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Research Article/Artículo de Investigación

SEMEN CHARACTERISTICS, SEMINAL BIOCHEMICAL AND OXIDATIVE STRESS MARKERS IN RABBITS DURING HEAT STRESS

CARACTERÍSTICAS SEMINALES, BIOQUÍMICA SEMINAL Y MARCADORES DE ESTRÉS OXIDATIVO EN CONEJOS DURANTE ESTRÉS TÉRMICO

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ABSTRACT

Heat stress hinders attainment of optimal reproductive ability and genetic improvement of rabbit in hot climate. There is genetic variation among animals for cooling capability, which suggests that more heat tolerant animals can be selected genetically. The success of continuous mating cycles in rabbits all year round is highly compromised by heat stress. Thus, contributions of heat stress to infertility in rabbits via oxidative stress requires investigation to assess the adaptability and productivity of exotic breeds of rabbit in tropics. Thirty adult rabbit bucks each of Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White, age range between 7-13 months were randomly selected from a larger flock at the season with the highest temperature-humidity index in Ibadan, Southwest Nigeria. After 9 weeks of exposure of the animals to the average daily temperature humidity index (THI) of the prevailing heat stress condition in the study area, semen ejaculate was collected weekly for assessment; semen characteristics, seminal biochemical and oxidative stress markers using standard procedures were assessed. Results obtained indicate that mass activity and motility of spermatozoa in New Zealand White bucks was significantly ($p<0.05$) higher than Fauve de Bourgogne bucks. Functional sperm membrane integrity of 54.35% (Fauve de Bourgogne), 48.43% (Chinchilla), 49.56% (British Spot) and 58.94 % for New Zealand White bucks was obtained. Seminal total antioxidant activity was significantly ($p<0.05$) highest in New Zealand White bucks (0.60mmol/litre) and significantly ($p<0.05$) least values was obtained in British Spot (0.17mmol/litre). The seminal lipid peroxidation was significantly ($p<0.05$) lower in British Spot (1.84 TBARS/mg protein). New Zealand white bucks had the best semen quality which could be due to its high antioxidant status. However, British spot bucks had the least seminal lipid peroxidation.

Keywords: antioxidant enzyme, bucks, libido, lipid peroxidation, temperature-humidity index.

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RESUMEN

El estrés térmico dificulta alcanzar una optima capacidad reproductiva y el mejoramiento genético del conejo en un clima cálido. Existe una variación genética entre los animales para la capacidad de enfriamiento, lo que sugiere que los animales con mayor tolerancia al calor pueden seleccionarse genéticamente. El éxito de los ciclos de apareamiento continuo en conejos durante todo el año se ve altamente comprometido por el estrés térmico. Por lo tanto, las contribuciones del estrés térmico a la infertilidad en conejos a través del estrés oxidativo requieren investigaciones para evaluar la adaptabilidad y la productividad de las razas exóticas de conejos en los trópicos. Se seleccionaron aleatoriamente treinta conejos adultos de las siguientes razas: Fauve De Bourgogne, Chinchilla, British Spot y New Zealand White, de entre 7 y 13 meses de un lote más grande, y durante la época con el índice de temperatura y humedad más alto en Ibadan, en el suroeste de Nigeria. Después de 9 semanas de exposición de los animales al índice temperatura-humedad diario promedio (THI) característico de estrés térmico en el área de estudio, se colectó semen semanalmente para su evaluación. Se evaluaron las características del semen, marcadores bioquímicos seminales y de estrés oxidativo utilizando procedimientos estándar. Los resultados obtenidos indican que la actividad masal y la motilidad de los espermatozoides en los conejos New Zealand White fue significativamente más alta ($p<0.05$) que los Fauve de Bourgogne. Se obtuvo una integridad funcional de la membrana espermática de 54.35% (Fauve de Bourgogne), 48.43% (Chinchilla), 49.56% (Mancha británica) y 58.94% para los New Zealand White. La actividad antioxidante total del semen fue significativamente más alta ($p<0.05$) en los conejos de raza New Zealand White (0.60 mmol / litro) y significativamente ($p<0.05$) menor en los British Spot (0.17 mmol / litro). La peroxidación lipídica seminal fue significativamente menor ($p<0.05$) en la British Spot (1.84 TBARS/mg de proteína). Los conejos de la raza New Zealand White tuvieron la mejor calidad seminal, lo que podría deberse al alto estatus antioxidante. Sin embargo, los conejos de raza British Spot tuvieron la menor peroxidación lipídica seminal.

