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# Allometric Growth of Testes in Relation to Age, Body Weight and Selected Blood Parameters in Male Japanese Quail (Coturnix japonica)

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Abstract: The Japanese quail is very a valuable animal model for research in a variety of biological disciplines. The purpose of this study was to characterize and interrelate age-dependent testicular parameters with various blood constituents: blood glucose, plasma proteins and packed cell volume that are developing concurrently in the growing bird. Another objective of the study was to identify selective physioanatomical markers for predicting the testicular growth and the onset of sexual maturity. Male Japanese quail hatchlings were raised in temperature controlled brooders for up to 3 weeks of age under a constant light and then shifted to hanging cages in an air conditioned room set at ~73° F under a 14L: 10D lighting system and ad libitum access to feed and water. Starting d8, a group of 8-10 birds of uniform size and weight were selected randomly at 4-day intervals up to d52 of age for the project. The birds were weighed and blood sampled using the brachial vein and Blood Glucose (BGL), Total Plasma Proteins (PP) and Packed Cell Volume (PCV) levels were measured prior to euthanization. The testes were removed and measured for weight, length, width and Volume (VOL). All the testicular measurements were then correlated with age and body weight. The left testes were larger than the right testes and their differences were evident at d36 of age. Testicular measurements also reflected two distinct growth surges at d28, d32 and d36 of age. Combined Testes Weight (CTW) and Combined Testes Volume (CTV) revealed a strong positive correlation with PCV and PP and a negative correlation with Blood Glucose Level (BGL). Accordingly, these measurements could serve as reliable markers of growth rate and sexual maturation in male Japanese quail.

Key words: Body weight, testes. Japanese quail, blood constituents, sexual maturity

#### INTRODUCTION

Japanese quail is widely being used as a model (Huss et al., 2008) in research in a variety of disciplines including physiology, nutrition, endocrinology, pathology, embryology, reproduction and immunology. Physiological and anatomical parameters serve as valuable tools for predicting outcomes consequences (Tilgar et al., 2008; Vatsalya and Arora, 2011). Growth rate, a key morphological characteristic, has been positively correlated with robustness, physiology and behavior of birds (Gebhardt-Henrich et al., 1998; Starck and Ricklefs, 1998). The changes in epochs and phases could also reveal changes in physical development, retardation, growth, malnutrition, weight gain, puberty and other age-related events (Spencer et Similarly, al., 1968). testicular growth measurements in males could be used as important indicators of age-based growth modifications (Bennett, 1947; Noirault et al., 2006). This study on the development of testes could provide additional understanding of the role of gonads, age, body weight and blood constituents in the development of male sexual maturity. This would also provide baseline information on the growth of testes and hence assist investigators in their age-related studies with male Japanese quail.

## **MATERIALS AND METHODS**

A breeding colony of Japanese quail having an overall fertility of ~90% provided material for this study. The birds ~70 days of age were housed in suspended cages in a temperature controlled room at ~73° F under a 14L: 10D lighting regimen with ad libitum access to feed and water. The eggs were collected between 3:00 to 6:00 PM daily and refrigerated overnight. Prior to transferring the eggs into the incubator the following day, the eggs were allowed to sit at room temperature for approximately 3 hours. The eggs were incubated in an auto-turner incubator at a temperature of ~99.5°F and ~60-65% relative humidity. The eggs were hatched in four sequential batches to obtain sufficient males for this study and were handled in a similar fashion. The hatchlings were weighed and transferred to temperature controlled brooders for 21 days with continuous light and free access to feed and water. For identification purpose small pieces of numbered adhesive tape were applied on the underside of the right wing and finally tagged with metal wing bands on day 8 (d8). Starting d8, a group of

