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AGE-RELATED PLASMA CHEMISTRY CHANGES IN HOUBARA AND KORI BUSTARDS IN THE UNITED ARAB EMIRATES

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ABSTRACT: Plasma chemistry parameters were measured in adult (>1.5 yr) and juvenile (4–8 and 9–16 wk) captive houbara bustards (*Chlamydotis undulata macqueenii*) and from adult (>1.5 yr) and juvenile (4–8, 9–16, 17–24, 25–32, 33–40 and 41–52 wk) captive kori bustards (*Ardeotis kori*) to study age-related changes. A comparison of the values obtained was made between adult and juvenile bustards of both species and from the literature with other bustard species. Significant differences between adult and juvenile bustards of both species were found for glucose, total protein, alkaline phosphatase (ALKP), aspartate amino transferase (AST), lactate dehydrogenase, and calcium. Some parameters, such as calcium, showed comparable age-related changes in both species. In contrast, other parameters showed clear differences in the type (ALT, AST) or magnitude (ALKP) of age-related change between the species, demonstrating the importance of determining normal values for individual species. The results obtained from this study provide blood chemistry values for these species and demonstrate age-related differences between adult and juvenile birds.

Key words: Age-related differences, *Ardeotis kori, Chlamydotis undulata macqueenii,* Houbara bustard, Kori bustard, plasma chemistries.

INTRODUCTION

Management of bustards in captivity is handicapped by the paucity of biomedical data on these birds (Bailey et al., 1996), and blood chemistry studies have been instigated at the National Avian Research Center (NARC) (D'Aloia, 1996; D'Aloia et al., 1996a, b; Bailey et al., 1997, 1998). Blood biochemical analysis is an important supplement to history and clinical examination in determining the health status of animals, and previous investigations in juvenile psittacines (Clubb et al., 1990; Joyner and Duarte, 1994), storks (Montesinos et al., 1997) and bustards (D'Aloia, 1996) have described age-related changes in values. There are no published blood chemistry values for either juvenile houbara (*Chlamydotis undulata macqueenii*) and kori bustards (*Ardeotis kori*). This report describes the results of an investigation on age-related blood chemistry changes in captive houbara and kori bustards

MATERIALS AND METHODS

Blood samples were obtained from 13 male and eight female captive-reared houbara bustard chicks and six male and 11 female kori bustard chicks hatched in the 1995 (June to October 1995) and 1996 (June 1996 to June 1997) breeding seasons. Samples were collected from two age classes of houbara bustard chicks; 4–8 and 9–16 wk, and six age classes of kori bustard chicks; 4–8, 9–16, 17–24, 25–32, 33–40 and 41–52 wk after hatching. The birds were maintained at breeding facilities of the National Avian Research Center (NARC; 24°N, 55°E; Al Ain, Abu Dhabi Emirate, United Arab Emirates). Chicks were kept indoors in coops for the first 3 wk and moved to intermediate-stage rearing facilities with air-conditioned indoor units with outdoor access until 24 wk. After this period, they were moved to outdoor pens with natural vegetation. Their diet consisted of a proprietary pellet supplemented with alfalfa and invertebrates (Sleigh and Samour, 1996). These birds were part of a radiographic study to monitor the normal skeletal development of bustards (Naldo et al., 1997). The birds were captured by hand and were anaesthetised with a mixture of 3% isoflurane (Abbott Laboratories Ltd, Maidenhead, UK) in 2 l of oxygen for the sampling procedure, during which 0.5–1.5 ml of blood (depending on the size of the bird) was obtained. Sampling occurred before the first meal of the day, between 7 to 9 A.M., and within 30 min of capture. It was possible to collect an additional blood sample from individual birds, 4 wk apart, for some age classes.

Blood samples were obtained from 15 male and 13 female adult houbara bustards and 13

male and 15 female adult kori bustards during routine health checks in October 1996 to January 1997 at the same NARC breeding facility. The birds were 1.5- to 6-yr-old and were maintained in outdoor aviaries with natural vegetation. Their diet consisted of a proprietary pellet supplemented with vegetables, fruit, mice and invertebrates (Sleigh and Samour, 1996). All birds had been vaccinated against Newcastle disease 1 yr previously (Newcavac Nobilis, Intervet, Cambridge, UK) and had received prophylactic treatment against gastro-intestinal helminth (Droncit, Bayer plc, Bury St Edmunds, UK; Ivomec, MSD AGVET, Hoddesdon, UK) and protozoan parasites (Emtryl, Rhône Mérieux Ltd, Harlow, UK) at least 3 mo prior to sampling. The houbara bustards were captured by hand between 7 to 10 AM, placed in a transport box and taken to a nearby (<1 km) veterinary clinic at the same NARC site. They were manually restrained for the sampling procedure, during which approximately 2 ml of blood was obtained within 1 to 2 hr of capture. The kori bustards were captured by hand and were manually restrained for the sampling procedure which took place in the aviary within 15 min of capture.

