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ORIGINAL ARTICLE

Serum biochemical profiling in different varieties of Japanese quail, *Coturnix coturnix japonica* (Temminck and Schlegel, 1849)

Kananbala Patra[1] · Sujata Puspamitra[1] · Aryadhara Das[1] · Bandi K. Mallik[2] · Prafulla K. Mohanty[1]

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Abstract

A good quantum of reports is in record on serum biochemical analysis of Japanese quail. But the serum biochemical parameter of different varieties of healthy Japanese quail is poorly understood, and reference values are sparsely available. The investigation focuses to assess serum biochemical profiles of three different varieties of Japanese quail, i.e. brown-feathered, grey-feathered, and white-feathered at different age groups with sexual dimorphism. The five parameters taken for the analyses include glucose, cholesterol, total protein, albumin, and globulin. Two millilitre of blood was collected from the ulnar vein of 90 individual birds, comprising of 05 males and 05 females, from three age groups and three varieties of Japanese quail. For biochemical analyses, three age groups were considered, i.e. chick (0–3 weeks), grower (4–5 weeks), and adult (above 5 weeks), respectively. Data were analysed through standard statistical methods such as ANOVA and test of significance by using Paleontological Statistics (PAST) version 2.17. The data were presented as mean ± standard error, and the significant difference both in age and sex has been recorded at $p < 0.01$. Throughout the study, a decrease in serum glucose level was observed with increase in age. The serum cholesterol level increased with age, and more serum cholesterol concentration is seen in adult females. The results were similar for serum total protein, albumin, and globulin. The results of serum biochemical analysis reflect a difference at $p < 0.01$ in experimental birds which were similar to that of chickens and other poultry birds.

Keywords *Coturnix coturnix japonica* · Biochemical profile · Blood · Japanese quail · Serum

Introduction

Japanese quail, which belongs to family Phasianidae and order Galliformes, is an avian species used in poultry sector for the production of eggs and meat. Japanese quails were originally introduced to North America by US Fish and Wildlife Service as game birds in 1817, and releases continued into the late 1950s (Standford 1957). They were originally kept for their song (Howes 1964), and particular call types were bred for use in song contests (Taka-Tsukasa 1935). In India, the Central Avian Research Institute (CARI) at Izatnagar first introduced quails by procuring hatching eggs from University of California, USA, in 1974 under the United Nations Development Project (Shrivastav and Panda 1999). In this present investigation, three varieties, namely white-feathered Japanese quail (Fig. 1), brown-feathered Japanese quail (Fig. 2), grey-feathered Japanese (Fig. 3) quail, have been taken into account because of their abundance in the farm of the government of India. In brown-feathered Japanese quail, the plumage is completely brown with an average body weight of 150–160 g at fourth week. It attains sexual maturity within 38–41 days posthatching. Eggs are tinted and are about one-fifth the size of chicken eggs with an average weight of 11 g per egg (Huss et al. 2008). The plumage colour of grey-feathered Japanese quail is grey or pharaoh with an average body weight of 150–155 g at fourth week and 220 g at fifth

✉ Kananbala Patra
kananpatra462@gmail.com

Sujata Puspamitra
puspamitrapm@gmail.com

Aryadhara Das
aryadharads@gmail.com

Bandi K. Mallik
bandim64@gmail.com

Prafulla K. Mohanty
prafulla.mohanty3@gmail.com

[1] Cytogenetics Laboratory, Postgraduate Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar 751 004, Odisha, India

[2] Central Poultry Development Organisation (CPDO), Eastern Region, Government of India, Bhubaneswar 751 012, Odisha, India