8-10 birds of uniform size and weight were selected for evaluations at 4-day intervals up to d52. The gender of the birds was determined at necropsy since the baby chicks did not exhibit plumage-based sexual dimorphism until d20. The birds were weighed to the nearest 0.1 g and approximately 0.2 mL of blood was collected from the brachial vein using a lancet into EDTA-coated vials to determine Packed Cell Volume (PCV), Blood Glucose Level (BGL) and Total Plasma Proteins (PP) levels. PCV was determined with microhematocrit tube centrifugation at 12,000 RPM for five minutes (UNICCO C-MH30) and total plasma proteins were measured using clinical refractometer (T2-Ne Atago Co). Blood glucose was measured with a glucometer (Elite XL) at the time of blood collection. This procedure continued at 4-day intervals until the birds reached d52. Following blood collection, the birds were euthanized with CO2 gas in small animal anesthesia chambers. Beyond d20, the males could easily be identified with their male plumage color. Once the euthanasia was complete, the birds were dissected and their testicular development was quantified with respect to their weight, length, width and volume. The testes were weighed to the nearest milligram (mg) using a Sartorius precision weighing scale and dimensions, namely length (mm) and width (mm) were collected with a Vernier Caliper (Tekton 7159). Volume of the testis (mm<sup>3</sup>) was calculated using the following equation (4/3x  $3.5414 \times a b^2$ , where a = 0.5 of long axis and b = 0.5 of short axis (Chaturvedi et al., 1993; Moller, 1994). The data collected on body weight, testes and blood constituents were analyzed for correlations, regression and ANOVA analyses using SPSS version 19.0 and MS Office Excel 2010 statistical tools.

# **RESULTS**

The following parameters were measured in male Japanese quail (age 8 to d52): body weight, testes (weight, length, width and volume), PCV, PP, BGL and shank length.



Fig. 1: Left testis (L) is wider and more rounded than the Right testis (R) which echoes to its larger volume

Growth of testes, body weight and shank: The growth of body weight was linear between d12 and d32, remained the same between d32 and d36, demonstrated rapid growth to d44 and then plateaued at d52 (Fig. 2 and 6). In comparison, the growth of the testes was very small during d8-d24, moderate up to d32, very rapid to d44, leveling off thereafter (Fig. 2-4). During the early growth period, the testes appeared vellowish-brown in color and ellipsoidal or pointed in shape. The color of the testes then changed to grayish white and finally to pinkish-red due to the copious supply of blood vessels on the surface. The contour of testes changed as the birds matured. The left testes were larger than the right testes particularly after d24 (Table 1, Fig. 2). The combined weight of both testes was 0.01% of total body weight at d8, 0.02% at 16d, 0.13% at d20,

Table 1: Body weight, testicular measures and blood constituents (PCV, PP, BGL) values as a function of age

		Weight	of testes							
Age (days)	Body weight (gm)	LT weight (mg)	RT weight (mg)	Left and right testes weight differences (mg)	Combined testes weight (mg)	Combined weight as % of BW	Combined testes volume (mm³)	PCV %	Plasma protein (gm/dl)	Blood glucose (mg/dl)
8	18.7	1.4	1.4	0.00	2.80	0.01	4.3	38.3	2.5	275.1
12	23.9	1.7	1.5	0.20	3.20	0.01	3.9	37.1	2.5	276.2
16	40.2	4.8	4.3	0.50	9.10	0.02	11.8	38.1	2.6	259.1
20	63.2	42.1	42.2	-0.10	84.30	0.13	70.0	34.8	2.6	269.9
24	71.2	39.8	30.7	9.10	70.50	0.10	91.0	35.2	2.6	278.8
28	80.1	292.8	263.9	28.90	556.70	0.70	499.0	37.3	2.7	273.8
32	84.9	528.0	483.6	44.40	1011.60	1.19	835.7	38.0	2.7	268.7
36	84.2	673.3	546.4	126.93	1219.74	1.45	1137.3	40.0	2.7	252.2
40	93.3	998.0	903.3	94.70	1901.30	2.04	1602.9	45.6	2.8	215.1
44	94.9	1255.0	1248.0	7.00	2503.00	2.64	2500.2	47.2	3.0	184.6
52	98.3	1219.2	1166.4	52.80	2385.60	2.43	2226.0	48.5	3.2	182.2

BW = Body Weight; LT = Left testis; RT = Right testis

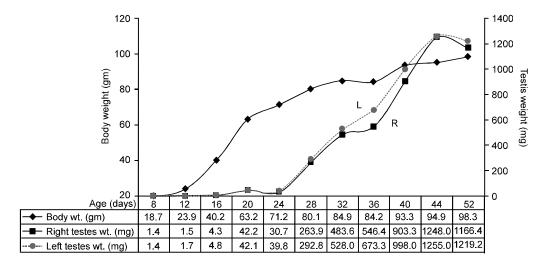


Fig. 2: Mean weight of left and right and combined testicular weight as a function of age and body weight

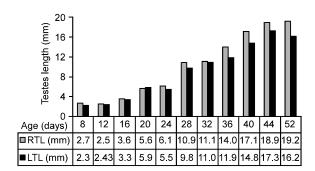


Fig. 3: Mean length of left and right testes in relation to age. RTL = Right testes length, LTL = Left testes length