Prior to sampling, all birds were examined by a veterinarian and declared clinically normal. After collection from the brachial (basilic) vein using either 1 ml or 3 ml disposable syringes and a 25 g butterfly cannulae, all samples were mixed immediately with the anticoagulant lithium heparin (1.8 mg/ml of blood) in commercially available storage tubes (Sarstedt, Numbrecht, Germany). These were immediately centrifuged at 10,000 *g* for 10 min using a bench-top centrifuge (Hawksley, Lancing, UK). Plasma was removed and stored in microcentrifuge tubes at −20 C until analyses were carried out (a maximum period of 6 mo). Part (0.5 ml) of the blood sample from juvenile birds was collected in the anticoagulent ethylene diamine tetra-acetic acid (EDTA 1.5 mg/ml of blood) for full haematological analysis at NARC. These results were used to determine normal reference ranges for these species (Howlett et al., 1998).

A wet chemistry Hitachi 91111 Analyser (Boehringer Mannheim, Mannheim, Germany) at the Central Veterinary Research Laboratory (CVRL, Dubai) was used to analyse the plasma. Total plasma protein and albumin were measured using the biuret and bromocresol green method respectively. Globulin values and albumin globulin ratios were derived from total protein and albumin values. The limits for detection of albumin and total protein were 0.1 to 5 and 0.1 to 15 g/dl respectively. The activity of aspartate aminotransferase (AST, EC 2.6.11), alanine aminotransferase (ALT, EC 2.6.1.2), alkaline phosphatase (ALKP, EC 3.1.3.1), lactate dehydrogenase (LDH) and creatine kinase (CK, EC 2.7.3.2) were measured at 37 C using commercial kits (Boehringer Mannheim, Mannheim, Germany). Magnesium and calcium were determined by the xylidyl-blue reaction and o-Cresolphthalein methods respectively and uric acid and cholesterol were determined by the enzymatic colorimetric test using commercial kits (Boehringer Mannheim, Mannheim, Germany). Quality control of all procedures was carried out using reference plasma (Precinorm U, Precipath U, Precinorm UPX, Boehringer Mannheim, Mannheim, Germany).

Statistical analysis was performed using the computer program MEDCALC (Medcalc Software, Mariakerke, Belgium). An analysis of variance (ANOVA) was used to determine significant differences between groups. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Most biochemical values varied significantly with age, except uric acid in both species, cholesterol in houbara bustards (not measured in kori bustards), and ALT in kori bustards (Tables 1, 2). Glucose, ALKP and LDH decreased, while total protein and calcium increased with age in both species. Creatine kinase and magnesium increased with age in houbara bustards, but were not measured in kori bustards. ALT increased with age in houbara bustards. Analysis of albumin levels in the plasma of the chicks proved problematic (most values were reported as below the analyser range) and were not included in Tables 1 and 2.

DISCUSSION

Blood chemistry reference values for adult houbara and kori bustards determined by dry chemistry analysis have been recently reported (D'Aloia et al., 1996a, b). A preliminary study by D'Aloia (1996), also using dry chemistry analysis, showed age-related blood chemistry changes in four young kori bustards, but no values (mean, SEM, range) were presented, due to the small sample size. Apparently, our study giving blood chemistry values and

TABLE 1. Biochemical values for clinically normal adults and two age classes of juvenile houbara bustards.

