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Semen characteristics and seminal oxidative status of four breeds of rabbit in South west, Nigeria

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Abstract

Background: Within the rabbit population in South West of Nigeria, four exotic breeds of rabbits consisting of Fauve de Bourgogne, Chinchilla, British Spot, and New Zealand White were evaluated for their reproductive response. This investigation was carried out within July and August, when the least temperature-humidity index (THI) (ranged between 23.41 and 25.30) is observed in Ibadan, South West, Nigeria, which depicts the highest thermal comfort in the study area. Thirty bucks per breed, housed individually and randomly allotted to experimental units, were used for this study. All bucks were made to serve an artificial vagina, libido was evaluated, and the collected ejaculates were assessed for semen characteristics, seminal biochemical parameters, and oxidative stress indices weekly for 4 weeks. Two ejaculates per buck were collected weekly. The first ejaculate for spermiogram and the second ejaculate was centrifuged at 4000 rpm for 15 min to separate seminal plasma and used for biochemical analysis using standard procedures.

Result: The result obtained revealed that sexual urge (reaction time) in the four breeds was similar statistically. British Spot and New Zealand White breeds despite recording a lower semen volume had apparently higher mass motility, individual sperm motility, and significantly higher ($P < 0.05$) sperm concentration compared with the other breeds. Genetic differences in breeds were evident in most oxidative stress markers, except for the total antioxidant activity of seminal fluid. New Zealand White had the highest antioxidant enzyme activity.

Conclusion: New Zealand White had the best oxidative status among the four breeds, and this enhanced its semen quality parameters among the rabbit breeds.

Keywords: Spermiogram, Seminal plasma, Rabbit breeds, Thermal comfort, Oxidative stress

Background

The rabbit is more tolerant to low temperatures than to high temperatures; thus, if the environmental temperature is above 25–30 °C, the behaviour and several physiological changes reduce productivity. Comparative study on breeds of rabbit for production superiority will lead to the discovery of breed to be considered for improved breeding, and crosses between these breeds and with other rabbit breeds will improve the production efficiency of rabbit breeds with less production efficiency (El-Sheikh & Seleem, 2010). Abd El-Azim and El-kamash (2015) reported that breed of rabbit bucks differ in reaction time, semen pH, semen density, semen colour, mass motility and advanced motility. Seminal plasma also contains other particles of different sizes which affect the spermatozoa behaviour during the transit along the female reproductive tract (Castellini, 2008). Variation in the seminal characteristics contribute to the large variability in semen quality traits (Alvariño, 2000), it is important to evaluate seminal inclusions to improve semen output.Oxidative stress results from increased production of free radicals and reactive oxygen species and a decrease in antioxidant defense (Trevisan, Browne, Ram, Muti, & Freudenheim, 2001; Williams, Kronfeld, Hess, Saker, & Waldron, 2002). Ganaie, Shanker, Bumla, Ghasura, and Mir (2013) reported that oxidation is essential to nearly all cells in the body to provide energy for vital functions. Approximately

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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-reactive oxygen species, that may damage the DNA of genes and contribute to degenerative changes (Ganaie et al., 2013). Studies have shown that antioxidants are uniquely different from each other, and each has a specific function in the body. It has been suggested that reactive oxygen species (ROS) and lipid peroxide products in various clinical diagnoses of infertility are associated with high oxidative stress and whether any group of infertile animal is more likely to have high seminal oxidative stress. ROS play an important role in sperm physiological functions, but elevated levels of ROS or normal cell functions and integrity of cell structures may be broken via considerable reactivity of ROS. The body system of animals has enzymatic, e.g. superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic, e.g., vitamin E antioxidant mechanisms that work as a 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 (El-Tohamy, Kotp, El-Nattat, Amira, & Soliman, 2012). The contributions of thermal discomfort (heat stress) to infertility in rabbits via oxidative stress necessitated investigation to assess the reproductive performance and seminal oxidative stress markers of four exotic breeds of rabbit at the least thermal discomfort condition in Ibadan, South west, Nigeria. It is therefore important to determine and document the semen characteristics as well as the oxidative status of semen ejaculates produced by rabbit bucks at their peak of thermal comfort.

