



## **Oxidative Stress Markers in Exotic Breeds of Rabbit during Peak of Heat Stress in Ibadan, Nigeria**

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### **Authors' contributions**

Author OAJ designed and carried out the study, performed the statistical analysis and wrote the first draft of the manuscript. Author EOE wrote the protocol and supervised the study, vetted the drafts of the manuscript. Author ASB managed the laboratory analysis and field trial. All authors read and approved the final manuscript

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### **ABSTRACT**

The study assesses oxidative stress markers in exotic rabbit bucks at peak of heat stress in Ibadan, Nigeria. Four rabbit breeds were considered; Fauve De Bourgogne, Chinchilla, British Spot and New Zealand White. Adult rabbits (10-12 months old) were randomly selected per breed and randomly allotted to experimental units at highest temperature-humidity index. Blood samples were collected through the ear vein and assessed for serum biochemicals and oxidative stress markers; malondialdehyde, total antioxidant activity, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase at 7 weeks of exposure to peak of thermal discomfort. The results obtained indicate that serum glucose, sodium and potassium were significantly affected by breed. Serum lipid peroxidation was also significantly lower in British Spot rabbits and highest in Fauve De Bourgogne. Serum SOD of British Spot rabbits (1.47 U/min/mg protein) was significantly highest compared with New Zealand White (1.20U/min/mg protein), Chinchilla (0.92 U/min/mg protein) and Fauve De Bourgogne (0.88 U/min/mg protein). British Spot had significantly highest serum catalase (130.73 nm H<sub>2</sub>O<sub>2</sub> / min/mg protein) activities and an apparently highest total antioxidant activity (0.99mmol/litre) and GPx (40.32 µgGSH/min/mg protein). This suggests that British Spot breed of rabbit had better oxidative stability among the breeds of rabbits assessed.

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## 1. INTRODUCTION

The exposure of rabbits to extreme of heat leads to the decomposition of normal physiological and biological mechanisms with a consequent damage of many organs [1,2]. 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. It occurs when animals are exposed to high ambient temperatures, high humidity (60 – 90%), low wind speed and high direct and indirect solar radiation [3]. Nigeria is located within humid tropics and is subjected to extended periods of high ambient temperature and humidity.

Heat stress is one of the wide varieties of factors which cause oxidative stress *in-vivo* [4] during summer and/or in tropics. Reactive oxygen species (ROS), the major culprits for causing oxidative stress, are constantly generated *in vivo* as an integral part of metabolism. Oxidative stress results from increased production of freeradicals and ROS, and a decrease in antioxidantdefense [5,6]. Ganaie et al. [7] reported that oxidation is essential to nearly all cells in the body to provide energy for vital functions. Approximately 95 to 98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products - ROS, that may damage the DNA of genes and contribute to degenerative changes [7]. Animals have enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g. vitamin E) antioxidant mechanisms that work as scavenger for this harmful ROS. Radical scavenging antioxidants are consumed by the increased free radical activity associated with several conditions, and the total antioxidant response has been used to indirectly assess the free radical activity [8].

The levels of ROS are determined by production and the rate of ROS degradation and/or inactivation; an appropriate balance is important for maintaining cellular homeostasis [9]. Although low levels of ROS are essential in many biochemical processes, accumulation of ROS may damage biological macromolecules i.e. lipids, proteins, carbohydrates and DNA [4]. External factors such as heat, trauma,

ultrasound, infections, radiations, toxins etc. can lead to increased free radicals and other ROS and may lead to oxidative stress [10]. Altan et al. [11] have demonstrated that heat stress increased lipid peroxidation which was associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids.

The most obvious limitation to exotic rabbit production in regions with hot climate is the susceptibility of this species to heat stress. Climatic heat stress had deleterious effect on exotic temperate breeds, such as Flemish Giant and New Zealand white rabbits more than indigenous tropical breeds, such as Egyptian Baladi rabbits [2]. Thus, a study was conducted to assess the oxidative stress indicators of four exotic breeds of rabbit at peak of heat stress in Ibadan, Nigeria.

