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Source: Journal of Wildlife Diseases, 38(4) : 804-816

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.4.804

*Journal of Wildlife Diseases,* 38(4), 2002, pp. 804–816


# AGE-RELATED HEMATOLOGIC CHANGES IN CAPTIVE-REARED HOUBARA, WHITE-BELLIED, AND RUFOUS-CRESTED BUSTARDS

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ABSTRACT: Blood samples were obtained at monthly intervals between April 1994 and March 1996 from captive-bred houbara (*Chlamydotis undulata macqueenii),* rufous-crested (*Eupodotis ruficrista gindiana*), and white-bellied (*Eupodotis senegalensis*) bustards from 4–24 wk of age. Hematology investigations were conducted to determine age-related changes and to establish reference values for growing chicks of these species. There were significant age-related changes in hematocrit, hemoglobin, and red cell count in young birds compared with those of adults. White cell counts (lymphocytes and monocytes) were higher in juvenile birds, compared with adult values.

*Key words:* Age-related changes, *Chlamydotis undulata macqueenii*, houbara bustard, hematology, *Eupodotis ruficrista gindiana, Eupodotis senegalensis,* rufous-crested bustard, white-bellied bustard.

## INTRODUCTION

Bustards are members of the Otididae family of which there are 25 species. Habitat loss, agricultural changes, overgrazing, hunting and trapping are threatening many bustard populations (Johnsgard, 1991). The National Avian Research Center (NARC) in the United Arab Emirates (UAE) maintains a captive breeding program for houbara (*Chlamydotis undulata macqueenii*); rufous-crested (*Eupodotis ruficrista gindiana*), and white-bellied (*Eupodotis senegalensis*) bustards and therefore the veterinary care of these species is of importance.

Hematology is widely recognized as an important diagnostic tool in the veterinary care of avian species, both in diagnosing disease and clinical monitoring of the patient (Hawkey et al., 1983, 1984a, b; Dein, 1986; Hawkey and Samour, 1988). There have been a number of hematologic investigations of bustard species (Alonso et al., 1990; Jimenez et al., 1991; Mikaelian, 1993; Samour et al., 1994, 1996; Flach, 1995; D'Aloia et al., 1995, 1996; Howlett et al., 1995, 1998). However, the majority of these studies have focused on adult bustards with only limited reference to bustard chicks, these include the great bustard (Alonso et al., 1990); rufous-crested bustard (D'Aloia et al., 1995) and kori bustard (Howlett et al., 1998). The purpose of this study is to report hematology values in developing bustard chicks up to the age of 6 mo in order to establish reference values and to determine any significant age-related changes.

## MATERIALS AND METHODS

The hematologic survey was carried out on chick and juvenile bustards from 4–24 wk of age. Blood samples were collected between April 1995 and March 1996 from clinically normal captive-reared bustard chicks hatched during the 1995 breeding season. The birds included seven houbara, five white-bellied, and 10 rufous-crested bustards hatched from eggs that had been artificially incubated. The chicks were hand-reared at the NARC breeding facilities (24°15′N, 55°43′E; Al Ain, Abu Dhabi, United Arab Emirates) as described for the kori bustard (Howlett et al., 1998). The birds used for the study appeared clinically normal and were within normal weight ranges for their respective species. Samples were obtained between 0700 and 0900 hours when the birds were handled for a radiographic study to monitor normal skeletal development of bustard chicks (Naldo et al., 1997). Samples were obtained under 3% isoflurane (Abbot Laboratories Ltd., Maidenhead, UK) within 5 min of the onset of anesthesia. Unfortunately there were a limited number of chicks from which we could obtain samples. The rufous-crested bustards, due to their small size not all were sampled at 4 wk and the majority had ceased being part of the radiographic study at 5 mo. Additionally, during this investigation the houbara of which there was a 2 mo range between hatch dates, where translocated en masse from the Al Ain site to breeding facilities at another NARC facility, where they became part of the breeding flock and sampling was discontinued.

The samples were collected from the ulnaris or jugular vein, using a 23 or 25 gauge×16 mm needle and a 1 or 2 ml syringe. A volume of 0.3 ml of blood was deposited into a commercially available pediatric blood tube (Sarstedt, Nümbrecht, Germany) and mixed well with the anti-coagulant, ethylenediaminetetraacetic acid (EDTA 1.5mg/ml of blood).

The hematology samples were processed in the laboratory on the same day of collection. The laboratory techniques used for this study are described only briefly here, full details have been reported by Hawkey and Samour (1988) and Hawkey and Gulland (1988). We have used these techniques in previous bustard studies at the NARC laboratory using the same equipment.

Red blood cells (RBC) were counted manually after mixing 20 μl of whole blood with 4 ml of formal citrate solution (10 ml of 20% formaldehyde plus 990 ml of 32 g/l trisodium citrate solution) using an Improved Neubauer Brite-line hemocytometer (Weber Scientific International, Teddington, Middlesex, UK) and examined under phase contrast microscopy (Dacie and Lewis, 1975). Hemoglobin (Hb) was measured as oxyhemoglobin by diluting 20 μl of whole blood into 4 ml of 0.04% ammonia solution (Dacie and Lewis, 1975) and read using a spectrophotometer at 540 mm (Spectronic 501, Bausch & Lomb, Rochester, New York, USA). Spectrophotometer readings were converted into Hb g/dl by reference to a calibration curve prepared from a commercially available hemoglobin standard (Diagnostic Reagents Ltd., Thame, Oxford, UK; Hawkey and Samour, 1988). Hematocrit (Hct) estimation was obtained by filling plain microcapillary tubes (Hawksley and Sons Ltd., Sussex, UK) with whole blood which were centrifuged using a microhematocrit centrifuge (Hawksley and Sons Ltd.) at 10,000 G for 5 min. Hematocrit readings were carried out using a microhematocrit reader (Hawksley and Sons Ltd.). Fibrinogen was estimated as protein precipitated at 56 C by heating the same microhematocrit tube used for Hct measurement in a waterbath at 56 C for 3 min. The amount of fibrinogen present related to the increase in the optimal density of the plasma (Hawkey and Gulland, 1988). The red cell indices, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were determined using standard formulae (Dacie and Lewis, 1975). White blood cells (WBC) were examined under phase contrast microscopy and counted manually after mixing 100 μl of blood into 1.9 ml of 1% ammonium oxalate solution (Hawkey et al., 1983) using an improved Neubauer Brite-line hemocytometer (Weber Scientific International, Teddington, Middlesex, UK). Morphologic assessment of red and white blood cells and differential white cell and thrombocyte counts were carried out on blood films after staining and fixing as described by Samour et al. (1994) and Howlett (2000).