Methods

Experimental site

The 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, South west, Nigeria. They are situated in the 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 average temperature are 22.87 ± 3.92 °C and 34.57 ± 4.01 °C, rainfall 184.85 ± 4.82 mm and 8.03 ± 0.21 mm, and relative humidity 87.20 ± 3.19% and 63.06 ± 5.28%, during the dry and wet season, respectively. The study was approved by our institutional committee on the care and use of animals for research.

Experimental animals, design and management

Four exotic breeds of adult rabbit 10–14 months old with an average weight of 1245 ± 30.56 g consisting of Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White. The bucks used for the study were 12–18 months old with an average weight of 1826 ± 8.35 g. At the commencement of the trial, animals were confirmed to be of good health status, without abnormalities and conform to the breed categorization.

This experiment was carried out within July and August, when least THI is observed (range between 23.41 and 25.30) in the experimental site as reported by Jimoh and Ewuola (2018). Temperature and relative humidity of the rabbitry microclimate was recorded at 08:00 h every morning and 16:00 h in the evening during the study period using a thermohygrometer. The ambient temperature and relative humidity were used to compute the temperature-humidity index. Readings were taken daily. The temperature-humidity index (THI), an indicator of thermal comfort level for animals in an enclosure, was calculated as modified by Marai et al. (2001) and given as follows:

$$\mathrm{THI} = t - [(0.31 - 0.31 \times \mathrm{RH}\, t - 14.4)]$$

where RH = relative humidity/100 and t = ambient temperature.

The values of THI obtained for rabbit are 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
Above 30 °C = very severe heat stress (Marai et al., 2001)

In the morning, the THI of July and August were 24.82 and 24.05 °C, respectively, and the afternoon THI for July and August were 30.50 and 26.05 °C, respectively. The average monthly THI for July and August were 27.66 and 25.05, respectively.

Thirty bucks per breed were used for this study. Animals were housed individually and allotted randomly into experimental units. The experimental design was a completely randomised design. The animals were fed 5% of their bodyweight, with diets containing crude protein 17.05%, digestible energy 2592.06 Kcal/kg, crude fibre 10.02%, calcium 0.45% and phosphorus 0.21%. Fresh water was made available to the animals always. Other routines and periodic management practices necessary for rabbit production were carried out. All bucks were used for assessment of semen characteristics, seminal biochemical parameters and oxidative stress indices.

Semen characteristics

Adult males were trained to serve an artificial vagina (designed and constructed for rabbits), two weeks prior

to the experimental period, and two ejaculates per male were collected weekly, with an interval of 3–4 days between successive ejaculation over a period of 4 weeks from all males within the experiment. The first ejaculate for spermiogram and the second ejaculate were centrifuged at 4000 rpm for 15 min to separate seminal plasma and stored at − 20 °C, until further analysis. Briefly, libido was measured in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck ejaculated (Daader, Gabr, & Seleem, 1999). Semen volume for each buck was measured using tuberculin syringe to the nearest 0.1 ml. For mass motility, a drop of fresh semen was placed on a clean glass slide and examined with a microscope under × 10 objective lens to determine mass motility. The mass motility 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 (+++). For individual 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 as may be required before it was covered with a glass cover slip and examine at a magnification of × 400. The percentage of progressively motile spermatozoa was estimated, and the score is subjectively between 0 and 100. At least 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 haemocytometer slide. Formal saline (1%) was used to dilute the semen (*v*/*v*) to immobilize the cells and make an enumeration of the sperm cells easier. The diluted semen was then charged on each of two chambers of the haemocytometer slide using a micropipette. The charged haemocytometer was placed on the microscope at a magnification of × 400. The concentration of spermatozoa per volume was determined using the formula $C = 32{,}000 \times N \times D$, where C is the concentration of the sperm cell per milligram of the semen, N is the number of spermatozoa counted and D is the dilution rate.The structural membrane integrity of spermatozoa is an assay of the livability of spermatozoa. Thus, it was determined by live to dead ratio of sperm cells. It involved adding a drop of the staining solution Nigrosin–Eosin 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 × 400 magnification. The functional membrane integrity was determined in semen samples in a 1:10 dilution of hypo-osmotic solution 75 mOsmol/L (Moce, Vicente, Lavara, Lavara, & Marco-Jiménez, 2004). To a warm 1-ml swelling solution in a closed Eppendorf tube at 37 °C, 0.1-ml liquefied semen was added and mixed gently within the tube. The mixture was kept at 37 °C for at least 30 min and sperm cells examined at × 400 magnification. The swelling of the sperm cell was identified with the changes in the shape of the tail. Counting in duplicate, the number of swollen cells in a total of 200 spermatozoa was counted and expressed as a percentage. The total motile sperm cell was calculated as the product of sperm concentration/milligram and percentage motility of semen sample per animal. Total live sperm cell was obtained by a multiple of sperm concentration per milligram and percentage livability.

