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Serum and Testicular Antioxidant Potentials of White Male Japanese Quails at Three Different Physiological Age Groups

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author VEU managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Label Problem: Bird spermatozoa are characterised by high proportions of fatty acids which make them very vulnerable to oxidative stress due to over production of free radicals.
Aim: This study aimed at assessing the antioxidant potentials of Japanese quails at three different physiological age groups and to evaluate the correlation between antioxidant capacity in the blood and testes of white male quails at the three age groups.
Study Design: The design of the study was completely randomised design.
Place of Study: The research was carried out at the Poultry Section (Quailery Unit), Teaching and Research Farm of the University of Ibadan, Ibadan, Oyo State, Nigeria.
Methodology: A study was carried out on the assessment of serum and testicular antioxidant potentials of white strains of male Japanese quails at three different physiological age groups: Pubertal (7-10 weeks), matured (14-20 weeks), and adult (≥24 week). Fifty-four white male quails

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were used and blood was sampled for antioxidant analysis. The animals were sacrificed, testes excised and homogenized for antioxidant determination. Total antioxidant capacity, catalase, glutathione peroxidase and superoxide dismutase were evaluated in the serum and testicular homogenate.

Results: Only glutathione peroxidase in the blood was significantly ($p<0.05$) higher in matured and adult quails than the pubertal quails. The testicular catalase and glutathione peroxidase activity were not significantly different across the age groups. There was a positive significant correlation between serum and testicular catalase ($r = 0.78$). Serum glutathione peroxidase was positively correlated with testicular total antioxidant capacity ($r = 0.20$).

Conclusion: The blood glutathione peroxidase activity was optimal in the matured age group than other age groups. Antioxidant activity in the blood and testes were positively correlated.

Keywords: Antioxidant status; glutathione peroxidase; superoxide dismutase; quail blood and testes.

1. INTRODUCTION

Specific antioxidants are reported to be produced as protective mechanisms against the free radicals which allowed survival in an atmosphere with increasing oxygen concentration [1]. Several compounds exist in nature that possess antioxidant properties, for examples fat-soluble (vitamin E and carotenoids, etc.) and water-soluble (ascorbic acid, glutathione, bilirubin, etc.) antioxidants, which can be synthesised in the body (ascorbic acid, glutathione) or are delivered with food/feed (vitamin E, carotenoids, Se etc.) [1].

Increased formation of reactive oxygen species (ROS) and or decreased antioxidant defence can be defined as oxidative stress. Body tissues including blood and testes remain vulnerable to oxidative stress due to the abundance of highly unsaturated fatty acids and the presence of potential reactive oxygen species (ROS)-generating systems. ROS generation can be from the mitochondria and a variety of enzymes including the xanthine- and NADPH- oxidases [2], and the cytochrome P450s [3]. These enzymes specialize in the professional generation of ROS as an inadvertent consequence of their biochemical activity. However, it has been reported that cells and biological fluids have an array of protective antioxidant mechanism comprising both enzymatic and non-enzymatic constituents for preventing the production of free radicals and for repairing oxidative damage [4]. Spermatozoa from all animal species are characterised by extremely high proportions of those fatty acids and as a result they become very vulnerable to oxidative stress due to over production of free radicals [5]. Concerning the enzymatic constituents of this defence system, it was observed that the induction of oxidative stress in the testes precipitates a response which is mediated by induction of mRNA species for superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities [6]. However, before any supplement can be encouraged to boost antioxidant capacity in attempt to improve reproductive efficiency of the birds, there is need to understand the antioxidant status in quails [7]. Possibly to also establish the relationship between the antioxidant enzymes in the blood and testes, peradventure if having knowledge of one can be used to predict the other.

There is no reported baseline information on the antioxidant properties in the serum and testes of white male quails strain and also information on the relationship between antioxidant properties in the testes and the blood in the white male quails has not been adequately documented. This study aimed at assessing the antioxidant potentials of Japanese quails at three different physiological age groups and to evaluate the correlation between antioxidant capacity in the blood and testes of white male quails at the three age groups.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was carried out at the Teaching and Research Farm (Quailery Unit) of the University of Ibadan, Ibadan, Oyo State, Nigeria. All the procedures used were according to Principles of laboratory animal care and animal ethics committee of the institution consented to the investigation.

2.2 Experimental Animal and Management

A total number of 54 white male quails were used for this research and all birds were kept in wire

cages under similar and standard hygienic, environmental and management condition. All the quails were given fresh water and corn-soybean meal based feed (23% crude protein and 2900 kcal/kg) *ad libitum* daily from the day old.

