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ORIGINAL RESEARCH ARTICLE

Serum and testicular glucose, total protein and sex hormone profile of mottled brown male Japanese quails

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ABSTRACT

This study was carried out to assess the total protein, glucose and sex hormone concentration in the serum and testes of mottled brown male Japanese quails at different physiological ages. Fifty-four mottled-brown male quail birds with average weight of 128.33±28.38g were allotted according to their age to three physiological age groups: pubertal (7 to 10 weeks), mature (15 to 20 weeks) and adult (24 weeks and above). Blood and testicular homogenates were used for total protein, glucose and sex-hormones determination. Testes were excised after sacrificing the birds and homogenised. The same parameters measured in the serum were determined in the testicular homogenates. Statistical comparisons were made between the serum and testicular total protein, glucose and hormonal indices. Glucose in the testes and serum were significantly ($p<0.05$) affected by age. There was significant ($p<0.05$) increase in serum glucose in the mature than in the pubertal male quails. Total protein concentration was not significantly different across the 3 age groups both in the testes and blood. There was no significant ($p>0.05$) difference in the serum and testicular testosterone level across the 3 age groups. However, it was observed that the level of testosterone concentration was apparently higher in the testes when compared with testosterone concentration in the serum of the male quails. This study suggests that the age difference in mottled brown male quails did not affect the level of gonadotropin hormones and subsequent testosterone responsible for fertility in male animals. Mature quails had higher glycaemic index values than other age groups thereby indicating an enhanced reproductive efficiency.

Keyword: Quail reproduction, brown quail blood, Physiological age, sex hormones

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 resistant (Kovach, 1974). Changes in physiological parameters could become very important markers in identifying growth patterns thereby serving as useful tools for predicting both physiological and pathological consequences (Tilgar *et al*., 2008). Various physiological characteristics reflect on the capability and performance of anatomical structures in health and in disease. For example, plasma protein functions as building blocks for 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).

Various anatomical and physiological parameters are regularly used in growth evaluations from birth to adult age. Additional indicators are being identified for assessing dynamics of growth and associated physiological functions for normal and anomalous developments in birds (Druyan *et al*., 2009). Development can be altered and is observed in physical development, retarded growth rate, rapid growth periods, nutrition-related deformities, causes of poor weight gain, onset of puberty and other age-related variations. The disruptions of such are important phases in identifying progression towards adulthood as well as adult life (Spencer *et al*., 1968; Arora, 2010).

Testicular androgen is essential for the stimulation and maintenance of sex related morphology and behaviour in the male Japanese quails (Nagra *et al*., 1959; Beach and Inman, 1965; Sachs, 1967; Adkins and Nock, 1976). Sexual behaviour and sex related characters can be stimulated in castrates by administration of testosterone (Sachs, 1969; Adkins, 1977). Testicular stimulation that occurs with photoperiodic stimulation is associated with increased concentrations of peripheral Luteinising hormone (LH), Follicle stimulating hormone (FSH) and testosterone (Gibson *et al.*, 1975; Follett, 1976; Follett and Davies, 1977). Furthermore, testosterone implantation in intact males reduces serum luteinizing hormone and follicle stimulating hormone and in high doses maintains spermatogenesis (Brown and Follett, 1977, Desjardins and Turek, 1977). During maturation process, the testicular development follows a period of rapid growth which occurs between 2 and 5 weeks of age (Yamoto, 1964; Mather and Wilson, 1964). While spermatogenesis is associated with a testicular weight of 1000 mg with an age of 36 days, increasing androgen concentrations may be expected with increasing testicular weight during maturation. A rise in serum testosterone from 0.59ng/ml to approximately 3.0ng/ml occurs with photoperiodic stimulation of somatically mature males (Follett, 1976). Reports for other avian and mammalian species also indicate that testosterone concentrations rise during maturation (Furr and Thomas, 1970; Schrocksnadel *et al.*, 1971; August *et al*., 1972; Davidson, 1974).

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, procured from University quails, were used for this research. The birds were of three different age groups (7 to 10 weeks), mature (15 to 20weeks) and adult (24 weeks and above) with average weight of 128.33±28.38g. The quail birds were housed in separate pens according to the different age groups in three replicates with 6 birds per replicate and 18 birds per group. Feed (containing 22% crude protein and 2500kcal/kg Metabolisable energy) and clean water were supplied twice daily.