The results were compared with data derived from reported adult values for each species (Samour et al., 1994; D'Aloia et al., 1995, 1996). Statistical analyse was conducted using a Student's unpaired *t*-test (Microsoft Excel Analysis Toolpak, Microsoft Corporation, Redmond, Washington, USA). A value of $P<0.05$ was considered significantly different.

## RESULTS

Hematology values obtained for houbara, rufous-crested, and white-bellied bustard chicks are presented in Tables 1, 2, and 3. The published reference values for clinically normal adults of the three species are presented in Table 4 for comparison.

Blood cell morphology of growing bustard chicks was similar to that described for adult houbara bustards (Samour et al., 1994) and as illustrated by Howlett (2000). The erythrocytes of bustards are oval cells with a slightly basophilic cytoplasm and containing a relatively large nucleus with evenly distributed chromatin clumps. Varying numbers of immature red cells at different stages of development were observed. Many cells exhibited polychromasia and anisocytosis; the less mature cells were more round in shape and basophilic. Poikilocytosis, characterized by spindle-shaped cells, was observed. Hemoparasites were not detected.

Heterophils of bustards are relatively large round cells with a colorless cytoplasm containing a bilobed nucleus with

TABLE 1. Hematologic findings in houbara bustard (*Chlamydotis undulata macqueenii*) of different ages.

| Parameter | <4 wk | $n$[a] | $P$ value[b] | 4–8 wk | $n$ | $P$ value[c] | 8–12 wk | $n$ | $P$ value[d] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells ($\times 10^{12}$/l) | 1.42 ± 0.06[e] (1.24–1.57)[f] | 7 | <0.0001 | 1.62 ± 0.07 (1.22–1.88) | 7 | <0.0001 | 1.70 ± 0.07 (1.44–1.96) | 7 | <0.0001 |
| Hemoglobin (g/dl) | 7.69 ± 0.25 (6.70–8.30) | 7 | <0.0001 | 8.97 ± 0.27 (7.20–10.00) | 7 | <0.0001 | 9.50 ± 0.48 (8.30–11.40) | 7 | <0.0001 |
| Hematocrit (l/l) | 0.23 ± 0.59 (0.22–0.26) | 7 | <0.0001 | 0.29 ± 0.86 (0.23–0.32) | 7 | <0.0001 | 0.31 ± 1.42 (0.26–0.37) | 7 | <0.0001 |
| Mean cell volume (fl) | 165.35 ± 4.47 (146.50–185.48) | 7 | <0.005 | 178.08 ± 7.47 (146.28–229.51) | 7 | NS | 182.20 ± 9.19 (156.08–221.15) | 7 | NS |
| Mean cell hemoglobin (pg) | 54.55 ± 1.87 (50.33–65.32) | 7 | NS | 56.02 ± 2.39 (47.87–73.77) | 7 | NS | 56.35 ± 3.00 (48.28–71.15) | 7 | NS |
| Mean cell hemoglobin concentration (g/dl) | 33.01 ± 0.79 (30.80–36.09) | 7 | NS | 31.47 ± 0.28 (30.65–32.78) | 7 | NS | 30.94 ± 0.58 (28.57–32.55) | 7 | NS |
| Thrombocytes ($\times 10^9$/l) | 14.18 ± 1.35 (8.90–19.95) | 7 | NS | 12.09 ± 2.13 (5.30–25.61) | 7 | NS | 8.73 ± 1.0 (4.97–12.33) | 7 | NS |
| Fibrinogen (g/l) | 1.82 ± 0.21 (1.10–2.75) | 7 | NS | 1.70 ± 0.18 (1.45–1.96) | 7 | NS | 2.06 ± 0.19 (1.60–2.76) | 7 | NS |
| White blood cells ($\times 10^9$/l) | 8.84 ± 0.72 (5.45–10.50) | 7 | <0.001 | 8.77 ± 0.58 (6.25–10.80) | 7 | <0.0001 | 8.77 ± 0.55 (6.80–10.50) | 7 | <0.0001 |
| Heterophils ($\times 10^9$/l) | 3.62 ± 0.56 (1.36–5.56) | 7 | NS | 3.57 ± 0.4 (1.17–5.18) | 7 | NS | 3.87 ± 0.60 (2.41–6.72) | 7 | NS |
| Lymphocytes ($\times 10^6$/l) | 4.74 ± 0.38 (3.65–6.20) | 7 | NS | 4.75 ± 0.37 (2.50–6.37) | 7 | <0.001 | 4.19 ± 0.36 (2.94–5.38) | 7 | <0.001 |
| Monocytes ($\times 10^9$/l) | 0.23 ± 0.05 (0.00–0.38) | 7 | NS | 0.28 ± 0.05 (0.00–0.43) | 7 | <0.05 | 0.42 ± 0.07 (0.20–0.63) | 7 | <0.05 |
| Eosinophils ($\times 10^9$/l) | 0.08 ± 0.03 (0.00–0.20) | 7 | NS | 0.14 ± 0.04 (0.00–0.32) | 7 | NS | 0.14 ± 0.07 (0.00–0.41) | 7 | NS |
| Basophils ($\times 10^9$/l) | 0.17 ± 0.04 (0.00–0.38) | 7 | NS | 0.10 ± 0.03 (0.00–0.22) | 7 | NS | 0.16 ± 0.04 (0.00–0.30) | 7 | NS |

