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Hematological and Cytometrical Parameters of Japanese Quails *Coturnix Coturnix Japonica*, Temminck & Schlegel, 1848 as Per Sex and Different Stages of Growth

Ananya Bhattacherjee (ananyabhattacherjee86@gmail.com)
Utkal University https://orcid.org/0000-0002-3617-2125
Prafulla Kumar Mohanty
Khallikote University
Bandi Kumar Mallik
Central Poultry Development Organisation Eastern Region, Bhubaneswar

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Abstract

This study delineates influence of sex and age on haematological and cytometrical parameters in Japanese quail. Sixty birds were sampled, out of which 20 belong to each age-group (chicks, growers and adults). Haemoglobin, Total Erythrocyte Count, Packed Cell Volume, Erythrocyte indices, Total Leukocyte Count and Differential Leukocyte Count for all 3 age groups and cytometry of blood cells for chicks were analysed. Haemoglobin and packed cell volume of chicks differs significantly with other age-groups at $p < 0.01$. Significant differences at $p = 0.01$, $p < 0.01$ and $p < 0.05$ observed in lymphocytes, monocytes and Heterophil to Lymphocyte ratio between chicks and growers. Length, length to breadth, nuclear length, breadth, area and Nucleus to Cytoplasm of erythrocytes, length and area of lymphocytes, breadth and area of heterophils also vary significantly at $p < 0.01$ between Japanese quail chick male and female. Moreover, breadth of erythrocyte and length of heterophil differs significantly at $p < 0.05$ between two groups. Thus, age and sex influence physiology and hence haematology and may therefore affect egg and meat production.

Introduction

The Japanese quail (JQ), *Cortunix coturnix japonica*, belongs to the family Phasianidae and the genus *Cortunix* (Mizutani 2003). The bird originally domesticated round the 11th century as a pet song bird, is now highly valued as an animal food in several parts of the planet, because it constitutes a crucial source of eggs and meat (Kayang et al. 2004; Babangida and Ubosi 2006). Rearing of JQ is promoted due to its low maintenance and high disease resistance capacity as per Deka and Borah (2008) compared to chickens and other poultry birds. JQ are hardy birds that thrive in small cages and are inexpensive to keep. Though they suffer from common poultry diseases but are fairly disease resistant. JQ mature in about 6 weeks and are usually in full egg production by 50 days of age. Life expectancy is only two to 2½ years. The adult male quail will weigh about 100–140g, while the females are slightly heavier, weighing from 120–160 g. The females can be clearly distinguished from males by their light tan feathers having black dots on the throat and upper breast. The throat in case of males is rusty brown in colour and bear breast feathers. Males also have a cloacal gland, a bulbous structure on the upper edge of the vent that secretes a white and foamy material. This unique gland can be used to assess the reproductive fitness of the male JQ as mentioned by Randall and Bolla (2008).

Haematology and cytometry (measuring the size of the cell and nucleus) facilitates management and treatment of diseases by disclosing facts regarding them (Narkkong et al. 2011). Various factors which affect haematological and cytometrical parameters are age, sex, species, breed, rearing conditions, nutrition, methodology of sampling, storing blood samples, microbial infections and egg-laying (Sparling et al. 1999). Therefore, these parameters can be used as valuable tools to determine physiological, environmental, nutritional and pathological factors. But comparison of these parameters among and between different age – groups of JQ with respect to sexual dimorphism reared in backyard are rare. So, an attempt has been made to study the influence of sex and age (chick, growers and adults) on haematological parameters and influence of sex (only chick) on cytometrical parameters in JQ.

Materials And Methods

The present study was conducted on three age groups of JQ, viz., chick, growers and adults reared by local farmers near Bhubaneswar. Chick stage includes 1st to 3rd week old birds; Grower stages include 4th to 5th week and above 5th week were considered adults (Puspamitra et al. 2014). A total of 60 birds were sampled, out of which 20 (10 males and 10 females) belong to each age-group (chicks=20, growers=20 and adults=20). Here, males and females of each age-group are abbreviated as chick male (CM), chick female (CF), grower male (GM), grower female (GF), adult male (AM) and adult female (AF).

Blood sampling was done by random sampling method during summer season at morning (8.00am to 10.00am) using sterile 2ml syringes and 25-gauge needles from ulnar vein of birds (Clark et al. 2009) and collected in Ethylene Diamine Tetra-acetic Acid (EDTA) vials and kept in ice box. Blood films were made immediately and allowed to dry and fixed in methanol.