Blood and testes collection and analysis

Blood (3ml) was sampled from the jugular vein (using a 5ml agary-ject sterile syringe and needle) of the mottled brown male Japanese quails and dispensed into a 5ml sterile sample bottle. Blood was allowed to clot and serum separated by centrifuging it at 3000rpm for 15minutes using an IEC centra-4B centrifuge. The serum was used for determination of glucose, total protein and sex hormones (luteinizing hormone, follicle stimulating hormone and testosterone). The birds were sacrificed, dissected, testes excised, macerated and homogenized in a 1ml of 0.154M NaCl (Physiological saline). The homogenate was then sieved using gauze and was centrifuged (using a centrifuge model of BOSCH 90-2) at 4000rpm for 15minutes and the supernatant was decanted into a sterile sample bottle for glucose, total protein and sex hormone determination as was with the serum samples.

Serum concentration of Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone were determined by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Acosta and Wright, 1983).

Statistical Analysis

All data obtained from the study were subjected to correlation analysis and One-Way Analysis of Variance procedures of the Statistical Analysis System (SAS, 2003). Treatment means were separated using Duncan Multiple range test of the same software.

RESULTS

Serum glucose, total protein and hormones in mottled brown male quails at different age groups

Serum glucose, total protein and hormones in mottled brown male quails at different age groups are shown in Tables 1 and 2. The glucose concentration in mature male quails was not significantly different from that of adult male quails, but was significantly ($p<0.05$) higher than that of the pubertal male quails. The glucose concentration was highest (25.89±5.93mmol/L) in mature male quails, while the least value (20.23±6.15mmol/L) was recorded in the pubertal male quails. The serum total protein concentration of the male quails was not significantly different across the 3 age groups that is, pubertal male quail, mature male quail and adult male quails, respectively.

However, the highest (80.55±28.79g/L) serum total protein concentration was observed in mature male quails, while the least value (67.94±23.64g/L) was recorded in the pubertal male quails. The Luteinizing hormone concentration of the male quails was not significantly different across the treatments. The LH ranged from 7.25±0.63 I.U/L to 7.50±0.65 I.U/L in the serum. The Follicle Stimulating Hormone concentration in the serum of the brown male quails was not significantly different across the age groups. The FSH ranged between 5.25±0.50 I.U/L (Adult) and 6.25±1.26 I.U/L (Mature quails). The serum testosterone concentration across the age groups ranged from 0.73±0.17 I.U/L (Pubertal quail) to 0.80±0.12 I.U/L (Adult) and was not significantly different.

Table 1: Testicular and Serum Glucose and Total protein concentration of mottled brown male quails at different age groups

	Treatments		
Parameters	**Pubertal**	**Mature**	**Adult**
Testicular Glucose (mmol/L)	1.20±0.55[a]	0.98±0.64[a]	2.60±1.47[a]
Testicular Total Protein (g/L)	95.90±35.55	74.81±28.56	101.23±40.37
Serum Glucose (mmol/L)	20.23±6.15[b]	25.89±5.93[a]	24.28±6.32[ab]
Serum Total Protein (g/L)	67.94±23.64	77.69±14.34	80.55±28.79

[a b] Means with different superscripts are significantly different (P<0.05).

Table 2: Reproductive hormone level in mottled brown male quails at different age groups

	Treatments		
Parameters (IU/L)	**Pubertal**	**Mature**	**Adult**
Luteinizing Hormone	7.25±0.96	7.50±1.29	7.25±1.26
Follicle Stimulating Hormone	6.00±0.82	6.25±1.26	5.25±0.50
Testosterone	0.73±0.17	0.78±0.10	0.80±0.12
Testicular Testosterone	1.05±0.19	0.95±0.19	1.18±0.26

Testicular Glucose, Total Protein and Hormones in brown male quails at different age groups

Testicular Glucose, Total Protein and Hormones in brown male quails at different age groups are presented in Tables 1 and 2. It was observed that the glucose level of the adult brown male quails was significantly (p<0.05) different from that of the mature and pubertal brown male quails. The glucose concentration was highest (2.60±1.47mmo/L) in adult male quail while the least value (0.98±0.64mmol/L) was recorded in mature male quails. The total protein level across the age groups was not significantly different. The total protein concentration was highest (101.23±40.37g/L) in the adult male quail while the least (74.81±28 56g/L) was recorded in the mature brown male quail. It was observed that the mature brown male quails had the least (0.95±0.19 I.U/L) level of testosterone concentration while the adult brown male quail had the highest (1.18± 0.26 I.U/L) testosterone concentration in the testes.

Correlation coefficient between the serum and testicular hormones of mottled brown male quails at different age groups.

