Morphometrical Analysis of Blood Cells of Japanese quail (*Coturnix coturnix japonica*) in two different Age Groups

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Abstract

Japanese quail is an important farm bird in today's poultry industry and, therefore, study of its blood cells can reveal the physiological condition of the bird. Reports on the dimensions of blood cells with respect to age (fifth week and ninth week) and sex are not adequate which the objective of this study is. Blood of these birds were collected; smears were prepared and stained for morphometrical analysis. Significant difference (P < 0.01) was recorded between fifth week (before maturity) male and ninth week (egg- laying) female with respect to erythrocyte length and lymphocyte breadth. Females of both age groups differ significantly (P < 0.01) with respect to length of erythrocyte nucleus. These differences are believed due to difference in physiological conditions in these two age- groups. The impact of environmental and physiological factors on these birds can be revealed through this type of study.

Keywords: Morphometry, blood cell, Japanese quail, age group.

Introduction

In India, breeding of Japanese quail (*Coturnix coturnix japonica*) has been introduced very recently for egg and meat production. In near future Japanese quails may acquire an important segment in rapidly expanding Indian poultry industry as they are rapidly gaining popularity for commercial exploitation[1]. The Japanese quail has the potential to serve as an excellent and cheap source of animal protein and therefore now being bred for meat and eggs[2]. Cytomorphometry of blood cells, an important aspect of hematology, can reveal the physiological condition of the organism. Investigations on measurement of blood cells are well described in fishes[3], amphibians[4,5] and reptiles[6]. The measurements of erythrocytes are available in case of fishes, amphibians and reptiles[7]. Blood cell morphology has been undertaken on several species of herpetofauna[8]. Cytomorphometry of erythrocytes only have been reported in some birds[9-13]. The morphology and morphometry of both erythrocytes and leukocytes are discussed by in adult male ostrich[14]. But the studies on nucleus morphometry of blood cells are inadequate in birds[15]. Measurement of both cellular and nuclear length and breadth of erythrocytes, cellular and nuclear diameter of lymphocytes and monocytes and cellular diameter of granulocytes and cellular dimensions of thrombocytes are reported in different chickens[16]. But comparison of these parameters between those birds especially with respect to age and sex are not reflected in their study. In case of Japanese quails, data on blood cell morphometry are scanty. Moreover, according to physiological condition such as age, cell morphology also changes[17]. So, in this study an attempt has been made to report age-wise differences in cytomorphometry of erythrocytes and leukocytes of Japanese quail.

Material and Methods

The investigation was conducted on Japanese quail (*Coturnix coturnix japonica*) being maintained at Central Poultry Development Organization (CPDO), Eastern Region (ER), Government of India, Bhubaneswar, Odisha, India under standard managemental practices. Blood samples were collected from a total of eight birds. Out of which four (2 males and 2 females) were of fifth week and another four (2 males and 2 females) ninth week old. Samples were taken out with the help of sterile 25 gauge needles from the wing vein known as ulnar vein of the birds' aseptically[18]. Blood smears were prepared at site on clean grease free slides [Blue Star Pic-2, Polar Industrial Corporation, Mumbai, Maharashtra, India] and air dried and fixed in methanol [Qualigens Product No.34457, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Maharashtra, India] for staining[19]. The slides were stained with Giemsa stain[20] prepared from Giemsa powder [Qualigens CAS NO. 51811-82-6 Product No. 39382, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Maharashtra, India] as per protocol in Cytogenetics laboratory for cytomorphometrical analyses on subsequent days[21]. Cytomorphometry of cells and nuclei of blood cells of both age groups with sexual dimorphism was undertaken with the help of Microscope Eyepiece Digital Camera [CatCam130 – 1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India] attached to Hund Wetzlar Microscope [MICROSCOPE H 600 WILOZYT PLAN, Serial No. 1024980, Helmut Hund GmbH, Wetzlar-Nauborn, Germany] and computer. The entire data (30 observations per type of cell, which varied according to availability on the smeared slides) from males and females of both age-groups were subjected to Paleontological Statistics (PAST) Version 2.17 [Natural History

Museum, University of Oslo] for One-Way Analysis of Variance (ANOVA) followed by Tukey's pair wise comparison tests. Differences were classified as significant at $P < 0.05$ and $P < 0.01$.

