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Antioxidant Activity in the Blood and Testes of the Mottled Brown Male Japanese Quails at Different Age Groups

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Abstract

This study was carried out to assess the antioxidant activity in the blood and testes of mottled brown male Japanese quails at different physiological age groups. Fifty four mottled-brown male quail birds with average weight of 128.33±28.21g were randomly allotted to three age groups: pubertal (7 to 10 weeks old), mature (15 to 20 weeks old) and adult (above 24 weeks old). Eighteen birds were allocated per age group; each treatment had 3 replicates with 6 birds each. Blood sample was collected from all the birds and the birds were sacrificed, dissected, testes exiced and processed into testicular homogenate. Both serum and homogenate fluids were centrifuged separately to harvest the supernatants and analysed for the antioxidant indices. Statistical comparisons were made between the serum and testicular antioxidant indices. Result showed that the activities of catalase, superoxide dismutase, glutathione peroxidase and total antioxidant capacity in the serum and testis were not significantly (p□0.05) different among the age groups except for the glutathione peroxidase activity which was significantly (p<0.05) higher in the mature male quail compared to the adult and pubertal age groups. The result from this study suggests that the age difference in mature mottled brown male quails had improved glutathione peroxidase than other age groups, indicating an enhanced reproductive efficiency than pubertal and adult age groups in Japanese quails.

Key words: Reproductive potentials, Mottled brown male quail, Age groups, Antioxidant indices.

Introduction

Japanese quails are hardy birds that thrive in small cages and are inexpensive to keep. They are affected by common poultry diseases but are fairly disease resistant. Japanese quails attain puberty in about 6 weeks (Maurice and Gerry, 2008, Huss *et al.,* 2008) and are usually in full egg production by 50 days of age (Maurice and Gerry, 2008). With proper care, hens should lay 200 eggs in their first year of lay. Life expectancy is only 2 to 2½ years. If the birds have not been subjected to genetic selection for body weight, the adult male quail will weigh about 100–140g, while the females are slightly heavier, weighing from 120–160g (Maurice and Gerry, 2008). In breeding quails, it has been demonstrated that the number of fertile eggs drops sharply when males are removed from the group (Sittman and Abplanalp, 1965) which confirms that spermatozoa can survive in the female genital tract for more than 14 days, they are able to fertilize only 45% of the eggs eight days after the removal of the males (Reddish *et al*., 1996).

Changes in physiological parameters could become very important markers in identifying growth patterns thereby can be very useful tools for predicting both physiological and pathological consequences (Tilgar *et al*., 2008). Models for growth rate evaluations developed in two strains of

chicken (Gavin *et al*., 1998) and Gull-billed Tern chicks (Albano *et al*., 2011), were very useful tools for comparative growth studies. Anatomy and physiology are intertwined and various physiological characteristics, undoubtedly, reflect on the capability and performance of anatomical structures in health and in disease such as the function of plasma proteins, the building blocks of body tissues, in production of hormones and antibodies, carriers of numerous blood constituents, maintenance of osmotic pressure, controlling acid-base balance of the blood and production of series of enzymes associated with performance and maintenance of different body activities (Harper *et al*., 1993; Druyan *et al*., 2007; Kiani *et al*., 2011).

Despite the low oxygen tensions that characterize the testicular micro-environment, this tissue remains vulnerable to oxidative stress due to the abundance of highly unsaturated fatty acids (particularly 20:4 and 22:6) 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, (Bangi *et al.*, 2001, Kumagai *et al.*, 2002) and the cytochrome P450s (Zangar *et al.*, 2004). These enzymes specialize in the professional generation of ROS or produce these toxic metabolites as an inadvertent consequence of their biochemical activity. In order to address this risk, the testes have developed a sophisticated array of antioxidant systems comprising both enzymatic and non-enzymatic constituents. Concerning the enzymatic constituents of this defence system, the induction of oxidative stress in the testes precipitates a response which is mediated by induction of mRNA species for superoxide dismutase (SOD), glutathione peroxi-dase (GPx) and glutathione-S-transferase (GST) activities (Kaur *et al.*, 2006).