Assay	*P* value	Age		
		4–8 wks (*n* = 10–11)	9–16 wks (*n* = 24–26)	>1 yr (adult) (*n* = 28)
Glucose (mmol/l)[a]	0.016	20.70 ± 2.45[b] (14.93–39.63)[c]	16.90 ± 0.56 (13.21–24.37)	16.89 ± 0.26 (14.04–19.59)
Uric acid (μmol/l)	0.816	393.76 ± 55.61 (190.34–713.76)	402.32 ± 42.75 (107.1–856.51)	432.42 ± 39.79 (202.23–1,005.21)
Total protein (g/l)[a]	0.006	32.0 ± 0.97 (927.0–36.0)	33.12 ± 1.07 (23.0–48.0)	37.93 ± 0.9 (30.0–48.0)
Albumin (g/l)	ND[d]	ND	ND	14.5 ± 0.28 (11.0–18.0)
Globulin (g/l)	ND	ND	ND	2.38 ± 0.09 (1.7–3.7)
Albumin : globulin ratio	ND	ND	ND	0.64 ± 0.03 (0.32–0.84)
ALKP (U/l)[a]	0.044	622.8 ± 82.43 (257–1,131)	278.72 ± 23.97 (122–622)	80.39 ± 7.24 (17–175)
ALT (U/l)[a]	0.018	21 ± 1.31 (11–26)	22.2 ± 1.33 (14–42)	45.14 ± 3.27 (22–97)
AST (U/l)[a]	0.007	376.36 ± 16.21 (293–466)	342.2 ± 15.08 (247–528)	467.9 ± 24.93 (246–774)
LDH (U/l)[a]	0.006	934.6 ± 69.54 (676–1,284)	690.72 ± 46.31 (406–1,467)	609.57 ± 43.42 (246–774)
CK (U/l)[a]	0.01	228.1 ± 42.76 (55–427)	141.04 ± 24.52 (14–479)	778.4 ± 122.2 (12–2,309)
Magnesium (mmol/l)[a]	0.015	0.75 ± 0.05 (0.41–1.03)	0.80 ± 0.02 (0.58–1.03)	1.01 ± 0.03 (0.81–1.27)
Calcium (mmol/l)[a]	0.013	2.07 ± 0.13 (1.5–2.75)	1.81 ± 0.08 (1.1–2.73)	2.49 ± 0.08 (1.46–3.01)
Cholesterol (mmol/l)	0.273	6.29 ± 0.33 (4.65–7.76)	6.08 ± 0.28 (1.09–8.83)	6.78 ± 0.33 (4.06–10.65)

[a] Values significantly different among the groups ($P < 0.05$) (ANOVA).
[b] Mean ± standard error of the mean.
[c] Minimum–maximum.
[d] ND = not determined.

age-related changes using wet chemistry analysis in captive houbara and kori bustards is the first to be reported. Significant differences in a number of blood chemistry values between adult and juvenile birds of both species were found (Tables 1, 2).

The requirement of calcium for skeletal growth may explain the low circulating levels detected in juvenile birds in the current study. Skeletal maturation in houbara and kori bustards is still incomplete at 22 and 52 wk respectively (J. Naldo, unpubl. data), the age when samples were collected from the oldest juvenile bustards used in the current study. Although Hawkey et al (1991) reported that birds with calcium levels <1.8 mmol/l are likely to be hypocalcaemic, our results demonstrate that low levels in bustard chicks are a normal age-related difference and are not a sign of abnormal health. These findings are consistent with previous studies in young psittacines and kori bustards (Joyner and Duarte, 1994; Hochleithner, 1995; D'Aloia, 1996). Further studies to correlate plasma calcium with skeletal maturation in bustards and other birds are warranted.

The low total protein levels in our birds are consistent with previous studies in young psittacines, white storks (Montesinos et al., 1997) and kori bustards (Joyner

TABLE 2. Biochemical values for clinically normal adult and six age classes of juvenile kori bustards.