Samples were analysed at Cytogenetics Laboratory, PG Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar. Haemoglobin (Hb %) was recorded by using Sahli's acid haematin method (Sonia et al. 2012). Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) were recorded by using Neubauer's chamber as mentioned by Dacie and Lewis (1991). Packed Cell Volume (PCV) was determined by centrifuging Wintrobe haematocrit tube at 3,500 rpm for 15 minutes. Erythrocyte indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated by using appropriate formula (Campbell 1988).

Methanol fixed blood films were stained with Giemsa stain and used for Differential Leukocyte Count (DLC) by following Nowaczewski and Kontecka (2012) to find out percentages of Lymphocytes (L%), Monocytes (M%), Eosinophils (E%), Heterophils (H%) and Basophils (B%) and to calculate Heterophil to Lymphocyte (H/L) ratio.

Cytometry of RBC, Lymphocytes [Cell length (CL), Cell Breadth (CB), Cell Area (CA), Cell Length/Cell Breadth (CL/CB), Nuclear Length (NL), Nuclear Breadth (NB), Nuclear Area (NA), Nuclear Length/Nuclear breadth (NL/NB) and Nucleus to Cytoplasmic ratio (N:C)], Monocytes, Eosinophils and Heterophils (30 observations per type of cell) and basophils (10 observations) [Cell length (CL), Cell Breadth (CB), Cell Area (CA) and Cell Length/Cell Breadth (CL/CB)] was undertaken with the help of Microscope Eyepiece Digital Camera attached to Hunds Weltzar photomicroscope and computer and calculated wherever necessary.

CA and NA (μm^2) for elliptical cells and nuclei were calculated by the formula Length × Breadth × 0.785 or L×B× (as used by Arserim and Mermer (2008). The CA and NA of circular cells and nuclei were calculated using the formula . CL/CB and NL/NB were calculated by the formula Length/Breadth (L/B). N:C were calculated by the formula NA/CA. All the above calculations were done in MS-Excel 2010.

The haematological and cytometry data were statistically analysed by using Paleontological Statistics (PAST) Version 2.17 for One-Way Analysis of Variance (ANOVA) followed by Tukey's pair wise comparison tests and by using MS-Excel for 't – test'. Significant differences were observed at $P<0.01$ and $P<0.05$.

Results

Hb% of chicks differs significantly with all other groups at $p<0.01$. PCV of CM and CF differ significantly at $p<0.01$ with both growers and AM. Significant differences at $p=0.01$, $p<0.01$ and $p<0.05$ occur in case of L% (both chicks with GM), M% (CF with GM which differs with both adults) and H/L (CM and GM). TEC, MCV, MCH, MCHC, TLC and H%, E% and B% do not differ significantly (Table 1).

CL, CL/CB, NL, NB, NA and N:C of erythrocyte, CL and CA of lymphocyte, heterophil CB and CA differ significantly at $p<0.01$ between Japanese quail CM and CF. Similarly, lymphocyte CB and heterophil CL differs significantly at $p<0.05$. No significant sexual dimorphism is reflected by morphometry of monocytes, eosinophils and basophils of JQ chicks (Table 2).

Discussion

Hb%, PCV and leucocytes (except, M% and E%), in DLC of growers and adults are within the normal range for JQ mentioned by Ritchie et al. (1994). Also, long and short diameter of erythrocytes of common quail reported by above author is similar to this study which may be due to common features between these two species. Except, MCV of adults, all other erythrocytic parameters of this study are within the range mentioned for adult JQ by Agina et al. (2017). Hb% and PCV are higher in males irrespective of age group, except PCV of CM. This may be due to presence of androgens in males as mentioned by Aengwanich et al. (2007) and CM has negligible androgen. Hb% and PCV of JQ chicks and TEC of growers are consistent with 1 month old Fayoumi and local chicks from Sylhet, Bangladesh studied by Islam et al. (2004). TEC and PCV of adult JQ are comparable to 6 to 12 month's old chicken studied by Sharmin and Myenuddin (2004), adult helmeted guinea fowl (Nalubamba et al. 2010), adult female indigenous chicken (Hachesoo et al. 2011) and eight weeks old JQ from Tamilnadu (Pandian et al. 2012). Growers and adults of this study, also have TEC like that of 6^{th} and 7^{th} week JQ from Aizawl reported by Ali et al. (2012). Freely moving birds considered in this study show very different erythrocytic parameters compared to Cage-raised JQ reported by Aina and Ajibade (2014) which may be due to difference in rearing process. Hb% content of chick and adult JQ in our present study is consistent with earlier records of Puspamitra et al. (2014) for same categories. JQ CM and GM and both adults have comparable PCV, Hb & MCV and TEC respectively with 4^{th} week male, 7^{th} week male & 8^{th} week female and 25^{th} week NN chicken respectively studied by Sen et al. (2017). Hb% content of JQ adults matches with the Hb% content of 8 weeks old three lines of quail and their crosses. Eight weeks old purebred dark brown quail can be compared to GF with respect to PCV values observed by Meshabaz et al. (2017). TEC and PCV of adult JQ match with adult IR and MY ducks mentioned by Acharya et al. (2019). Consistency with the same parameters of ducks may have occurred as both belong to same clade Galloanserae as per Livezey and Zushi (2007). Moreover, changes due to storage related artefact increase size of RBC which results into increase in PCV and MCV. Wild type quails studied by Aydin et al. (2008) have similar RBC content and MCHC with that of grower female and male respectively. Onyeyili et al. (1992) worked on six to eight months old grey breasted Guinea fowl which corroborates with adult quails with respect to PCV. Findings of Olayemi (2009) about MCH of adult Guinea fowls is in accordance with adult JQ from this study.

