

Hematological and Plasma biochemical reference values in Chukar Partridge (*Alectoris chukar*) under captive facilities

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ABSTRACT

The current study was conducted to determine hematology and blood chemistry of Chukar Partridge kept in captive conditions in Punjab Province of Pakistan. During this study, total 28(10 male, 18 female) blood samples of healthy birds were collected from four different captive facilities under the control of Punjab Wildlife and Parks Department, Government of Punjab, Pakistan. Hematological parameters including Total Red Blood Cell Count, Total White Blood Cell Count, Packed Cell Volume, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Platelets, Lymphocytes, Monocytes and Neutrophils were determined. The overall red blood cells count, hemoglobin and hematocrit values were $2.00 \pm 0.054 \times 10^6/\mu\text{L}$, $11.40 \pm 0.350 \text{ g/dL}$ and $31.30 \pm 0.830\%$, respectively. White blood cells count $220.10 \pm 0.857 \times 10^3/\mu\text{L}$ and platelets values $5.56 \pm 1.431 \times 10^3/\mu\text{L}$ were recorded. The sex wise hematological parameter was statistically non-significant ($p > 0.05$) except MPV and P-LCP values which were significantly different ($p < 0.05$). The plasma chemical values including the Urea, Alanine aminotransferase, Aspartate aminotransferase, Creatinine, Total Protein and Albumin were determined. The overall biochemical blood values of Urea $282.06 \pm 37.18 \text{ mg/dL}$, ALT $13.61 \pm 2.597 \mu\text{L}$, AST $48.23 \pm 28.157 \mu\text{L}$, Creatinine $61.04 \pm 12.658 \text{ mg/dL}$, Total protein $9.29 \pm 1.228 \text{ mg/dL}$ and Albumin $2.44 \pm 0.108 \text{ mg/dL}$ and were recorded. All these parameters between male and female were non-significant ($p > 0.05$). As a pioneer work, these hematological and blood chemistry values may serve as reference range in male and female Chukar Partridge in captivity.

Keywords: Hematological parameter, Plasma chemical, Blood profiling, Captive chukar partridge.

Original Research Article

INTRODUCTION

Chukar Partridge (*Alectoris chukar*; Order Galliformes; Family Phasianidae) is the national bird of Pakistan and has natural habitat ranging from Pakistan to Afghanistan in Asia. *Alectoris chukar* shows great similarity to the Red-legged partridge (*Alectoris rufa*) which exists in Western areas of the world and was first time introduced to North America in 1893 as a game bird

(Christensen, 1996; Shen *et al.*, 2010). Globally it is present in Palestine, Turkey, Iran, Lebanon, India, Central Nepal, Middle East, Afghanistan, Pakistan and Western Himalayas. In Pakistan, it is distributed in Sindh, Chitral, Swat, Kohistan, Gilgit, Punjab, Baluchistan, and Azad Jammu Kashmir (Pathan *et al.*, 2014). It is mainly found at an altitude of 2000 to 4000 meters except in Pakistan, where it occurs at 600 meters (Rasmussen & Anderton 2005) and adaptable to all kind of arid,

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rocky and high mountain valleys (Awan *et al.*, 2013). It's a medium sized bird with 13-15 inches in length. Wings and back are light greyish brown; belly, throat, cheeks are whitish in colour; however throat is surrounded by a band of black colour. Its eyelids, legs and feet are pink or dark red. The tail has fourteen feathers. Both sexes look alike as far as physical characteristics are concerned (Thiollay *et al.*, 1994). It breeds once in a year depending on environmental conditions, usually their breeding season ranges from April to July. Chukar partridges are herbivores and eat leaves, seeds, grains, nuts and fruits, and sometimes eat insects if available (Christensen, 1996) and is polytypic species with 16 reported subspecies in the world Song & Liu, 2013).