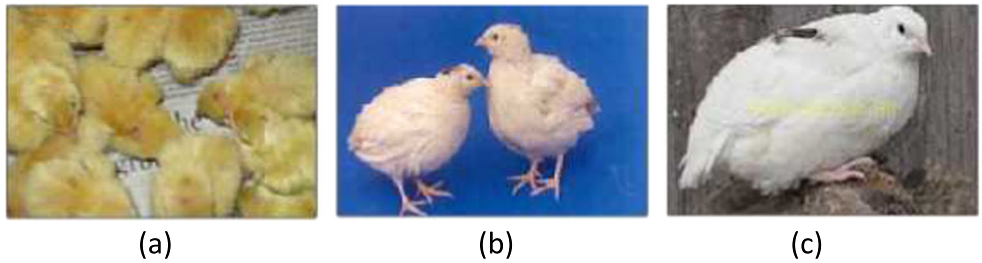

Fig. 1 **a** Chick, **b** grower, **c** adult of white-feathered Japanese quail

week. It attains sexual maturity at the age of 42–46 days. Eggs are tinted with an average weight of 14 g per egg. White-feathered Japanese quail is a dark-eyed white-coloured bird. The chicks are usually cream-yellow in colour, similar to that of white Leghorn chick. As adults, these birds are pure white and both sexes are similar. Occasionally, few black down feathers appear on the top of the head. Since Japanese quail is suitable for egg and meat production, commercial quail farming in low-income regions can be a great source of money and employment (Santhi and Kalaikannan 2017). Its short life-span together with its physiological similarity with humans has made it as an excellent model organism in studying various fields of biology such as immunology, endocrinology, and reproductive biology (Holmes and Ottinger 2003; Ottinger et al. 2004). It has also been used to study photoperiodism and circadian control of brain function, circulating levels of sex hormones, and reproductive activity (Watson et al. 1990; Follett et al. 1992; Ottinger et al. 2005). Clinical signs in birds are often nonspecific, and the information gained by physical examination is limited with regard to specific and detailed diagnosis. Evaluation of serum biochemistry profile provides useful information about their physical condition making them useful tools in differentiating apparently healthy birds from abnormal or diseased ones.

Materials and methods

The present study was conducted on three varieties of Japanese quail which were reared and maintained at a well-planned poultry farm called Japanese quail farm unit of Central Poultry Development Organisation (CPDO), Eastern Region, Government of India, located in Bhubaneswar, Odisha, under systematic farming practice. Two millilitre of blood was syringed aseptically by professional veterinary doctors of the farm from three age groups, i.e. chick (3rd week), grower (5th week), and adult (9th week) including both the sexes, without sacrificing or harming the birds. Blood was collected from 90 individual birds which comprise five from each sex of each age group and each variety. After collection, the blood was analysed and assayed. The present investigation was carried out during September–December 2017 when the average temperature and humidity was 13–28 °C and 33–98%, respectively. The weights of chick, grower, and adult were 63.34–101.70 g, 148.35–186.31 g, and 186.31−203.9 g, respectively.

Blood was collected from the prominently visible ulnar vein or the wing vein (Fowler 1978) into the Eppendorf tube with the help of 2-ml syringe with 25 gauge needles of Dispovan make, Faridabad, India. The tubes were left undisturbed for 5 h. The upper clear liquid or the serum was pipetted and transferred to another Eppendorf tube and then stored at 4 °C. The serum biochemical profiling for five parameters, i.e. serum glucose, serum cholesterol, serum total protein, serum albumin, and globulin, was carried out by commercial kit manufactured by Coral Clinical Systems, Uttarakhand, India.

Glucose is the major carbohydrate present in blood. Its oxidation in the cells is the source of energy for the body. Increased level of glucose is found in diabetes mellitus, hyperparathyroidism, pancreatitis, and renal failure. Decreased level is found in insulinoma, hypothyroidism, and extensive liver disease (Burtis et al. 2007). The glucose content in serum was estimated by glucose oxidase/peroxidise method, commonly known as GOD/POD method (Trinder 1969), and absorbance was measured in UV spectrophotometer at 505 nm.

$$\text{Total glucose (mg/dl)} = \text{absorbance of test sample} \div \text{absorbance of standard glucose} \times 100$$