The total antioxidant capacity is not a simple sum of the activities of the various antioxidative substances as it may appear. But rather, it is a dynamic equilibrium that is influenced by the interactions between the serum antioxidative constituents. It is however thought that the cooperation of antioxidants in animal serum provides greater protection against attacks by free radicals than any antioxidant alone (Wayner *et al*., 1987). Relevant antioxidants are classified into two groups: the preventive and chain breaking antioxidants (Wayner *et al*., 1987, Woodford *et al*., 1998). Preventive antioxidants prevent the initiation of radical chain reactions by reducing hydroperoxides to molecular species without the formation of free radicals or by sequestering transition metals (iron, copper) thereby preventing them from participating in the reactive radical production. Examples of preventive antioxidants of biological systems are Catalase, glutathione peroxidase and metal binding proteins (albumin, transferrin). Chain-breaking antioxidant can trap free radicals directly thereby interrupting chain propagating reactions. Examples of chain breaking antioxidants are superoxide dismutase (SOD), uric acid, ascorbate, □-tocopherol, reduced glutathione (GSH). For example superoxide dismutase exerts antioxidant activity (as a chain breaking agent) by scavenging oxygen radicals once they are produced (Anmes *et al*., 1981) and by forming a stable complex with iron ions (Davies *et al*., 1986) as a preventive antioxidant. With the above in mind, the antioxidant status of male Japanese quails at different physiological age groups was investigated as a measure of reproductive stability and efficiency of the bird.

Materials and methods

Experimental site and Animals

The experiment was carried out at the Quailery unit of Teaching and Research Farm, University of Ibadan, Ibadan, Oyo State. A total of 54 mottled-brown male quail birds were used. The quail birds were housed in separate pens according to the different age groups in three replicate per group. Feed and clean cool water was supplied *ad libitum* twice a day, in the morning and late afternoon. The birds were randomly, in a completely

randomized design, assigned to three different age groups: pubertal (7 to 10 weeks old), mature (15 to 20 weeks old) and adult (above 24 weeks old) with average weight of 132±23.50g, 136±38.46g and 117±23.18g respectively. The experimental diet is shown in Table 1 and was fed to the birds from day old.

Blood Sample Collection

Blood was sampled (3ml) from the jugular vein of the mottled-brown male Japanese quail and dispensed into a sterile sample bottle. It was allowed to clot and centrifuged at 3000rpm for 15minutes using an IEC centra-4B centrifuge, after which the serum was separated for catalase, superoxide dismutase, glutathione peroxidase and total antioxidant capacity determination. For the testicular homogenate, mottled-brown quail birds were sacrificed, dissected and testes excised using a pair of scissors and forceps. The testes were further macerated in a 1ml of 0.154M NaCl (Physiological Saline). The homogenate was centrifuged (using a centrifuge model of BOSCH 90-2) at 4000rpm for 15 minutes and the supernatant was decanted into a sterile sample bottle for antioxidant determination.

Antioxidant analysis in serum and testicular homogenates

Total Antioxidant Capacity was determined using a Fenton-type reaction method and appropriate formula as described by Gutteridge *et al*. (1990), Winterbourn (1979) and Yamazaki *et al*. (1990). A peroxidative activity method was used to determine the Catalase with appropriate formula as estimated by Beers and Sizer (1952) and as time duration was modified to 0 and 5minutes reading. Superoxide dismutase activity was determined using an inhibition method and appropriate formula as described by Marklund and Marklund (1974) and modified by Soon and Tan (2002) and the time duration was modified to 0 and 3minutes reading. Glutathione Peroxidase activity was estimated with appropriate formula as described by Rotruck *et al*. (1973).

Statistical Analysis

All data obtained from the experiment were subjected to correlation analysis and One-Way Analysis of Variance of Statistical Analysis System at α0.05. Treatment means were separated using Duncan Multiple range test of the same software.