There were 3 age groups with 18 birds per group, each age group was replicated thrice with 6 birds per replicate in a completely randomised design and the experimental layout is shown below;

Age group 1: Pubertal quail (7-10 weeks)
Age group 2: Mature quail (14-20 weeks)
Age group 3: Adult quail (24 weeks and above)

2.3 Sample Preparation

Blood was collected from the experimental animals (white male Japanese quails), via the jugular vein; and was centrifuged at 4000 rpm for 15 minutes using an IEC centra-4B centrifuge (International Equipment Co., Needham Heights, MA, USA). The serum was then collected after the separation for antioxidant analysis.

The birds were sacrificed and dissected. Testes were excised, weighed and homogenized in 0.154 M NaCl (physiological saline). The mixture was then sieved using gauze wrapped in a funnel and pressed little to get the homogenate (filtrate) in a sample bottle for the antioxidant assay. The homogenate was centrifuged at 3000 rpm for 15 minutes using a Bosch 90-2 centrifuge (Jiangsu Jinyi Instrument Technology Company, Jiangsu, China). The supernatant was then collected after the separation for antioxidant analysis.

2.4 Antioxidant Assay in the Blood Serum and Testicular Homogenate

2.4.1 Catalase (CAT)

Catalase activity was estimated by Beers and Sizer [8] method. The assay system contained 1.9 ml of 0.05 M buffer (pH 7.0) and 1.0 ml of 0.059 MH_2O_2. The reaction was initiated by addition of 0.1 ml test sample. The decrease in absorbance was monitored at 1min interval for 5 min at 240 nm and activity was expressed as moles of H_2O_2 decomposed/min/mg protein. The CAT was estimated using the formula below.

$Y = 6.5103x – 0.2653$
$Y = H_2O_2$ concentration
x = absorbance

Catalase activity = Y-- H_2O_2 Remaining = H_2O_2 decomposed/min/mg protein.

2.4.2 Glutathione peroxidase (GPx)

Glutathione peroxidase activity was estimated as described by Rotruck et al. [9] and Ellman [10]. 0.2 ml test sample, 0.2 ml of 2 mM Glutathione (GSH), and 0.1 ml of 0.2 mM H_2O_2 were added to 0.5ml of 0.4 M buffer (pH 7.0) and incubated at room temperature for 10 min along with a control tube that contained all reagents except enzyme source. The reaction was arrested by adding 0.5ml of 10% Trichloroacetic acid (TCA), and then centrifuged at 4000 rpm for 5 min. The supernatant was harvested and the GPx content in 0.5 ml of the supernatant was estimated at 412 nm. The activity was expressed as µg of GSH consumed/min/mg protein. The GPx in the sample was estimated using the formula below.

GSH consumed: $Y= 143.05x + 5.7469$

Where

Y = GSH concentration
X = absorbance

Therefore, GSH remaining =

$$\frac{245.34 - Y}{\text{Concentration of protein sample}}$$

GPx = GSH concentration – GSH Remaining

2.4.3 Superoxide dismutase (SOD)

Superoxide dismutase activity was estimated by the method of Marklund and Marklund [11] adopted by Soon and Tan [12]: 0.02 ml of test sample and 0.86 ml of distilled water were added to 2.1 ml of 50 mM buffer. The reaction was initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance was measured at 420 nm. One unit of SOD was defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in standard assay system of 3 ml. the specific activity was expressed as units/min/mg protein. The SOD was estimated as follow.

$$\text{SOD Assay (\% inhibition)} = \frac{A_3 - A_0}{3 \text{ minutes}}$$

Where

A_3 = absorbance at 3 minutes
A_0 = absorbance at 0 minutes

Therefore,

$$\% \text{ Inhibition} = \frac{\text{Change in absorbance of sample}}{\text{Absorbance of Blank}} \times 100$$

2.4.4 Total antioxidant capacity (TAC)

The TAC was determined by measuring the antioxidative activity in the serum sample as described by Koracevic et al. [13]. 0.01 ml of the test sample was taken into a clean dry test tube and 0.01 uric acid was also taken into another standard test tube. 0.49 ml phosphate buffer was added to each test tube containing the test sample and the standard (as a negative control containing total of 0.50 ml of the buffer with the uric acid). The positive control contained only 0.49 ml of phosphate buffer in a separate test tube. 0.50 ml of sodium benzonate was added into all the test tubes and read using spectrophotometer at 532 nm.

Antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (mmol/L)} = (C_{UA})\ (K\text{-}A)\ (K - UA)$$

Where;

K = absorbance of control ($K_1 - K_0$)
A = absorbance of sample ($A_1 - A_0$)
UA = absorbance of uric acid solution ($UA_1 - UA_0$)
C_{UA} = concentration of uric acid (in mmol/liter)

2.5 Data Analysis

The data obtained were analysed using correlation analysis and one-way analysis of variance of SAS [14] package; a value of $p<0.05$ was considered statistically significant. The means were separated using Duncan multiple range test (DMRT) of the same software.

3. RESULTS

3.1 Antioxidant Activities in the Serum of the White Male Quails at Different Age Groups

The serum antioxidant activities (Catalase, glutathione peroxidase, superoxide dismutase and total antioxidant capacity) in the white male quails across the age groups are shown in Table 1. The catalase activity was not significantly different among the age groups. The glutathione peroxidase level of T3 (Adult age group) was not significantly different from that of T2 (Matured age group). However, the glutathione peroxidase level of T1 (Pubertal age group) was significantly ($P < 0.05$) lower than that of T2 and T3.

There was no significant difference in the total antioxidant capacity (TAC) across the age groups. However, treatment 2 (matured age group) recorded apparently highest (6.25±6.42 mmol/litre Protein) activity while Treatment 1 (the pubertal age group) had the least value (4.12±1.78 mmol/litre Protein) recorded among the age groups. The Serum Superoxide Dismutase (SOD, units/min/mg) showed no significant differences across the age groups.

3.2 Antioxidant Indices in the Testes of the White male Quails at Different Age Groups

The catalase, glutathione peroxidase, superoxide dismutase and total antioxidant capacity activity in the testes of white Japanese quails are shown in Table 2. The testicular catalase revealed that there was no significant difference in the testicular catalase activity across the age groups. The Testicular Glutathione peroxidase activity was also not significantly different among the three age groups of the animals. The activity was highest (3.24±2.22 µg/min/mg) in the matured age group of the white male quail while the least (1.97±0.67 µg/min/mg) activity was observed in the adult age group (T3) of the white male quails. There was no significant difference in the testicular total antioxidant capacity and superoxide dismutase across the age groups.

3.3 Correlation between Antioxidants in the Blood and Testis of White Male Japanese Quails

The Pearson correlation coefficient between the antioxidant activity in the serum and in the testes of pubertal age group is represented in Table 3. A positive correlation existed between serum and testicular catalase ($r = 0.78$) which was significant ($P<0.05$). The glutathione peroxidase in serum was significantly ($p<0.05$) different and positively correlated with serum superoxide dismutase. Serum superoxide dismutase ($r = 0.14$) and testicular catalase ($r = 0.08$) were not significantly correlated with serum total antioxidant activity, however, testicular total antioxidant capacity ($r = -0.70$) was significantly correlated with serum total antioxidant capacity.

The Pearson correlation in the matured white quails between serum antioxidants and testicular antioxidant is shown in Table 4. No significant correlation existed between the serum total antioxidant capacity (r= -0.08) and testicular superoxide dismutase (r= -0.03) and serum catalase. Also no significant correlation existed between serum glutathione peroxidase (r =0.44), testicular glutathione peroxidase (r =0.43) and serum catalase. However, Positive and very significant correlation was observed between serum and testicular glutathione peroxidase (r=0.94). There was a significant difference (P<0.05) and positive correlation observed between serum and testicular superoxide dismutase.

The correlation between antioxidant in the serum and testes in adult white male quails is shown in Table 5. Among all the parameters, only testicular catalase positively and significantly correlated with serum total antioxidant capacity, while only testicular super oxide dismutase negatively and significantly with testicular glutathione peroxidase. Other parameters that were not significantly correlated were serum superoxide dismutase and catalase, serum superoxide dismutase and glutathione peroxidase, Glutathione Peroxidase and serum total antioxidant capacity, testicular glutathione peroxidase and catalase, and testicular superoxide dismutase and total antioxidant capacity.

4. DISCUSSION

In this study, antioxidant assessment in the blood serum for catalase showed that both catalase level in serum and testes were not significant, this reflected that the catalase activity in white male quails is not age dependent. This result agreed with the findings of Sakr et al. [15] who assayed biochemical's and antioxidant activity in the liver and spleen of albino rats. In the Glutathione peroxidase (GPx) assay, the level of GPx in the serum which was highest in the adult and matured age groups of the white male Japanese quails is in line with the report of Kheradmand et al. [16] who also observed increased level of total antioxidant activity and glutathione peroxidase in the rat's testes which can be attributed to age related reasons.