Palabras clave: enzima antioxidante, conejos, libido, peroxidación lipídica, índice temperatura-humedad.

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INTRODUCTION

Heat stress, often blamed for suboptimal reproductive efficiency is a worldwide problem which inflicts heavy economic losses on farmers, due to its limiting the breeding season of rabbits (Jimoh, 2016). Improving semen quality of rabbit bucks under heat stress is linked to improving reproductive efficiency of rabbits (Kamel, 2012). The negative effects of heat stress on rabbit bucks include decreased semen quality and essential biochemical constituents of seminal plasma (Attia et al., 2010).

Various factors facilitate the accumulation of free radicals, but common stressors (heat stress and high physiological demands) increase both the cell's metabolic rate and the accumulation of free radicals (Lohrke et al., 2005). Cells can tolerate moderate oxidative load by increasing gene expression to up-regulate their reductive defense systems and restore the oxidant/antioxidant balance but when this increased synthesis cannot be achieved due to damage to enzymes, or substrate limitations, or when the oxidative load is overwhelming, an imbalance persists and the result is oxidative stress. Reactive oxygen species (ROS) participate in normal physiological event in various organs including the testis. Although high concentrations of ROS cause sperm pathology (ATP depletion, leading to insufficient axonemal phosphorylation, lipid peroxidation and loss of motility and viability), low and controlled concentrations of ROS play an important role in sperm physiology. Mild and even undetectable oxidative stress for extensive periods could alter the condensation state of sperm DNA, an effect that would not be visible before fertilization (De Lamirande et al., 1997).

Many factors affect seminal traits and thus, it is crucial to define suitable protocols to improve spermatozoa characteristics during peak periods of heat stress. Hence, it is possible to produce more doses of semen with higher fertility and with less variability all year round. Therefore, semen characteristics and seminal oxidative status of breeds of rabbit were investigated during the peak of heat stress in Ibadan, Nigeria.

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 the 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 the institution's ethics committee on the care and use of animals for research and in accordance with the guide for the care and use of laboratory animals.

Four Breeds of Rabbit consisting of Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White used for this study were obtained from the rabbit unit of teaching and research farm, University of Ibadan. The animals were fed ad libitum with diets containing crude protein 17.05%, digestible energy 2592.06 Kcal/kg, crude fibre 10.02%. A total of 120 bucks consisting of thirty bucks per breed were randomly selected from a population, they were housed individually and allotted randomly into experimental units and the experimental design was completely randomised design.

This experiment was carried out at a period of the year when the highest temperature-humidity index is observed (February and March as reported by Jimoh (2016), after 12 weeks of exposure to prevailing environmental condition in the study area indicate heat stress. Bucks were trained to serve an artificial vagina two weeks prior to sample collection, as the preliminary period in order to assure that the males were reproductively normal and also to establish the same regular semen collection schedule. Semen was collected using artificial vagina from all bucks and were assessed for semen characteristics, seminal biochemical indices, and oxidative stress markers. Two ejaculates per male were collected weekly, with an interval of 3-4days between successive ejaculates over a period of 3 weeks from all males within the experiment. The first ejaculate for spermogram and the second ejaculate was centrifuged at 4000 rpm for 10 min to separate seminal plasma, and stored at -20^{0}C, until further analysis.

Libido (sexual desire) measured in terms of reaction time in seconds and estimated from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe. Semen volume from each of the buck was measured using a tuberculin syringe to the nearest 0.1ml. For mass activity, a drop of fresh semen was placed on a clean glass slide and examined with a microscope under x10 objective lens to determine mass activity. The mass activity was scored subjectively according to the intensity of the wave motion seen in the medium by the collective activities of spermatozoa, from the absence of wave motion (+) to very turbulent motions (+++) scored in percentage. For sperm motility, a drop of semen with the aid of a micropipette was placed on a pre-warmed microscope slide and a drop of the diluent sodium citrate), was added before it was covered with a glass coverslip and examine at a magnification of ×400. The percentage of progressively motile spermatozoa was estimated and score subjectively between 0 and 100. Five microscopic fields were examined for each semen sample.

The sperm concentration was measured by the direct sperm cell count method, using an improved Neubauer hemocytometer slide. Formal saline was mixed with semen at v/v dilution. The diluted semen was then charged on each of two ends of the hemocytometer using a micropipette. The charged hemocytometer was placed on the microscope at a magnification of ×400. The concentration of sperm per volume was determined using the formula: C = 32,000 × N × D. Where: C = concentration of sperm cell per ml of semen; N = Number of spermatozoa counted; D = Dilution rate.