Results and Discussion

The age-wise morphometrics of various types of blood cells of Japanese quail are recorded in micron meter (µm) (table 1) and analyzed. The findings of this study revealed the effect of age on size of erythrocytes and leukocytes (lymphocytes, monocytes, eosinophils, heterophils and basophils) of Japanese quail with sexual dimorphism. Difference between age groups and sex are shown by red blood cells, lymphocytes, heterophils and basophils and eosinophils at $P < 0.05$. Monocytes are not significantly different between or among the groups. The cell length of erythrocytes shows high significant difference ($P = 0.008$) between fifth week old male and ninth week old female. The nucleus length of erythrocyte differs ($P = 0.001$) between the females of both age groups. But the cell breadth and nucleus breadth of erythrocytes do not differ significantly between and within groups. The dimensions of erythrocytes (both cell and nucleus) agree with the earlier studies[22] and corroborates with that of common quail[23] but differ with other such studies[10]. Further, the breadth of erythrocytes is larger in females of both groups which are against the previous findings[13]. In present study, erythrocytes are larger in ninth week female, which corroborates with the data[11] where increase in size of erythrocytes with increase in age of female ostrich is observed. During egg- laying stage, females become anaemic[24]. The sizes of erythrocytes in peripheral blood increase during macrocytic anaemia[25]. From this, it is apparent that since birds in egg-laying stage are prone to anaemia, erythrocytes become large in size. The measurement of nucleus breadth of red blood cells is according to that of pheasants[12]. The length of erythrocytes are considerably larger compared to leukocytes but breadth-wise they are smaller. This may be due to their elliptical shape. Dimensions of erythrocytes, lymphocytes and granulocytes of these birds are related to the range for chickens except that of monocytes[16].

The length and breadth of leukocytes do not vary much due to their round shape. Among agranulocytes (lymphocytes and monocytes), lymphocytes are larger than monocytes. The cell length, nucleus length and nucleus breadth of lymphocytes do not show significant differences between age- groups and among males and females of those age groups. The fifth week male and ninth week female reflect difference ($P = 0.003$) in their breadth of lymphocytes.

Likewise among granulocytes (eosinophil, heterophil and basophil), heterophils are larger than eosinophils followed by basophils. Cellular measurements of erythrocytes and eosinophils of fifth week bird approximately match with the work earlier[17]. Eosinophil differs significantly among the rows with respect to cell length at $P < 0.01$ and cell breadth at $P < 0.05$. This is the only parameter which differs at $P < 0.05$ among the row. A significant sexual dimorphism ($P = 0.004$) is shown for the length of eosinophil of ninth week birds. The dimensions of eosinophils of Japanese quail recorded in this study corroborate with the reports of previous authors[26]. According to their findings, the size of granulocytes decreases with increase in age. But present report shows the cellular dimensions of these cells increase with age in case of females, except basophils. The size of eosinophil is considerably smaller than sarus crane[15] and adult male ostrich[14]. ANOVA showed the difference in cell length of heterophil is significant ($P < 0.01$) in the row. But Tukey's comparison test does not find any difference within and between groups with respect to heterophil cell length. The females of these two age groups reflect difference ($P = 0.001$) in the breadth of heterophil. All the values of basophils differed significantly ($P < 0.01$) within the rows.

The basophil morphometrics also differed according to sexual dimorphism in fifth week individuals and not between age groups. Since, numbers of basophils are very few, apparent significant difference was shown by them. Therefore, no Standard Error of Mean (SEM) was reflected by female of fifth week age. Morphometric measurements of monocytes, heterophils and basophils corroborate with other birds[15].

The number of observations (30) in each case varied upon the availability of that particular type of cells as reflected by basophils. Sexual dimorphism was reflected by fifth week Japanese quail in case of basophils which may be due to above stated reasons and ninth week Japanese quail in case of eosinophil cell length. Similarly, age-wise difference of parameters in the same sex was expressed in few cases, viz., erythrocyte nucleus length and heterophil cell breadth. Significant differences noticed between males of fifth week age (just before sexual maturity) and females of ninth week age (in peak egg-production) may be due to their difference in physiological condition[27]. According to them, quails attain sexual maturity at sixth week age and are in full egg production by 50 days, i.e., almost seventh week.

Conclusion

The study revealed that as a whole, effect of age on morphometry of some blood cells of Japanese quails (*Coturnix coturnix japonica*) reared in farm conditions of CPDO, ER, Bhubaneswar exists. But, certain genetic and non-genetic factors such as, onset of maturity, sexual dimorphism, breeding or egg- laying and environment affect the shape and size of blood cells. Finally, it is important to consider these factors and detailed investigation is suggested to arrive at accurate clinical and physiological interpretations.