		Age						
Assay	*P* value	4–8 wk (*n* = 2–4)	9–16 wk (*n* = 6–8)	17–24 wk (*n* = 7–14)	25–32 wk (*n* = 6–13)	33–40 wk (*n* = 8–10)	41–52 wk (*n* = 6–7)	> 1 yr (adult) (*n* = 28)
Glucose (mmol/l)[a]	<0.0001	20.87 ± 0.33[b] (20.26–21.21)[c]	17.61 ± 0.57 (15.32–19.43)	17.74 ± 0.45 (15.43–19.87)	18.14 ± 0.49 (16.38–20.26)	18.26 ± 0.72 (14.65–22.37)	16.43 ± 0.52 (15.38–18.87)	14.16 ± 0.33 (11.1–17.65)
Uric acid (μmol/l)	0.057	233.76 ± 46.57 (148.7–309.30)	422.31 ± 63.12 (178.44–797.03)	548.11 ± 46.04 (333.08–808.93)	433.07 ± 33.49 (273.61–594.8)	480.0 ± 36.69 (297.4–612.64)	518.43 ± 67.27 (321.19–785.14)	534.25 ± 38.36 (237.92–969.52)
Total protein (g/l)[a]	<0.0001	17.25 ± 1.25 (14.0–20.0)	24.63 ± 1.22 (19.0–28.0)	35.86 ± 1.98 (24.0–51.0)	35.84 ± 1.22 (29.0–43.0)	37.0 ± 1.64 (28.0–43.0)	32.83 ± 1.25 (29.0–38.0)	30.0 ± 0.82 (23.0–40.0)
Albumin (g/l)	ND[d]	ND	ND	ND	ND	ND	ND	11.0 ± 0.3 (8.0–15.0)
Globulin (g/l)	ND	ND	ND	ND	ND	ND	ND	1.9 ± 0.06 (1.5–3.1)
Albumin/globulin ratio	ND	ND	ND	ND	ND	ND	ND	0.58 ± 0.01 (0.29–0.73)
ALKP (U/l)[a]	<0.0001	272.5 ± 34.5 (238–307)	182.75 ± 17.24 (117–239)	147.5 ± 16.35 (55–219)	130.75 ± 3.51 (119–144)	95.25 ± 5.92 (75–119)	56.67 ± 4.35 (47–78)	65.9 ± 2.6 (37–98)
ALT (U/l)	0.556	27 *n* = 1	27.29 ± 1.99 (20–35)	27.71 ± 2.69 (20–39)	28 ± 2.27 (20–34)	33.7 ± 1.54 (24–39)	25.33 ± 1.31 (23–31)	34.4 ± 3.43 (20–120)
AST (U/l)[a]	<0.0001	221.5 ± 8.5 (213–230)	273 ± 21.94 (208–408)	323.57 ± 16.39 (285–409)	294.67 ± 16.88 (231–400)	282.75 ± 13.94 (224–355)	228.67 ± 12.08 (191–271)	207 ± 7.1 (168–369)
LDH (U/l)[a]	0.001	1,114 ± 17 (1,124–1,158)	1,080.5 ± 48.29 (874–1,172)	1,315.14 ± 167.47 (1,008–2,307)	996.88 ± 35.80 (840–1,117)	994.88 ± 37.1 (848–1,163)	887.83 ± 42.91 (771–1,050)	818.2 ± 50.8 (543–1,921)
CK (U/l)	ND	ND	ND	ND	ND	ND	ND	275 ± 87.07 (35–2,527)
Magnesium (mmol/l)	ND	ND	ND	ND	ND	ND	ND	1.05 ± 0.02 (0.85–1.27)
Calcium (mmol/l)[a]	<0.0001	0.83 ± 0.1 (0.7–1.03)	1.41 ± 0.12 (1.0–1.8)	1.42 ± 0.07 (1.1–1.83)	1.67 ± 0.08 (1.25–2.2)	1.98 ± 0.1 (1.5–2.4)	1.94 ± 0.1 (1.5–2.28)	2.34 ± 0.07 (1.71–3.44)
Cholesterol (mmol/l)	ND	ND	ND	ND	ND	ND	ND	3.7 ± 0.13 (2.4–5.15)

[a] Values significantly different among the groups ($P < 0.05$) (ANOVA).
[b] Mean ± standard error of the mean.
[c] Minimum–maximum.
[d] ND = not determined.

and Duarte, 1994; D'Aloia, 1996). The reason for the high total protein levels in 17 to 40 wk kori bustards is not known.

The high glucose levels found in our birds is similar to a study in budgerigars (*Melopsittacus undulata*) by Hochleithner (1995) who found higher levels in juveniles than adults, but differs with a study in the brown pelican (*Pelecanus occidentalis*) by Wolf (1984) who found an increase in serum glucose from fledging to juvenile stages. The reason for these differences are not known, but could be related to differences in sampling methods and/or temperament between adult and juvenile birds. In the case of the bustard chicks, samples were collected in the morning before the first meal of the day and although adults were also captured in the morning they had access to food in the aviaries.

The high ALKP in both bustard species is consistent with previous studies in young psittacines and kori bustards (Clubb et al., 1990; Joyner et al., 1990; D'Aloia, 1996) and is considered to be associated with normal bone growth and development.