The structural membrane integrity of spermatozoa was evaluate with eosin-nigrosin stain. It involved adding a drop of the staining solution eosin-nigrosin on a clean slide and a drop of undiluted semen, mixed gently to prepare a smear. The slide was air-dried and examined with a microscope at x 400 magnification. The functional membrane integrity was determined in semen samples in a 1:10 dilution of hypo-osmotic solution 75 mOsmol/L. To a warm 1ml swelling solution in a closed Eppendorf tube at 37°C, 0.1 ml of liquefied semen was added and mixed gently within the tube. The mixture was kept at 37°C for at least 30 minutes and sperm cells examined at X 400 magnification. Swelling of sperm cell was identified as changes in the shape of the tail. Counting in duplicate the number of swollen cells in a total of 200 spermatozoa counted and expressed as a percentage. The total motile sperm cell was calculated as the product of sperm concentration/ml and percentage motility of semen sample per animal. Total live sperm cell was obtained by a multiple of sperm concentration per ml and percentage livability.

Seminal biochemical indices

Seminal biochemical was determined using the spectrophotometric procedure of Randox commercial assay kit; total protein according to Lowry et al., (1951), glucose and total cholesterol concentration were determined as described by Lindner and Mann (1960). The spectrophotometry as explained by Quinn et al. (1966) was used to determine sodium, chloride, phosphorus, magnesium and potassium contents of the samples.

Seminal Oxidative Status

Determination of seminal total antioxidant activities was carried out according to Korecevic (2001); The reactive mixture contained 0.5mL of a (10 mmol/L) Na-Benzoate, 0.2mL 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 Na2HPO4 (100 mmol/L), then adjusted the pH to 7.4) and 0.2 mL of Fe-EDTA complex (2mmol/L) (prepared freshly by mixing equal volumes of EDTA (2mmol/L) and ammonium ferrous sulfate (2mmol/L), then left to stand 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 50mmole/L NaOH) was 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:

Total antioxidant activities (mmol/L) = (CUA) (K – A) / (K – UA)

Where: (CUA): Concentration of uric acid (mmol/L).
K: Absorbance of control (K1 - K0).
A: Absorbance of the sample (A1 - A0).
UA: Absorbance of uric acid solution (UA1 - UA0).

Superoxide dismutase (SOD) activity is estimated by the method of Soon and Tan (2002) by adding 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source and 0.86 ml of distilled water. The reaction is initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance monitored at 420 nm. One unit of SOD is defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50 % in the standard assay system of 3 ml. The specific activity is expressed as units/min/mg protein.

Glutathione peroxidase activity (GPx) is estimated by Rotruck et al. (1973) by adding to 0.5 ml 0.4 M buffer pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM GSH, 0.1 ml 0.2 mM H2O2 added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as µg of GSH consumed/min/mg protein.

Catalase activity is estimated by Beers and Sizer (1952) assay system contains 1.9 ml 0.05 M buffer pH 7.0 and 1.0 ml 0.059 M H_2O_2. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm and activity is expressed as nmoles of H_2O_2 decomposed/ min/mg protein.

Serum lipid peroxidation assay is determined according to Ohkawa et al. (1979); the reaction mixture in a total volume of 3.0ml contained 1.0 ml serum, 1.0 ml of TCA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 minutes. The tubes were shifted to

the ice bath and then centrifuged at 2500rpm for 10 minutes. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm.

Statistical Analysis

Data obtained in this study were subjected to descriptive statistics, analysis of variance of the general linear model procedure to detect significant effects with a confidence level of 95%. Means were separated with New Duncan's multiple range test of statistical analysis software.

RESULTS

Semen Characteristics of Four Exotic Breeds of Rabbit

Semen characteristics of four exotic breeds of rabbit at highest THI of Ibadan are shown in Table 1. Semen volume, sperm concentration, total motile sperm cells, and total live sperm cells were similar across the breeds. Libido of New Zealand White bucks was significantly ($p<0.05$) higher than British Spot and Fauve de Bourgogne bucks but Chinchilla bucks share similar libido with other breeds. The mass activity of spermatozoa in New Zealand White bucks was significantly ($p<0.05$) higher than Fauve de Bourgogne bucks but Chinchilla and British Spot bucks had statistically ($p>0.05$) similar values with New Zealand White and Fauve de Bourgogne bucks. Sperm motility follows a similar trend as the mass activity.