TLC of chick JQ is in accordance with young Nigerian ducks as per Olayemi et al. (2003) and quails as per Kabir (2012). H/L ratio of JQ chicks is similar with 6-8 months old Thai indigenous chicken mentioned by Simaraks et al. (2004), as this parameter depends upon calculation based on L% and H%. H/L is useful to gauge the level of stress of the animals. Heteropenia can occur during severe stress. More L% and less H% recorded in this study are consistent with previous studies of Islam et al. (2004) and Aengwanich et al. (2007). Smaller birds are reported to have fewer leukocytes. But if the TLC goes below 3000, then it's not normal. Moreover, the most ignored fact is that avian leukopenia can occur due to artefacts in sample which may result into alterations. Other factors such as, clotting of blood before pushing into anticoagulant vials, lysis of blood cells before counts, transport and storage technique and duration may result into pseudo leukopenia (false shortage of leukocytes). Preparation of blood smears also effect DLC. Real leukopenia occurs due to pathological conditions such as severe bacterial, fungal, protozoan or viral infection or due to physiological stress like exposure to toxic substances, crowding etc as suggested by Clark et al. (2009). As age increases, L% come down and H% goes up and which may be a possible reason for increase in H/L ratio. Mostly, birds contain high H% but there are exceptions also whose L% are higher in DLC as described by Schmidt et al. (2009). Heterophils are at the front line while defencing against antigens or pathogens and are good at physiological stress management as explained by Lazăr et al. (2012) which may be the reason of low H% in this study. The likeliness of chick stage JQ and cage-raised chick JQ studied by Aina and Ajibade (2014), with respect to H/L may be calculative as L% and H% does not match. The exceptionally large L% and M% are indication of some sort of infection by parasites or by antigens according to Charles-Smith et al. (2014). This may be because of free – ranging of the birds as they are reared by local farmers in backyard. Aydin et al. (2008) studied recessive white quail which have a likeness in H/L ratio with that of GF, which may be mere calculative as their study do not reflect the H% and L%. Earlier study performed by Mihailov et al. (1999) on 150 days old JQ and by Nalubamba et al. (2010) on adult Zambian helmeted Guinea fowl reflects higher L% and lower H% as observed in this report, though all these studies do not have exact similarity in terms of their percentages but the trend is same. Consistency exists between L% and E% of adult female JQ with previous studies of Dalai et al. (2015). Similarly, GM JQ have similar H% with that of 7^{th} week female NN chicken and B% of both growers and AM JQ have similarity with all groups

of NN chicken reported by Sen et al. (2017). TLC of AM JQ matches with adult IR ducks of both sexes. Moreover, AM JQ is in accordance with adult IR female with respect to L%. Similarly, E% and B% of adult JQ matches with that of adult IR ducks studied by Acharya et al. (2019).