[a] Total number of samples collected.
[b] Statistical analysis 4 wk chick compared with adult houbara (Table 4).
[c] Statistical analysis 4–8 wk chick compared with adult houbara (Table 4).
[d] Statistical analysis 8–12 wk chick compared with adult houbara (Table 4).
[e] Mean±standard error of mean.
[f] (Minimum–maximum).
[g] Statistical analysis 12–16 wk chick compared with adult houbara (Table 4).
[h] Statistical analysis 16–20 wk chick compared with adult houbara (Table 4).
[i] Statistical analysis 20–24 wk chick compared with adult houbara (Table 4).

TABLE 1. Continued.

| Parameter | 12–16 wk | $n$ | $P$ value[g] | 16–20 wk | $n$ | $P$ valued[h] | 20–24 wk | $n$ | $P$ value[i] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells (×10$^{12}$/l) | 1.93 ± 0.09 (1.67–2.28) | 7 | <0.0001 | 1.80 ± 0.08 (1.70–2.05) | 4 | <0.0001 | 1.93 ± 0.11 (1.73–2.17) | 4 | <0.01 |
| Hemoglobin (g/dl) | 11.47 ± 0.2 (10.80–12.10) | 7 | <0.0001 | 12.85 ± 0.46 (12.10–14.10) | 4 | <0.01 | 13.63 ± 0.39 (12.70–14.40) | 4 | NS |
| Hematocrit (l/l) | 0.36 ± 0.8 (0.32–0.38) | 7 | <0.0001 | 0.41 ± 1.17 (0.38–43.0) | 4 | <0.01 | 0.43 ± 1.02 (0.42–0.46) | 4 | <0.01 |
| Mean cell volume (fl) | 188.54 ± 7.4 (160.09–216.76) | 7 | NS | 226.88 ± 13.04 (195.12–252.94) | 4 | NS | 232.26 ± 7.25 (211.98–242.78) | 4 | <0.01 |
| Mean cell hemoglobin (pg) | 60.02 ± 2.2 (51.75–69.94) | 7 | NS | 72.10 ± 4.87 (59.51–82.94) | 4 | NS | 72.10 ± 2.05 (66.36–76.02) | 4 | <0.01 |
| Mean cell hemoglobin concentration (g/dl) | 31.83 ± 0.4 (30.41–33.75) | 7 | NS | 31.72 ± 0.51 (30.50–33.79) | 4 | NS | 31.06 ± 0.27 (30.24–31.36) | 4 | NS |
| Thrombocytes (×10$^9$/l) | 7.29 ± 2.2 (2.00–17.47) | 7 | NS | 3.09 ±0.7 (2.00–5.04) | 4 | NS | 4.06 ± 0.30 (2.70–5.04) | 4 | NS |
| Fibrinogen (g/l) | 2.13 ± 0.2 (1.27–3.15) | 7 | NS | 1.70 ± 0.16 (1.36–2.01) | 4 | NS | 1.54 ± 0.12 (1.36–1.89) | 4 | NS |
| White blood cells (×10$^9$/l) | 8.44 ± 1.1 (4.70–12.70) | 7 | <0.001 | 9.08 ± 0.60 (7.70–10.10) | 4 | <0.05 | 7.90 ± 0.82 (6.50–10.05) | 4 | <0.05 |
| Heterophils (×10$^9$/l) | 3.79 ± 0.74 (1.10–6.86) | 7 | NS | 3.41 ± 0.51 (2.23–4.55) | 4 | NS | 3.23 ± 0.24 (2.82–3.92) | 4 | NS |
| Lymphocytes (×10$^9$/l) | 3.84 ± 0.3 (3.10–5.33) | 7 | <0.01 | 4.79 ± 0.35 (3.94–5.63) | 4 | <0.05 | 4.15 ± 0.14 (2.99–5.63) | 4 | <0.05 |
| Monocytes (×10$^9$/l) | 0.32 ± 0.1 (0.00–0.99) | 7 | <0.05 | 0.31 ± 0.06 (0.30–0.54) | 4 | <0.05 | 0.32 ± 0.05 (0.25–0.46) | 4 | <0.05 |
| Eosinophils (×10$^9$/l) | 0.21 ± 0.06 (0.00–0.51) | 7 | NS | 0.31–0.11 (0.00–0.05) | 4 | NS | 0.04 ± 0.02 (0.00–0.08) | 4 | NS |
| Basophils (×10$^9$/l) | 0.27 ± 0.12 (0.00–0.81) | 7 | NS | 0.34 ± 0.17 (0.08–0.47) | 4 | NS | 0.17 ± 0.07 (0.00–0.33) | 4 | NS |

TABLE 2. Hematologic findings in rufous-crested bustard (*Eupodotis ruficrista gindiana*) of different ages.