Table 1. Semen Characteristics of Four Breeds of Rabbit at highest temperature humidity index in Ibadan

	Fauve de Bourgogne	Chinchilla	British Spot	New Zealand White	SEM
Volume (ml)	0.47	0.50	0.51	0.33	0.04
Libido (sec)	7.78^{b}	9.92^{ab}	9.21^{b}	11.9^{a}	0.40
Mass Activity (%)	63.3^{b}	83.3^{ab}	77.0^{ab}	87.7^{a}	3.67
Motility (%)	59.5^{b}	74.0^{ab}	67.1^{ab}	79.9^{a}	2.93
Sperm Concentration (x 10^{8}/ml)	8.06	11.8	10.3	6.64	0.94
Total Motile Sperm Cells (x10^{8}/ml)	5.11	8.17	7.67	5.41	0.70
Total Live Spermatozoa(x10^{8}/ml)	7.47	11.0	8.37	6.12	0.86

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

Structural and Functional Spermatozoa Membrane Integrity of Four Exotic breeds of Bucks

Structural and functional spermatozoa membrane integrity of four exotic breeds of rabbit at highest THI of Ibadan is shown in Figure 1. Structural membrane integrity of Fauve de Bourgogne, Chinchilla, British Spot, and New Zealand White were 92.29%, 94.33%, 85.28% and 89.93% respectively. Functional sperm membrane integrity of 54.35% Fauve de Bourgogne), 48.43% Chinchilla), 49.56% British Spot and 58.94 % New Zealand White were recorded in bucks.

Seminal Biochemistry of Four Exotic Breeds of Rabbit

Seminal biochemistry of four exotic breeds of the rabbit during highest THI in Ibadan is shown in Table 2. All seminal indices assessed were significantly ($p<0.05$) influenced by the breed except cholesterol. Seminal glucose was significantly ($p<0.05$) highest in Chinchilla and the significantly ($p<0.05$) least value was obtained in British Spot and Fauve de Bourgogne. New Zealand White bucks had significantly ($p<0.05$) higher seminal glucose compared with British Spot and Fauve de Bourgogne bucks. Seminal protein of Fauve de Bourgogne and British Spot bucks were similar but significantly ($p<0.05$) higher than Chinchilla and New Zealand White. Seminal magnesium follows similar trends with seminal protein. Seminal phosphorus of Fauve de Bourgogne bucks was significantly ($p<0.05$) highest, while Chinchilla bucks had significantly ($p<0.05$) higher seminal phosphorus than New Zealand White bucks. Sodium was statistically similar in Fauve de Bourgogne, British Spot, and New Zealand White bucks but they were significantly ($p<0.05$) higher than Chinchilla bucks. The potassium in Fauve de Bourgogne bucks semen were significantly ($p<0.05$) highest and that of British Spot and New Zealand White were statistically similar. Seminal chloride of Chinchilla and New Zealand White bucks was significantly ($p<0.05$) highest and the significantly ($p<0.05$) least value was observed in British Spot bucks.

Seminal Oxidative Status of Four Exotic Breeds of Rabbit

Seminal lipid peroxidation of four exotic breeds of the rabbit during HTHI in Ibadan is shown in Figure 2. Seminal lipid peroxidation was significantly ($p<0.05$) least in British Spot. Chinchilla bucks had a significantly ($p<0.05$) higher seminal lipid peroxidation than Fauve de Bourgogne, while New Zealand White had statistically similar values with Fauve de Bourgogne and Chinchilla. Seminal total antioxidant activity was significantly ($p<0.05$) highest in New Zealand White bucks and least ($p<0.05$) values was obtained in British Spot as shown in Figure 3. Chinchilla bucks had significantly ($p<0.05$) higher TAA values than Fauve de Bourgogne. Seminal SOD activity was similar in Fauve de Bourgogne, Chinchilla, and New Zealand White bucks but significantly ($p<0.05$) lower than British Spot bucks as shown in Figure 4. An inverse trend was obtained in seminal catalase activity as shown in Figure 5. Seminal glutathione peroxidase activity was significantly ($p<0.05$) highest in Fauve de Bourgogne and New Zealand White bucks, while Chinchilla had significantly ($p<0.05$) higher value than British Spot bucks as shown in Figure 6.