Cholesterol is the main lipid found in blood, bile, and brain tissues. It is the main lipid associated with arteriosclerotic

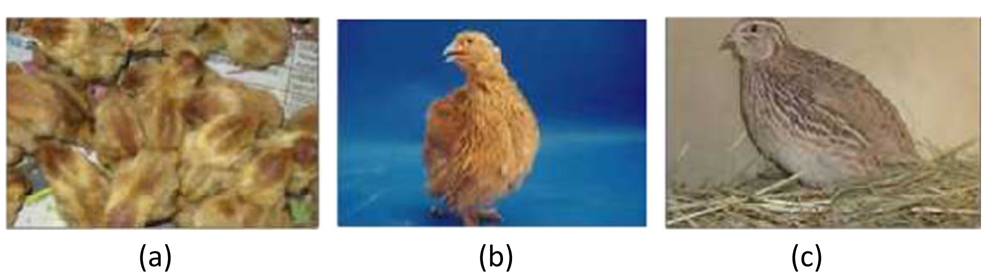

Fig. 2 **a** Chick, **b** grower, **c** adult of brown-feathered Japanese quail

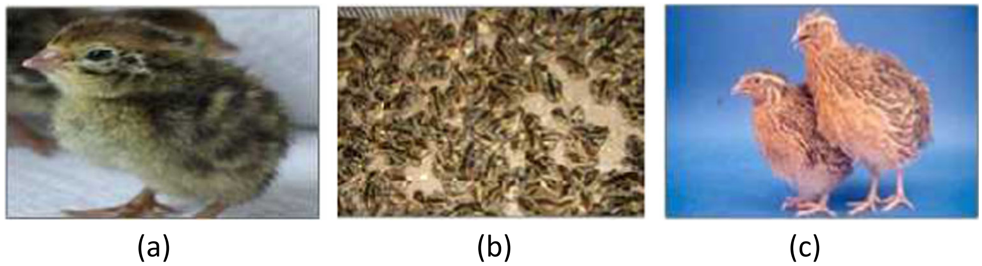

Fig. 3 **a** Chick, **b** grower, **c** adult of grey-feathered Japanese quail

vascular diseases. It is required for the formation of steroids and cellular membranes. The liver metabolises the cholesterol, and it is transported in the bloodstream by lipoproteins. Increased level is found in hypercholesterolemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephritic syndrome, and cirrhosis. Decreased level is found in malnutrition, hyperthyroidism, anaemia, and liver diseases (Burtis et al. 2007). Serum cholesterol was measured by cholesterol oxidase/phenol 4-aminoantipyrine method (Trinder 1969; Allain et al. 1974; Flegg 1973) at 505 nm.

$$\text{Total cholesterol (mg/dl)} = \text{absorbance of test} \div \text{absorbance of standard cholesterol} \times 200$$

Plasma proteins are important complementary constituents in the diagnosis of gastrointestinal, hepatic, renal, or infectious diseases (Burtis et al. 2007). According to Lumeij and McLean (1996), the correlation is highly significant ($p < 0.000001$; $R = 0.99$; $n = 50$). In female birds, a considerable increase in plasma total protein concentration occurs just before egg laying because of an oestrogen-induced increase in the globulin fractions (Griminger 1976). The proteins are the yolk precursors (e.g. vitellogenin and lipoproteins), which are synthesised in the liver and transported to the ovary, where they are incorporated in the oocyte (Griffin et al. 1984). Serum total protein was measured by the Biuret method (Gornall et al. 1949), and absorbance was measured at 530 nm.

$$\text{Total protein (g/dl)} = \text{absorbance of test} \div \text{absorbance of standard} \times 8$$