## 2. MATERIALS AND METHODS

This research was carried out at the rabbit unit of the Teaching and Research Farm and the Animal Physiology Laboratory, Department of Animal Science, both of University of Ibadan, Ibadan, Nigeria. They are situated in rainforest agro-ecological zone of Nigeria, between lat. 7° 27' 18.74"N and 7° 27' 19.17"N and Long. 3° 53' 13.98"E and 3° 53' 32.69"E. The study was approved by our institutional committee on the care and use of animals for experiment, and in accordance with NIH guide for the care and use of laboratory animals.

This experiment was carried out at period of the year, when highest temperature humidity index (THI) is observed (February and March). This was obtained by the review of the meteorological data of University of Ibadan over six years and computation of temperature humidity index according to Marai et al. [12] on monthly basis, as reported by Jimoh [13].

Four Exotic Breeds of Rabbit consisting of Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White. Before commencement of trial, animals were confirmed to be of normal health status, without abnormalities and conform to the breed.

Fifteen bucks per breed housed individually were allotted randomly into experimental units and used for this study. The experimental design

was completely randomised design. The animals were fed ad libitum. The animals were fed with same diet containing crude protein (17.05%), digestible energy (2592.06 Kcal/kg) and crude fibre (10.02%). Fresh water was made available to the animals always. Other routine and periodic management practices schedule necessary for rabbit production were carried out.

At seven weeks of exposure to peak THI (March, 2014), Blood was collected from all rabbit bucks through the Ear vein into sample bottle containing ethylene diamine tetra acetic acid (EDTA) for serum biochemical assay. Serum total protein was determined using the SP400UV/VIS spectrophotometer [14], serum glucose and Serum total cholesterol concentration were determined using the colorimetric procedure as described by Lindner and Mann [15]. The spectrophotometer as explained by Quinn et al. [16] was used to determine sodium, chloride, phosphorus, magnesium and potassium contents of the samples.

Serum total antioxidant activity was carried out according to Koracevic et al. [17], superoxide dismutase (SOD) was determined by the method adopted by Soon and Tan [18], glutathione peroxidase (GPx) was determined as described by Rotruck et al. [19] and Ellman's [20], and catalase was determined by Beers and Sizer [21] method, serum lipid peroxidation was determined using thiobarbituric acid assay according to Ohkawa et al. [22].

Data obtained was subjected to descriptive statistics and ANOVA to detect significant effects at  $p=0.05$ , Duncan's multiple range test was used for post hoc test using SAS 2001 software package.

### 3. RESULTS

#### 3.1 Temperature Humidity Index of Rabbit Pen during HTHI of Ibadan

In the morning, THI range from 24.86°C in February to 26.51°C in March. In the evening THI obtained in February and March are 35.02°C and 30.66°C, respectively. The average daily THI obtained in February and March are 29.94 and

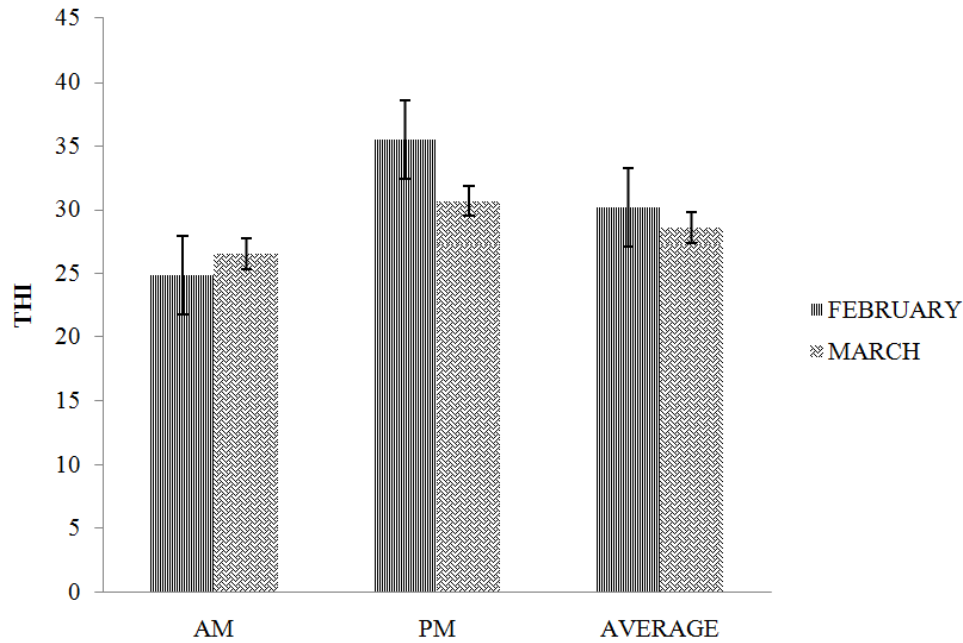
28.59, respectively are higher than the range of THI obtained in University of Ibadan from 2009 to 2014 reported by Jimoh [13]; 26.35°C – 27.73°C and 26.75°C - 27.67°C in February and March, respectively.