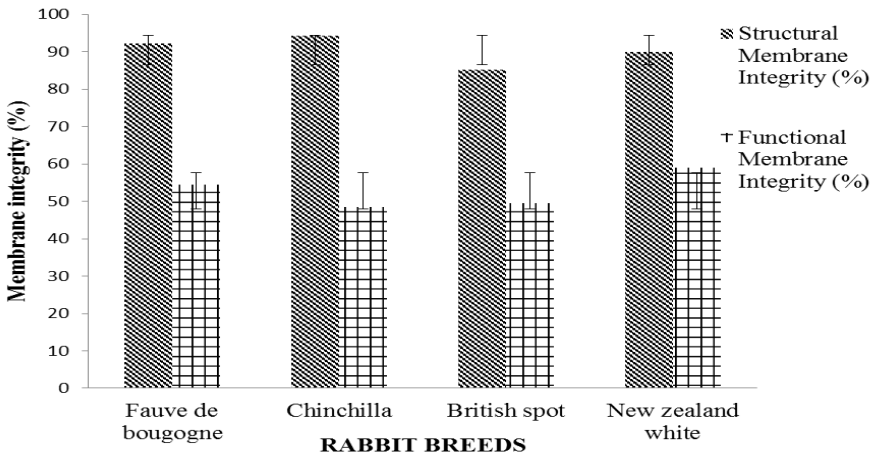

Figure 1: Structural and Functional Spermatozoa Membrane Integrity of Four breeds of Rabbit Bucks during highest temperature humidity index of Ibadan

Table 2: Seminal Biochemistry of Four Breeds of Rabbit during highest temperature humidity index in Ibadan

	Fauve de Bourgogne	Chinchilla	British Spot	New Zealand White	SEM
Glucose (mmol/L)	0.98^{c}	3.18^{a}	1.05^{c}	2.47^{b}	0.39
Protein (g/L)	100^{a}	39.4^{b}	150^{a}	39.3^{b}	21.6
Cholesterol (mmol/L)	2.57	2.46	2.62	2.72	0.06
Magnesium (mmol/L)	0.61^{b}	0.69^{a}	0.65^{a}	0.6^{b}	0.03
Phosphorus (mmol/L)	2.75^{a}	2.01^{b}	1.92^{bc}	1.73^{c}	0.23
Sodium (mmol/L)	56.6^{a}	21.2^{b}	57.3^{a}	54.9^{a}	7.03
Potasium (mmol/L)	63.2^{a}	28.5^{c}	38.8^{b}	39.89^{b}	4.26
Chloride (mmol/L)	76.5^{b}	91.9^{a}	62.5^{c}	91.8^{a}	9.33

abc: means in the same row with different superscripts are significantly different. SEM: Standard Error of Mean.

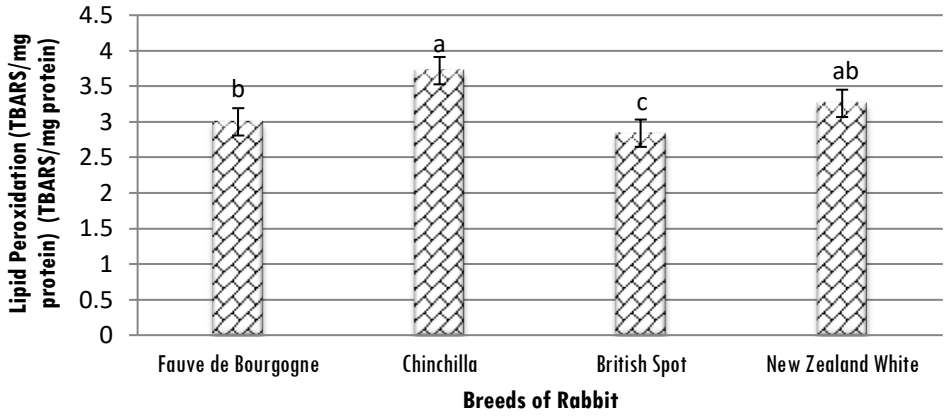

Figure 2: Seminal Lipid Peroxidation of Four Breeds of Rabbit during the highest temperature humidity index