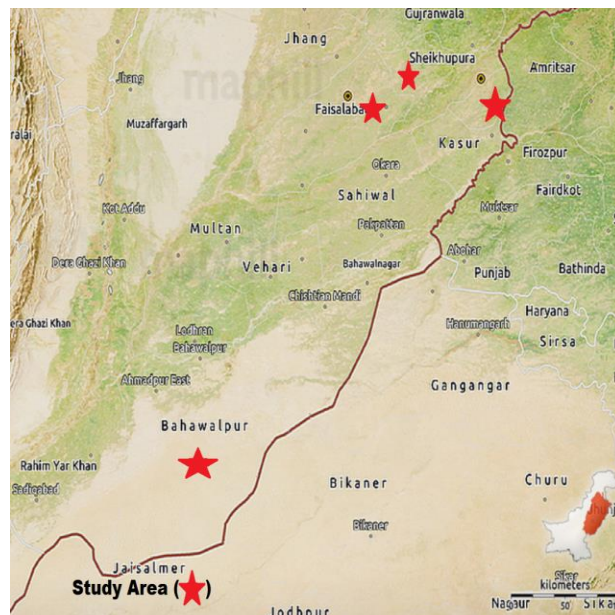
In recent years this bird has been reared extensively and farm-reared partridges are raised annually for releasing into the environment and for meat and eggs production as a source of protein and also for hunting. Hematology and biochemical analysis provide information to monitor health as well as the general condition of the birds. Further, blood biochemical analysis in several avian species have been used for the diagnosis and treatment of diseases for a long time (Kaneko *et al.*, 1997; Opara *et al.*, 2006). Although there have been several studies about plasma chemistry values for some species of genus *Alectoris* (Lloyd & Gibson, 2006; Ozbey & Esen, 2007; Perez-Rodriguez *et al.*, 2008), published data of the serum biochemistry of *Alectoris chukar* is limited (Woodard *et al.*, 1983; Ozek and Bahtiyarca, 2004; Suchy *et al.*, 2010). The purpose of the present study was to investigate reference values for hematology and serum biochemistry parameters of chukar partridges and to determine the variation in these values between male and female birds kept in captivity in Punjab, Pakistan.

MATERIALS AND METHODS

Study area

Blood samples of Chukar Partridge were collected from four different Wildlife breeding centres of Punjab including three Government breeding sites and one private breeding site registered under Punjab Wildlife and Parks Department, Government of Punjab, Pakistan: Bahawalpur Zoo, Bahawalpur (29°24'8.7"N 71°40'54.5"E), Gatwala Wildlife Breeding Centre,

Faisalabad (31°28'42.7"N 73°12'36.7"E), Jallo Wildlife breeding farm, Lahore (31°34'21"N 74°28'38"E) and Wali private breeding farm, Sheikhupura (30°59'06.4"N 74°39'35.0"E).



Collection of blood sample

A total of 28 blood samples (10 male and 18 female) were collected from healthy adult Chukar Partridge during field visit in months of January to March 2018. Feed was withdrawn 2h before blood collection. Blood collection was performed between 10:00 am and 12:00 am. After capturing the bird properly and gently, the Basilic vein was venipuncture for collecting the blood by using disposable syringes and observing safety rules. Animal ethics were ensured by any means. Blood was collected in two vacutainers; one vacutainer having EDTA an anticoagulant for hematology and another vacutainer without EDTA for blood serum analysis.

Blood analysis

Heparinized blood samples were subjected to hematological analysis viz., Total Red Blood Cell Count, Total White Blood Cell Count, Packed Cell Volume, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Platelets, Granulocytes, Lymphocytes, Monocytes and Neutrophils using XP-100 Sysmex, Japan (Sripad *et al.*, 2014).

Sera of the blood samples were separated by centrifugation at 750 g (2500rpm) for 15 minutes and stored at -20° C until further analysis of the samples. These samples were analyzed to determine total protein by using the Biuret method, creatinine was determined by using Jaffe method, uric acid was determined by using Phosphotungstic acid method, In additions to these, enzyme Alanine aminotransferase (ALT) activity was determined by using the modified Bowers and McComb method, enzyme aspartate aminotransferase (AST) activity was determined by the colorimetric method of Reitman and Frankel. These enzyme activities were measured at 37°C (Burtis & Ashwood, 1994). Further, biochemical parameters were measured by using the standard auto analyzer with up to dated veterinary software (Cobas-Mira, ABX-Diagnostics, Japan).