| Parameter | <4 wk | n[a] | P value[b] | 4–8 wk | n | P value[c] | 8–12 wk | n | P value[d] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells (×$10^{12}$/l) | 1.76 ± 0.08[e] (1.51–1.89)[f] | 5 | <0.0001 | 1.87 ± 0.09 (1.38–2.10) | 8 | <0.0001 | 1.97 ± 0.09 (1.61–2.41) | 9 | <0.0001 |
| Hemoglobin (g/dl) | 7.72 ± 0.45 (6.80–9.30) | 5 | <0.0001 | 9.55 ± 0.17 (7.90–11.30) | 8 | <0.0001 | 10.64 ± 0.36 (8.90–12.00) | 9 | <0.0001 |
| Hematocrit (l/l) | 0.30 ± 2.18 (0.26–0.37) | 5 | <0.0001 | 0.35 ± 1.57 (0.28–0.42) | 8 | <0.0001 | 0.38 ± 1.26 (0.37–0.43) | 9 | <0.0001 |
| Mean cell volume (fl) | 172.35 ± 7.50 (150.54–195.77) | 5 | NS | 185.30 ± 5.25 (162.30–205.00) | 8 | NS | 193.42 ± 6.18 (164.14–217.88) | 9 | NS |
| Mean cell hemoglobin (pg) | 43.93 ± 1.77 (37.04–49.21) | 5 | <0.0001 | 51.47 ± 20.65 (42.70–63.35) | 8 | <0.05 | 54.81 ± 1.87 (43.22–65.92) | 9 | <0.001 |
| Mean cell hemoglobin concentration (g/dl) | 25.76 ± 1.75 (18.92–28.93) | 5 | <0.001 | 27.70 ± 0.86 (24.31–30.91) | 8 | <0.0001 | 28.37 ± 0.77 (21.45–33.65) | 9 | <0.0001 |
| Thrombocytes (×$10^9$/l) | 8.74 ± 2.46 (3.40–17.88) | 5 | NS | 6.50 ± 0.99 (2.20–9.72) | 8 | NS | 9.22 ± 1.21 (3.07–17.92) | 9 | NS |
| Fibrinogen (g/l) | 1.68 ± 0.24 (1.02–2.50) | 3 | NS | 1.88 ± 0.21 (1.50–3.30) | 8 | NS | 1.73 ± 0.18 (1.20–3.52) | 9 | NS |
| White blood cells (×$10^9$/l) | 10.55 ± 1.51 (5.20–14.00) | 5 | <0.05 | 9.15 ± 0.94 (5.20–12.95) | 8 | <0.05 | 10.27 ± 0.69 (5.70–14.00) | 9 | <0.001 |
| Heterophils (×$10^9$/l) | 5.2 ± 1.17 (0.94–7.56) | 5 | NS | 3.72 ± 0.53 (1.64–5.97) | 8 | NS | 3.31 ± 0.46 (0.51–6.44) | 9 | NS |
| Lymphocytes (×$10^9$/l) | 4.25 ± 0.53 (3.02–5.85) | 5 | <0.005 | 4.33 ± 0.56 (2.50–7.90) | 8 | <0.001 | 5.49 ± 0.66 (2.16–2.82) | 9 | <0.0001 |
| Monocytes (×$10^9$/l) | 0.29 ± 0.03 (0.21–0.39) | 5 | NS | 0.31 ± 0.09 (0.00–0.75) | 8 | NS | 0.57 ± 0.10 (0.11–1.47) | 9 | NS |
| Eosinophils (×$10^9$/l) | 0.55 ± 0.15 (0.13–0.98) | 5 | NS | 0.47 ± 0.05 (0.15–0.62) | 8 | NS | 0.66 ± 0.17 (0.11–1.41) | 9 | NS |
| Basophils (×$10^9$/l) | 0.27 ± 0.11 (0.00–0.63) | 5 | NS | 0.34 ± 0.07 (0.00–0.63) | 8 | NS | 0.20 ± 0.07 (0.00–0.64) | 9 | NS |

[a] Total number of samples collected.
[b] Statistical analysis 4 wk chick compared with adult rufous-crested (Table 4).
[c] Statistical analysis 4–8 wk chick compared with adult rufous-crested (Table 4).
[d] Statistical analysis 8–12 wk chick compared with adult rufous-crested (Table 4).
[e] Mean±standard error of mean.
[f] (Minimum–maximum).
[g] Statistical analysis 12–16 wk chick compared with adult rufous-crested (Table 4).
[h] Statistical analysis 16–20 wk chick compared with adult rufous-crested (Table 4).
[i] Statistical analysis 20–24 wk chick compared with adult rufous-crested (Table 4).

TABLE 2. Continued.