0.70% at d28, 1.19% at d32, 1.45% at d36 and 2.64% at d44 (maximum). There was an increase of 120 folds from d8 to d32 and 1.66 folds from d36 to d44 during the faster growth rate of testes. Testicular growth spikes were observed d16-20, d28-32 and d40-44, which corresponded with age and growth of body weight (Fig. 2-4). CTW and body weight percent deviations during on d12-16, d16-20 and d24-28 are provided in Fig. 7. Growths differential of CTW during each of the 4-day intervals during the entire period are provided in Fig. 9. CTW with respect to age and body weight showed positive association, which can be characterized with the following regression equation: CTWn = 1062 In (AGEn) -803.65,  $R^2 = 0.6412$  (Fig. 8) and CTWn = 1189.6 In (BWn) - 3996,  $R^2$  = 0.4877, respectively. During the accelerated growth period between d24 and d44 (n) stage, CTW showed a highly significant positive correlation with age and body weight and can be characterized with the following regression equation: CTWn = 1282.3 ln (AGEn) - 3717.3,  $R^2$  = 0.8225 and body weight, CTWn = 1722.2 In (BWn) - 3723.3,  $R^2$  =

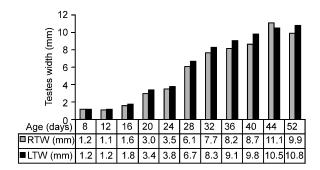


Fig. 4: Mean width of left and right testes in relation to age. RTW = Right testes width, LTW = Left testes Width

0.8771, respectively. Shank length increased progressively from d8 to d24 and then leveled off.

Left and right testes: Data on the left and right testes deviations between and the two and measurements are presented in Table 1 and Fig. 2-4. The left testes were larger than the right testes starting at d20 with differences peaking at d36 (129.6 mg) and diminishing significantly at d44. Body weight was more positively correlated with the left testes (LTW; r = 0.788) than the right testes (RTW; 0.771). Percentage differences in body weight had a correlation of 0.508 with LTW and 0.476 with RTW. Right testes were comparatively longer than the left testes (Fig. 3) although the left testes were wider than the right testes (Fig. 4). Also, the lengths of the Left Testis (LTL) reflected a higher correlation with body weight (r = 0.918) compared to the length of the right testis (r = 0.902). A similar relationship between body weight and length of testes was evident during the growth surges mentioned earlier. The correlation between age and the length of the Right Testes (RTL) was greater than the length of the Left

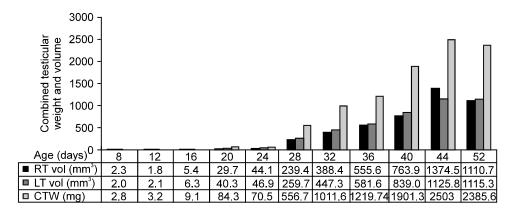


Fig. 5: Mean volume of left and right testes as a function of age. Combined weight of both testes is also shown

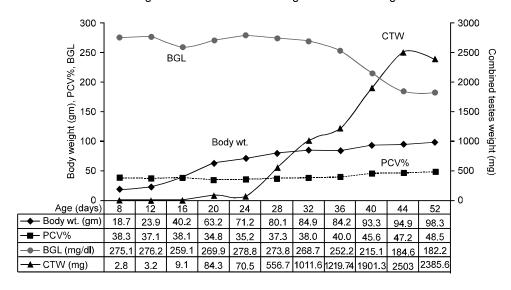


Fig. 6: Combined Testicular Weight (CTW) as a function of age and body weight. Associated blood parameters (PCV% and BGL) are also shown

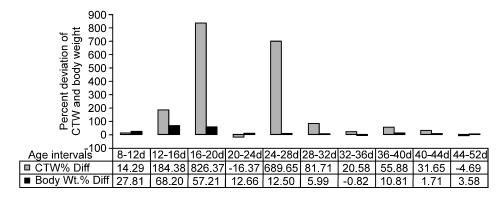


Fig. 7: Percentage deviations of CTW and body weight as a function of age intervals

Testes (LTL), which became more evident as the birds approached sexual maturity. Body weight demonstrated a higher correlation with LTWd (r = 0.780) compared to RTWd (r = 0.771; p<0.01).