Erythrocytic dimensions and monocyte CL of JQ chicks are in accordance with the study of Tadjalli et al. (2003) on 3 weeks old JQ. Nuclear dimensions of red blood cells of capture and culture Asian eel mentioned by Ponsen et al. (2009) match with this study. Erythrocyte CL/CB of CF have similarity with 13-30 months old female ostriches reflected in the work of Sabino et al. (2011) which may be due to same sex or may be due to same formula by which this parameter is calculated as length and breadth are different in these cases. CL of erythrocytes of JQ chicks corroborates with chicken broilers of 44 days age studied by Nowaczewski and Kontecka (2012). From the study of Narkkong et al. (2011) it is clear that diameter of large lymphocyte Grus Antigone Sharpii has some similarity with dimensions of lymphocyte CL with present study, but in this study, lymphocytes are not distinguished as large or small. Also, monocyte CL and heterophil dimensions of chick JQ reflects likeliness with Eastern Sarus Crane. Monocyte dimension of marine teleost porcupine fish reported by Radhakrishnan et al. (1976) is similar to this study. Moreover, measurement of all the granulocytes is within the range mentioned by Lucas and Jamroz (1961). CL and CB of RBC and heterophils of CM and CF corroborates with 2-month-old Sikhar chicken mentioned by Mayengbam et al. (2020). The CB of basophil of CM is in accordance with size of basophil of quail studied by Shalini et al. (2018). Erythrocyte CL and CB of CM and CF respectively are in accordance with adult Rhea reported by Gallo et al. (2015) and adult Uttara fowl by Khan et al. (2016). Also, the mean diameter of heterophil of Uttara fowl matches with heterophil CL of CM from this study. Erythrocyte CL and CB of CF corroborates with female caged chicken and CM has similarity with female caged and free-roaming ducks for RBC length reported by Smith and Hattingh (1979). CB and NB of red blood cell of CF corroborate with that of mallards mentioned by Driver (1981). NB of CF is in accordance with Imperial and Golden Eagle researched by Polo et al. (1992) and pheasant chicks recorded by Keçeci and Çöl (2011).

Hb%, TEC, M% and H% are more in males compared to females when observed in all age groups which may be due to level of their sex hormones. Oestrogen in females lowers these parameters, whereas testosterone works just oppositely by enhancing these characteristics. Similar is the condition related to cytometry of RBC, monocyte and heterophil in chicks. But other parameters are more in females compared to males which may be calculative or physiological or pathological or environmental. The non-significant difference in haematological parameters between of three ages groups and sexes of JQ and cytometrical differences in CM and CF might have occurred because of physiology, pathology, nutrition, environment, duration of storage of blood samples, transport of blood samples sexually immaturity in chick stage birds or calculative. The current study establishes that haematological parameters change with respect to age and sex in JQ and similarities or dissimilarities with studies undertaken by other researchers and by us earlier might occur due to age, sex, climate, temperature, stress, hormone etc. or artefactual. This study may shed lime light for assessing the effect of above parameters on the quality of JQ poultry products and may help future researchers to take into consideration the above factors while interpreting scientific data.

Declarations

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Conflicts of interest/Competing interests: The authors declare that there is no conflict or competing interests.

Availability of data and material: Data may be available upon reasonable request.

Code availability: Not applicable

Authors' contributions: 1st (Corresponding) Author: Performed research (Collected samples, estimated different parameters) and wrote the paper (manuscript).

2nd Author: Designed the study (made plan for the research), Provided facilities for estimation of different parameters and guided in writing results and discussion.

3rd Author: Analyzed data (Performed statistical analysis).

Ethical approval: Not applicable, since the research do not involve any type of experimentation or sacrifice of the bird species concerned.

Consent to participate: Not applicable

Consent for publication: All authors give their consent for publication of the manuscript.

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Ethical Standards: The manuscript does not contain clinical studies or patient data.

