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ABSTRACT: Vitamin E (measured as α-tocopherol) and cholesterol concentrations were determined in plasma samples collected from 86 clinically healthy captive adult bustards of six species and 23 captive juveniles (6–12 mo old) of two of these species. Adult houbara bustards (*Chlamydotis undulata macqueenii*) had higher plasma α-tocopherol concentrations than juveniles (adult: mean ± SE, 11.07 ± 0.41 μg/ml, $n = 32$; juvenile: 6.33 ± 0.48, $n = 12$) and higher α-tocopherol : cholesterol ratios (adult: 6.09 ± 0.44, $n = 12$; juvenile: 2.94 ± 0.22, $n = 11$). No age difference was evident for kori bustard (*Ardeotis kori*) plasma α-tocopherol concentrations (adult: 4.43 ± 0.42, $n = 21$; juvenile: 4.46 ± 0.26, $n = 11$) or α-tocopherol : cholesterol ratios (adult: 3.67 ± 0.44, $n = 20$; juvenile: 3.71 ± 0.36, $n = 11$). Adult houbara bustards had significantly higher ($P < 0.01$) α-tocopherol concentrations compared with adult rufous-crested (*Eupodotis ruficrista*; 6.64 ± 0.33, $n = 19$) and white-bellied (*Eupodotis senegalensis*; 7.75 ± 0.81, $n = 8$) bustards, but similar α-tocopherol : cholesterol ratios (rufous-crested: 5.56 ± 0.32, $n = 18$; white-bellied: 5.83 ± 0.43, $n = 8$). Juvenile houbara bustards had higher plasma α-tocopherol concentrations than juvenile kori bustards but similar α-tocopherol : cholesterol ratios. Adult houbara bustard plasma α-tocopherol levels and α-tocopherol : cholesterol ratios did not differ significantly between sexes. The vitamin E status of adult bustards appeared to be influenced by environmental conditions that varied due to species-specific husbandry regimes, but no clear relationship was seen with dietary vitamin E levels. Juvenile bustards did not have higher vitamin E levels than adults, despite being maintained on four-fold dietary vitamin E concentrations and in similar environmental conditions. This paper presents the first published data for plasma vitamin E concentrations in bustards. The plasma α-tocopherol and cholesterol concentrations and α-tocopherol : cholesterol ratios of captive bustards were similar to those previously reported for omnivorous avian species. Further research is required to determine which components of the identified environmental conditions affect bustard vitamin E status and to confirm whether differences exist between species independent of the variation in their management regimes.

Key words: Alpha-tocopherol, *Ardeotis,* bustard, *Chlamydotis,* diet, *Eupodotis, Neotis,* nutrition.

INTRODUCTION

Adequate levels of vitamin E are necessary for good health and successful reproduction in birds. Vitamin E deficiency in captive avian species is associated with low fertility, low hatchability, immunosuppression, and specific clinical abnormalities such as encephalomalacia and muscular myopathies (Kling and Soares, 1980; Scott et al., 1982; Dierenfeld, 1989; Dierenfeld et al., 1993; Ullrey, 1993).

Normal ranges of vitamin E values for avian species assist with the diagnosis of morbidity and reproductive failure and assist with assessment of the adequacy of diets for captive species. Plasma vitamin E levels are one method of assessing overall vitamin E status, although other dietary and environmental factors need to be taken into account when interpreting results (Dierenfeld, 1989). Vitamin E is dependent on lipid components for absorption and transportation, so it is important to evaluate blood vitamin E levels relative to blood lipid levels (Horwitt et al., 1972). Since blood cholesterol assay is a routine procedure, cholesterol levels are commonly used as an index of blood lipid levels, with blood α-tocopherol levels standardized by calculating α-tocopherol : cholesterol ratios. Plasma vitamin E and choles-

TABLE 1.   Management regime of each bustard species and age group at the time of blood sample collection.

Bustard species	Age[a]	Diet	Availability of natural food[b]	Pen area (m^2)	Social grouping
Houbara	Adult	Maintenance	Limited	25	Singles
	Juvenile	Production	Limited	108	Small groups (≤5)
Kori	Adult	Maintenance	Available	14,400	Large group (~25)
				100	Trio
	Juvenile	Production	Available	1,800	Large group (~15)
Rufous-crested	Adult	Production	Available	108	Pairs, singles
White-bellied	Adult	Production	Available	5,184	Pair
				108	Singles
Black	Adult	Maintenance	Negligible	25	Singles
Heuglin's	Adult	Production	Available	5,184	Small group (5)

[a] Adult: >12 mo; juvenile: 6–12 mo.
[b] Negligible: only had access to invertebrates that may have entered their pens; Limited: had access to invertebrates and small lizards that may have entered their pens, some adult pens had cultivated alfalfa beds, juvenile pens contained shrubs and cultivated alfalfa beds; Available: pens contained shrubs, varying quantities of grasses and herbaceous vegetation, cultivated alfalfa beds (except for the kori bustard pens), invertebrates, and small lizards.

terol concentrations have been measured in a wide range of captive avian species (Gulland et al., 1988; Dierenfeld et al., 1989, 1993; Schweigert et al., 1991; Dierenfeld and Traber, 1992), but vitamin E levels have not previously been reported in bustards.