Correlation coefficient between the serum and testicular hormones of mottled brown male quails at different age groups is shown in Table 3. A positive correlation existed between serum luteinizing hormone and serum follicle stimulating hormone (r = 0.85, p> 0.05), serum luteinizing hormone and serum testosterone (r = 0.15, p> 0.05), serum luteinizing hormone and testicular testosterone (r=0.46) of the pubertal brown male quails. Also, a positive correlation existed between serum FSH and serum testosterone (r = 0.24), serum testosterone and testicular testosterone (r = 0.35), however, the correlations were not significant. In mature

brown male quail (Table 4), result indicated a positive correlation between the blood serum luteinising hormone and serum follicle stimulating hormone (r = 0.92), serum luteinising hormone and serum testosterone (r = 0.94) and serum luteinising hormone and testicular testosterone (r = 0.32). A positive correlation also exist between serum Luteinizing hormone and each of serum testosterone (r = 0.90) and testicular testosterone (r = 0.32). Table 5 showed the result for adult brown male quails indicating a positive relationship between serum LH and serum follicle stimulation hormone (r 0.93) of the adult brown male quails. A negative correlation existed between serum LH and testosterone (-0.69), LH and testicular testosterone (r-0.23), FSH and serum Testosterone (r -0.58), FSH and testicular testosterone (r -0.58).

Table 3: Correlation between serum and testicular hormones of the pubertal brown male quails

Parameters	Serum Luteinizing Hormone	Serum Follicle Stimulating Hormone	Serum Testosterone	Testicular Testosterone
Serum Luteinizing Hormone	1	0.86^{ns}	0.15^{ns}	0.46^{ns}
Serum Follicle Stimulating Hormone		1	0.24^{ns}	0.00^{ns}
Serum Testosterone			1	0.35^{ns}
Testicular Testosterone				1

ns: non-significant

Table 4: Correlation between serum and testicular hormone of the mature brown male quails

Parameters	Serum Luteinizing Hormone	Serum Follicle Stimulating Hormone	Serum Testosterone	Testicular Testosterone
Serum Luteinizing Hormone	1	0.92^{ns}	0.94^{ns}	0.32^{ns}
Serum Follicle Stimulating Hormone		1	0.90^{ns}	0.32^{ns}
Serum Testosterone			1	0.65^{ns}
Testicular Testosterone				1

ns: non-significant

Table 5: Correlation between serum and testicular sex hormones of the adult brown male quails

Parameters	**Serum Luteinizing Hormone**	**Serum Follicle Stimulating Hormone**	**Serum Testosterone**	**Testicular Testosterone**
Serum Luteinizing Hormone	1	0.93^{ns}	-0.69^{ns}	-0.23^{ns}
Serum Follicle Stimulating Hormone		1	-0.58^{ns}	-0.58^{ns}
Serum Testosterone			1	0.00^{ns}
Testicular Testosterone				1

ns: non-significant

Correlation coefficient between serum and testicular glucose and total protein of mottled brown male quails at different age groups.

Correlation coefficient between serum and testicular glucose and total protein of mottled brown male quails at different age groups is shown in Tables 6, 7 and 8**.** In pubertal quails, a positive correlation existed between serum glucose and testicular total protein (r = 0.17) and between testicular glucose and testicular total protein (r=0.62), while a negative correlation exist between serum glucose and serum total protein (r = -0.04), serum glucose and testicular glucose (r = -0.29), serum total protein and testicular total protein (r = -0.37). Result for mature brown male quails (Table 7) showed that correlation coefficient were positive for serum total protein and testicular glucose (r = 0.48), serum total protein and testicular total protein (r = 0.26) and between testicular glucose and testicular total protein (r = 0.82). There existed a negative correlation between the serum glucose and serum total protein (r=-0.18), serum and testicular glucose (r = -0.23) and serum glucose and testicular total protein (r = -0.06). In the adult brown male quails (Table 8), positive correlation was found between serum glucose and serum total protein (r = 0.72), serum and testicular glucose (r = 0.43), serum glucose and testicular total protein (r = 0.61), and significant correlation between testicular glucose and testicular total protein (r = 0.58).