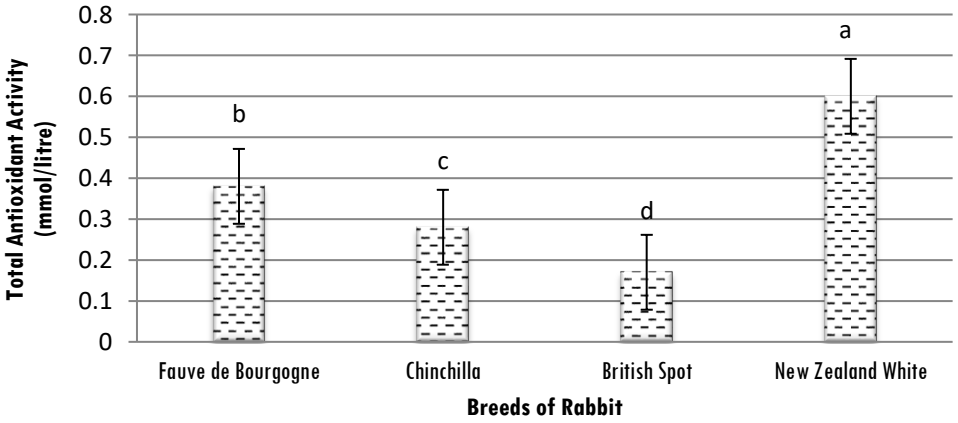

Figure 3: Seminal Antioxidant Activity of Four Breeds of Rabbit during the highest temperature humidity index

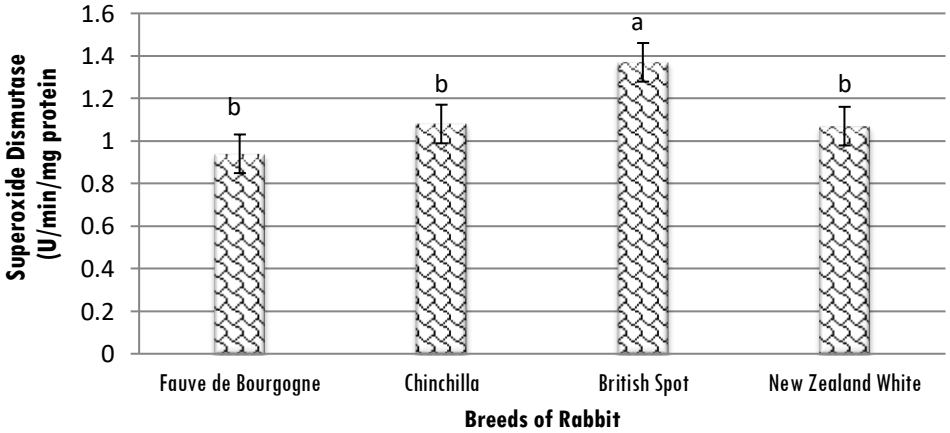

Figure 4: Seminal Superoxide Dismutase of Four Breeds of Rabbit during the highest temperature humidity index

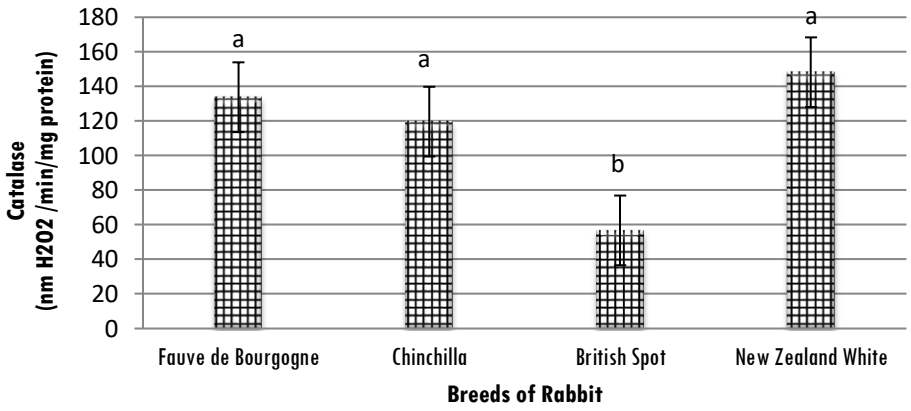

Figure 5: Seminal Catalase of Four Breeds of Rabbit during the highest temperature humidity index