| Parameter | 12–16 wk | $n^g$ | $P$ value[b] | 16–20 wk | $n$ | $P$ value[h] | 20–24 wk | $n$ | $P$ value[i] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells (×$10^{12}$/l) | 2.37 ± 0.10 (1.93–2.67) | 8 | <0.05 | 2.59 ± 0.05 (2.32–2.74) | 8 | NS | 2.86 ± 0.18 (2.82–3.45) | 5 | NS |
| Hemoglobin (g/dl) | 13.15 ± 0.31 (11.90–14.50) | 8 | <0.0001 | 14.34 ± 0.29 (13.60–16.20) | 8 | <0.0001 | 14.24 ± 0.61 (12.40–16.20) | 5 | <0.001 |
| Hematocrit (l/l) | 0.43 ± 0.99 (0.40–0.47) | 8 | <0.005 | 0.48 ± 0.73 (0.46–0.51) | 8 | <0.05 | 0.50 ± 0.55 (0.49–0.51) | 5 | NS |
| Mean cell volume (fl) | 185.18 ± 8.68 (150.94–229.06) | 8 | NS | 185.14 ± 4.24 (169.71–202.43) | 8 | NS | 176.04 ± 10.54 (147.83–211.30) | 5 | NS |
| Mean cell hemoglobin (pg) | 55.47 ± 2.10 (48.99–66.99) | 8 | <0.05 | 55.59 ± 1.46 (49.64–62.07) | 8 | <0.05 | 50.65 ± 3.75 (41.74–57.74) | 5 | <0.05 |
| Mean cell hemoglobin concentration (g/dl) | 30.12 ± 0.9 (26.23–34.35) | 8 | <0.0001 | 30.04 ± 0.51 (25.20–32.09) | 8 | <0.0001 | 28.71 ± 2.0 (25.57–32.40) | 5 | <0.0005 |
| Thrombocytes (×$10^9$/l) | 8.40 ± 1.54 (4.40–17.85) | 8 | NS | 6.28 ± 1.04 (2.28–11.60) | 8 | NS | 9.20 ± 2.11 (3.90–16.80) | 5 | NS |
| Fibrinogen (g/l) | 2.21 ± 0.14 (1.80–2.60) | 6 | NS | 2.39 ± 0.28 (1.15–3.33) | 8 | NS | 2.20 ± 0.32 (1.60–3.33) | 5 | NS |
| White blood cells (×$10^9$/l) | 8.81 ± 1.23 (3.10–14.75) | 8 | <0.05 | 9.44 ± 0.56 (7.50–11.70) | 8 | <0.001 | 10.71 ± 1.10 (7.50–14.00) | 5 | <0.05 |
| Heterophils (×$10^9$/l) | 3.37 ± 1.13 (0.56–9.44) | 8 | NS | 2.80 ± 0.10 (2.31–3.16) | 8 | NS | 2.77 ± 0.54 (1.04–4.20) | 5 | NS |
| Lymphocytes (×$10^9$/l) | 4.14 ± 0.61 (1.92–6.50) | 8 | <0.001 | 4.89 ± 0.55 (3.17–7.14) | 8 | <0.001 | 6.27 ± 0.56 (4.50–7.70) | 5 | <0.0005 |
| Monocytes (×$10^9$/l) | 0.53 ± 0.10 (0.19–0.95) | 8 | NS | 0.63 ± 0.13 (0.20–1.18) | 8 | NS | 1.00 ± 0.26 (0.30–1.80) | 5 | <0.05 |
| Eosinophils (×$10^9$/l) | 0.29 ± 0.10 (0.10–0.92) | 8 | NS | 0.83 ± 0.33 (0.08–2.29) | 8 | NS | 0.54 ± 15 (0.47–0.84) | 5 | NS |
| Basophils (×$10^9$/l) | 0.48 ± 0.16 (0.00–1.48) | 8 | NS | 0.34 ± 0.08 (0.11–0.70) | 8 | NS | 0.13 ± 0.07 (0.0–0.36) | 5 | <0.05 |

TABLE 3. Hematologic findings in white-bellied bustard (*Eupodotis sensgalensis*) of different ages.

| Parameter | <4 wk | n[a] | P value[b] | 4–8 wk | n | P value[c] | 8–12 wk | n | P value[d] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells ($\times 10^{12}$/l) | 1.45 ± 0.13[e] (1.16–1.68)[f] | 4 | <0.001 | 1.76 ± 0.12 (1.47–2.04) | 4 | <0.05 | 1.95 ± 0.04 (1.88–2.05) | 4 | <0.05 |
| Hemoglobin (g/dl) | 8.75 ± 0.96 (7.00–10.70) | 4 | <0.005 | 10.53 ± 0.8 (9.30–12.90) | 4 | <0.05 | 11.31 ± 0.6 (9.40–12.30) | 4 | <0.001 |
| Hematocrit (l/l) | 0.26 ± 2.98 (0.20–0.32) | 4 | <0.001 | 0.31 ± 1.7 (0.27–0.35) | 4 | <0.005 | 0.35 ± 2.25 (0.29–0.40) | 4 | <0.001 |
| Mean cell volume (fl) | 175.21 ± 6.21 (159.09–187.50) | 4 | <0.0 | 177.73 ± 4.1 (169.12–187.88) | 4 | <0.05 | 182.52 ± 13.47 (146.46–210.11) | 4 | NS |
| Mean cell hemoglobin (pg) | 60.05 ± 1.83 (56.13–64.67) | 4 | NS | 60.03 ± 2.3 (55.91–64.63) | 4 | NS | 58.23 ± 3.85 (47.47–65.43) | 4 | NS |
| Mean cell hemoglobin concentration (g/dl) | 34.33 ± 0.98 (31.43–35.67) | 4 | NS | 33.86 ± 1.7 (30.00–37.39) | 4 | NS | 31.96 ± 0.35 (31.14–32.52) | 4 | NS |
| Thrombocytes ($\times 10^9$/l) | 9.45 ± 2.70 (4.80–15.00) | 4 | NS | 8.22 ± 2.4 (4.60–15.20) | 4 | NS | 7.20 ± 2.07 (3.64–12.10) | 4 | NS |
| Fibrinogen (g/l) | 1.13 ± 0.09 (1.00–1.30) | 3 | <0.05 | 2.02 ± 0.3 (1.37–2.48) | 3 | NS | 2.19 ± 0.33 (1.40–2.72) | 4 | NS |
| White blood cells ($\times 10^9$/l) | 8.10 ± 0.86 (6.15–10.10) | 4 | NS | 9.01 ± 2.0 (5.60–14.78) | 4 | NS | 9.53 ± 2.5 (5.20–16.00) | 4 | NS |
| Heterophils ($\times 10^9$/l) | 3.80 ± 0.21 (3.30–4.20) | 4 | NS | 4.77 ± 1.1 (2.69–7.97) | 4 | NS | 3.83 ± 1.51 (2.20–8.36) | 4 | NS |
| Lymphocytes ($\times 10^9$/l) | 3.61 ± 0.54 (2.58–5.15) | 4 | NS | 3.39 ± 0.8 (1.85–5.61) | 4 | NS | 4.17 ± 0.98 (2.20–6.36) | 4 | <0.05 |
| Monocytes ($\times 10^9$/l) | 0.15 ± 0.06 (0.00–0.30) | 4 | NS | 0.25 ± 0.09 (0.06–0.44) | 4 | NS | 0.79 ± 0.38 (0.10–1.73) | 4 | NS |
| Eosinophils ($\times 10^9$/l) | 0.27 ± 0.08 (0.06–0.44) | 4 | NS | 0.36± 0.06 (0.27–0.53) | 4 | NS | 0.40 ± 0.15 (0.00–0.71) | 4 | NS |
| Basophils ($\times 10^9$/l) | 0.27± 0.15 (0.00–0.62) | 4 | NS | 0.24 ± 0.07 (0.09–0.44) | 4 | NS | 0.33 ± 0.12 (0.14–0.64) | 4 | NS |