The albumin consists approximately 60% of the total proteins in the body, the other major part being globulin. The serum globulin increases with the severity of pathological changes of amyloidosis and that the globulin is also found in the amyloidotic tissue in ducks. Gastrointestinal and renal diseases can also lead to severe hypoproteinaemia. In birds, protein malnutrition may lead to hypoproteinaemia (Leveille and Sauberlich 1961). Increased total protein (TP) concentration with a normal albumin (A) to globulin (G) ratio can be expected in dehydrated birds if the primary disease does not cause hypoproteinaemia. Serum albumin was measured by the bromocresolgreen (BCG) method (Doumas and Watson 1971) at 630 nm.

$$\text{Albumin (g/dl)} = \text{absorbance of test} \div \text{absorbance of standard} \times 4$$
$$\text{Globulin (g/dl)} = (\text{total protein in g/dl}) - (\text{albumin in g/dl}).$$

Data obtained were expressed as mean ± standard error. These were subjected to Paleontological Statistics (PAST) version 2.17 for one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparison tests. The significance was set at $p < 0.01$.

Results

Throughout the study, a decrease in serum glucose level was observed with increase in age (Table 1 and Fig. 4a). The brown chick males are significant with brown chick females, brown grey males, brown grey females, brown adult males, and brown adult females, whereas brown chick females are significant with brown adult males, and brown adult females. No significance is observed between brown grower males and brown grower females. These were, however, significant with other groups such as brown adult males and brown adult females. Grey grower females are significant with growers and adults. Moreover, white chick males are significant with white adult males, and white chick females are significant with white grower females. The serum glucose concentration is higher in grey chick females (455.08 ± 1.84 g/dl) and lower in grey adult males (74.04 ± 1.14 g/dl).

The serum cholesterol level increased with age, and more serum cholesterol concentration is seen in adult females (Table 2 and Fig. 4b). In brown-feathered Japanese quails, adults and chicks of both the sexes differed significantly at $p < 0.01$. The grower males and females, however, differed non-significantly at $p < 0.01$. Similarly, the grey- and white-feathered Japanese quails differed significantly with other age groups. In all the three varieties of Japanese quail, females showed higher value than the males and this value has been observed to increase with the increase of age. The serum cholesterol content was found to be 109.10 ± 0.38 g/dl in brown chick males and 146.14 ± 2.56 g/dl in brown adult males. Higher value was recorded in females (e.g. brown chick females 117.82 ± 1.19 and brown adult females 150.51 ± 3.08) as compared with males (brown chick males 109.10 ± 0.38; brown adult males 146.14 ± 2.56).

Total serum protein concentration differed significantly ($p < 0.01$) in both the sexes of brown-, grey-, and white-feathered Japanese quails (Table 3 and Fig. 4c). The observed

Table 1 Serum glucose (mg/dl) profile of different varieties of Japanese quail at different age groups

Sl No.	Age	Chick (0–3 weeks)		Grower (4–5 weeks)		Adult (> 5 weeks)		*F* value
	Sex	Male (05)	Female (05)	Male (05)	Female (05)	Male (05)	Female (05)	
1	Brown	$147.55 \pm 3.18^{a,1}$	$327.06 \pm 1.40^{a,b,1,2}$	$323.86 \pm 0.77^{a,c,1}$	$316.14 \pm 1.77^{a,d,2}$	$94.27 \pm 7.18^{a,b,c,d}$	$109.47 \pm 4.85^{a,b,c,d,2}$	8.53**
2	Grey	$288.02 \pm 17.57^{a,1,3}$	$455.08 \pm 1.84^{a,b,1,2,3,4}$	$307.44 \pm 1.74^{b,c}$	$324.25 \pm 2.1^{a,b,d,4}$	$74.04 \pm 1.14^{a,b,c,d,2}$	$90.2 \pm 7.85^{a,b,c,d,3}$	3.40**
3	White	$269.42 \pm 9.08^{a,1,4,5}$	$378.19 \pm 0.8^{a,b,1,2,3,5}$	$285.83 \pm 7.10^{b,c,1,2,4,5}$	$352.06 \pm 10.57^{a,c,d,1,2,4,5}$	$92.18 \pm 6.73^{a,b,c}$	$102.4 \pm 5.62^{a,b,c,d,3}$	279.7**
4	*F* value	1.28**		16.47**		4.04**		

Figures in the parentheses represent number of observations; mean ± SE bearing similar alphabetical and numerical superscript differ in rows and columns respectively at $p < 0.01$, **significant at $p < 0.01$

serum total protein value differed significantly between and among different age groups. The serum total protein was found to be increased with age in both the sexes.