RESULTS

The detail studies of hematological parameters of chukar partridge are summarized (Table 1). The mean value of Red Blood Cells (RBC) was $2.00 \pm 0.284 \times 10^6 / \mu\text{L}$. The mean values of hemoglobin (HGB) and Haematocrit (HCT) were $11.40 \pm 1.850 \text{ g/dL}$ and $31.30 \pm 4.394\%$ respectively. The mean value of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular HGB Concentration (MCHC) were $156.45 \pm 7.297 \text{ fL}$, $57.41 \pm 8.567 \text{ pg}$ and $36.65 \pm 4.844 \text{ g/dL}$, respectively. The mean value of Red blood cell distribution width (RDW-SD) was $64.72 \pm 17.918 \text{ fL}$ and Red blood cell distribution width (RDW-CV) was $12.56 \pm 3.209\%$. The sex wise hematological parameters of Chukar partridge are summarized in Table 2.

Table I: Over all Haematological values of Chukar Partridge (n=28) in Captivity in Punjab, Pakistan

Parameter	Range(Min-Max)	Mean± SE
RBC (x10 ⁶ /μL)	1.32 (1.16-2.48)	2.00±0.054
HGB (g/dL)	12.30 (5.20-17.50)	11.40±0.350
HCT (%)	21.30 (16.60-37.90)	31.30±0.830
MCV (fL)	30.10 (139.90-170.00)	156.45±1.379
MCH (pg)	38.20(44.80-83.00)	57.41±1.619
MCHC (g/dL)	19.10(30.90-50.00)	36.65±0.915
PLT (x10 ³ /μL)	20.00(1.00-21.00)	5.56±1.431

WBC (x10 ³ /μL)	47.40 (186.90-234.30)	220.10±1.857
LYM (%)	7.20(88.60-95.80)	93.45±0.561
NEUT (%)	7.20(4.20-11.40)	6.55±0.561
LYM (x10 ³ /μL)	32.10(179.10-211.20)	200.01±2.895
NEUT (x10 ³ /μL)	17.20(7.80-25.00)	14.08±1.297
RDW-SD (fL)	66.20(35.10-101.30)	64.72±3.448
RDW-CV (%)	12.60(6.00-18.60)	12.56±0.629
PDW (fL)	3.30(5.00-8.30)	6.70±0.822
MPV (fL)	4.50(6.20-10.70)	8.63±0.233
P-LCR (%)	28.00(8.70-36.70)	21.74±1.421

Table II: Sex-wise hematological values of Chukar Partridge (n=28) in Captivity in Punjab, Pakistan

Para-meter	Male (n= 10) Mean± SE (Min-Max)	Female (n= 18) Mean± SE (Min-Max)	t-value	P-value
RBC (x10 ⁶ /μL)	2.03±0.074 (1.66-2.43)	1.98±0.074 (1.16-2.48)	0.46 ^{NS}	0.651
HGB (g/dL)	11.77±0.279 (9.7-13)	11.2±0.522 (5.2-17.5)	0.78 ^{NS}	0.445
HCT (%)	32.34±1.015 (28-37.9)	30.72±1.16 (16.6-34.9)	0.93 ^{NS}	0.359
MCV (fL)	158.87±0.867 (155.8-162.7)	155.11±2.044 (139.9-170)	1.33 ^{NS}	0.196
MCH (pg)	58.33±1.664 (52.9-66.9)	56.91±2.371 (44.8-83)	0.42 ^{NS}	0.682
MCHC (g/dL)	36.68±0.957 (33.5-41.4)	36.63±1.342 (30.9-50)	0.03 ^{NS}	0.979
PLT (x10 ³ /μL)	2.2±0.735(1-5)	6.85±1.857(1-21)	-1.51 ^{NS}	0.151
WBC (x10 ³ /μL)	224.34±2.254 (206.3-234.3)	217.61±2.483 (186.9-231.6)	1.83 ^{NS}	0.079
LYM (%)	93.5±0.361 (93-94.2)	93.44±0.777 (88.6-95.8)	0.05 ^{NS}	0.963
NEUT (%)	6.5±0.361(5.8-7)	6.56±0.777 (4.2-11.4)	-0.05 ^{NS}	0.963
LYM (x10 ³ /μL)	203.73±5.971 (191.8-210.1)	198.61±3.408 (179.1-211.2)	0.77 ^{NS}	0.460
NEUT (x10 ³ /μL)	14.1±0.624 (12.9-15)	14.08±1.807 (7.8-25)	0.01 ^{NS}	0.994
RDW-SD (fL)	60.25±3.348 (46.2-84.2)	67.35±5.081 (35.1-101.3)	-0.99 ^{NS}	0.330
RDW-CV (%)	11.76±0.663 (8.04-14)	13.05±0.93 (6-18.6)	-0.99 ^{NS}	0.330