Bustards are maintained in zoological and private collections throughout the world; they are especially common in collections in the Middle East. The National Avian Research Center (NARC) in Abu Dhabi, United Arab Emirates, runs captive breeding programs for houbara (*Chlamydotis undulata macqueenii*), kori (*Ardeotis kori*), and rufous-crested (*Eupodotis ruficrista*) bustards and maintains small numbers of white-bellied (*Eupodotis senegalensis*), black (*Eupodotis afra*), and Heuglin's (*Neotis heuglinii*) bustards.

Vitamin E deficiency has been implicated in captive adult bustards with capture myopathy (Bailey et al., 1996) and in vitamin E-responsive encephalomalacia in juvenile bustards (Bailey et al., 1997). However, α-tocopherol levels were not determined for these individuals, and values from clinically healthy bustards were not available for comparison. The aim of this study was to establish normal plasma vitamin E ranges for the six bustard species

maintained by NARC to assist with detection and diagnosis of abnormalities.

MATERIALS AND METHODS

All of the captive bustards were maintained in Abu Dhabi Emirate at NARC's Sweihan (adult houbara) and Al Ain (juvenile houbara and all other bustard species) sites (24°13′N, 55°46′E). Table 1 summarizes differences in management of each species and of age groups within species. All species and age groups were maintained on the stated diet for 3–6 mo prior to sample collection. The maintenance diet consisted of, on an as fed basis, 75% bustard maintenance pellet (Abu Dhabi Flour & Animal Feed Factory, Abu Dhabi, UAE), 10% 'animal matter' (cultured mice or mealworm larvae [*Tenebrio molitor*], or pre-thawed raw minced meat when mice and mealworm larvae were not available), 10% diced apple, 5% chopped cabbage, plus a mineral-vitamin supplement (SA-37; Intervet UK Ltd., Cambridge, UK), and additional calcium (calcium carbonate granules); the production diet was identical except bustard productioner pellet was used. Pellet vitamin E concentrations were assayed by reverse-phase high pressure liquid chromatography (HPLC) at a commercial laboratory (Aspland & James Ltd., Chatteris, UK) in 1995; the maintenance pellet contained, on an as fed basis, 51.30 mg/kg vitamin E (measured as dl-α-tocopherol acetate) and the productioner pellet contained 212.00 mg/kg. Vitamin E was not assayed in the other dietary components.

Blood samples were collected from clinically healthy birds at routine pre-breeding season

health checks (kori bustards April–May 1997, all other species November–December 1996). Single blood samples were collected from 86 adults of the six species maintained in the collection and from 23 juveniles of two species. The birds were all caught early in the morning; none had food withheld prior to capture. The two largest bustard species (kori and Heuglin's) were manually restrained and blood samples collected within 15 min of capture; the other species were placed in pet carrier crates and transported to on-site veterinary clinics for examination and sample collection. After collection from the brachial (basilic) vein using 3 ml disposable syringes and 25 gauge butterfly cannulae, all blood samples were mixed immediately with the anticoagulant lithium heparin (1.8 mg/ml of blood) in commercially available storage tubes (Sarstedt, Numbrecht, Germany). Samples were wrapped in aluminum foil and stored on ice in a cool box, then centrifuged at $10,000 \times G$ for 10 min at room temperature using a bench-top centrifuge (Hawksley, Lancing, UK) within 30 min of collection. Plasma was removed, divided into two 500 µl aliquots, and then stored in separate microcentrifuge tubes at -20 C. Samples for α-tocopherol assay were transferred to a -80 C freezer within 24 hrs; the second set of samples remained stored at -20 C until analyzed for cholesterol content.

Plasma samples from three additional adult houbara bustards were provided by a private collection in Dubai, United Arab Emirates. These birds were housed in a mixed-sex group of adults in a 32 m^2 pen without vegetation and offered a diet consisting of commercial poultry pellets, minced meat, hard-boiled eggs, and lettuce.