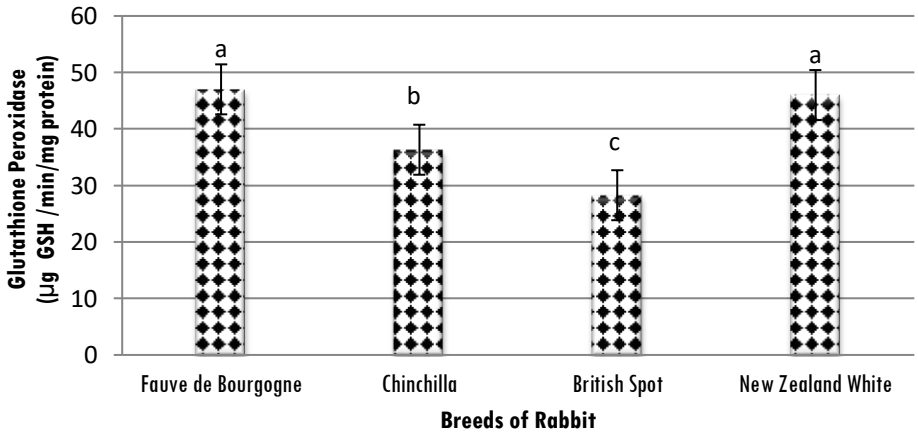

Figure 6: Seminal Glutathione Peroxidase of Four Breeds of Rabbit during the highest temperature humidity index

DISCUSSION

At highest THI, slowest reaction time was observed in New Zealand White and fastest in Fauve de Bourgogne. Semen volume, sperm concentration, total motile sperm cells and total live sperm cells showed similarities among the breeds (Table 1). The trend of higher mass activity and sperm motility despite low volume observed in New Zealand White could be attributed to the report of Jimoh and Ewuola (2018) that New Zealand Rabbits have the least susceptibility to heat stress, amongst the four breeds of rabbits assessed. However, the range of reaction time observed in this study (7.78-11.88 seconds) indicates that the breeds of rabbit used in this study have a higher libido than New Zealand White rabbits (12.11 to 23.39 seconds) in the summer of Egypt reported by El Tohamy *et al.* (2012). Sexual desire was found to significantly decrease with increasing ambient temperature (Kamel, 2012; Daader and Seleem, 1999). Tharwat *et al.* (1994) found that libido was delayed by 11.9–18.5 min and by up to 40 min in New Zealand White rabbit bucks exposed to 40^0C temperature with a relative humidity of 60–65%. This delay may be due to the decrease in testosterone concentration, minimal spermatogenesis (Zeidan *et al.,* 1997) and/ or the low-quality semen (El-Kelawy et al., 1997), occurring in a hot climate.

Lower ejaculate volume and higher concentration, total motile sperm cells and motility was inherent in the breed than New Zealand White bucks reported by El-Tohamy et al. (2012). The result obtained in this study suggests that the breeds of rabbit assessed had better sperm quality than Baladi and New Zealand White bucks reported by Safee et al. (2008); this is evident in faster reaction time, higher sperm concentration and motility observed in this study. The range of sperm motility (59.50-79.88%) obtained in this study is close to that reported by Attia et al. (2010), 70.4 - 82.5% in New Zealand White bucks administered oral glucose supplementation. However, structural sperm membrane integrity, sperm concentration and total live spermatozoa obtained in this study are higher than those obtained by Attia et al. (2010). The range of ejaculate volume, sperm concentration, motility and livability obtained in this study is higher than those reported in V-line buck subjected to heat stress by Zeweil et al. (2013). Higher ejaculate volume, lower concentration, and total motile spermatozoa values were obtained by Kamel (2012) in V-line rabbits supplemented with selenium and folic acid under heat stress compared to values obtained in this study, but reaction time, sperm motility and sperm livability values were similar. Functional membrane integrity of spermatozoa (48.43-58.94%) obtained in this study (Figure 1), is lower than those obtained in summer heat stressed bucks supplemented with antioxidant (El-Tohamy et al., 2012) but higher than those obtained in summer heat stressed Baladi and New Zealand White bucks 33.42% and 19.75%, respectively (2008).

Seminal plasma is usually an isotonic neutral medium and it is a detrimental factor to sperm cell survival (White, 1976). All seminal biochemical assessed were affected by breed differences except cholesterol. The range of seminal cholesterol observed in this study is higher than the range of values reported by Zeweil et al., (2013) in male rabbit subjected to summer heat stress and supplemented with pomegranate peel but similar values were obtained by Attia et al. (2010) in New Zealand White bucks supplemented with glucose during summer heat stress. The range of seminal total protein observed in this study is higher than values reported by Kamel (2012) in V -line rabbits supplemented with organic selenium and folic acid during summer heat stress and Attia et al. (2010) in New Zealand White bucks administered oral glucose under summer heat stress conditions.