Seminal biochemical indices

Seminal biochemical parameter values were determined using spectrophotometric procedure using Randox commercial assay kits. Total protein, according to Lowry, Rosebrough, Farr, and Randall (1951), glucose and total cholesterol concentration were determined as described by Lindner and Mann (1960). The spectrophotometry was used to determine sodium, chloride, phosphorus, magnesium and potassium contents of the samples.

Seminal oxidative status

Determination of seminal total antioxidant capacity activities was carried out according to (Koracevic, Koracevic, Djordjevic, Andrejevic, & Cosic, 2001). The reactive mixture contained 0.5 mL of a (10 mmol/L) Na-Benzoate, 0.2 mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH = 7.4) (prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na_2HPO_4 (100 mmol/L), then adjusted the pH to 7.4) and 0.2 mL of Fe-EDTA complex (2 mmol/L) (prepared freshly by mixing equal volumes of EDTA (2 mmol/L) and ammonium ferrous sulfate (2 mmol/L), then left to stand at 25 °C for 60 min). Ten microliters of the blood serum was added to the latter reactive mixture and was incubated at 37 °C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% *w*/*v* in 100 mL of 50 mmole/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:

$$\text{TA capacity (mmol/L)} = (\text{CUA})\,(K - A)/(K - \text{UA})$$

where (CUA) is the concentration of the uric acid (mmol/L), K is the absorbance of the control (K1–K0), A is the absorbance of the sample (A1–A0) and UA is the absorbance of the uric acid solution (UA1–UA0).

Glutathione peroxidase activity is estimated as described by Rotruck et al. (1973) and Ellman's (1959). Briefly, the sample was added to 0.5 ml 0.4 M buffer (pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM GSH, and

0.1 ml 0.2 mM H_2O_2 and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 ml of 10% trichloroacetic acid TCA centrifuged at 4000 rpm for 5 min, and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as microgram of GSH consumed/min/mg protein.

Catalase activity is estimated by Beers and Sizer (1952) method. The assay system contains 1.9 ml and 0.05 M buffer (pH 7.0) and 1.0 ml and 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 the activity is expressed as nanomoles of H_2O_2 decomposed/min/mg protein. Serum lipid peroxidation was determined using thiobarbituric acid assay according to Ohkawa, Ohishi, and Yagi (1979).

Statistical analysis

Data obtained in this study was subjected to descriptive statistics and analysis of variance of the general linear model procedure, and significant effects were detected with a confidence level of 95%. The new Duncan multiple range test was used to separate means.

The statistical model is the following:

$$Y_{ijl} = \mu + B_i + e_{ijl}$$

where Y_{ijl} represents the value of semen characteristics, biochemical parameters and oxidative status measured in the lth animal, μ is the overall mean for each character, B_i is the fixed effect of ith breed with four levels (i = Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White) and e_{ijl} is the random residual effect.

Results

Reaction time and semen characteristics of four breeds of rabbit are presented in Table 1. Libido, sperm mass motility and motility were not significantly ($p > 0.05$) affected by breeds of rabbit. However, the semen volume of Chinchilla was significantly ($p < 0.05$) higher than that of British Spot and New Zealand White. British Spot and New Zealand White had similar semen volume with Fauve de Bourgogne rabbits. Sperm concentration, total live spermatozoa of British Spot and New Zealand White were significantly ($p < 0.05$) higher than that of Chinchilla rabbits. Fauve de Bourgogne rabbits had similar values with other breeds. The total motile spermatozoa of New Zealand White was significantly ($p < 0.05$) higher than Chinchilla rabbits. Fauve de Bourgogne and British Spot had similar total motile sperm with Chinchilla and New Zealand White rabbits.