#### 3.2 Serum Biochemistry of Four Exotic Breeds of Rabbit during HTHI

Serum biochemistry of four exotic breeds of rabbit during highest THI is presented in Table 1. Serum glucose, sodium and potassium were significantly ( $p<0.05$ ) affected by differences in breed. Fauve de Bourgogne bucks had significantly ( $p<0.05$ ) highest serum glucose, while Chinchilla, British Spot and New Zealand White bucks had similar serum glucose. Chinchilla and British Spot bucks had significantly ( $p<0.05$ ) higher serum sodium than Fauve de Bourgogne and New Zealand. However, Fauve de Bourgogne bucks had significantly ( $p<0.05$ ) higher serum sodium than New Zealand White bucks. British Spot bucks had significantly ( $p<0.05$ ) lower serum potassium than Chinchilla and New Zealand White, while serum potassium of Fauve de Bourgogne was not significantly different from other breeds.

#### 3.3 Serum Oxidative Status of Four Exotic Breeds of Rabbit during HTHI

Serum oxidative status of four exotic breeds of rabbit during Highest THI is presented in Figs. 2-6. Serum total antioxidant activity and glutathione peroxidase activity of rabbit were not significantly ( $p>0.05$ ) influenced by breed. Serum lipid peroxidation in Fauve de Bourgogne and New Zealand White rabbits were significantly ( $p<0.05$ ) higher than other breeds, while serum lipid peroxidation in Chinchilla rabbits was significantly ( $p<0.05$ ) higher than British Spot rabbits. The SOD activity in British Spot rabbits was significantly ( $p<0.05$ ) higher than other breeds. The SOD activity in New Zealand White rabbits was also significantly ( $p<0.05$ ) higher than Fauve de Bourgogne and Chinchilla rabbit which had statistically ( $p>0.05$ ) similar values. Serum catalase activity in Fauve de Bourgogne, Chinchilla and New Zealand White bucks was not significantly ( $p>0.05$ ) different from one another but they were significantly ( $p<0.05$ ) lower than serum SOD activity in British Spot bucks.



**Fig. 1. Temperature humidity index of rabbit pen during HTHI of Ibadan**

**Table 1. Mean values of serum biochemistry of four exotic breeds of rabbit during HTHI in Ibadan**

	Fauve de Bourgogne	Chinchilla	British spot	New Zealand White	SEM
Glucose (mmol/l)	46.73 <sup>a</sup>	38.04 <sup>b</sup>	39.31 <sup>b</sup>	41.88 <sup>b</sup>	2.53
Protein (g/l)	65.00	61.80	61.40	62.00	1.10
Cholesterol (mmol/l)	2.44	2.45	2.53	2.65	0.05
Magnesium (mmol/l)	0.46	0.47	0.59	0.47	0.07
Phosphorus (mmol/l)	1.26	1.22	1.05	1.27	0.09
Sodium (mmol/l)	80.49 <sup>b</sup>	93.30 <sup>a</sup>	91.01 <sup>a</sup>	72.74 <sup>c</sup>	5.66
Potassium (mmol/l)	6.37 <sup>ab</sup>	6.99 <sup>a</sup>	5.83 <sup>b</sup>	7.04 <sup>a</sup>	0.39
Chloride (mmol/l)	85.97	82.61	84.65	78.45	1.28

*abc: means in the same row with different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean*