The serum total antioxidant capacity showed that the antioxidant activity was not significantly different from the testes among the three age groups which were at variance to the result obtained by Kheradmand et al. [16]. However, high antioxidant activity in the different age groups given varying results can be attributed to different dietary intake due to age differences [1,17]. Testicular superoxide dismutase showed the same trend like the total antioxidant capacity which was similar between serum and testes among all the age groups [18]. This result was at variance to the findings of Kaur et al. [19], who reported increased level of superoxide dismutase in adult rats. The significant increase in the glutathione peroxidase in the blood of mature and adult quails than the pubertal age group probably implies that the activity of the antioxidant is high and effective in the two age groups [7,20] probably because of their increasing demand for sexual activities.

Table 1. Antioxidant activities in the serum of the white male quail at different age groups

Parameters	Age group 1 (Pubertal)	Age group 2 (Matured)	Age group 3 (Adult)
Catalase (nmol/min/mg)	0.0020±0.0019	0.0037±0.0055	0.0024± 0.0011
Glutathione peroxidase (µg/min/mg)	2.33±0.74[b]	4.26±2.58[a]	4.10±1.41[a]
Total antioxidant capacity (mmol/litre)	2.70±2.99	6.25±6.42	4.12±1.78
Superoxide dismutase (units/min/mg)	110.08±113.94	87.28±49.57	157.21± 251.40

ab: Means along the same row with different superscripts are significantly (P<0.05) different

Table 2. Antioxidant activity indices in the testes of the white male quail at different age groups

Parameters	Age Group 1 (Pubertal)	Age Group 2 (Matured)	Age Group 3 (Adult)
Testicular catalase (nmol/min/mg)	0.001±0.002	0.001±0.001	0.001±0.0004
Testicular glutathione peroxidase (µg/min/mg)	2.38±1.25	3.24±2.22	1.97±0.67
Testicular total antioxidant capacity (mmol/litre)	0.65±0.157	0.66±0.17	0.75±0.21
Testicular superoxide dismutase (units/min/mg)	187.00±86.76	221.96±81.69	222.22±112.83

Table 3. Correlation coefficient values obtained between the serum antioxidant and testicular antioxidant in the pubertal age group of white Japanese quails

Parameter	Serum catalase	Serum glutathione peroxidase	Serum total antioxidant capacity	Serum superoxide dismutase	Testicular catalase	Testicular glutathione peroxidase	Testicular total antioxidant capacity	Testicular superoxide dismutase
Serum catalase	1.00	-0.17^{ns}	-0.10^{ns}	-0.01^{ns}	0.78*	-0.18^{ns}	0.06^{ns}	-0.15*
Serum glutathione peroxidase		1.00	0.10^{ns}	0.69*	-0.56^{ns}	-0.01^{ns}	0.20^{ns}	0.46^{ns}
Serum total antioxidant capacity			1.00	0.14^{ns}	0.08^{ns}	-0.22^{ns}	-0.70*	-0.09^{ns}
Serum superoxide dismutase				1.00	-0.26^{ns}	-0.43^{ns}	0.002^{ns}	0.81*
Testicular catalase					1.00	-0.08^{ns}	0.01^{ns}	-0.33^{ns}
Testicular glutathione peroxidase						1.00	0.29^{ns}	-0.62^{ns}
Testicular total antioxidant							1.00	0.07^{ns}
Testicular superoxide dismutase								1.00

*ns= Non significant, * = Significant (p<0.05), ** = Very significant (p<0.01)*

Table 4. Correlation coefficient values obtained between the serum antioxidant and testicular antioxidant in the matured age group of white Japanese quails

Parameter	Serum catalase	Serum glutathione peroxidase	Serum total antioxidant capacity	Serum superoxide dismutase	Testicular catalase	Testicular Glutathione peroxidase	Testicular total antioxidant capacity	Testicular superoxide dismutase
Serum catalase	1.00	0.44^{ns}	-0.08^{ns}	-0.48^{ns}	0.08^{ns}	0.43^{ns}	0.14^{ns}	-0.03^{ns}
Serum glutathione peroxidase		1.00	-0.03^{ns}	-0.28^{ns}	-0.05^{ns}	0.94**	0.49^{ns}	-0.17^{ns}
Serum total antioxidant capacity			1.00	-0.34^{ns}	0.39^{ns}	-0.02^{ns}	0.28^{ns}	-0.42^{ns}
Serum superoxide dismutase				1.00	0.20^{ns}	-0.45^{ns}	0.10^{ns}	0.66*
Testicular catalase					1.00	-0.15^{ns}	0.26^{ns}	0.40^{ns}
Testicular glutathione peroxidase						1.00	0.22^{ns}	-0.44^{ns}
Testicular total antioxidant							1.00	0.38^{ns}
Testicular superoxide dismutase								1.00