[a] Total number of samples collected.
[b] Statistical analysis 4 wk chick compared with adult white-bellied (Table 4).
[c] Statistical analysis 4–8 wk chick compared with adult white-bellied (Table 4).
[d] Statistical analysis 8–12 wk chick compared with adult white-bellied (Table 4).
[e] Mean±standard error of mean.
[f] (Minimum–maximum).
[g] Statistical analysis 12–16 wk chick compared with adult white-bellied (Table 4).
[h] Statistical analysis 16–20 wk chick compared with adult white-bellied (Table 4).
[i] Statistical analysis 20–24 wk chick compared with adult white-bellied (Table 4).

TABLE 3. Continued.

| Parameter | 12–16 wk | *n* | *P* value[g] | 16–20 wk | *n* | *P* value[h] | 20–24 wk | *n* | *P* value[i] |
|---|---|---|---|---|---|---|---|---|---|
| Red blood cells ($\times 10^{12}$/l) | 2.04 ± 0.10 (1.76–2.30) | 5 | <0.05 | 2.31 ± 0.13 (2.05–2.72) | 5 | NS | 2.58 ± 0.13 (2.15–2.98) | 5 | NS |
| Hemoglobin (g/dl) | 11.84 ± 0.27 (11.20–12.60) | 5 | <0.001 | 13.98 ± 0.86 (11.90–16.30) | 5 | NS | 14.24 ± 0.35 (13.20–14.90) | 5 | NS |
| Hematocrit (l/l) | 0.39 ± 0.46 (0.38–0.40) | 5 | <0.05 | 0.42 ± 0.86 (0.40–0.44) | 5 | <0.05 | 0.44 ± 0.93 (0.41–0.46) | 5 | NS |
| Mean cell volume (fl) | 191.95 ± 9.75 (171.74–215.91) | 5 | NS | 183.62 ± 9.88 (159.93–213.59) | 5 | NS | 171.23 ± 6.69 (147.65–188.37) | 5 | <0.05 |
| Mean cell hemoglobin (pg) | 58.94 ± 3.74 (50.87–71.59) | 5 | NS | 60.91 ± 3.68 (55.23–75.59) | 5 | NS | 55.57 ± 2.51 (49.3–63.26) | 5 | <0.05 |
| Mean cell hemoglobin concentration (g/dl) | 30.69 ± 1.03 (28.00–33.16) | 5 | NS | 33.32 ± 1.43 (29.38–37.47) | 5 | NS | 32.45 ±0.66 (30.00–33.58) | 5 | NS |
| Thrombocytes ($\times 10^9$/l) | 7.60 ± 1.08 (5.10–10.50) | 4 | NS | 6.34 ± 1.18 (3.70–8.80) | 5 | NS | 9.14 ± 1.12 (7.00–11.9) | 5 | NS |
| Fibrinogen (g/l) | 2.44 ± 0.58 (1.60–4.80) | 5 | NS | 1.88 ± 0.40 (1.00–2.90) | 4 | NS | 2.20 ± 0.70 (1.06–4.00) | 4 | NS |
| White blood cells ($\times 10^9$/l) | 11.10 ± 0.99 (8.75–14.13) | 5 | <0.005 | 10.88 ± 0.84 (9.35–14.15) | 5 | <0.005 | 12.26 ± 1.15 (9.10–14.75) | 5 | <0.005 |
| Heterophils ($\times 10^9$/l) | 4.61 ± 1.06 (1.39–7.21) | 5 | NS | 5.43 ± 0.91 (3.77–8.77) | 5 | NS | 4.91 ± 0.81 (1.91–6.81) | 5 | NS |
| Lymphocytes ($\times 10^9$/l) | 5.08 ± 0.66 (2.74–6.50) | 5 | <0.005 | 4.23 ± 0.37 (2.81–4.85) | 5 | <0.005 | 5.66 ± 0.62 (4.20–7.32) | 5 | <0.005 |
| Monocytes ($\times 10^9$/l) | 0.68 ± 0.33 (0.09–1.71) | 5 | NS | 0.69 ± 0.13 (0.40–1.17) | 5 | NS | 0.97 ± 0.26 (0.39–1.77) | 5 | NS |
| Eosinophils ($\times 10^9$/l) | 0.40 ± 0.18 (0.12–1.07) | 5 | NS | 0.29 ± 0.17 (0.00–0.92) | 5 | NS | 0.13 ± 0.06 (0.00–0.30) | 5 | NS |
| Basophils ($\times 10^9$/l) | 0.32 ± 0.10 (0.09–0.64) | 5 | NS | 0.25 ± 0.09 (0.00–0.52) | 5 | NS | 0.56 ± 0.20 (0.00–1.05) | 5 | NS |

TABLE 4. Published reference values for clinically normal adult houbara, rufous-crested, and white-bellied bustards.