#### 4. DISCUSSION

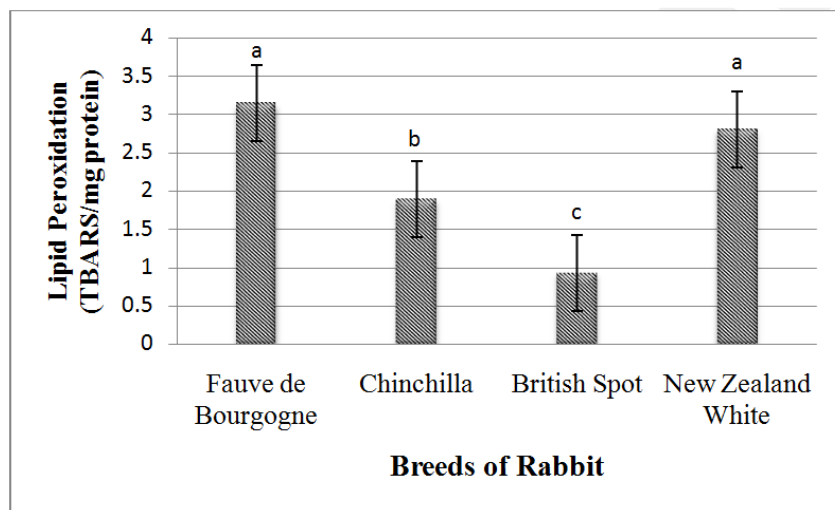
Temperature-humidity index (THI) is an indicator of thermal comfort level for animals in enclosure. The THI values of 29.94 and 28.59 obtained in February and March respectively indicates that rabbits were exposed to severe heat stress in February and moderate heat stress in March during the study period, according to Marai et al. [12] THI classification.

Serum glucose, sodium and potassium of bucks were affected by breed differences. This suggests differences in breed biochemical component in response to thermal comfort.

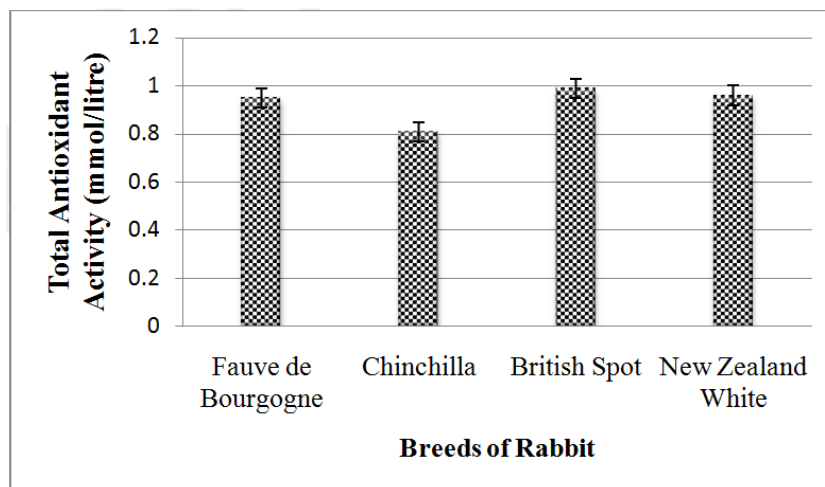
However, the trend of serum protein and cholesterol of rabbits obtained in this study is similar to values obtained by Ondruska et al. [23] in rabbit bucks exposed to heat stress for one month. However, higher serum glucose value was reported by Ondruska et al. [23] compared to values obtained in this study. This could be due to the period of exposure. Serum biochemical analysis was carried out after rabbits were exposed to seven weeks of heat stress in Ibadan. The effects of reduction in feed intake and metabolic rate from heat stress could differ depending on length of exposure and would affect serum glucose. Increase in serum glucose and potassium due to heat stress in goat as

reported by Okoruwa, [24] probably led to stress induced activation of cortisol secretion and the consequent stimulation of gluconeogenesis and potassium with inhibition of cellular glucose uptake and utilization [25]. Decrease in plasma glucose could be due to the increase in glucose utilization to produce more energy for greater cellular expenditure required for high respiratory activity and/or the marked dilution of blood and body fluids as a whole caused by increase in water consumption [26]. The consequence of decrease in glucose concentration or increase in body water content results from increase in utilization of fatty acids for energy production