Similarly, the serum albumin and globulin were found to be increased with increase in age, i.e. adults showed higher value than chicks and grower in all the three varieties of Japanese quails (Table 4 and Fig. 4d; Table 5 and Fig. 4e). The chick males differed significantly with all other age groups at $p < 0.01$.

Discussion

With the advancement of age, serum glucose concentration in both the sexes of Japanese quail showed a decreasing tendency. Young quails, i.e. chicks, had a lower erythrocyte count than the adults (Kundu et al. 1993). The glucose content in the red blood cell is very low. As the number of erythrocytes increases with the advancement of age, the glucose concentration in whole blood also gets decreased. The increase in red blood cell count (Kundu et al. 1993) may be the reason for the decreasing of glucose in adult Japanese quails. Females have comparatively high serum glucose level than males at all the age groups which is in agreement with Poyraz (1988) who has reported higher blood glucose level in female Japanese quails. Male birds had a higher erythrocyte count under the influence of the testosterone (Greenman and Zarrow 1961) which may be due to their lower blood glucose concentration when compared with females. Another reason for such a high level of serum glucose in chicks and growers in all the three varieties may be due to the presence of more amount of glucagon-secreting alpha cells in islets of Langerhans (El-Ghalid 2009). The decreasing trends of glucose level were accompanied by increased liver glycogen indicating a stimulated pancreatic activity which comes in agreement with the findings of Schulz (1940) who opines that, in pigeons, the pancreatic islets of Langerhans increase in size and number during egg-laying period of the females (Hassan 2010).

Hassan (2010) in his studies on variation in egg performance and plasma constituents at different ages of female Japanese quails observes a gradual increase in cholesterol level with age, and the highest value was observed at 30th week while the lowest at 8th week. During the laying period, the hepatic syntheses of triglycerides, phospholipids, and cholesterol have been observed to be increased (Walzem et al. 1999). Laying hens, therefore, have extraordinarily high circulating concentration of triglycerides and cholesterol in contrast to male birds (König et al. 2007). Females demonstrate a marked increase in total protein concentration during egg production (Urist et al. 1958; Coenen et al. 1994). This can be explained as a result of increased secretion of oestrogen which causes an increase in production of egg yolk precursor's vitellogenin and lipoproteins (Lumeij 1997), thus leading to an increase