Results

Serum Antioxidant Indices

The antioxidant activity in blood of mottled brown quails at different age groups is shown in Table 2. It was observed that catalase, superoxide dismutase, glutathione peroxidase and total antioxidant capacity activities were not significantly ($p>0.05$) different among the different age groups of mottled brown quails.

Testicular Antioxidant Indices

The antioxidant indices in the testes of the mottled brown male quails at the different age groups are shown in Table 3. The testicular catalase activity, Superoxide dismutase and Total antioxidant capacity were not significantly ($p>0.05$) different among the age groups. Superoxide dismutase activity was apparently reduced in the adult age group. The glutathione peroxidase activity in the mature brown male quail was not significantly ($p>0.05$) different from the pubertal age group but was significantly ($p<0.05$) higher (3.66±1.74 g/min/mg) than that of the adult age group which had the least (2.44±0.80 g/min/mg) activity.

Correlation coefficient between serum and testicular antioxidant parameters of mottled brown male quails at different age groups

The results on correlation coefficients between the serum and testicular antioxidant parameters (Catalase, Superoxide dismutase, Glutathione peroxidase and Total antioxidant capacity) in pubertal, mature and adult brown male quails are shown in Tables 4, 5 and 6.

Within the pubertal age group, a positive correlation existed between the activities of serum catalase and other antioxidant such as serum superoxide dismutase (r = 0.35), serum total antioxidant (r = 0.25), testicular catalase (r = 0.08), testicular superoxide dismutase (r = 0.12) and testicular glutathione peroxidase (r = 0.04) but serum catalase negatively correlated with serum glutathione peroxidase (r=-0.50) and testicular total antioxidant (r=-0.32) the values were not significantly ($p>0.05$) different among the parameters. However, it was observed that testicular catalase positively and significantly ($p<0.05$) correlated with testicular superoxide dismutase (r = 0.69), testicular glutathione peroxidase (r = 0.78). A high level of significance of negative correlation between testicular superoxide dismutase and testicular glutathione peroxidase (r=-0.62) at the pubertal age was also observed.

In mature age brown male quail as shown in Table 5, a positive correlation existed between serum catalase and serum superoxide dismutase (r = 0.01), serum glutathione peroxidase (r = 0.43), serum total antioxidant (r = 0.41), testicular superoxide dismutase (r = 0.45) as well as between testicular superoxide dismutase (r = 0.45) and serum total antioxidant (r = 0.16), testicular superoxide dismutase (r = 0.24) and between testicular glutathione peroxidase and testicular total antioxidant (r = 0.54). A negative correlation was observed between testicular catalase and testicular superoxide dismutase (r=-0.13), testicular glutathione peroxidase (r-0.26) and testicular total antioxidant (r=-0.29) and as well as between serum total antioxidant and testicular catalase (r= -0.17), testicular glutathione peroxidase (r-0.52) and testicular total antioxidant (r= -0.68). However, no significant ($p>0.05$) difference was observed among the serum and testicular antioxidant indices in the mature age group.

There existed a high level of significance between serum catalase and testicular glutathione peroxidase (r = 0.67), serum superoxide dismutase and testicular glutathione peroxidase (r = 0.09) which were positively correlated in the adult quails (Table 6). Other positively correlated indices were between serum glutathione peroxidase and serum total antioxidant (r = 0.36), testicular catalase (r = 0.41), testicular glutathione peroxidase (r = -0.003) and testicular total antioxidant (r = 0.31) as well as between testicular superoxide dismutase and testicular glutathione peroxidase (r = 0.57), between testicular glutathione peroxidase and testicular total antioxidant (r = 0.30) but were not significantly different among the adult male quails. A non-significant ($p>0.05$) negative correlation existed between serum catalase and serum glutathione peroxidase (r = -0.32), testicular catalase and testicular superoxide dismutase (r= -0.54) and between testicular superoxide dismutase and testicular total antioxidant (r = -0.21) in the adult age group.