References

1. Acharya, C.P., Bhattacherjee, A., Mallik, B.K. and Mohanty, P.K., 2019. Erythrogram, leukogram and blood cell measurement of Muscovy and Indian Runner ducks – a comparison, International Research Journal of Biological Sciences, 8, 8–16.
2. Aengwanich, W. and Tanomtong, A., 2007. Blood cell characteristics and hematological values of free ranging red jungle fowl *(Gallus gallus*) in northeastern Thailand, Journal of Biological Sciences, **7,** 689–692.
3. Agina, O.A., Ezema, W.S. and Iwuoha, E.M., 2017. The haematology and serum biochemistry profile of adult Japanese Quail (*Coturnix coturnix japonica*), Notulae Scientia Biologicae, 9, 67–72.
4. Aina, O.O. and Ajibade, T., 2014. Age-related changes in haematologic parameters of cage-raised Japanese quails (*Cortunix japonica*), Journal of Veterinary Medicine and Animal Health, 6, 104–108.
5. Ali, M.A., Hmar, L., Devi, L.I., Prava, M., Lallianchhunga, M.C., Tolenkhomba, T.C. 2012. Effect of age on the haematological and biochemical profile of Japanese quails (Coturnix coturnix japonica), International Multidisciplinary Research Journal, 2, 32–35.
6. Aydin, C., Petek, M. and Çibik, R., 2008. Effect of recessive colour mutation on haematological characteristics of Japanese quail (Coturnix coturnix japonica), Archiv für Geflügelkunde – European Poultry Science (EPS), 72, S.164—167.
7. Babangida, S. and Ubosi, C.O., 2006. Effects of varying dietary protein levels on the performance of laying Japanese quail (Coturnix coturnix japonica) in a semi- arid environment, Nigerian Journal of Animal Production, 33, 45–52.
8. Campbell, T.W., 1988. Avian Hematology and Cytology, (Iowa State University Press, Iowa)
9. Charles-Smith, L.E., Rutledge, M.E., Meek, C.J., Baine, K., Massey, E., Ellsaesser, L.N., DePerno, C.S., Moorman, C.E. and Degernes, L.A., 2014. Hematologic parameters and hemoparasites of nonmigratory Canada geese (*Brantacanadensis*) from Greensboro, North Carolina, USA, Journal of Avian Medicine and Surgery, 28, 16–23.
10. Clark, P., Boardman, W.S.J and Raidal, S.R., 2009. Atlas of Clinical Avian Hematology, (Wiley- Blackwell, West Sussex, United Kingdom)
11. Dacie, J.V. and Lewis, S.M., 1991. Practical Haematology, 7th edn., (Edinburgh: Churchill Livingstone, London)
12. Dalai, M., Puspamitra, S., Bhattacherjee, A., Acharya, D., Acharya, G. and Mohanty, P.K., 2015 Comparative haematology of *Anas platyrhynchos* (Anseriformes) and *Coturnix coturnix japonica* (Galliformes), Journal of Entomology and Zoology Studies, 3, 50–53.
13. Deka, K. and Borah, J., 2008. Haematological and biochemical changes in Japanese Quails *Coturnix coturnix japonica* and chickens due to *Ascaridia galli* infection, International Journal of Poultry Science, 7, 704–710.
14. Driver, E.A., 1981. Haematological and blood chemical values of mallard, Anas p. platyrhynchos, drakes before, during and after remige moult, Journal of Wildlife Diseases, 17, 413–421.
15. Gallo, S.S.M., Ederli, N.B., Bôa-Morte, M.O. and Oliveira, F.C.R., 2015. Hematological, morphological and morphometric characteristics of blood cells from rhea, *Rhea Americana* (Struthioniformes: Rheidae): a standard for Brazilian birds, Brazilian Journal of Biology, 75, 953–962.
16. Hachesoo, B.A., Talebi, A. and Razaei, S.A. 2011. Comparative study on blood profiles of indigenous and Ross-308 broiler breeders, Global Veterinaria, 7, 238–241.
17. Islam, M.S., Lucky, N.S., Islam, M.R., Ahad, A., Das, B.R., Rahman, M.M. and Siddiui, M.S.I., 2004. Haematological parameters of Fayoumi, Assil and local chickens reared in Sylhet region in Bangladesh, International Journal of Poultry Science, 3, 144–147.
18. Kabir, A., 2012. Haematological studies in chicken and a group of birds, International Journal of Medicine and Applied Science, 1, 30–38.
19. Kayang, B.B., Inoue-murayama, V.M., Miwa, M., Monovoisin, J.L., Ito, S. and Minvielle, F., 2004. A first-generation micro satellite linkage map of the Japanese quail, Animal Genetics, 35, 195–200.
20. Keçeci, T. and Çöl, R., 2011. Haematological and biochemical values of the blood of pheasants (*Phasianus colchicus*) of different ages, Turkish Journal of Veterinary and Animal Sciences, 35, 149–156.
21. Khan, I.M., Meena, M., Singh, I., Singh, B., Arora, N. and Choudhary, O.P., 2016. Cytomorphological Study on Blood Cells of Uttara Fowl (*Gallus Domesticus*), Indian Veterinary Journal, 93, 32–36.
22. Lazăr, R., Boişteanu, P.C., Muntean, C., Apetroaei (Petrescu), C-A. and Ciobanu, M.M., 2012. Characterisation of the haematological profile of the hybrid B.U.T 6 turkey raised in Romania. Lucrări Ştiinţifice – Seria Zootehnie, 58, 254–257.
23. Livezey, B.C. and Zushi, R.L., 2007. Higher order phylogeny of modern birds (Therapoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion, Zoological Journal of Linnean Society, 49, 1–95.
24. Lucas, A.M. and Jamroz, C., 1961. Atlas of Avian Haematology, (Agricultural Monograph 25, United States Department of Agriculture, Washington)
25. Mayengbam, P., Tolenkhomba, T.C. and Ali, M.A., 2020. Blood cell morphometry and leukocyte-thrombocyte profile of indigenous chicken Sikhar of Mizoram, Indian Journal of Animal Sciences, 90, 3–5.
26. Meshabaz, R.A., Sulaiman, K.M., Hussen, S.H., Berwary, S., Saleh, J.H., Ahmed, S.A. and Mohammed, N.J., 2017. Hematological Study in three lines of quail and their crosses, Science Journal of University of Zakho, 5, 249–253.
27. Mihailov, R., Lasheva, V. and Lashev, L., 1999. Some Hematological Values of Japanese Quails, Bulgarian Journal of Veterinary Medicine, 2, 137--139.
28. Mizutani, M., 2003. The Japanese quail. Laboratory Animal Research Station, (Nippon Institute for Biological Science, Kobuchizawa, Yamanashi, Japan)