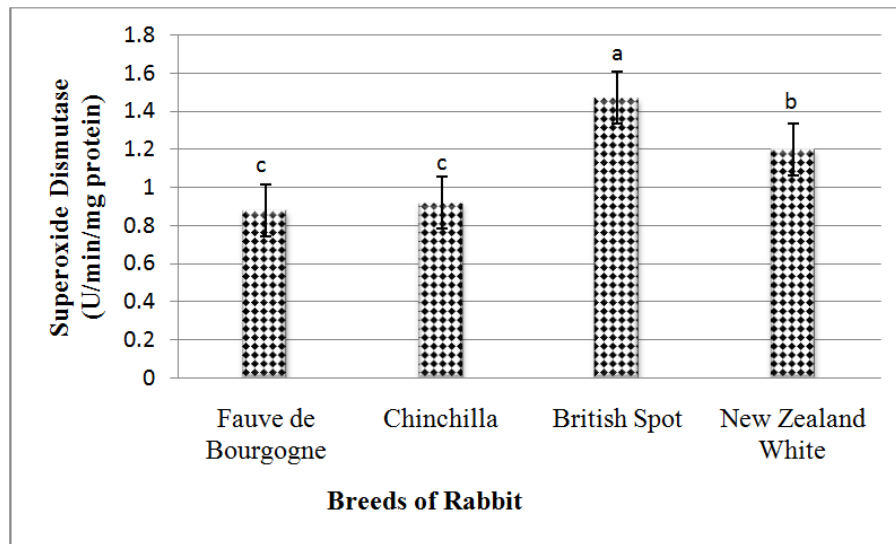
leading to a decline of serum total lipids concentrations due to exposure to high heat stress [26,27]. The range of serum cholesterol, protein and glucose obtained in this study is similar to the values reported in New Zealand White rabbits administered oral glucose supplementation by Attia et al. [28]. The range of serum Sodium and Potassium values obtained in this work is similar to that reported by Okoruwa [24] in WAD goats exposed to heat stress, while the total protein values obtained in this work is lower than the values obtained in Goats on heat stress in Southern Nigeria.



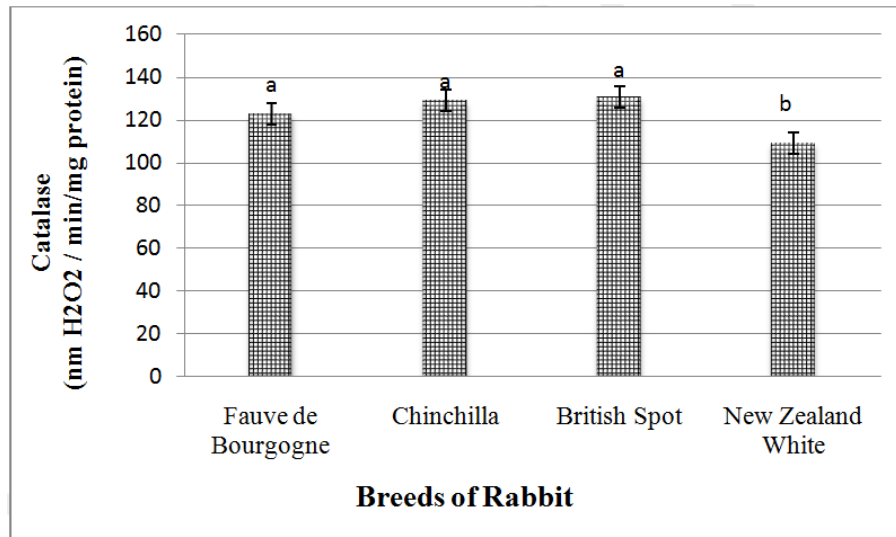
**Fig. 2. Serum lipid peroxidation of four exotic breeds of rabbit during HTHI**  
 The error bars signifies the standard deviation within the groups and the letters signifies mean separation according to the post hoc tests



**Fig. 3. Serum antioxidant activity of four exotic breeds of rabbit during HTHI**  
 The error bars signifies the standard deviation within the groups



**Fig. 4. Serum superoxide dismutase of four exotic breeds of rabbit during HTHI**  
 The error bars signifies the standard deviation within the groups and the letters signifies mean separation according to the post hoc tests