| Parameter | Houbara bustard[d] | $n$[c] | White-bellied bustard[e] | $n$ | Rufous-crested bustard[f] | $n$ |
|---|---|---|---|---|---|---|
| Red blood cells ($\times 10^{12}$/l) | 2.51 ± 0.09[a] | 3 | 2.31 ± 0.08 | 3 | 2.89 ± 0.19 | 14 |
| | (1.95–3.36)[b] | 4 | (2.16–2.47) | | (2.00–4.27) | |
| Hemoglobin (g/dl) | 14.9 ± 0.16 | 3 | 15.23 ± 0.17 | 3 | 17.62 ± 0.52 | 14 |
| | (13.3–16.3) | 4 | (14.9–15.5) | | (5.50–22.20) | |
| Hematocrit (l/l) | 0.47 ± 0.52 | 3 | 0.47 ± 0.01 | 3 | 0.47 ± 0.01 | 14 |
| | (0.42–0.55) | 4 | (0.45–0.50) | | (0.40–0.50) | |
| Mean cell volume (fl) | 191.3 ± 6.89 | 3 | 205.7 ± 8.3 | 3 | 172.85 ± 9.36 | 14 |
| | (146.3–259.1) | 4 | (190.2–218.6) | | (105.39–222.0) | |
| Mean cell hemoglobin (pg) | 60.4 ± 2.19 | 3 | 66.23 ± 3.06 | 3 | 65.21 ± 4.46 | 14 |
| | (42.3–75.1) | 4 | (60.3–70.8) | | (40.71–97.50) | |
| Mean cell hemoglobin concentration (g/dl) | 31.52 ± 0.47 | 3 | 32.1 ± 1.0 | 3 | 37.63 ± 1.18 | 14 |
| | (26.1–35.3) | 4 | (30.6–34.0) | | (31.31–47.56) | |
| Thrombocytes ($\times 10^{9}$/l) | 5.81 ± 0.25 | 3 | 6.26 ± 0.7 | 3 | 5.66 ± 0.38 | 14 |
| | (3.2–7.85) | 4 | (5.2–7.6) | | (4.00–9.80) | |
| Fibrinogen (g/l) | 3.73 ± 0.24 | 3 | 2.73 ± 0.75 | 3 | 3.32 ± 0.32 | 14 |
| | (1.7–5.7) | 4 | (1.92–4.25) | | (1.44–5.88) | |
| White blood cells ($\times 10^{9}$/l) | 1.83 ± 0.15 | 3 | 2.51 ± 0.17 | 3 | 1.11 ± 0.20 | 14 |
| | (0.8–3.3) | 4 | (2.18–2.73) | | (0.31–3.03) | |
| Heterophils ($\times 10^{9}$/l) | 0.14 ± 0.05 | 3 | 0.45 ± 0.14 | 3 | 0.42 ± 0.10 | 14 |
| | (0.0–1.15) | 4 | (0.22–0.72) | | (0.04–1.30) | |
| Lymphocytes ($\times 10^{9}$/l) | 0.06 ± 0.02 | 3 | 0.24 ± 0.09 | 3 | 0.24 ± 0.04 | 14 |
| | (0.0–0.39) | 4 | (0.06–0.36) | | (0.00–0.62) | |
| Monocytes ($\times 10^{9}$/l) | 0.06 ± 0.02 | 3 | 0.3 ± 0.13 | 3 | 0.44 ± 0.08 | 14 |
| | (0.0–0.34) | 4 | (0.07–0.54) | | (0.10–1.23) | |
| Eosinophils ($\times 10^{9}$/l) | 6.85 ± 0.86 | 3 | 5.99 ± 2.9 | 3 | 8.81 ± 1.04 | 14 |
| | (2.09–8.45) | 4 | (3.06–11.8) | | (4.00–15.0) | |
| Basophils ($\times 10^{9}$/l) | 2.0 ± 0.20 | 3 | 2.0 ± 0.15 | 3 | 1.70 ± 0.23 | 14 |
| | (0.68–4.8) | 4 | (1.7–2.19) | | (0.66–4.31) | |

[a] Mean ± standard error of mean.
[b] (Minimum–Maximum).
[c] Total number of samples collected.
[d] Samour et al. 1994.
[e] D'Aloia et al. 1996.
[f] D'Aloia et al. 1995.

rod-shaped shaped slightly basophilic granules. The basophils are small round cells with a round nucleus surrounded by a colorless cytoplasm often obscured by overlying irregular round and strongly basophilic granules. The eosinophils are large round cells containing a bilobed nucleus with a slightly basophilic cytoplasm almost completely covered by large disc-like and slightly basophilic granules. Monocytes are the largest white cells. They are often irregular in shape with a lace-like pattern in the cytoplasm and contain a large irregular kidney shaped nucleus. Both small and large lymphocytes were in most of the blood films examined, which was in accordance with findings in juvenile kori bustards (Howlett et al., 1998). The small lymphocytes are round with a high nucleus/cytoplasm ratio, dark cytoplasm and a central nucleus. The large lymphocytes have an irregular cell border, light blue cytoplasm, and sometimes contained magenta granules.

In all three species the mean RBC, Hct, and Hb levels were significantly lower in juveniles when compared with adults, (Samour et al., 1994; D'Aloia et al., 1995, 1996). These parameters increased as the chicks developed. These findings are similar to other studies on juvenile birds (Hawkey et al., 1984a; Levi et al., 1989; Puerta et al., 1989, 1990; Alonso et al., 1990; Clubb et al., 1991a, b; Palomeque et al., 1991; D'Aloia et al., 1995; Howlett et al., 1998). In contrast, at 24 wk of age, the mean RBC count of $1.9\times10^{12}$/l for houbara bustards was still significantly lower ($P< 0.01$) than the established adult houbara mean RBC of $2.51\times10^{12}$/l (Samour et al., 1994). However the white-bellied and rufous-crested bustards RBC counts increased steadily up to the age of 24 wk. These values were not significantly different from values presented for the adult white-bellied ($2.31\times10^{12}$/l; D'Aloia et al., 1996) and rufous-crested bustards ($2.89x10^{12}$/l; D'Aloia et al., 1995). The Hb values for the houbara and white-bellied bustards continued to rise steadily until 24 wk of age to 13.6 g/dl and 14.2 g/dl, respectively. These values compare with the mean values established for the adult houbara (14.9 g/dl; Samour et al., 1994) and white-bellied bustard (15.2 g/dl; D'Aloia et al., 1996). For the rufous-crested bustard the mean Hb value at 24 wk was still significantly lower ($P<0.001$) at 14.3 g/dl, than the mean adult value 17.6 g/dl (D'Aloia et al., 1995). At 24 wk there was no significant difference in Hct levels between the juveniles and the adults in all three bustard species.