Discussion

The antioxidant activities in the blood of the brown male quail across the age groups were not significantly different. It was observed that the activities were not age dependent in the male quails. This was supported by this work that since both spermatogenesis (Peltola *et al.,* 1994) and Leydig cell steroidogenesis (Quinin and Payne, 1984 and Chen *et al.,* 2005) are vulnerable to oxidative stress, the low oxygen tension that characterised this tissue may be an important component of the mechanisms by which the testes protect itself from free radical-mediated damage. This work was at variance with the work of Zhai (2007), who established that, free radicals or reactive oxygen species (ROS) are harmful to cell membranes, in which, it promotes lipid peroxidation, causing membrane breakdown and loss of function. Cell membrane damage results from lipid peroxidation, production and accumulation in the plasma membrane which occurs when the intracellular production of ROS rises above the antioxidant defence mechanisms utilized by cells (Zhai *et al.,* 2007). It has been established that the spermatozoa that are prone

to the lipid peroxidation, and despite the low oxygen tensions that characterize the testicular micro-environment, this tissue remains vulnerable to oxidative stress due to the abundance of highly unsaturated fatty acids (particularly 20:4 and 22:6) 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, (Bangi *et al.,* 2001, Kumagai *et al.,* 2002) and the cytochrome P450s (Zangar *et al.,* 2004). Furthermore, the antioxidant indices from the result of this study were not significantly different except for testicular Glutathione peroxidase which might be due to high level of enzymatic constituents and can be related to the work reported by Kaur *et al.* (2006) about the enzymatic constituents of the spermatozoa defence system, which inhibits 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 (Kaur *et al.,* 2006).

Conclusion

All age groups of mottled brown male quails exhibited similar circulatory antioxidant activities which indicate potentials for enhanced reproductive efficiency in the animal. The antioxidant activity was not different as observed in testes and blood serum across the age groups, however, mature quails had improved glutathione peroxidase than other age groups, indicating an enhanced reproductive efficiency than pubertal and adult age groups in Japanese quails which suggest that the cells and biological fluids have an array of protective antioxidant mechanism, both for preventing the production of free radicals and repairing the oxidative damages.

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Table 1: Gross composition of the diet fed to the animals during the experimental period

Feed ingredient	**Percentage composition**
Maize	50.00
Soyabean meal	30.00
Fish Meal	4.00
Wheat offal	12.00
Palm kernel cake	2.00
Oyster shell	1.00
Methionine	0.25
Lysine	0.25
Premix	0.25
Salt	0.25
Total	100.00
Calculated Nutrients	
Metabolizable energy (kcal/kg)	2500
Crude protein (%)	23.00
Methionine (%)	0.58
Arginine (%)	1.93
Calcium (%)	1.00
Available Phosphorus (%)	0.52
Sodium (%)	0.16

Table 2: Serum antioxidant indices of mottled brown male quails at different age groups

Parameters	**Pubertal**	**Mature**	**Adult**
Catalase (nmolesH_2O_2/min/mgprotein)	0.002±0.02	0.002±0.002	0.005±0.01
Superoxide Dismutase (units/min/mg)	30.49±14.86	35.74±33.93	25.64±24.29
GlutathionePeroxidase (g/min/mgprotein)	3.71±2.32	2.38±0.48	3.54±4.20
TotalAntioxidant Capacity (mmole/litre)	1.22±0.59	1.13±0.75	1.52±0.80

*Means without superscripts are not significantly different (P>0.05).

Table 3: Testicular antioxidant indices in mottled brown male quails among the different age groups

Parameters	**Pubertal**	**Mature**	**Adult**
Catalase(nmolesH_2O_2/min/mgprotein)	0.0004±0.0005	0.0010±0.0027	0.0002±0.0002
Superoxide Dismutase (units/min/mg)	43.90±30.73	41.57±33.73	25.84±16.19
Glutathione Peroxidase (g/min/mgprotein)	2.73 ± 1.07^{ab}	3.66 ± 1.74^{a}	2.44 ± 0.80^{b}
Total Antioxidant Capacity (mmole/litre)	0.79±0.31	0.80±0.15	0.74±0.27

*ab: means along the same row with different superscript are significantly (P < 0.05) different

Table 4: Correlation between antioxidant parameters in the blood and testis of pubertal mottled brown male quails

