 8:00 am to 4:00 pm followed by exposure to the normal air temperature as in the control group from 4:00 pm to 8:00 am. The temperature, relative humidity and temperature humidity index (THI) were as follow: T0 (control): 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, 62±0.8, 29.5±0.6, T3: 35-36, 32.9±0.6.

The THI was calculated following the formula developed 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 follow: < 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). The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Anatomy and Physiology of the University of Nairobi (REF: FVM BAUEC/2019/244).

Oxidative stress biomarkers biochemical analysis. At the end of the experimental period (30 days), all animals were fasted for 24h and humanly sacrificed. The blood was collected directly

by cardiac puncture before sacrificing, put in tubes free from anticoagulant, centrifuged at 3000 rpm for 15 min and supernatant separated as serum and preserved at -20°C for the evaluation of serum content in terms of total cholesterol, albumin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), urea, creatinine, protein and glucose using commercial assay kits. The kidney was quickly homogenized and the homogenate was then centrifuged at 4800 rpm for 60 min at 4 C and the supernatant was stored at –20 °C till further oxidative stress biomarkers estimations. Protein content in the supernatant was determined by the method using bovine serum albumin as standard as described by Lowry *et al*. (1951). The kidney activities of catalase (CAT) and reduced glutathione (GSH) as well as the levels of superoxide dismutase (SOD) and lipid peroxidation (MDA) were assessed in kidney homogenates using a spectrophotometer (GENESYS 20.0) following the methods described respectively by Dimo *et al*. (2006), Habbu *et al*. (2008), Sajeeth *et al*. (2011) and Kodjo *et al*. (2016).

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 and controls assessed using one-way ANOVA. The differences in mean values were compared using the Tukey HSD post hoc test at 5% significance level.

Results and Discussion

As shown in Table 1, creatinine, urea and Aspartate Aminotransferases (ASAT) were significantly increased in animals submitted to 31-32°C and 35-36°C while the level of total protein decreased in animals of these groups compared to those submitted to 27-28°C and the control group ($p< 0.05$). There was no significant difference on cholesterol, Alanine Amino Transferase (ALAT), glucose and total albumin in treated groups compared to controls. These results are in agreement with findings of Okab *et al*. (2008) in New-Zealand White rabbit males submitted to 26.5ºC-32.2ºC corresponding to summer conditions in Egypt.

Table 1. Biochemical parameters (mean ± s.d.) for doe rabbits, as affected by different ranges of temperature

Parameters	T0 (n = 6)	T1 (n = 6)	T2 (n = 6)	T3 (n = 6)	p-value
Cholesterol (mg/dl)	122.12±6.08	123.40±9.13	111.93±9.11	108.42±10.21	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}	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}	0.003
ALAT (U/L)	49.22±3.70	46.29±6.02	51.93±5.15	53.01±7.19	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}	0.036
Glucose (mmol/L)	7.26±0.08	6.55±0.16	6.61±0.12	5.81±0.39	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}	0.014
Total albumin (g/dl)	4.51±0.23	4.60±0.19	4.48±0.31	4.55±0.33	0.131

a, b, c: means with different letters are significantly different at $p<0.05$; n denotes number of animals in each group. T0 control group, T1: 27-28 C, T2: 31-32 C, T3: 35-36 C. ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase

In female rabbits submitted to 31-32°C and 35-36°C, the proteins level in the kidney significantly decreased as compared to control and 27-28°C animals. The opposite trend was recorded for MDA concentration (Table 2). The activities of CAT, SOD and GSH were significantly lower ($p<0.05$) in animals submitted to 35-36 C than in other ranges of temperature. Similar results have been reported by Jimoh *et al*. (2019) in exotic breeds of rabbit during peak of heat stress in Nigeria during seven consecutive weeks. The increased amount of MDA level 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 defence of animal tissues by catalysing the reduction of hydrogen and lipid peroxides. The SOD catalyses the dismutation of superoxide to hydrogen peroxide (H_2O_2) and it is considered the first defence against pro-oxidants (Halliwell and Chirico, 1993) while CAT is known for its facile ability to convert hydrogen peroxide into water and oxygen, reducing therefore H_2O_2 concentration in animal cells.

Table 2. Oxidative stress biomarkers (mean±s.d.) for doe rabbits, as affected by different ranges of temperature

Parameters	T0 (n = 6)	T1 (n = 6)	T2 (n = 6)	T3 (n = 6)	p-value
Protein (mg/ml)	10.65±0.55^a	10.50±0.67^a	9.3±0.84^b	6.62±0.43^c	<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	<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	<0.001
SOD (UI/tissues)	6.30±0.52^a	6.73±0.42^a	5.02±0.41^b	4.77±0.69^b	<0.001
GSH (mmol/mg of tissue wet)	9.55±0.57^a	9.55±0.39^a	6.38±0.60^b	6.27±0.75^b	<0.001

a, b, c: means with different letters are significantly different at $p<0.05$; n denotes number of animals in each group. T0 control group, T1: 27-28 C, T2: 31-32 C, T3: 35-36 C. CAT: catalase, GSH: reduced glutathione, SOD: superoxide dismutase, MDA: lipid peroxidation

Conclusions

In this study, exposition of female rabbits at 31-32°C and 35-36°C for 30 consecutive days altered their oxidative status and biochemical parameters through oxidative stress following the exposition to the heat. However, further work is needed to establish alternatives to alleviate the effects of heat stress on oxidative stress and biochemical damages in animals.

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