MPV (fL)	9.31±0.317 (7.9-10.7)	8.23±0.282 (6.2-10.5)	2.57*	0.017
P-LCR (%)	26.05±2.151 (14.1-36.7)	19.2±1.611 (8.7-32.6)	2.32*	0.035

NS = Non-significant ($P>0.05$); * = Significant ($P<0.05$); ** = Highly significant ($P<0.01$) SE = Standard error

DISCUSSION

The mean value of RBC, HCB, HCT, MCV, MCH, MCHC were higher (Table 2) in males as compared to females. In agreement with present results, Xiao-xia *et al.* (2015) observed a similar pattern of hematological values in apparently healthy and normal adult peafowl reared in China. A similar pattern of results was also reported by Samour *et al.* (2010) who examined the hematological profile in Blue Peafowl reared in India. However, the hematological examination of peafowl reared in Bulgaria showed that the concentration of RBC ($3.48\pm0.35 \times 10^{12}/L$) was slightly higher and the concentration of HGB (108.1 ± 1.35 g/L) was significantly lower compared with the data published for wild peafowl reared in India and China, and the values were closely related with those of pheasants and various breeds of domestic fowl (Lashev *et al.*, 2013; Lashev *et al.*, 2015). These data demonstrate that the hematological values of the same species vary with respect to the region or environment where they are reared.

In the present study, the comparison of erythrocytes indices between male and female of chukar partridge showed no significant difference between male and female however, the values were slightly higher in male than in the female. The hematological profiles of peafowl in relation to gender differences have shown variable reports. In agreement with our results, Xiao-Xia *et al.* (2015) found that the concentrations of RBC, MCV and MCH were significantly higher in male compared to female peafowl. On the contrary, Lashev *et al.* (2015) observed no significant differences in erythrocytes indices between male and female peafowl, however, most of the values were slightly higher in male compared to those of female birds. The important role of RBC and HGB is to transport oxygen from lungs to the body cells, which is required to derive energy through oxidative metabolism of fuels to maintain the biochemical activities (Roberson & Bennett-Guerrero, 2012).

The increased concentrations of RBC, MCV and MCH could result from increasing oxygen demand due to frequent and intensive activities of male birds. This could be another possible explanation for higher concentrations of RBC's, MCV and MCH in males to meet the oxygen requirement of increasing body tissues.

The mean value of Platelet (PLT) was $5.56\pm6.070 \times 10^3/\mu L$, platelet distribution width (PDW) was 6.70 ± 1.643 fL and the Mean platelet volume (MPV) was 8.63 ± 1.210 fL. The mean value of Platelet Large Cell Ratio (P-LCR) was $21.74 \pm 7.385\%$. The mean value White Blood Cell (WBC) was $220.10\pm9.648 \times 10^3/\mu L$. The mean value of Lymphocytes (LYM), Neutrophils (NEUT), Lymphocytes (LYM) and Neutrophils (NEUT) were $93.45\pm1.859\%$, $6.55\pm1.859\%$, $200.01\pm9.600 \times 10^3/\mu L$ and $14.08\pm4.303 \times 10^3/\mu L$, respectively. The mean value of LYM, NEUT, MPV and P-LCR were higher in males as compared to females. Whereas the mean value of PLT, NEUT (%), RDW-SD, RDW-CV were higher in females as compared to males. The sex wise hematological parameter were statistically non-significant ($P>0.05$) except MPV and P-LCP values which differed significantly ($p<0.05$). Xiao-xia *et al.* (2015) reported that WBC's, LYM and LYM % are not significantly different ($p > 0.05$) between male and female. It showed that the immunity of male and female is basically equal. The platelets are activated at, and seal the site of vascular injury through a complex system of clotting factors and thus prevent the loss of blood (Meseguer *et al.*, 2002; Khandekar *et al.*, 2012). In agreement with our results, a study of peafowl in China (Xiao-xia *et al.*, 2015) and hematological profile determined in Blue Peafowl reared in India showed a similar trend of results (Samour *et al.*, 2010). However, the hematological examination of peafowl reared in Bulgaria showed that the concentration of WBC's was significantly higher. These values were closely related to those of pheasants and various breeds of domestic fowl (Lashev *et al.*, 2013; Lashev *et al.*, 2015).