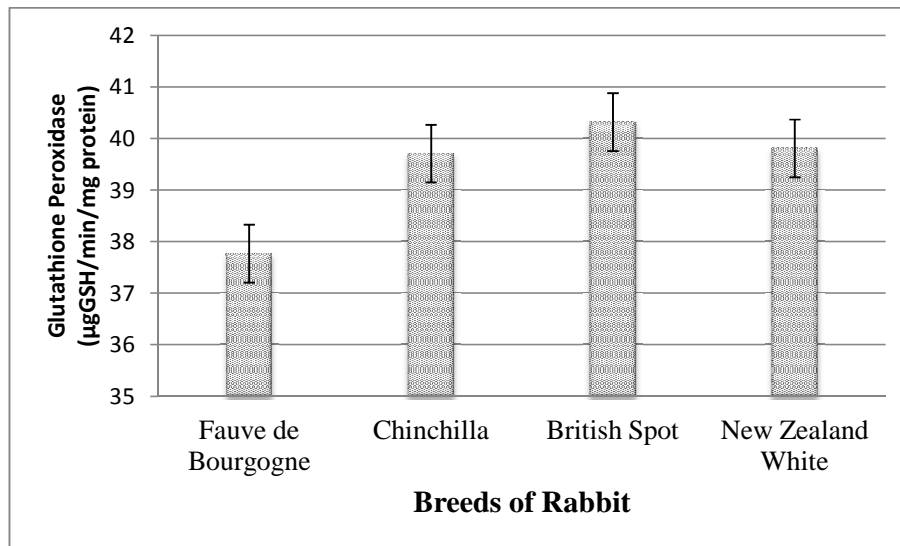
**Fig. 5. Serum catalase of four exotic breeds of rabbit during HTHI**  
 The error bars signifies the standard deviation within the groups and the letters signifies mean separation according to the post hoc tests

Enhanced heat dissipation during heat stress may also lead to electrolyte losses through sweat, saliva, polypnea and urine [29]. Marai et al. [25] and Okab et al. [2] report that New Zealand White rabbits were adversely affected by heat stress causing deterioration, in line with similar trend observed in this study, it could suggest that the exotic rabbit bucks are adversely affected by heat stress as revealed by the serum biochemical indices of the rabbits.

Increased serum total protein and sodium in goat has been reported to occur as a result of increased breathing rate [24].

#### 4.1 Serum Oxidative Status of Four Exotic Breeds of Rabbit in Ibadan

Serum total antioxidant and glutathione peroxidase activity were not significantly influenced by breed differences. However, it was



**Fig. 6. Serum glutathione peroxidase of four exotic breeds of rabbit during HTHI**  
*The error bars signifies the standard deviation within the groups*

observed that British Spot rabbit had significantly higher serum SOD, catalase and apparently higher GPx values and least lipid peroxidation than other breeds at peak of heat stress in Ibadan. This suggests that a superior Serum SOD and catalase activity was responsible for British Spot rabbits' lower serum lipid peroxidation compared to other breeds at peak heat stress in Ibadan. This could be attributed to report that SOD and catalase protects the cell from the damage due to the secondary generation of highly reactive hydroxyl group from superoxide ion to  $H_2O_2$  [7,30]. Because peroxisome catalase takes care of the cytosolic and mitochondrial peroxides formed during urate oxidation [31] and Mitochondrial SOD readily converts the bulk of mitochondrial superoxide ions to  $H_2O_2$  [7]. Evidence of breed differences in catalase activity is in line with report of Kumar [32] that higher catalase activity was registered in KF than Murrah buffaloes. Higher GPx activity in British Spot rabbits could be due to increased production of  $H_2O_2$  due to increased activity of SOD during heat stress resulting in a coordinated increase in GPx [7].

## 5. CONCLUSION

It was revealed that British spot had best oxidative stability among the four breeds of rabbit and serum biochemical components of rabbits were influenced by heat stress in Ibadan, Nigeria. It could be concluded that British Spot breed of rabbit could be better adaptable to

prevailing environmental conditions in Nigeria. This implies that British spot rabbits can be better managed in the prevailing heat stress condition of Nigeria. It is recommended that mitigating strategies to ameliorate the adverse effects of heat stress on these breeds, and stress markers in other extracellular and intracellular fluid compartments should be evaluated.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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