**Testicular volume:** The testes were enlarged and appeared bulky and rounded upon maturity. The values for Combined Testicular Volume (CTV) conformed to simultaneous changes in age and body weight (Table 1

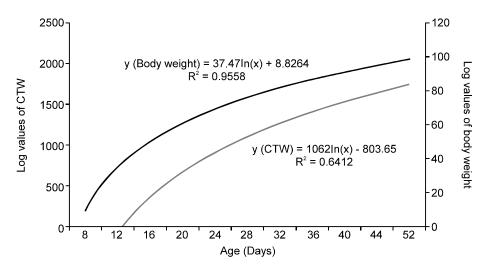


Fig. 8: Logarithmic curves demonstrating body weight and CTW in relation to age

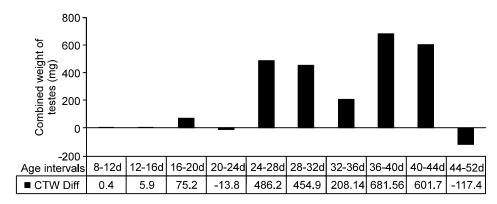


Fig. 9: Growth differential of CTW within each 4-day interval

and Fig. 5). The CTV was linear with age from d28 to d44 and was highly correlated with body weight. The volume of the Left Testis (LTV) was larger than the volume of the Right Testes (RTV), reversing during d40-44. RTV was highly correlated with age (0.893) and body weight (0.745) as compared to LTV of 0.928 and 0.785. respectively. The growth spikes observed during the early growth period also showed a sudden increase in testicular volumes due to an increase in testicular mass. LTV was more positively related with age and body weight than RTV during sexual maturation, demonstrating a positive linear relationship from d28 to d44 and a high correlation with body weight. A close association of CTV with age and body weight could be characterized with the following regression equation: CTVn = 1340.18 ln (AGEn) - 3485.9,  $R^2$  = 0.6961 and CTVn = 1097.3 In (BWn) - 3686.7,  $R^2 = 0.466$ respectively.

Association of testicular measures with physiological parameters: The data on testicular measurements, Packed Cell Volume (PCV) and Blood Sugar Level (BGL)

Table 2: Correlations of testicular measures with PCV and blood glucose (BGL)

gldCo3C (DGL)				
Parameters	PCV%	p-value	BGL	p-∨alue
Combined testes weight	0.936	0.010	-0.933	0.01
Right testes				
Weight (RTW)	0.941	0.050	-0.946	0.05
Length (RTW)	0.864	0.010	-0.868	0.05
Width (RTWd)	0.799	0.054	-0.807	0.05
Left testes				
Weight (LTW)	0.941	0.050	-0.939	0.05
Length (LTW)	0.828	0.063	-0.841	0.05
Width (LTWd)	0.788	0.010	-0.789	0.01
Testes Volume (Derived)				
Right Testes (RTV)	0.933	0.00	0.953	0.00
Left Testes (LTV)	0.943	0.00	0.946	0.00
Combined Volume (CTV)	0.935	0.00	0.943	0.00

are presented in Table 1 and Fig. 6. PCV increased gradually during early growth, followed by rapid growth after d36 (42.2%) reaching the highest level at sexual maturity (48.5%; p<0.01). All the testicular measurements were positively correlated with PCV and negatively correlated with Blood Sugar Level (BGL). Both CTW and LTWd were highly correlated with PCV

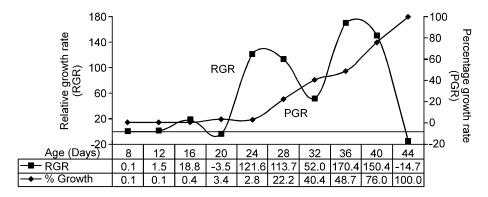


Fig. 10: Relative growth rate and percentage growth rate of CTW between d8 and d44 at 4-day intervals

(Table 2). Plasma Proteins (PP) increased from d8 to d28 and then gradually leveled off at d52. Blood sugar level continued to decrease on way to sexual maturity which was quite opposite to the increase of PCV during the same period. MANOVA analyses confirmed highly significant correlations (p≤0.01) with respect to all the testicular measurements with body weight and physiological parameters (Table 2).