Plasma α-tocopherol concentrations were determined by HPLC at the Department of Pediatrics laboratory at the United Arab Emirates University (Al Ain, Abu Dhabi). Plasma α-tocopherol was extracted with n-hexane and the extract evaporated to dryness under nitrogen. The extract was mixed with diethyl ether followed by ethanol prior to injection on to the HPLC column. Alpha-tocopherol concentration was determined using an HPLC system (Hewlett Packard model 1090, Hewlett-Packard Co., Palo Alto, California, USA) with a diode array detector. A C-18, 250 × 4.6 mm reversed-phase column with 5-µm packing was used for separation and the plasma α-tocopherol was measured by absorbance at 290 nm (Bieri et al., 1979). For quantification, standard curves were prepared. Reproducibility was monitored prior to, during, and at the end of each run. Concentrations of total cholesterol (high, low, and very low density lipids) were determined at NARC. Plasma was deposited on cholesterol reagent slides and cholesterol concentration was measured colorimetrically at 555 nm using a dry-chemistry system (Kodak Ektachem DT II, Eastman Kodak Co., Rochester, New York, USA). Quality control was carried out for the α-tocopherol and cholesterol assays using standard reference plasma.

Results are expressed as mean ± standard error (SE). Statistical analyses were performed using SYSTAT software (Wilkinson, 1992). Means were compared between sexes (houbara bustard only) using Student's t-test, and between species (houbara, rufous-crested, and white-bellied bustards only) using one-way analysis of variance (ANOVA) with probability levels adjusted using the Tukey HSD multiple comparison procedure (Zar, 1984). For the ANOVA, the values from each pair of bustards housed together were averaged prior to analysis, and the data from all three species were log-transformed to stabilize variances. Most of the kori bustards were housed in a large group of similar-aged birds; consequently this species was excluded from statistical analyses due to non-independence of samples. The black bustards and Heuglin's bustards were excluded from analyses due to small sample size.

RESULTS

Plasma α-tocopherol and cholesterol concentrations in the six bustard species maintained by NARC are presented in Table 2. The mean α-tocopherol value for the three adult houbara bustards in Dubai was 12.22 ± 0.69 µg/ml (cholesterol was not assayed).

Intra-species age differences were evident for houbara bustards; adults generally had higher plasma α-tocopherol levels and α-tocopherol : cholesterol ratios than juveniles (Table 2), but cholesterol levels did not differ with age. Kori bustards did not show age-related variation in plasma α-tocopherol concentrations, cholesterol levels, or α-tocopherol : cholesterol ratios (Table 2). Alpha-tocopherol levels, cholesterol levels, and α-tocopherol : cholesterol ratios did not differ significantly between sexes for adult houbara bustards.

Inter-species differences were evident for adults and juveniles (Table 2). Adult houbara bustards had significantly higher plasma α-tocopherol and cholesterol concentrations than adult rufous-crested and

TABLE 2.   Plasma α-tocopherol and cholesterol concentrations (mean ± SE, with range in parentheses) in captive bustards in Abu Dhabi, United Arab Emirates.

Bustard species	Age[a]	α-tocopherol		Cholesterol		α-tocopherol: cholesterol (µg/mg)
		n[b]	(µg/ml)	n[b]	(mg/ml)	
Houbara	Adult	32	11.07 ± 0.41[c] (6.20–15.20)	12	1.93 ± 0.10[c] (1.38–2.58)	6.09 ± 0.44[d] (3.31–9.20)
	Juvenile	12	6.33 ± 0.48 (4.17–9.56)	11	2.08 ± 0.09 (1.61–2.74)	2.94 ± 0.22 (1.93–4.55)
Kori	Adult	21	4.43 ± 0.42 (2.47–10.13)	20	1.23 ± 0.25 (0.90–1.83)	3.67 ± 0.44 (1.72–10.03)
	Juvenile	11	4.46 ± 0.26 (2.64–5.65)	11	1.28 ± 0.11 (0.71–2.03)	3.71 ± 0.36 (2.29–6.38)
Rufous-crested	Adult	19	6.64 ± 0.33[d] (4.77–10.40)	18	1.22 ± 0.05[d] (0.90–1.65)	5.56 ± 0.32[d] (3.52–8.20)
White-bellied	Adult	8	7.75 ± 0.81[d] (5.32–10.98)	8	1.35 ± 0.13[d] (0.98–1.92)	5.83 ± 0.43[d] (3.61–7.87)
Black	Adult	2	10.08 ± 0.06 (10.01–10.14)	2	1.37 ± 0.04 (1.33–1.41)	7.36 ± 0.17 (7.19–7.53)
Heuglin's	Adult	4	6.08 ± 0.64 (4.87–7.85)	4	1.16 ± 0.09 (0.99–1.33)	5.39 ± 0.86 (4.24–7.93)

[a] Adult: >12 mo; juvenile: 6–12 mo.
[b] Number of birds.
[c,d] Means within columns with different superscripts differ significantly ($P < 0.01$). Means without superscripts could not be statistically compared.

white-bellied bustards ($P < 0.01$ for all significant pairwise comparisons) but their α-tocopherol : cholesterol ratios did not differ. Adult houbara bustards also generally had higher plasma α-tocopherol and cholesterol concentrations and α-tocopherol : cholesterol ratios than adult kori bustards. Juvenile houbara bustards generally had higher plasma α-tocopherol and cholesterol concentrations than juvenile kori bustards but similar α-tocopherol : cholesterol ratios.