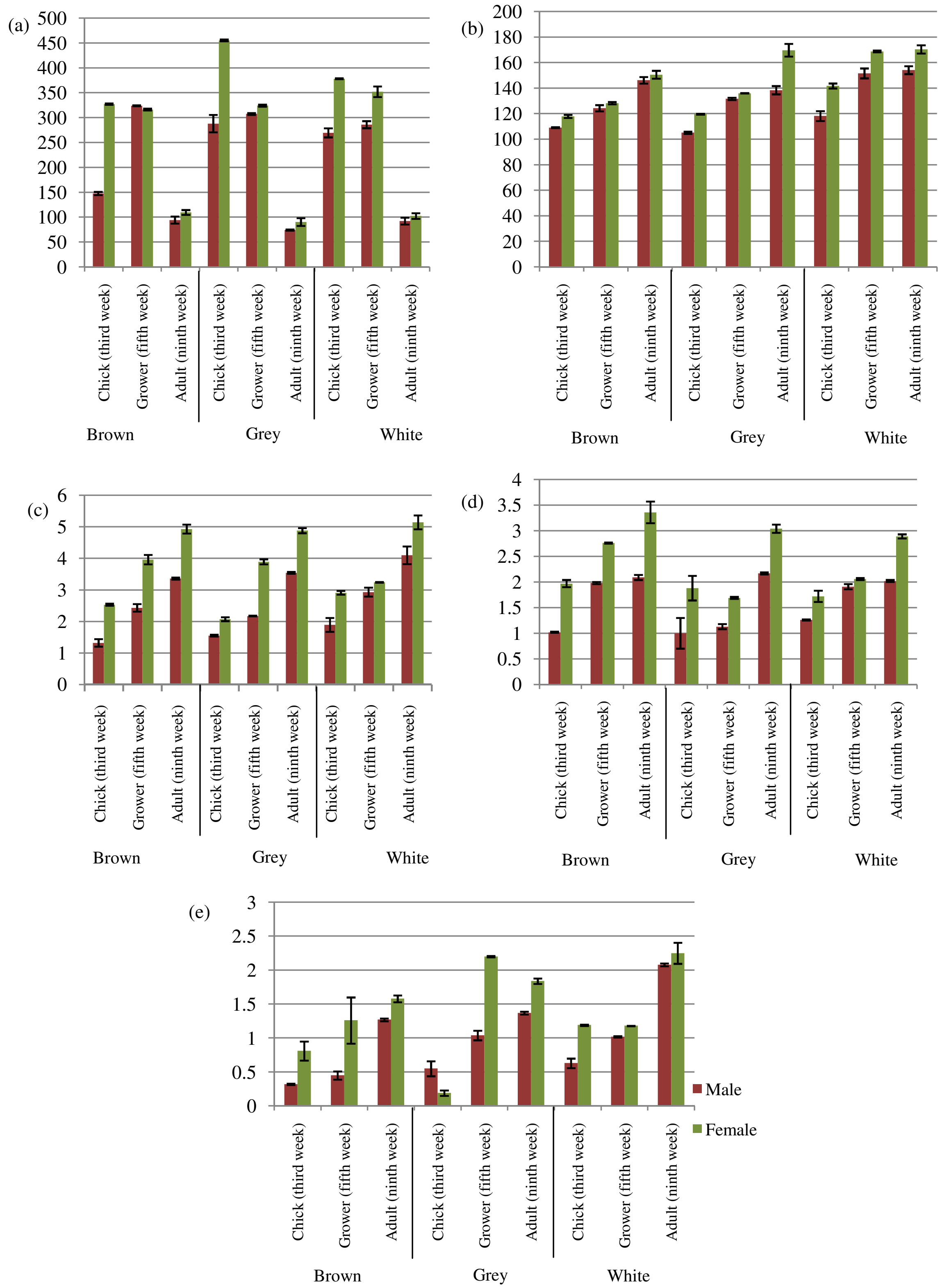

Fig. 4 Comparative analysis of **a** glucose (mg/dl); **b** cholesterol (mg/dl); **c** total protein (g/dl); **d** albumin (g/dl) and **e** globulin (g/dl) in brown, grey, and white variety of Japanese quail at different age groups

in the concentration of total protein. Such an elevated concentration of total protein in egg-laying birds is accompanied by a significant increase in levels of serum albumin and globulin (Hunt and Hunsaker 1965; Agina et al. 2017).

Table 2 Serum cholesterol (mg/dl) profile of three varieties of Japanese quail at different age groups