The structural and functional spermatozoa membrane integrity of four exotic breeds of rabbit buck is presented in Fig. 1. The four breeds had normal spermatozoa structural membrane integrity ranging from 98.01 to 99.28%. The sperm functional membrane integrity of the breeds is 69.65%, 70.55%, 72.78%, and 69.8% for Fauve de Bourgogne, Chinchilla, British Spot and New Zealand White, respectively.

The seminal biochemistry of four exotic breed of rabbit buck is presented in Table 2. Seminal glucose, cholesterol, magnesium, potassium and chloride were not significantly ($p > 0.05$) influenced by breeds of rabbit. However, the seminal protein of British Spot rabbit was significantly ($p < 0.05$) higher than New Zealand White, but both were not significantly ($p > 0.05$) different from Fauve de Bourgogne and Chinchilla rabbit. Seminal sodium of Fauve de Bourgogne, New Zealand White and British Spot rabbits were significantly ($p < 0.05$) higher than Chinchilla rabbits. Seminal phosphorus of Fauve de Bourgogne and Chinchilla were statistically similar, but both were significantly ($p < 0.05$) higher than seminal phosphorus of British Spot and New Zealand White.

The seminal oxidative status of four exotic breeds of rabbit bucks is shown in Table 3. Rate of seminal lipid peroxidation in British Spot and New Zealand White are similar but were significantly ($p < 0.05$) higher than Fauve de Bourgogne bucks. However, the rate of seminal lipid

Table 1 Libido and semen characteristics of four exotic breeds of rabbit

Parameters	Fauve de Bourgogne	Chinchilla	British Spot	New Zealand White	SEM	*p* value
Libido (reaction time in seconds)	6.13	5.40	5.70	7.50	0.54	NS
Volume (ml)	0.55^{ab}	0.70^{a}	0.41^{b}	0.34^{b}	0.04	*
Mass activity (%)	89.00	83.33	93.33	95.33	2.00	NS
Motility (%)	82.63	84.60	88.30	85.00	1.27	NS
Sperm concentration ($\times 10^8$/mL spermatozoa)	19.7^{ab}	16.91^{b}	23.68^{a}	22.78^{a}	2.49	*
Total motile sperm cells ($\times 10^8$/mL spermatozoa)	16.75^{ab}	15.90^{b}	19.48^{ab}	21.98^{a}	2.22	*
Total live spermatozoa ($\times 10^8$/mL spermatozoa)	19.52^{ab}	16.81^{b}	22.53^{a}	24.06^{a}	2.51	*

Means in the same row with different superscript letters are significantly ($p < 0.05$) different
SEM standard error of mean, *NS* not significant ($p > 0.05$)
*Significant difference ($p < 0.05$)

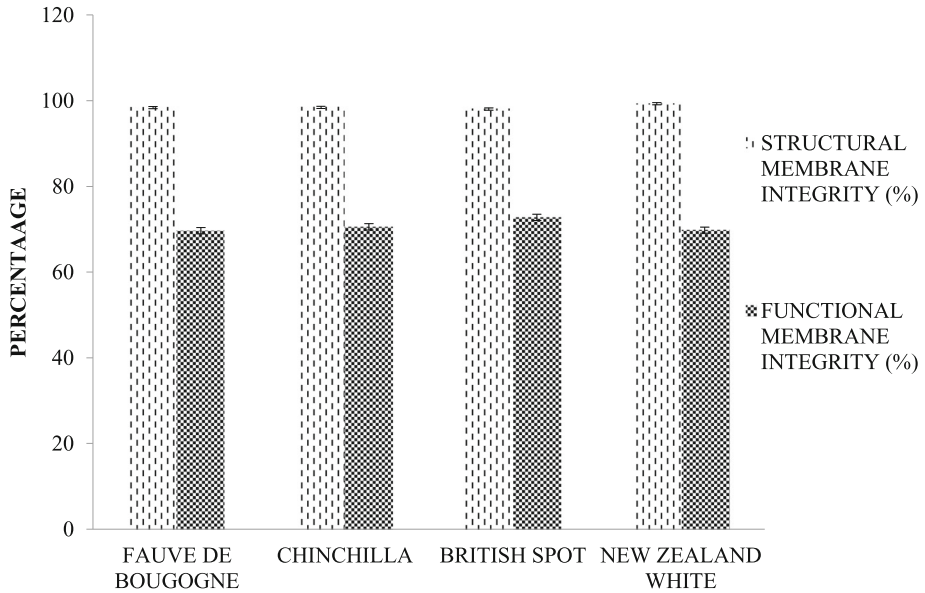

Fig. 1 Structural and functional spermatozoa membrane integrity of four exotic breeds of rabbit buck. Values are means ± SEM. Number of ejaculates per breed.30. Level of significance was ($p > 0.05$)

peroxidation in Chinchilla is not significantly ($p > 0.05$) different from other breeds.