The overall biochemical blood values including Urea, ALT, AST, Creatin, Total protein and Albumin values were 282.06 ± 37.18 , 13.61 ± 2.597 , 48.23 ± 28.157 , 61.04 ± 12.658 , 9.29 ± 1.228 and 2.44 ± 0.108 , respectively. All these parameters between male and female were non-

significant ($p>0.05$) (Table-4).

In agreement with present results, Samour *et al.* (2010) reported blood biochemical characteristics in normal captive Indian Blue Peafowl reared in India and Similar pattern of biochemical values was also shown by Xiao-Xia *et al.* (2015) in Indian Peafowl reared in China. In addition, Samour *et al.* (2010) compared these results with published data of other taxonomically related species and found a close relationship for most of the studied biochemical parameters with the other avian species.

Table III: Overall biochemical blood values of Chukar Partridge (n=28) in Captivity in Punjab, Pakistan

Parameter	Range(Min-Max)	Mean \pm SE
UREA (mg/dl)	595(35-630)	282.06 \pm 37.18
ALT (μ l)	35.8(2.61-38.41)	13.61 \pm 2.597
AST (μ l)	519.44(2.62-522.05)	46.23 \pm 28.157
CREAT (mg/dl)	147(0.75-147.75)	61.04 \pm 12.658
T. Protein (g/dl)	23.42(6.16-29.57)	9.29 \pm 1.228
ALBUMIN (g/dl)	2.14(1.2-3.34)	2.44 \pm 0.108

Table IV: Sex-wise Biochemical Blood Values of Chukar Partridge (n=28) in Captivity in Punjab, Pakistan

Parameter	Male (n= 10) Mean \pm SE (Min-Max)	Female (n= 18) Mean \pm SE (Min-Max)	t-value	P-value
UREA (mg/dl)	363.13 \pm 60.59 9 (140-630)	210 \pm 31.413 (35-315)	-0.10 ^{NS}	0.919
ALT (μ l)	13.31 \pm 3.879(2 .62-38.41)	13.87 \pm 3.711 (2.61-37.53)	-0.87 ^{NS}	0.395
AST (μ l)	18.55 \pm 4.681(5 .24-47.14)	68.38 \pm 50.59(2 .62-522.05)	-1.11 ^{NS}	0.284
CREAT (mg/dl)	45.47 \pm 15.609(1 1.5-121.5)	73.5 \pm 18.825(0 .75-147.75)	-0.90 ^{NS}	0.383
T. Protein (g/dl)	8.05 \pm 0.372(6. 19-9.34)	10.28 \pm 2.19(6. 16-29.57)	-0.38 ^{NS}	0.706
ALBUMIN (g/dl)	2.39 \pm 0.221(1. 2-3.34)	2.48 \pm 0.095(1. 99-2.98)	-0.88 ^{NS}	0.376

NS = Non-significant ($P>0.05$); * = Significant ($P<0.05$); ** = Highly significant ($P<0.01$) SE = Standard error

Alanine aminotransferase enzymes are

involved basically in the metabolism of proteins, fats and glucose. The clinical diagnosis could be done by checking the ALT level in serum. Under normal clinical conditions, ALT level kept very low. High level of ALT is a sign for liver tissue damage or disease. TP content of serum reflects the protein metabolism of poultry. Protein metabolism activity through high TP content of serum is conducive to improve the protein absorption and promote the animal's growth (Xu Liang-Mei, 2013). To certain extent dietary protein content also influenced the TP level. Physiological state, age and health status also have a relationship with TP (Liu Hua-zhong & Shen Qiu-gu, 2000).

CONCLUSION

The present reference ranges of hematology and plasma chemistry are the first reported for chukar partridge in captivity. These reference ranges provide valuable baseline information for the evaluation of fitness and health status of this species. The results do not account for various factors that could influence hematological and plasma chemistry values such as age, season, reproductive status, geographical location and management standards in captivity. Therefore, further studies should be under taken to determine the effect of these factors for this species.

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