29. Nalubamba, K.S., Mudenda, N.B. and Masuku, M. 2010. Indices of health; clinical haematology and body weights of free-range Guinea fowl (*Numida meleagris*) from the southern province of Zambia, International Journal of Poultry Science, 9, 1083–1086.
30. Narkkong, N.A., Aengwanich, W. and Tanomthong, A. 2011. Morphology and morphometrics of hematological cells from eastern sarus crane, *Grus antigone sharpie*, Comparative Clinical Pathology, 20, 299–304.
31. Nowaczewski, S. and Kontecka, H., 2012. Haematological indices, size of erythrocytes and haemoglobin saturation in broiler chickens kept in commercial conditions, Animal Science Papers and Reports, 30, 181–190.
32. Olayemi, F., Oyewale, J., Rahman, S. and Omolewa, O., 2003. Comparative assessment of the white blood cells, plasma volume and blood volume in the young and adult Nigerian duck (Anas platyrhynchos), Veterinary Arhiv, 73, 271–276.
33. Olayemi, F.O., 2009. Haematological changes in Guinea fowls (*Numida meleagris galeata*, Pallas) following haemorrhage, International Journal of Poultry Science, 8, 1093–1095.
34. Onyeyili, P.A., Egwu, G.O., Jibike, G.I., Pepple, D.J. and Ohaegbulam, J.O., 1992. Seasonal variations in haematological indices in the Grey-breasted Guinea fowl (Numida Meleagris Galeata Pallas), Nigerian Journal of Animal Production, 18, 108–110.
35. Pandian, C., Pandiyan, M.T., Sundaresan, A. and Omprakash, A.V., 2012. Haematological profile and erythrocyte indices in different breeds of poultry, International Journal of Livestock Research, 2, 89–92.
36. Polo, F.J., Celdran, J.F., Peinado, V.I., Viscor, G, and Palomeque, J., 1992. Hematological values for four species of birds of prey, The Condor, 94, 1007–1013.
37. Ponsen, S., Narkkong, N.A., Pamok, S. and Aengwanich, W., 2009. Comparative hematological values, morphometric and morphological observation of the blood cell in capture and culture Asian Eel, *Monopterus albus* (Zuiew), American Journal of Animal Veterinary Science, 4, 32–36.
38. Puspamitra, S., Mohanty, P.K. and Mallik, B.K., 2014. Haematological analyses of Japanese quail (*Coturnix Coturnix Japonica*) at different stages of growth. International Research Journal of Biological Sciences, 3, 51–53.
39. Radhakrishnan, S., Stephen, M. and Nair, N.B., 1976. A study on the blood cells of a marine teleost fish, *Diodon Hystrix* together with a suggestion as to the origin of lymphocytes, Proceedings of Indian National Science Academy, 42, 212–226.
40. Randall, M. and Bolla, G. Raising Japanese quail. In: Primefacts, NSW DPI. 2008. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/145346/Raising-Japanese-quail.pdf. Accessed 31st December 2013
41. Ritchie, B.W., Harrison, G.J. and Harrison, L.R., 1994. Avian Medicine: Principles and Application, (Wingers Publishing, Inc., Lake Worth, Florida).
42. Arserim, S.K. and Mermer, A., 2008. Hematology of the Uludag frog, ˘*Rana macrocnemis* Boulenger 1885, in Uludag National Park˘ (Bursa, Turkey), EU Journal of Fisheries and Aquatic Sciences, 25, 39–46.
43. Sabino, A.J., Trevelin, S.C., Almeida, B.F.M., Peiró, J.R. and Ciarlini, P.C., 2011. Erythrogram and erythrocytes measurement of ostriches (*Struthio camelus*) in São José do Rio Preto-SP, Brazil, Brazilian Journal of Veterinary Research and Animal Science SãoPaulo, 8, 234-238.
44. Schmidt, E.M., Paulillo, A.C., Martins, G.R.V., Lapera, I.M., Testi, A.J.P., Junior, L.N., Denadai, J. and Fagliari, J.J. 2009. Hematology of the Bronze Turkey (*Meleagris gallopavo*): Variations with Age and Gender, International Journal of Poultry Science, 8, 752–754.
45. Sen, S., Bhattacherjee, A., Mohanty, P.K. and Mallik, B.K. 2017. Age-wise variation in haematological and cytomorphometrical analysis of naked-neck chicken, Proceedings of National Academy of Science India Section B Biological Sciences, 87, 1199–1207.
46. Shalini, S., Mehta, S. and Sinha, R., 2018. Comparative cytomorphological studies on granulocytes of domestic fowl, duck and quail, *J Exp Zool India*, 21, 151–155.
47. Sharmin, M.L. and Myenuddin, M., 2004. Hematological values of the indigenous chickens, Bangladesh Journal of Veterinary Medicine, 2, 163–164.
48. Simaraks, S., Chinrasri, O. and Aengwanich, W., 2004. Haematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in northeastern, Thailand, Songklarakarin Journal of Science and Technology, 26, 425–430.
49. Smith, E.M. and Hattingh, J., 1979. The liability of some haematological parameters in chickens and ducks, South African Journal of Animal Science, 9, 11–15.
50. Sonia, C., Rajini, R.A., Babu, M. and Vairamuthu, S., 2012. The effect of age, sex and rearing system on differential count in Guinea fowl. Indian Journal of Poultry Science, 47, 251–253.
51. Sparling, D.W., Dag, D. and Klein, P., 1999. Acute toxicity and sub lethal effects of white phosphorous in mute swans, *Cygnus olor*, Archives of Environmental Contamination and Toxicology, 36, 316–322.
52. Tadjalli, M., Nazifi, S. and Eemanparvar, A., 2003. Normal cellular morphology of the blood of Japanese quail (*Coturnix coturnix japonica*), Comparative Clinical Pathology, 12, 102–105.