Table-1
Age-wise blood cell morphometry (in µm) of Japanese quail (*Coturnix coturnix japonica*)

Type of cell	Cell/ Nucleus	Parameters	5th Week		9th Week		F-Value
			Male (30)	**Female (30)**	**Male (30)**	**Female (30)**	
Erythrocyte	Cell	Length	11.34±0.1[A]	10.63±0.19	10.90±0.20	10.46±0.17[B]	3.97[**]
		Breadth	6.00±0.21	6.13±0.07	5.75±0.19	6.02±0.13	0.97[NS]
	Nucleus	Length	3.93±0.16	3.56±0.14[A]	4.11±0.22	4.59±0.22[B]	4.97[**]
		Breadth	3.01±0.05	2.89±0.05	2.91±0.13	2.90±0.06	0.46[NS]
			Male (30)	**Female (28)**	**Male (30)**	**Female (25)**	
Lymphocyte	Cell	Length	8.40±0.24	9.32±0.25	8.77±0.31	9.14±0.22	2.38[NS]
		Breadth	7.00±0.21[A]	7.97±0.27	7.46±0.23	8.30+0.31[B]	4.85[**]
	Nucleus	Length	5.84±0.18	6.01±0.13	6.14±0.14	6.39±0.25	1.57[NS]
		Breadth	5.36±0.21	5.49±0.17	5.23±0.21	5.76±0.24	1.09[NS]
			Male (14)	**Female (23)**	**Male (27)**	**Female (25)**	
Monocyte	Cell	Length	9.04±0.45	8.20±0.23	8.04±0.28	8.48±0.29	1.63[NS]
		Breadth	7.73±0.38	7.47±0.27	7.33±0.26	7.52±0.27	0.26[NS]
			Male (21)	**Female (10)**	**Male (30)**	**Female (30)**	
Eosinophil	Cell	Length	8.58±0.23	8.92±0.38	8.35±0.19[A]	9.83±0.30[B]	7.22[**]
		Breadth	7.96±0.27	8.61±0.57	7.96±0.22	8.78±0.23	2.56[*]
			Male (16)	**Female (16)**	**Male (30)**	**Female (30)**	
Heterophil	Cell	Length	9.32±0.40	8.71±0.52	8.43±0.20	9.58±0.22	3.61[**]
		Breadth	8.63±0.31	7.39±0.52[A]	8.17±0.19	9.07±0.25[B]	5.28[**]
			Male (5)	**Female (1)**	**Male**	**Female (12)**	
Basophil	Cell	Length	8.42±0.93[A]	6.24±0.00[B]	5.82±0.76 (**6**)	6.60±0.25	4.86[**]
		Breadth	7.98±0.84 [A]	6.24±0.00 [B]	5.77±0.76 (**6**)	6.31±0.16	4.61[**]

[1]Mean± SE with different superscripts (A, B) in the same row differ significantly at p<0.01. [2]Significant at *p<0.05, highly significant at **p<0.01. [3]Figures in parentheses represent number of cells observed in each case.

Acknowledgements

References

1. Deka K and Borah J., Haematological and biochemical changes in Japanese Quails *Coturnix coturnix japonica* and chickens due to *Ascaridia galli* infection, *Int J Poult Sci.,* **7(7)**, 704-710 (**2008**)

2. Babangida S. and Ubosi C.O., Effects of varying dietary protein levels on the performance of laying Japanese quail (*Coturnix coturnix japonica*) in a semi- arid environment, *Niger J Anim Prod.*, **33(1)**, 45-52 (**2006**)

3. Homątowska A., Wojtaszek J. and Adamowicz A., Haematological indices and circulating blood picture in the sunbleak, *Leucaspius delineates* (Heckel, 1843), *Zool Pol.,* **47(3-4)**, 57-68 (**2002**)

4. Atatür M.K., Arikan H. and Çevik I.E., Erythrocyte sizes of some anurans from Turkey, *Turk J Zool.*, **23(2)**, 111-114 (**1999**)

5. Tok C.V., Tosunoğlu M., Ayaz D., Çiçek K. and Gül Ç., Hematology of Lycian Salamander, *Lyciasalamandra fazilae, North West J Zool.*, **5(2)**, 321-329 (**2009**)

6. Orós J., Casal A.B. and Arencibia A., Microscopic studies on characterization of blood cells of endangered sea turtles. Microscopy: Science, Technology, Applications and Education. Formatex Research Center, Badajoz, Spain., **1**, 75-84 (**2010**)