DISCUSSION

The overall mean and range of means observed for adult bustards in this study (α-tocopherol: mean 7.68 µg/ml, range 4.43–11.07 µg/ml; cholesterol: 1.38 mg/ml, 1.16–2.08 mg/ml; α-tocopherol:cholesterol: 5.65 µg/mg, 3.67–7.36 µg/mg) did not differ substantially from comparable figures reported for other captive Gruiform species (Dierenfeld et al., 1993) or for other captive omnivorous birds such as nondomestic Galliformes (Dierenfeld and Traber, 1992).

Although significant inter-species differences were observed for adult bustards there was also a strong association between plasma vitamin E status and environmental factors that varied depending on each species' husbandry regime (Table 1). At one end of the spectrum were adult houbara bustards, housed singly in small pens (the most practical strategy for individuals in an artificial insemination program) with limited access to natural food sources, and at the other end of the spectrum were adult kori bustards, housed in large groups in large pens with access to natural food sources. Because all species were not maintained under a similar regime, conclusions cannot be drawn as to whether the differences between species are real or whether they reflect management-imposed variation in factors such as social grouping (Dierenfeld, 1989), physical exercise levels (Quintanilha and Packer, 1983), and influence of dietary vitamin E intake by consumption of natural food items.

There was evidence of age-specific dif-

ferences in bustard vitamin E status. Despite being maintained on a diet with vitamin E levels four times higher than that of adult houbara and kori bustards, the juveniles had, respectively, lower and similar vitamin E levels compared with adults. The juvenile houbara bustards had larger pens than the adults and also were held in small groups. Both factors may have resulted in the juveniles having higher levels of physical activity than the adults and consequently may have contributed to increased vitamin E requirements over and above any age-specific difference. The juvenile kori bustards were maintained in similar environmental conditions to the adults and the similarity in their plasma vitamin E levels, despite a higher vitamin E intake, suggests they were using more of this vitamin as it was consumed. Given that it may take several months for the tissue stores of adults to become saturated in response to vitamin E supplementation (Dierenfeld et al., 1989), it seems likely that it would also take juveniles at least several months to establish tissue stores of vitamin E which would then enable circulating vitamin E levels to be maintained at a higher level.

Differences in plasma α-tocopherol concentrations compared with α-tocopherol : cholesterol ratios highlight the importance of considering variation in blood lipid levels when assessing vitamin E status. Juvenile houbara bustards, for example, had higher plasma α-tocopherol concentrations than juvenile kori bustards but they also had higher plasma cholesterol levels and consequently their vitamin E status relative to lipid status was similar.

Cholesterol is obtained from animal matter sources in the diet and is also synthesized by the liver. The observed differences in plasma cholesterol concentrations may therefore reflect differences in species metabolism, dietary intake, or physical exercise levels. Similar plasma cholesterol levels have been found in adult houbara, kori, rufous-crested, and white-bellied bustards (D'Aloia et al., 1996a, b; Bailey et al., 1998a, b, 1999), despite differences in cholesterol assay methods (wet- vs. drychemistry systems) and, for the houbara, differences in social grouping and other environmental conditions. In one study, adult houbara cholesterol levels were even higher than in the present study (Bailey et al., 1999).

Actual dietary vitamin E intake was not quantified or controlled in this study. Most of the bustards had access to varying quantities of natural vegetation and invertebrates as additional food sources, and they were offered a cafeteria-style diet. This was a major limitation of this study and precluded any meaningful analysis of relationships between bustard plasma α-tocopherol concentrations and dietary levels of nutrients that influence vitamin E status (vitamin E, selenium, and polyunsaturated fatty acids; Dierenfeld, 1989; Dierenfeld et al., 1993). However, none of the birds sampled in this study showed clinical signs of vitamin E deficiency or capture myopathy while maintained on this dietary regime, and all species for which males and females are held have reproduced successfully. The vitamin E status of bustards maintained under the current dietary and management regime can therefore be considered to be adequate, and the plasma α-tocopherol concentrations presented here can be considered representative of the normal range of values for clinically healthy non-fasting bustards maintained under this particular set of management conditions.

Further research is needed to confirm whether differences exist between bustard species independent of the variation in their husbandry regimes. It would be useful to monitor plasma vitamin E values for each species throughout the year, particularly to investigate whether the vitamin E status of adult houbara and kori bustards increases when they are maintained on the bustard productioner pellet. Plasma α-tocopherol and cholesterol values from freeranging individuals of each species would also be useful for comparative purposes.

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