Seminal antioxidant activity was not significantly ($p > 0.05$) affected by breeds of rabbit examined. Seminal superoxide dismutase activity in New Zealand White rabbits was significantly ($p < 0.05$) higher than that of Chinchilla; however, Fauve de Bourgogne and British Spot had statistically ($p > 0.05$) similar seminal SOD activity. Seminal catalase of New Zealand White bucks was significantly ($p < 0.05$) different from that of British Spot. However, Fauve de Bourgogne and Chinchilla were statistically similar to British Spot and New Zealand White rabbits. Seminal glutathione peroxidase of New Zealand White bucks was significantly ($p < 0.05$) higher than the three other breeds. Fauve de Bourgogne, British Spot and Chinchilla bucks were not significantly ($p > 0.05$) different in their seminal glutathione peroxidase activity.

Discussion

Libido (sexual desire) measured in terms of reaction time in seconds and estimated from the time the teaser doe was introduced to the buck's cage up to the point when the buck ejaculated (Daader et al., 1999). Reaction time in the four breeds was similar statistically. However, the range of values obtained in this study 5.40–7.5 s is lower than 11.49 s reported in the New Zealand White rabbit by El-Tohamy et al. (2012) and New Zealand White and Baladi rabbits (19.3 and 12.3 s, respectively) reported by Safaa, Emarah, and Saleh (2008) in winter of Egypt. This suggests high sexual urge in this breeds of rabbit at thermal comfort. Bagliacca, Camillo, and Paci

Table 2 Seminal biochemistry of four exotic breeds of rabbit

Parameters	Fauve de Bourgogne	Chinchilla	British Spot	New Zealand White	SEM	*p* value
Glucose (mmol/L)	1.12	0.75	0.58	0.54	0.17	NS
Protein (g/L)	28.80^{ab}	27.00^{ab}	39.90^{a}	12.20^{b}	3.50	*
Cholesterol (mmol/L)	0.87	1.86	0.83	0.97	0.21	NS
Magnesium (mmol/L)	1.01	1.11	1.06	1.03	0.03	NS
Phosphorus (mmol/L)	3.91^{a}	3.74^{a}	3.25^{b}	3.26^{b}	0.23	*
Sodium (mmol/L)	60.24^{a}	45.56^{b}	62.01^{a}	63.88^{a}	7.03	*
Potassium (mmol/L)	46.42	58.68	62.15	38.56	4.89	NS
Chloride (mmol/L)	0.30	0.63	0.52	0.44	0.08	NS

Means in the same row with different superscripts are significantly ($p < 0.05$) different
SEM standard error of the mean, *NS* not significant ($p > 0.05$)
*Significant difference ($p < 0.05$)

Table 3 Seminal oxidative status of four exotic breeds of rabbit

Parameters	Fauve de Bourgogne	Chinchilla	British Spot	New Zealand White	SEM	*p* value
Lipid peroxidation (TBARS/mg protein)	0.03^{b}	0.05^{ab}	0.07^{a}	0.06^{a}	0.01	*
Total antioxidant activity (mmol/litre)	0.78	0.82	0.79	0.60	0.07	NS
Superoxide dismutase (U/min/mg protein)	57.27^{ab}	29.09^{b}	48.18^{ab}	91.82^{a}	10.87	*
Catalase (nmoles of H_2O_2 consumed/min/mg protein)	345.69^{ab}	300.43^{ab}	165.99^{b}	817.73^{a}	62.13	*
Glutathione peroxidase (μgGSH/min/mg protein)	102.85^{b}	88.27^{b}	61.16^{b}	244.14^{a}	18.89	*

Means in the same row with different superscripts are significantly ($p < 0.05$) different
SEM standard error of the mean, *LTHI* least temperature-humidity index, *NS* not significant ($p > 0.05$), *TBARS* thiobarbituric acid reactive substances, *GSH* glutathione
*Significant difference ($p < 0.05$)

(1987) reported that the effect of heat stress on spermatogenesis can be observed in the decreased semen quality in autumn, since after heat exposure (in summer), the effects disappear by the end of the third spermatogenetic cycle (Tharwat, Khadr, Amin, Miukawy, & Kotby, 1994), or more than 70 days are required for recovery. Thus, best ejaculate from bucks could be obtained at thermal comfort, as post treatment effect of heat stress also declines semen quality.