7. Hartman F.A. and Lessler, M.A., Erythrocytes measurements in fishes, amphibia and reptiles, *The Biol Bull*., **126(1)**, 83-88 (**1964**)

8. Arikan H. and Çiçek K., Morphology of peripheral blood cells from various species of Turkish herpetofauna, *Acta Herpetol*., **5(2)**, 179-198 (**2010**)

9. Smith E.M. and Hattingh J., The lability of some haematological parameters in chickens and ducks, *S Afr J Anim Sci.,* **9(1)**, 11-15 (**1979**)

10. Pampori Z.A., and Iqbal S., Haematology, serum chemistry and electrocardiographic evaluation in native chicken of Kashmir, *Int J Poult Sci.,* **6(8)**, 578-582 (**2007**)

11. Sabino A.J., Trevelin S.C., Almeida B.F.M., Peiró J.R. and Ciarlini P.C., Erythrogram and erythrocytes measurement of ostriches (*Struthio camelus*) in São José do Rio Preto-SP, Brazil, *Braz J Vet Res Anim Sci. São Paulo*., **48(3)**, 234-238 (**2011**)

12. Keçeci[*] T. and Çöl R., Haematological and biochemical values of the blood of pheasants (*Phasianus colchicus*) of different ages, *Turk J Vet Anim Sci.,* **35(3)**, 149-156 (**2011**)

13. Nowaczewski S. and Kontecka H., Haematological indices, size of erythrocytes and haemoglobin saturation in broiler chickens kept in commercial conditions, *Anim Sci Pap Rep*., **30(2),** 181-190 (**2012**)

14. Tadjalli M., Nazifi S., Abbasabadi B.M. and Majidi B., Histomorphometric study on blood cells in male adult ostrich, *Vet Res Forum,* **4(3)**, 199-203 (**2013**)

15. Narkkong N.A., Aengwanich W. and Tanomthong A., Morphology and morphometrics of hematological cells from eastern sarus crane, *Grus antigone sharpii. Comp Clin Path*., **20(4)**, 299-304 (**2011**)

16. Lucas A.M. and Jamroz C., Atlas of Avian Haematology, United States Department of Agriculture, Washington, *Agricultural Monograph,* **25,** 211-215 (**1961**)

17. Tadjalli M., Nazifi S. and Eemanparvar A., Normal cellular morphology of the blood of Japanese quail (*Coturnix coturnix japonica*), *Comp Clin Path*., **12(2)**, 102-105 (**2003**)

18. Talebi A., Asri-Rezaei S., Rozeh-Chai R. and Sahraei R., Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian), *Int J Poult Sci*., **4(8)**, 573-579 (**2005**)

19. Sonia C., Rajini R.A., Babu M. and Vairamuthu S., The effect of age, sex and rearing system on differential count in Guinea fowl, *Indian J Poult Sci.,* **47(2)**, 251-253 (**2012**)

20. Clark P. Boadrman W.S.J. and Raidal S.R., Atlas of Clinical Avian Hematology. Wiley- Blackwell, West Sussex, United Kingdom, 19-22 (**2009**)

21. Lillie R.D., Methods for testing biological stains. H.J. Conn's Biological Stains. The Williams and Wilkins Company, Baltimore, United States of America **9th ed**., 606-607 (**1977**)

22. Hartman F.A. and Lessler M.A., Erythrocytes measurements in birds. *Auk.,* **80(4)**, 467-473 (**1963**)

23. Ritchie B.W., Harrison G.J. and Harrison L.R., Avian Medicine: Principles and Application, Wingers Publishing, Inc., Lake Worth, Florida., 1341 (**1994**)

24. Williams T.D., Challenger W.O., Christians J.K., Evanson M., Love O. and Vezina F., What causes the decrease in haematocrit during egg production? *Funct Ecol.,* **18(3)**, 330-336 (**2004**)

25. Aslinia F., Mazza J.J. and Yale S.H., Megaloblastic anemia and other causes of macrocytosis, *Clin Med Res*., **4(3)**, 236-241(**2006**)

26. Aengwanich W. and Tanomtong A., Blood cell characteristics and hematological values of free ranging-red jungle fowl *(Gallus gallus*) in northeastern Thailand, *J Biol Sci*., **7(4)**, 689-692 (**2007**)

27. Randall M. and Bolla G., Raising Japanese quail. *Primefacts, NSW DPI*., **2**, 1-4 (**2008**) Accessed 31st December 2013. http://www.dpi.nsw.gov.au/__data/assets/pdf_ file/0005/145346/Raising-Japanese-quail.pdf. (**2008**)