Cholesterol has been shown to be involved in the process of capacitation in mammalian spermatozoa and an important role in fluidity and structure of the plasma membrane (Langlais and Roberts, 1985; Blesbois et al., 2010). It has been reported that the higher the level of cholesterol, the lower the fertility (Jimoh, 2016).

Heat stress may affect osmotic equilibrium and ionic channels that are significant in the interplay between spermatozoa, its environment, and the egg, thus distorting spermatozoa homeostasis, its behavior or its metabolic machinery (Darszon et al., 1999). The activation of sperm motility depends on intracellular and extracellular calcium (Ca^{++}), extracellular sodium (Na^{+}) and potassium (K^{+}). Ion concentrations increased in whole semen when animals were heat stressed, which may be due to increase in sperm concentration (Karaca *et al.,* 2002). This corroborates the result of this study which discovers that a decrease in sodium, potassium, and phosphorus was accompanied with a reduction in sperm concentration. Karaca et al., (2002) claimed that change in sperm and plasma Ca^{++}, Chloride (Cl^{-}) and Na^{+} concentrations may be related to heat stress infertility, while it was also stated that seminal plasma from semen samples with heat stressed sperm contained lower Ca^{++}, Na^{+}, and Cl^{-} concentrations when compared with that from control sperm (Ilori et al., 2012), this is in line with the result obtained in this study.

Thermal stress is often accompanied by oxidative stress in which ROS compounds are produced in greater amounts (Heise et al., 2003). One of the most important factors contributing to poor quality semen has been reported to be oxidative stress (Bucak et al., 2010). The increase in the production of reactive oxygen species (ROS) determines semen characteristics and sperm-oocyte fusion (Akiyama, 1999). Also, an antioxidant mechanism is necessary to prevent free radical damage to the sperm cell as a result of secretion of hydrogen peroxide and the superoxide ion by rabbit spermatozoa (Holland et al., 1982). Literature regarding oxidative stress characteristics in semen in response to heat stress is very limited. The objective of most experiments was to evaluate the effects of prooxidants or antioxidants on the concentrations of TBARS on semen quality (Nichi et al., 2006).

Breed influenced oxidative stress markers at highest THI. British spot breed had least seminal lipid peroxidation despite recording the least total antioxidant activity, catalase, and GPx activities but the highest superoxide dismutase activity. This could be partly due to low serum lipid peroxidation and higher antioxidant activity observed at this period in the breed (Jimoh et al., 2017) or superoxide dismutase was able to mitigate superoxide induced lipid peroxidation to hydrogen peroxide which was also scavenged by the level of GPx and catalase. This could have accounted for the breed's low peroxidation during this period. New Zealand White, Chinchilla and Fauve de Bourgogne breeds had higher lipid peroxidation, total antioxidant activity, and catalase activities compared to British Spot rabbits.

Related existing studies have suggested that the higher concentrations of TBARS found in seminal plasma during the summer were apparently related to higher levels of ROS, and not due to a lower antioxidant capacity (El-Tohamy et al., 2012), but indications from the result of this findings signifies both increases in ROS production and a decrease in antioxidants, a situation that is referred to as oxidative stress. El-Tohamy et al. (2012) reported in the summer heat stress bucks supplemented with antioxidants showed a decrease in seminal plasma TBARS levels than

summer control group, thus suggesting a protective effect of antioxidant against ROS induced oxidative stress. Kamel (2012) reported that seminal plasma antioxidant GPx, SOD and Glutathione-S-Transferase activities of rabbit bucks increased significantly due to supplementation with organic selenium, folic acid, and their combinations through 12 weeks of summer heat stress, while seminal plasma TBARS concentration decreased significantly as compared with the control group. Most species have little protective catalase in their semen, but rabbit semen contains substantial amounts of catalase (Foote and Hare, 2000). This is corroborated by the report of El-Tohamy et al. (2012) and also that antioxidant supplemented bucks showed a significant increase in seminal plasma catalase activities in comparison with summer control bucks.

CONCLUSIONS

It could be concluded from this study that chronic heat stress induces oxidative stress in sperm cells of rabbit bucks. At peak of thermal discomfort in Nigeria, heat stress adversely affects semen biochemicals which deleteriously influence semen quality. New Zealand white bucks had better semen quality and British Spot bucks had better oxidative stability among the breeds, and are reliable breeders for genetic improvement for an uninterrupted breeding cycle for commercial enterprise.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests in the publication of this article.

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