This study is in agreement with the findings of Abd El-Azim and El-kamash (2015) that breed of rabbits bucks differs in reaction time, semen pH, semen density, semen color, mass motility and advanced motility.

Chinchilla rabbits had highest semen volume compared to other breeds, but its lower sperm concentration indicates that its semen output constitutes more of seminal fluid than spermatozoa. This is also evident in lower total motile spermatozoa and live spermatozoa compared to other breeds. British Spot and New Zealand White breeds despite recording a lower semen volume had apparently higher mass motility and individual sperm motility and significantly higher sperm concentration. The superiority of New Zealand White is also reported by Abd El-Azim and El-kamash (2015) that semen ejaculates of New Zealand White is better than Balady and Sinai under the environmental conditions of Egypt.

This corroborates with the findings of El-Tohamy et al. (2012) in which New Zealand White bucks in winter despite having high semen volume (1.02 ml) had lower sperm concentration 2.82×10^{8}/ml and total motile sperm cells 1.99×10^{8}/ejaculate. However, the semen volume of 1.02 ml obtained by El-Tohamy et al. (2012) is higher than the range of values obtained in this study (0.34–0.70 ml), but higher sperm concentration and total motile spermatozoa were obtained in this study compared to values obtained in New Zealand White bucks in winter of Egypt. However, the range of semen volume (0.34–0.70 ml) obtained in this study was similar to the result obtained by Safaa et al. (2008) in Baladi and New Zealand White rabbits 0.80 and 0.55 ml under winter conditions of Egypt. The New Zealand White that had better semen quality than other breeds in this study corroborates evidence that the reproductive and productive performances of New Zealand White rabbits reared in Egypt were superior to that of other imported breeds with regard to semen quality and reproductive performance (Daader, Gabr, Khadr, & Seleem, 2002; Safaa et al., 2008). This could be an inherent trait in this breed due to its low thermal susceptibility (Jimoh & Ewuola, 2016).

Daader and Seleem (2005) have reported that in rabbits, hypo-osmotic swelling test (HOS test; a measure of functional membrane integrity) is more reliable in assessing the outcome of in vitro fertilization than other semen parameters. This is perhaps the most reliable estimate of structural and functional integrity of the sperm acrosomal and tail membranes. Though, statistically similar values of structural and functional membrane integrity were obtained. British Spot had apparently highest functional membrane integrity (72.78%) among the breeds, and New Zealand White had apparently highest structural membrane integrity (99.28%). Season has a significant effect on sperm abnormalities of New Zealand White bucks (Bodnär, Szendrö, Nemeth, Eiben, & Radnai, 2000) as demonstrated in Pannon White and New Zealand White bucks, which showed higher ratio of intact spermatozoa in spring and winter (Bodnär et al., 2000), similar to Russian breed (Kadlecik, 1983). Bodnär et al. (2000) reported that Pannon White and New Zealand White males produce less abnormal spermatozoa throughout the year than Angora bucks.

If the suggestion on changes in sperm cells subjected to HOS test indicate a functionally intact plasma membrane is accepted, and that this is necessary for normal spermatozoa function such as motility and fertilization, then, within a semen sample, the more cells with swollen plasma membranes, the better the potential sperm quality (Neild et al., 2000). Daader and Seleem (2005) added that the abrupt drop in osmotic pressure may result in a malfunction in the physiological processes of spermatozoa. It was reported based on HOS test that the reproductive efficiency of Baladi bucks (44.83%) seems to be greater than that of the New Zealand White rabbit bucks (32.17%) under winter Egyptian condition

(Safaa et al., 2008). However, range of values (69.65–72.78%) obtained in this study is higher than that reported by Safaa et al. (2008) and El-Tohamy et al. (2012) in New Zealand White bucks in winter of Egypt. The evaluation of rabbit spermatozoa functional membrane integrity is a good indicator of the reproductive ability of rabbit bucks (El-Tohamy et al., 2012). Also, the incidence of sperm abnormalities was higher in summer compared with other seasons in ejaculates of Bouscat White and New Zealand White males (Amin, El-Fouly, El-Shobhy, & El-Sherbiny, 1987).