The low plasma AST levels in houbara bustard chicks is consistent with previous studies in young psittacines and storks (Clubb et al., 1990; Hochleithner, 1995; Montesinos et al., 1997), but the cause of this age-dependent increase in activity has not been established. Reasons for the apparently higher levels of AST in juvenile kori bustards in the current study are not known. In a previous study AST levels in juvenile kori bustard chicks were also higher compared with adults, but because there were only one to three samples for each age group statistics were not performed (D'Aloia, 1996).

Plasma LDH values were significantly higher in some age classes of juvenile kori and houbara bustards compared with adult birds. In contrast, plasma CK values in juvenile houbara bustards were lower compared with adults. The reasons for these differences in LDH and CK values have not been determined, although they could be related to differences in capture technique and the time that samples were collected after capture. Plasma CK values of adult houbara bustards in the current study were higher than levels (335.9 ± 24.2 U/l) previously reported in adult houbara bustards that were sampled within 10 min of capture (Bailey et al., 1997). Plasma CK is markedly elevated in birds, including mallard ducks (*Anas platyrhynchos*) and houbara bustards following routine capture and handling (Bollinger et al., 1989; Bailey et al., 1997), indicating that muscle damage may occur during these procedures. In the current study samples were collected from juvenile houbara bustards within 30 min of capture from small rearing pens, compared with the adults which were caught in large aviaries and sampled 1 to 2 hr after capture. Additionally, the temperament of adults and juveniles differed considerably, juveniles were hand-reared birds and very tame, while the adults were of a more nervous temperament and struggled more when restrained. This suggests that differences in CK between adult and juvenile houbara bustards is related to bird temperament and/or sampling time after capture, rather than being an age-related change.

Only three comparable values were available from 4- to 8-wk-old free-living great bustard (*Otis tarda*) chicks to compare with the juvenile kori and houbara bustard values (Alonso et al., 1990). Values for total protein (36.0 ± 1.0 g/l) and uric acid (1303.1 ± 124.9 μmol/l) in the great bustard were above the ranges that were obtained for 4 to 8 wk houbara and kori bustard chicks. The value for cholesterol (3.43 ± 0.24 mmol/l) in the great bustard was below the range of that obtained for 4 to 8 wk houbara bustard chicks. These differences may represent variations between species, analytical techniques, or captive-reared and free-living chicks. Results from the current study and previous investigations in young psittacines (Clubb et al., 1990; Joyner et al., 1990) and kori bustards (D'Aloia, 1996) are different from

the findings of Alonso et al (1990) who concluded that total protein values are not subjected to age-related changes in great bustards.

The range of values for glucose, uric acid, total protein, ALT, calcium and cholesterol in adult houbara and kori bustards in the current study were all within the range of values recently reported for adults of the same species (D'Aloia et al., 1996a, b). ALKP values in houbara bustards in the current study were also within the range of values previously reported for this species (D'Aloia et al., 1996a), while the ALKP values reported for adult kori bustards are the first to be reported for adults of this species.

Adult kori bustard values for AST in the current study were within the range recently reported for adult birds by D'Aloia et al. (1996a). These samples were derived from the same kori bustard flock under the same management two years after the study by D'Aloia et al. (1996a). In contrast, in the current study adult houbara bustard values for AST were higher (467.9 ± 24.93 U/l) than previously published values (372.91 ± 13.29 U/l) determined by dry chemistry (D'Aloia et al., 1996b). The adult houbara bustards sampled by D'Aloia et al. (1996b) were housed in a quarantine station and fed only a standard diet, while the adult birds used in the current study were kept in naturalistic aviaries where it is known that approximately 25% of the diet consists of natural food (annual plants and terrestrial invertebrates) from their surroundings, in addition to the same standard diet (Warren, 1996). It is possible that differences in AST between these two groups of adult houbara bustards is related to these dietary differences and further investigations are warranted.

Diagnosis of diseases in bustards, particularly juveniles, is hampered by the scant knowledge of the reference ranges for laboratory parameters, including blood chemistry values, that are found in healthy individuals. In the current study some parameters, such as calcium showed comparable age-related changes in both species. In contrast, other parameters, showed clear differences in the type (ALT, AST) or magnitude (ALKP) of age-related change between the species, demonstrating the importance of determining normal values for different age groups of each species. Although biochemical parameters assayed from stored samples should be interpreted with care (Thoresen et al., 1995), our results provide values for both clinically normal adult and juvenile kori and houbara bustards which may prove useful for the interpretation of some laboratory findings in clinical cases.

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