#### DISCUSSION

A cascade of body weight, testicular metrics and physiological parameters up to the age of maturity is presented in Table 1. The dimensions and shape of the testes changed as the birds approached sexual maturity, appearing greatly enlarged. The combined weight of testes was highly correlated with age (r = 0.940) and body weight (r = 0.802). At d20, we observed a typical male type brownish plumage at the throat region. Two peaks of accelerated growth of testes was observed during d24-28 and d28-36, however the most significant increase occurred d36 onwards to sexual maturity with an elevated relative growth rate from 24d stage onwards (Fig. 10). The male Japanese quail begin secreting LH hormones at 3-4 weeks of age which is crucial for initiating the secretion of testosterone which, in turn, stimulates the rapid growth of testes (Follett and Maung, 1978; Garamszegi et al., 2005; Denk and Kampenaers, 2005; Sedgvar et al., 2008). This explains the reason for appearance of male type plumage color in the chicks around d20 in this study. Several reports are available in the literature stating that left testes are larger than right testes in most avian species such as chickens (Hocking, 1992; Moller, 1994; Yu, 1998), turkeys (Burke, 1973), white-crown sparrow (Farner and Wilson, 1957) and humans (Gardner et al., 1975). Our studies also revealed that the left testes are larger than the right testes in the Japanese quail (Fig. 1); and the differences among testes were evident around d28, highest at d36, decreasing somewhat at d44 and then increasing slightly at d52.

Our observations also indicated that various quantitative testicular measurements (weight, length, width, volume) and PCV are positively correlated with age and body weight and negatively correlated with BGL, especially toward the onset of sexual maturity. These measures also tended to change in tandem with changes in age and body weight. A similar relationship was reported by Bhardwaj and Anushi (2006) in house sparrows (Passer domesticus) and by Deviche et al. (2001) in dark-eyed junco (Junco hyernalis) under the altered photoperiod regimens. A positive relationship among various physiological traits was also reported in growing chicks and pigeons (Gavin et al., 1998) and among developmental and biochemical indices in reptiles (Mohri et al., 2007), juvenile green turtle (Roark et al., 2009) and Japanese quail (Vatsalya and Arora, 2011). Furthermore, our observations also indicate that PCV was positively correlated and BGL negatively correlated with the testicular measures (p<0.01), particularly close to sexual maturity. An increase of PCV is an indication of enhanced erythropoiesis (Mirand et al., 1965) due to increased secretion of gonadotropins and testosterone. A close relationship of CTW and body weight with age is depicted as logarithmic curves (Fig. 8). There was a very close relationship between PCV and CTW as the body weight approached sexual maturity (Table 1), therefore, these two traits could be used as reliable biological markers for sexual maturity and functional maturity of the testes. Cecil and Bakst (1991) also recommended the use of hematocrit values in predicting sexual maturity in turkeys. Reduction of BGL during sexual maturity could be due to changes in the carbohydrate metabolism and production of energy required for various metabolic and synthesis processes (Cornblath et al., 1990).

Strong relationship between testicular size and body size has been reported in invertebrates (Poulin and Morand, 2000), Japanese quail (Sachs, 1969), turkeys (Bennett 1947) and domestic fowl (Tyler and Gous, 2009). Furthermore, the secretion of Leptin hormone during growth is also known to influence growth rate and

the onset of puberty in Japanese quail (Lamosova et al., 2003). For visual assessment of sexual maturity, we used various published indices and measurements particularly the growth of cloacal gland, foam production, vocalization and behavioral changes, which are positively correlated with testes size, sperm production, testosterone level. There is also a close relationship of cloacal gland size and foam production with the fertilizing ability of individual male birds (Sachs, 1969; Siopes and Wilson, 1975; Oishi and Konishi, 1983; Marin and Satterlee, 2004; McFarland et al., 1968; Ottinger and Brinkley, 1979; Biswas et al., 2007). Biswas and Arora (2008) reported that fertility in male Japanese quail can be assessed from the size of the cloacal gland larger than 13.5 mm. This was confirmed via the fertility of the eggs laid by the matted virgin females. Mather and Wilson (1964)reported that the stage spermatogenesis may be estimated on the basis of testes weight and showed d43 testes in full spermatogenesis in their publication. Smedley et al. (2002) predicted sexual maturity in male cyanomolgus macque on the basis of age, body weight and histological evaluation of the testes. In conclusion, this study reflects that, in addition to age, body weight, size of the cloacal gland, an increase in PCV and a decrease in blood sugar level during the pre-pubertal phase, individually or in combination can be used as predictors of maturation of testes and thus can help in the selection of potentially superior breeders. This study also presents baseline information on the growth and measurements corresponding with increase in age and body weight. This type of information would be useful to investigators engaged in various studies in growth, reproduction. endocrinology, nutrition, toxicology, photoperiods and physio-pathological responses to experimental treatments in male Japanese quail.

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