Kasa and Thwaites (1992) observed significant increases in the ratio of dead and piriform sperm after an increase in the level of heat stress.

Seminal plasma is usually an isotonic neutral medium, and it is a detrimental factor to sperm cell survival (White, 1976). Genetic differences did not affect seminal glucose, cholesterol, magnesium, potassium and chloride in the current study. However, genetic differences were observed in the breeds' seminal protein, sodium and phosphorus. This suggests breed differences in semen biochemical inclusions. The range of seminal protein values 1.22–3.99 g/dL observed in this study is lower than the range 2.88–4.50 g/dL reported by Attia, Abd El Hamid, Ismaiel, and El- Nagar (2013) in rabbit bucks. Cholesterol efflux from the plasma membrane is required for the cyclic adenosine monophosphate-dependent tyrosine phosphorylations that are correlated with sperm capacitation (Visconti et al., 1999). Changes in cholesterol content, phospholipid dynamics and protein mobility are intimately linked to capacitation (Flesch et al., 2001). Several studies have demonstrated that cholesterol inhibited the induction of the acrosome reaction (Davis, 1982) and also inhibits fertilization (inhibits or delays capacitation) in many species including the rabbit (Davis, 1982). The influx of cholesterol also reverses capacitation of sperm; indeed, vesicles from rabbit seminal plasma containing cholesterol and liposomes containing cholesterol render capacitated sperm incapable of fertilizing eggs in vivo (Gerena, Irikura, Urade, & Eguchi, 1998).

However, it was indicated that one of the most important factors contributing to poor quality semen has been attributed to oxidative stress (Bucak, Sariözkan, & Tuncer, 2010). The increase in the production of reactive oxygen species ROS determines semen characteristics and sperm-oocyte fusion (Akiyama, 1999). Also, the antioxidant mechanism is necessary to prevent free radical damage to the sperm cell as a result of generation of hydrogen peroxide and the superoxide ion by rabbit spermatozoa (Holland, Alvarez, & Storey, 1982). The literature on endogenous antioxidant contributions to oxidative stress protection of spermatozoa in the rabbit is scanty. The objective of most experiments was to evaluate the effects of prooxidants or antioxidants on the concentrations of TBARS or on semen quality (Nichi et al., 2006). Genetic differences in breeds were evident in most oxidative stress markers except total antioxidant activity of seminal fluid. New Zealand White had higher antioxidant enzyme activity at this period. However, the buck breeds had the different activity of various antioxidants in response to the antioxidant demand of the system for ROS scavenging, which is evident in low seminal lipid peroxidation. The antioxidant enzyme activity of the rabbits assessed was better than values reported by Jimoh, Ewuola, and Balogun (2017) at the peak of heat stress. This infers that the animals have better antioxidant status at thermal comfort compared to when exposed to heat stress. This claim is buttressed by the low lipid peroxidation recorded in this study compared to Jimoh et al. (2017) values recorded for seminal lipid peroxidation in heat stressed rabbits. It is thus inferred that rabbit oxidative stability which could influence immune status and productivity is better when rabbits are at thermal comfort giving the fact that oxidative stress due to hyperthermia apparently reduces the activity of antioxidant enzymes which could result in impairment of sperm membrane integrity.

Conclusion

New Zealand White had the best oxidative status across the four breeds, and this enhanced its semen quality parameters. However, British spot bucks had comparable high semen quality as New Zealand White bucks, and seminal biochemical parameters in the breeds were within a similar range.

Abbreviations

GPx: Glutathione peroxidase activity; LTHI: Least temperature-humidity index; ROS: Reactive oxygen species; SEM: Standard error of mean; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substance; THI: Temperature-humidity index

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Authors' contributions

JOA designed the study, carried out the field work and statistical analysis and wrote the first manuscript. EOE approved the study layout, supervised the research and read and corrected the first manuscript. Both authors read and approve the final manuscript.

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Ethics approval and consent to participate
The study was approved by the institutional committee on the care and use of animals for experiment and in accordance with the NIH guide for the care and use of laboratory animals.

Consent for publication
Not applicable

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

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