Parameters	Serum CAT	Serum SOD	Serum GPX	Serum TAC	Testicular CAT	Testicular SOD	Testicular GPX	Testicular TAC
Serum Catalase	1.00	0.35^{ns}	-0.50^{ns}	0.25^{ns}	0.08^{ns}	0.21^{ns}	0.04^{ns}	-0.32^{ns}
Serum Superoxide Dismutase		1.00	0.31^{ns}	0.25^{ns}	0.21^{ns}	-0.23^{ns}	0.23^{ns}	-0.16^{ns}
Serum Glutathione Peroxidase			1.00	-0.05^{ns}	-0.46^{ns}	0.54^{ns}	-0.36^{ns}	0.49^{ns}
Serum Total Antioxidant Capacity				1.00	0.21^{ns}	0.20^{ns}	-0.09^{ns}	-0.32^{ns}
Testicular Catalase					1.00	0.69**	0.78**	-0.20^{ns}
Testicular Superoxide Dismutase						1.00	-0.62*	0.11^{ns}
Testicular Glutathione Peroxidase							1.00	0.24^{ns}
Testis Total Antioxidant Capacity								1.00

ns: non- significant, *: significant, **: highly significant, CAT: catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidise, TAC: Total antioxidant activities

Table 5: Correlation between antioxidant parameters in the blood and testis of matured mottled brown male quails

Parameters	Serum CAT	Serum SOD	Serum GPX	Serum TAC	Testicular CAT	Testicular SOD	Testicular GPX	Testicular TAC
Serum Catalase	1.00	0.01^{ns}	0.43^{ns}	0.41^{ns}	-0.22^{ns}	0.45^{ns}	-0.15^{ns}	-0.23^{ns}
Serum Superoxide Dismutase		1.00	0.71^{ns}	0.25^{ns}	0.02^{ns}	-0.13^{ns}	0.31^{ns}	-0.15^{ns}
Serum Glutathione Peroxidase			1.00	0.16^{ns}	-0.56^{ns}	0.24^{ns}	-0.15^{ns}	-0.02^{ns}
Serum Total Antioxidant Capacity				1.00	-0.17^{ns}	0.12^{ns}	-0.52^{ns}	-0.68^{ns}
Testicular Catalase					1.00	-0.13^{ns}	-0.26^{ns}	-0.29^{ns}
Testicular Superoxide Dismutase						1.00	-0.25^{ns}	0.01^{ns}
Testicular Glutathione Peroxidase							1.00	0.54^{ns}
Testicular Total Antioxidant Capacity								1.00

ns: non- significant, *: significant, **: highly significant, CAT: catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, TAC: Total antioxidant activities

Table 6: Correlation between antioxidant parameters in the blood and testis of adult mottled brown male quails

Parameters	Serum CAT	Serum SOD	Serum GPX	Serum TAC	Testicular CAT	Testicular SOD	Testicular GPX	Testicular TAC
Serum Catalase	1.00	0.23^{ns}	-0.32^{ns}	0.20^{ns}	0.35^{ns}	0.48^{ns}	0.67*	0.14^{ns}
Serum Superoxide Dismutase		1.00	0.23^{ns}	0.17^{ns}	-0.30^{ns}	-0.31^{ns}	0.09*	0.35^{ns}
Serum Glutathione Peroxidase			1.00	0.36^{ns}	0.41^{ns}	-0.32^{ns}	0.003^{ns}	0.31^{ns}
Serum Total Antioxidant Capacity				1.00	0.25^{ns}	0.40^{ns}	0.30^{ns}	0.32^{ns}
Testicular Catalase					1.00	-0.54^{ns}	0.49^{ns}	-0.08^{ns}
Testicular Superoxide Dismutase						1.00	0.57^{ns}	-0.21^{ns}
Testicular Glutathione Peroxidase							1.00	0.30^{ns}
Testicular Total Antioxidant Capacity								1.00

ns: non- significant, *: significant, CAT: catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidise, TAC: Total antioxidant activities