*ns= Non significant, *= Significant (p<0.05), **= Very significant (p<0.01)*

Table 5. Correlation coefficient values obtained between the serum antioxidant and testicular antioxidant in the adult age group of white Japanese quails

Parameter	Serum catalase	Serum glutathione peroxidase	Serum total antioxidant capacity	Serum superoxide dismutase	Testicular catalase	Testicular glutathione peroxidase	Testicular total antioxidant capacity	Testicular superoxide dismutase
Serum catalase	1.00	0.08 ^{ns}	0.14 ^{ns}	-0.33 ^{ns}	-0.003 ^{ns}	0.11 ^{ns}	0.41 ^{ns}	0.23 ^{ns}
Serum glutathione peroxidase		1.00	0.22 ^{ns}	-0.47 ^{ns}	0.27 ^{ns}	0.32 ^{ns}	0.01 ^{ns}	0.15 ^{ns}
Serum total antioxidant capacity			1.00	0.35 ^{ns}	0.79 *	-0.05 ^{ns}	0.59 ^{ns}	0.07 ^{ns}
Serum superoxide dismutase				1.00	0.06 ^{ns}	0.11 ^{ns}	0.18 ^{ns}	-0.23 ^{ns}
Testicular catalase					1.00	-0.29 ^{ns}	0.64 ^{ns}	0.27 ^{ns}
Testicular glutathione peroxidase						1.00	0.11 ^{ns}	-0.69 *
Testicular total antioxidant							1.00	-0.07 ^{ns}
Testicular superoxide dismutase								1.00

*ns= Non significant, *= Significant (p<0.05), **= Very significant (p<0.01)*

The present study also revealed the correlation between antioxidant activities in the serum and in the testes. The result showed that in the pubertal age group; there were negative correlation between the serum glutathione peroxidase, serum total antioxidant capacity, serum superoxide dismutase, testicular glutathione peroxidase, testicular superoxide dismutase and serum catalase. Also, similar negative correlation was observed in the matured age group where serum catalase was negatively correlated with serum total antioxidant capacity, serum superoxide dismutase and testicular superoxide dismutase. It was observed that there was an existing negative correlation between serum total antioxidant capacity, serum superoxide dismutase, testicular catalase, testicular superoxide dismutase and serum glutathione peroxidase in the matured age group but there was a significant difference that existed between testicular glutathione peroxidase and serum glutathione peroxidase, also between testicular superoxide dismutase and serum superoxide dismutase in the adult age group. These findings were in line with the result of Jang et al. [18] who reported similar result in the small intestine and liver antioxidant enzyme activity in rats.

The Pearson correlation in the adult age group for the serum and testicular antioxidant activity showed a different trend unlike in the pubertal and matured age group where a positive correlation existed between serum glutathione peroxidase, serum total antioxidant capacity, testicular glutathione peroxidase, testicular superoxide dismutase and serum catalase in agreement with Kheradmand et al. [16] that reported similar trend in rat testis after the administration of ghrelin. However, there was a positive and significant correlation between testicular catalase and serum total antioxidant capacity. Also, negative significant correlation showed between testicular superoxide dismutase and testicular glutathione peroxidase, agreed with the reports of Kaur et al. [19] when supplements of sub-chronic selenium was given to calves in a study which also corroborate the findings of Hongqin et al. [21] on effect of lycopene supplementation on plasma lipid profile, lipid peroxidation and antioxidant defense system in Feedlot Bamei Lamb. Also, Ranawat and Bansal [20] reported a decrease in glutathione peroxidase when selenium was used in an in vitro study [22]. The clinical applications of these findings involve predicting the antioxidant status of the testes using the antioxidant level in the blood in vivo. It also established that the endogenous antioxidant level is not physiological age dependent except Gluthathione peroxidase in the blood [23,24] and that despite sexual activeness of the mature white quails, they are still less susceptible to oxidative stress because of rich antioxidant enzyme status than both pubertal and adult age groups.

5. CONCLUSION

Relationship between total antioxidant activity in the blood and catalase in the testes of adult quails was positive and significant, while Glutathione peroxidase activity in serum of mature quails can be used to predict status of same antioxidant in the testes. The highest glutathione antioxidant activity in the blood was observed in the matured and adult age groups which indicated that such good antioxidant status in mature quails has tendency to scavenge free radicals and reactive oxygen species thereby improve their semen quality and reproductive potential than other age groups. It was also established that the endogenous antioxidant level is not physiological age dependent in white quails except Gluthathione peroxidase in the blood.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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