Tables

Table 1 Red and White Blood Cell parameters of three age-groups of Japanese Quail

PARAMETERS	CM (10)	CF (10)	GM (10)	GF (10)	AM (10)	AF (10)	ANOVA	p-VALUE
Hb (%)	7.96±0.25[a]	7.46±0.24[b]	11.30±0.42[a,b]	10.85±0.50[a,b]	11.95±0.25[a,b]	11.05±0.45[a,b]	25.7**	3.59E-13
TEC (10^6/mm^3)	1.35±0.24	1.23±0.17	2.93±0.19	2.57±0.77	2.43±0.36	2.21±0.31	2.132 NS	0.07547
PCV (%)	26.70±1.70[a]	27.70±2.52[b]	44±1.39[a,b]	36.5±2.63[a]	39.35±2.82[a,b]	35.21±1.89	9.031**	2.77E-06
MCV(μ³)	647.30±346.86	265.91±43.26	200.71±25.76	210.84±28.41	195.33±27.04	188.61±28.69	1.548 NS	0.1905
MCH (pg)	211.01±116.36	78.06±16.19	51.28±5.70	64.70±9.70	61.86±9.73	60.03±9.75	1.58 NS	0.1814
MCHC (%)	30.88±2.02	28.45±2.02	25.95±1.36	30.89±2.34	31.96±2.54	31.68±0.95	1.41NS	0.2352
TLC (10^3/mm^3)	11705±2809.78	10985±1623.71	10120±930.32	12730±965.37	9735±1924.57	10055±933.64	2.132NS	0.09007
L (%)	50.70±3.23[a]	49.00±2.31[b]	33.4±2.84[a,b]	46.4±3.6	43.6±3.95	42.1±4.41	3.191*	0.0135
M (%)	17.00±2.95	12.00±1.19[a]	23.1±2.59[a,b]	15.8±2.15	13±1.54[b]	11±1.64[b]	4.442**	0.00184
H (%)	17.90±3.02	23.40±1.65	29.8±3.63	21.7±3.80	27.5±2.89	24.6±3.75	0.71 NS	0.1455
E (%)	11.40±1.33	12.80±1.48	12±1.27	14.7±3.71	14.4±2.53	9.4±2.63	1.721 NS	0.6144
B (%)	3.00±0.59	2.80±0.86	1.7±0.42	1.4±0.45	1.6±0.4	2.7±0.97	1.51 NS	0.3453
H/L	0.38±0.06[a]	0.49±0.04	1.14±0.35[a]	0.53±0.10	0.70±0.10	0.64±0.09	2.361*	0.03357