Sl No.	Age	Chick (0–3 weeks)		Grower (4–5 weeks)		Adult (> 5 weeks)		F value
	Sex	Male (05)	Female (05)	Male (05)	Female (05)	Male (05)	Female (05)	
1	Brown	$109.10 \pm 0.38^{a,1}$	$117.82 \pm 1.19^{b,1,2}$	$124.25 \pm 2.48^{a,c,1}$	$128.75 \pm 0.85^{a,b,d,1,2}$	$146.14 \pm 2.56^{a,b,c,d,1}$	$150.51 \pm 3.08^{a,b,c,d,2}$	64.41**
2	Grey	$105.10 \pm 0.82^{a,2,3}$	$119.65 \pm 0.3^{a,b,c,1,3,4}$	$131.59 \pm 0.86^{a,b,c,1,3}$	$136.00 \pm 0.15^{a,b,d,1,4}$	$138.39 \pm 3.20^{a,b,e,3}$	$169.68 \pm 4.97^{a,b,c,d,e,1,2,3,4}$	60.25**
3	White	$118.17 \pm 3.87^{a,1,3,5}$	$141.73 \pm 1.93^{a,b,1,2,3,4,5}$	$151.55 \pm 3.89^{a,c,1,2,3,4,5}$	$168.75 \pm 0.71^{a,b,c,d,1,2,3,4,5}$	$154.06 \pm 3.08^{a,d,e,3,4,5}$	$170.28 \pm 3.21^{a,b,c,e,1,2,3,5}$	41.13**
4	F value	46.09**		47.84**		13.72**		

Figures in the parentheses represent number of observations; mean ± SE bearing similar alphabetical and numerical superscript differ in rows and columns respectively at $p < 0.01$, **significant at $p < 0.01$

Table 3 Serum total protein (g/dl) profile of three varieties of Japanese quail at different age groups

Sl No.	Age	Chick (0–3 weeks)		Grower (4–5 weeks)		Adult (> 5 weeks)		F value
	Sex	Male (05)	Female (05)	Male (05)	Female (05)	Male (05)	Female (05)	
1	Brown	$1.32 \pm 0.12^{a,1}$	$2.53 \pm 0.03^{a,b,1,2}$	$2.43 \pm 0.12^{a,c,1}$	$3.96 \pm 0.15^{a,b,c,d,1,2}$	$3.37 \pm 0.03^{a,b,c,e,1}$	$4\ .93 \pm 0.14^{a,b,c,d,e,1,2}$	109.6**
2	Grey	$1.55 \pm 0.03^{a,2,3}$	$2.07 \pm 0.06^{a,b,1,3,4}$	$2.17 \pm 0.01^{a,c,2,3}$	$3.89 \pm 0.08^{a,b,c,d,1,3,4}$	$3.54 \pm 0.03^{a,b,c,d,e,1,2,3}$	$4.88 \pm 0.08^{a,b,c,d,e,1,2,3,4}$	541.8**
3	White	$1.89 \pm 0.22^{a,1,2,3,4}$	$2.91 \pm 0.06^{a,b,1,2,3,4}$	$2.93 \pm 0.14^{a,c,1,2,3,4}$	$3.24 \pm 0.01^{a,d,1,2,3,4}$	$4.10 \pm 0.28^{a,b,c,d,e,1,2,4,5}$	$5.14 \pm 0.22^{a,b,c,d,e,1,2,3,5}$	36.2**
4	F value	143.70**		44.03**		28.25**		

Figures in the parentheses represent number of observations; mean ± SE bearing similar alphabetical and numerical superscript differ in rows and columns respectively at $p < 0.01$, **significant at $p < 0.01$

Table 4 Serum albumin (g/dl) profile of three varieties of Japanese quail at different age groups

Sl No.	Age	Chick (0–3 weeks)		Grower (4–5 weeks)		Adult (> 5 weeks)		*F* value
	Sex	Male (05)	Female (05)	Male (05)	Female (05)	Male (05)	Female (05)	
1	Brown	$1.02 \pm 0.01^{a,1}$	$1.97 \pm 0.07^{a,b,1,2}$	$1.98 \pm 0.02^{a,c,1}$	$2.76 \pm 0.01^{a,b,c,d,1,2}$	$2.09 \pm 0.05^{a,d,e,1}$	$3.36 \pm 0.21^{a,b,c,d,e,1,2}$	70.32**
2	Grey	$1.00 \pm 0.30^{a,2,3}$	$1.88 \pm 0.24^{a,b,1,3}$	$1.90 \pm 0.05^{c,1,2,3}$	$2.06 \pm 0.02^{d,2,3,4}$	$2.17 \pm 0.02^{a,c,e,2,3}$	$3.04 \pm 0.08^{a,b,c,d,e,1,3,4}$	17.75**
3	White	1.26 ± 0.01^{a}	$1.72 \pm 0.11^{a,b}$	$1.91 \pm 0.05^{a,c,2,3}$	$2.06 \pm 0.02^{a,b,d,2,3,4}$	$2.02 \pm 0.02^{a,b,e,2,4,5}$	$2.89 \pm 0.04^{a,b,c,d,e,1,2,3,5}$	92.6**
4	*F* value	39.01**		44.23**		6.53**		