There was no significant difference between juvenile and adult rufous-crested bustard MCV (D'Aloia et al., 1995). For houbara and white-bellied bustards, the MCV was initially significantly lower in the juveniles than the adults (Samour et al., 1994; D'Aloia et al., 1996) and in subsequent months, there was no significant difference until 24 wk of age. For juvenile houbara and white-bellied bustards there was no significant difference in MCHC when compared to adults (Samour et al., 1994, D'Aloia et al., 1996). In the first 20 wk of age for these two species, there was no significant difference in MCH compared to adults. Although at 24 wk, values for juvenile white-bellied bustards were significantly lower than reported for adults (D'Aloia et al., 1996) and the houbara bustard was significantly higher than reported for the adults (Samour et al., 1994). However, the MCHC and MCH values for the juvenile rufous-crested bustards were both significantly lower than for adults (D'Aloia et al., 1995).

In our study, the mean total WBC counts were significantly higher and the observed range wider for houbara and rufous-crested bustards than mean values reported for adult birds (Samour et al., 1994; D'Aloia et al., 1995). Juvenile white-bellied bustards, WBC counts were similar to adult values up to 12 wk of age, when WBC counts became significantly higher than the adults (D'Aloia et al., 1996). For all three species of juvenile bustards mean lymphocyte counts were significantly higher than those reported for adult birds (Samour et al., 1994; D'Aloia et al., 1995, 1996).

The mean absolute heterophil, eosinophil, basophil, and thrombocyte counts for juvenile houbara, white-bellied and rufous-crested bustards were similar to values established for adults (Samour et al., 1994; D'Aloia et al., 1995, 1996). Similarly, the mean monocyte counts for white-bellied and rufous-crested bustard were comparable with established adult ranges. However, mean monocyte ranges for juvenile houbara bustards were significantly higher than those reported for the adults (Samour et al., 1994). Fibrinogen levels for juveniles of all three species were within the adult ranges.

## DISCUSSION

Lower RBC, Hb, and Hct levels and an increased WBC and lymphocyte count in our bustard chicks is similar to results for other juvenile birds. Age-related hematologic differences have been reported in captive Chilean (*Phoenicopterus chilensis*) and rosy (*P. ruber ruber*) flamingos (Hawkey et al., 1984a, b); domestic fowl, geese, and quail (Hodges, 1977); various psittacines (Clubb, 1990, 1991a, b); Masai ostriches (*Struthio camelus*; Levi et al., 1989; Palomeque et al., 1991; Fudge, 1996); white storks (*Ciconia ciconia*; Alonso et al., 1990b; Montesinos et al., 1997); white and black (*Ciconia nigra*) storks (Puerta et al., 1989); great bustards (*Otis tarda*; Alonso et al., 1990) and kori bustards (*Ardeotis kori*; Howlett et al., 1998).

The WBC counts in the juveniles were comparatively higher than in adults and the range of counts was wide. An increase in WBC count in the white-bellied and rufous-crested bustard chicks with age is in accordance with findings in juvenile kori bustards (Howlett et al., 1998). Reasons for the increase in WBC are not entirely clear, although it could possibly be due to changes in their housing, management, and social grouping. Campbell (1994) suggested that there is a wide variation in normal leukocyte counts among birds of the same species. There are a number of factors to take into account when interpreting leukocyte values in birds, including stress from capture, caging, social interactions, diet, environmental conditions, molt, sex, disease, temperature, social group, growth rate, and season. Mikaelian (1993) reported significant changes in the hematology values in houbara bustard depending on the time of year.

In our juvenile bustards, heterophil and lymphocyte counts were similar or the lymphocytes were the dominant leukocyte, as has been reported in juvenile kori bustards (Howlett et al., 1998). Conversely, in adult houbara, rufous-crested, and white-bellied bustards the heterophil was the predominant leukocyte (Samour et al., 1994; D'Aloia et al., 1995, 1996). In young great bustards, Alonso et al. (1990) reported lymphocytes and heterophils in similar numbers and high eosinophil levels. In adult great bustards, Jimenez et al. (1991) reported heterophils as the predominant leukocyte and higher WBC counts than those reported for chicks or any other bustards. Therefore, we concluded that there are age-related changes in the leukocyte differential count in these species. A similar conclusion was drawn in wintering common cranes (Puerta et al., 1990) and free-living white storks (Puerta et al., 1989). The immune response system could have a differential expression in young and in adult birds (Alonso et al., 1990).

This is the first study of age-related hematologic changes in white-bellied and houbara bustards and the most comprehensive study of rufous-crested bustards. Although the number of birds used in this study was relatively small, the findings demonstrate discernible age-related hematologic differences, some of which could hamper the interpretation of results from diseased individuals if not taken into account (Hawkey et al., 1984a). Whereas, D'Aloia et al. (1995) reported age-related findings in the rufous-crested bustard, only one group of 4–6 mo old chicks was assessed. The reference values we obtained are useful addition to data from other bustards (Alonso et al 1990; D'Aloia et al. 1995; Howlett et al., 1998) and other studies on adolescent birds. However further studies of hematologic responses of bustards to specific pathological conditions are warranted to assist in assessment of clinical cases.

## ACKNOWLEDGMENTS

We wish to thank H. H. Sheikh Khalifa bin Zayed al Nahyan, H. H. Sheikh Hamdan bin Zayed al Nahyan, and M. Al-Bowardi for their continued support of NARC. We also thank F. Launay, for reviewing the paper. Thanks go to A. Azur, M. Nafeez, I. Sleigh; N. Jarrett; A. Owen; B. Tarr; S. Anderson; N. Simpson, G. Clarkson; M. Black, and M. Fryer for the handling of the birds.

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*Received for publication 15 August 2001.*