**means significant at p<0.01, * means significant at p<0.05 and NS means not significant. Figures in brackets reflect number of observations.

Table 2 Blood Cell Cytometry of Japanese Quail chick

CELL TYPES	PARAMETERS	MALE (30)	FEMALE (30)	t- VALUE	p-VALUE
RBC	CL (µm)	11.25±0.20	10.41±0.14	3.28**	0.00171
	CB (µm)	5.88±0.29	6.28±0.02	1.31NS	0.19458
	CA (μm^2)	51.65±2.28	51.62±0.73	0.01NS	0.98839
	CL/CB	2.07±0.13	1.66±0.02	3.08**	0.00316
	NL (µm)	3.76±0.14	3.32±0.11	2.36*	0.02130
	NB (µm)	3.07±0.05	2.87±0.05	2.71**	0.00860
	NA (μm^2)	7.32±0.81	3.34±0.61	3.90**	0.00025
	NL/NB	1.22±0.04	1.18±0.05	0.61NS	0.54056
	N:C	0.14±0.01	0.06±0.01	3.88**	0.00026
LYMPHOCYTES	CL (µm)	8.29±0.26	9.75±0.24	4.06**	0.00014
	CB (µm)	6.92±0.19	7.71±0.28	2.29*	0.02548
	CA (μm^2)	42.36±2.88	56.84±4.15	2.86**	0.00586
	CL/CB	1.21±0.04	1.29±0.04	1.25NS	0.21411
	NL (µm)	5.76±0.22	5.82±0.15	0.22NS	0.82314
	NB (µm)	4.97±0.23	5.38±0.11	1.55NS	0.12458
	NA (μm^2)	18.76±2.17	19.24±1.90	0.16NS	0.86814
	NL/NB	1.21±0.05	1.09±0.04	1.68NS	0.09648
	N:C	0.49±0.05	1.49±0.79	1.25NS	0.21425
MONOCYTES	CL (µm)	9.02±0.29	8.42±0.21	1.64NS	0.10553
	CB (µm)	7.63±0.25	7.36±0.24	0.75NS	0.45141
	CA (μm^2)	37.68±5.28	37.43±4.66	0.03NS	0.97119
	CL/CB	1.21±0.05	1.16±0.03	0.78NS	0.43746
HETEROPHILS	CL (µm)	9.21±0.29	8.36±0.28	2.09*	0.04059
	CB (µm)	8.48±0.20	7.26±0.37	2.84**	0.00613
	CA (μm^2)	53.10±4.75	35.43±4.50	2.69**	0.00914
	CL/CB	1.09±0.03	1.24±0.09	1.49NS	0.14144
EOSINOPHILS	CL (µm)	8.34±0.19	8.71±0.22	1.25NS	0.21318
	CB (µm)	7.88±0.22	8.40±0.32	1.31NS	0.19363
	CA (μm^2)	34.08±4.64	43.99±6.13	1.28NS	0.20279
	CL/CB	1.07±0.02	1.06±0.03	0.27NS	0.78252
BASOPHILS	CL (µm)	8.42±0.62 (10)	8.58±0.66 (10)	0.17NS	0.86639
	CB (µm)	7.98±0.56 (10)	8.11±0.50 (10)	0.16NS	0.87221
	CA (μm^2)	22.13±10.35 (10)	24.74±12.19 (10)	0.16NS	0.87226
	CL/CB	1.05±0.02 (10)	1.05±0.02 (10)	0.15NS	0.87766

**means significant at p<0.01, * means significant at p<0.05 and NS means not significant. Figures in brackets reflect number of observations