Figures in the parentheses represent number of observations; mean ± SE bearing similar alphabetical and numerical superscript differ in rows and columns respectively at $p < 0.01$, **significant at $p < 0.01$

Table 5 Serum globulin (g/dl) profile of three varieties of Japanese quail at different age groups

Sl No.	Age	Chick (0–3 weeks)		Grower (4–5 weeks)		Adult (>5 weeks)		*F* value
	Sex	Male (05)	Female (05)	Male (05)	Female (05)	Male (05)	Female (05)	
1	Brown	$0.32 \pm 0.001^{a,1}$	$0.81 \pm 0.14^{b,1,2}$	$0.45 \pm 0.06^{c,1}$	$1.26 \pm 0.34^{a,c,1,2}$	$1.27 \pm 0.02^{a,c,1}$	$1.587 \pm 0.05^{a,b,c,1,2}$	10.21**
2	Grey	$0.55 \pm 0.11^{a,3}$	$0.19 \pm 0.04^{a,b,2,3,4}$	$1.04 \pm 0.007^{a,b,c,3}$	$2.20 \pm 0.01^{a,b,c,d,1,2,3,4}$	$1.37 \pm 0.02^{a,b,c,d,e,3}$	$1.84 \pm 0.04^{a,b,c,d,e,1,3,4}$	813.6**
3	White	$0.63 \pm 0.07^{a,1,4,5}$	$1.19 \pm 0.01^{a,b,1,2,3,4,5}$	$1.02 \pm 0.01^{a,c,4}$	$1.18 \pm 0.003^{a,d,1,4}$	$2.08 \pm 0.02^{a,b,c,d,1,2,3}$	$2.25 \pm 0.155^{a,b,c,d,1,2,3,4}$	80.34**
4	*F* value	25.58**		15.6**		32.2**		

Figures in the parentheses represent number of observations; mean ± SE bearing similar alphabetical and numerical superscript differ in rows and columns respectively at $p < 0.01$, **significant at $p < 0.01$

Conclusion

The present study provides five different serum biochemical values of male and female Japanese quails and its effect on different age groups which can be used as reference value for future investigation of quails and also other poultry birds. The changes in biochemical parameters may be due to the factors such as breeding or egg-laying, onset of maturity, sexual dimorphism, water deprivation, temperature variation, and other environmental parameters. Throughout the study, the brown-feathered Japanese quails were found to have more concentration of serum glucose than grey- and white-feathered ones. The highest concentration of serum cholesterol and serum total protein was observed in white-feathered quails. However, further research on serum biochemical parameters is suggested for pathological interpretation of avian species both in farming and non-farming condition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The guidelines applicable for the investigation has appropriately followed international, national, and/or institutional guidelines for the care and use of animals. As per the discussion with the members of the Animal Ethical Committee of the University, the permission of the ethical committee is not necessary since the animals are neither sacrificed nor killed nor anesthetised nor narcotized nor etherised nor harmed during the investigation. Further, for this particular investigation, only 2 ml of blood per species of different age groups and different sexes is required which is collected during the routine test of the birds of Central Poultry Development Organisation (CPDO), Government of India, Odisha. This causes neither any injury nor pain to the birds as blood is collected by professional veterinary doctors of the farm.

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