Table 6: Correlation between serum and testicular biochemicals in pubertal brown male quails

Parameters	Serum Glucose	Serum Total Protein	Testicular Glucose	Testicular Total Protein
Serum Glucose	1	-0.04^{ns}	-0.29^{ns}	0.17 ^{ns}
Serum Total Protein		1	-0.28^{ns}	-0.37^{ns}
Testicular Glucose			1	0.62^{ns}
Testicular Total Protein				1

ns: non-significant

Table 7: Correlation between serum and testicular biochemicals in mature brown male quails

Parameters	Serum Glucose	Serum Total Protein	Testicular Glucose	Testicular Total Protein
Serum Glucose	1	-0.18^{ns}	-0.23^{ns}	-0.06 ^{ns}
Serum Total Protein		1	0.48^{ns}	0.26^{ns}
Testicular Glucose			1	0.82^{ns}
Testicular Total Protein				1

ns: non-significant

Table 8: Correlation between serum and testicular biochemicals in adult brown male quails

Parameters	Serum Glucose	Serum Total Protein	Testicular Glucose	Testicular Total Protein
Serum Glucose	1	0.72ns	0.43^{ns}	0.61^{ns}
Serum Total Protein		1	0.26^{ns}	0.35^{ns}
Testicular Glucose			1	0.58*
Testicular Total Protein				1

ns: non-significant, *:significant

DISCUSSION

Glucose and total protein are essential biochemical necessary for adequate reproductive function in farm animals. In this study, significantly higher value of glucose was observed in the pubertal and mature male quails than that of the adult quails. This observation could possibly be attributed to the effect of ageing in the birds. A similar result was reported by Perez- Rodriguez *et al*. (2008) in red-legged partridges with males recording higher value for glucose concentration with ageing. However, in a previous descriptive study in red-legged partridges, sexual differences were reported in various blood parameters except in glucose (Rodriguez *et al.,* 2004; 2006). It was observed also that there was no significant difference in the total protein across the age groups of the brown male quails which indicated that age probably did not influence dietary protein absorption and protein synthesis that determine the total protein concentration in the blood. In comparing the testosterone level in the blood and testes, it was observed that there was no significant difference across the age groups. This could be attributed to attainment of puberty which cannot be easily affected except for deterioration in the health status of the male animal. Besides, all birds examined were those at sexual maturity and pre-pubertal age was not captured in this study. This implies that as soon as the quail attain puberty, the testosterone secretion becomes active and consistent to adulthood. Some authors earlier reported that age at sexual maturity is associated with highest testes weight and consequently with the highest blood concentration of testosterone and luteinizing hormone (Sturkie *et al.,* 1986; Gilbert, 2005). Gonzalez-Moran and Soria-Castro (2010) reported positive correlation between greater testosterone and luteinizing hormone production, as well as greater numbers and increases in the Leydig cells volume. These authors also reported an increase in number and volume of Leydig cells during sexual maturation which did not translate into significant increase in the testosterone as the quails aged. In addition to the secretions of luteinising hormone, the Follicle stimulating hormone (FSH) is a glycoprotein produced and secreted by the basophilic cells of the anterior lobe of the pituitary gland. Secretion of FSH is stimulated by gonadotropin releasing hormone (GnRH). Gonadal steroids like progesterone, oestrogen and androgen exerts both positive and negative feedback on FSH function. Although pulse-like secretion of FSH is more pronounced in female animals than in male animals, in male animals FSH and LH maintains spermatogenesis in the testes. FSH level at prepubertal or pubertal age in animals is low. From this study, it was observed that there was no significant difference in the FSH and LH in the blood across the age groups of the brown male quails. This finding is supported by the work of Deviche *et al.* (2011) who observed that changes in the blood concentration of FSH during sexual maturation are rare. The present study revealed that despite the age difference from puberty to adult, there is positive correlation between the serum and testicular biochemical as well as that of the hormone indices. The high positive correlation that existed between serum and testicular glucose in this study may be attributed to increase in blood glucose and total calcium level (Samara *et al*., 1996; Donaldson *et al*., 1999) as a typical response by adrenal gland against stress factor that occur as a result of high environmental temperature since this study was carried out during the early dry season. Glucose level has very important role to play in the regulation of glucose in the tissues and production of energy for use in the metabolic processes (Kiani *et al*., 2011).

CONCLUSION

The total protein concentration was similar among the age groups, while the glucose concentration was higher in matured quails than the quails at puberty. All age groups of the male quails exhibited similar circulatory sex hormones activities which indicate potentials for enhanced reproductive efficiency in the animal. The testosterone was observed to be significantly high in the testes than in the blood. Blood glucose, total protein and sex hormones positively correlated with that in the testes.

CONFLICTS OF INTEREST

The authors declare that there is no any conflict of interest as regards the results reported in this study.

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