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VITAMIN E IN CRANES: REFERENCE RANGES AND NUTRIENT INTERACTIONS

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ABSTRACT: Fat soluble vitamins E and A (quantified as α-tocopherol and all-trans retinol, respectively) were measured in plasma samples from 274 captive cranes from four institutions and five free-ranging birds. Ages ranged from 4 mo to 80 yr, and all 15 crane species were represented. Captive cranes had a mean ± standard error (SE) of 6.57 ± 0.82 μg/ml α-tocopherol; migrating greater sandhill cranes (*Grus canadenis tabida*) had a plasma concentration of 3.71 ± 0.22 μg/ml. Sex and age differences were not significant, but crane species that evolved in temperate habitats had higher circulating levels of α-tocopherol than tropical or subtropical species. Mean ± SE retinol values were 0.69 ± 0.05 μg/ml in captive cranes, and 0.66 ± 0.08 μg/ml in free-ranging cranes; values did not differ significantly by sex, age, or species. Dietary vitamin E concentrations were significantly correlated with plasma α-tocopherol levels in a logarithmic relationship. Dietary selenium at 0.5 mg/kg was associated with decreased circulating α-tocopherol concentrations.

Key words: Crane, diet, nutrition, vitamin A, vitamin E, selenium.

INTRODUCTION

Isolated cases of capture myopathy have been described in crane species including East African crowned cranes (*Balearica rugelorum gibbericeps*), and both greater sandhill (*Grus canadensis tabida*) and Mississippi sandhill (*G. canadensis pulla*) cranes (Windingstad et al., 1983; Carpenter et al., 1991). Clinical and pathological signs observed were similar to those documented in vitamin E-deficient species including cranes (Liu et al., 1985). Although vitamin E status influences oxidative tissue damage during physical exertion (Aikawa, 1984), capture myopathy has been recorded in species with apparently normal vitamin E levels (Spraker, 1980).

Vitamin E is the generic term for a group of eight tocol and tocotrienol derivatives. While each compound possesses some degree of vitamin activity, α-tocopherol is the most active isomer found in animal feeds and monitored in blood. Similarly, vitamin A is a generic term for compounds possessing the biological activity of all-trans retinol. One International Unit (IU) of vitamin E is equivalent to 1.1 mg racemic α-tocopherol (as measured in this study); 0.3 μg of all-trans retinol is equivalent to 1 IU of vitamin A (Machlin, 1984). Antagonistic interactions of dietary vitamins A and E have been reported in domestic poultry (Frigg and Broz, 1984); high levels of one may decrease intestinal absorption or utilization of the other.

A mean ± standard error (SE) plasma α-tocopherol concentration of 7.6 ± 0.5 μg/ml (n = 185) has been documented for healthy cranes (Dierenfeld, 1989), but α-tocopherol levels were not determined for any cranes with capture myopathy or vitamin E deficiency. The relation between plasma α-tocopherol and capture myopathy in avian species has not been adequately investigated. In this report, we compare plasma α-tocopherol concentrations among crane species, document differences in circulating levels of α-tocopherol in cranes fed known dietary concentrations of vitamin E, and provide limited comparative data obtained from free-ranging cranes. Interactions of dietary vitamin E and selenium (Se) also were examined through feeding trials over a 4-yr

period (1986 to 1989). Such baseline data should prove useful in assessing nutrient status in both captive and wild crane populations.

MATERIALS AND METHODS

Four hundred and eighty-five blood samples were obtained by jugular, tarsal, or ulnar venipuncture from 274 individuals at the International Crane Foundation (ICF), Baraboo, Wisconsin (USA) (n = 168); New York Zoological Park (NYZP), Bronx, New York (USA) (n = 48); St. Catherines Island Survival Center (SCISC), Midway, Georgia (USA) (n = 25); or Patuxent Wildlife Research Center (PWRC), Laurel, Maryland (USA) (n = 33). Bird ages ranged from 4 mo to 80 yr, and all 15 extant crane species were represented. All cranes were fed *ad libitum* on pelleted diets containing vitamin E as racemic α-tocopheryl acetate at concentrations of 22 (PWRC), 33 (SCISC), 55 (ICF), or 660 (NYZP) IU/kg. Pellets were either locally milled (ICF) based on published nutrient specifications (Carpenter, 1986) or commercially available (Crane Maintenance or Breeder Diet, Zeigler Bros., Inc., Gardners, Pennsylvania, USA [PWRC, SCISC]; Avi-Pels, Blue Seal Feeds, Lawrence, Massachusetts, USA [NYZP]). An additional five blood samples from free-ranging adult and subadult greater sandhill cranes were obtained in 1988 from the brachial vein of individuals captured at Seney National Wildlife Refuge, Seney, Michigan (USA) (45°15′N, 86°12′W).

Interactions of dietary vitamin E and Se upon circulating concentrations of α-tocopherol were evaluated by altering pellet composition over a 4-yr period at the ICF. The original diet, manufactured by a local feed mill as described above, contained 55 IU/kg vitamin E (as α-tocopheryl acetate, Hoffmann LaRoche, Inc., Nutley, New Jersey, USA) and 0.1 mg/kg Se as Na_2SeO_3. A high-Se pellet (0.5 mg/kg Se; 55 IU/kg vitamin E) was fed for 1 year (1987), followed by pellets containing 0.3 mg/kg Se and 200 IU/kg vitamin E for 2 yr (1988 to 1990). No other nutrient concentrations in pellets were altered during this time frame. Diet changes were implemented in January of each year, and all cranes were bled by jugular venipuncture in October or November of the same year. Thus serum values represent effects of ≥10 months' feeding trials.

In all cases, plasma was separated by centrifugation and frozen immediately. Samples were extracted following the methods of Storer (1974), with modifications as described by Dierenfeld and McGuire (1991). Alpha-tocopherol and retinol concentrations were determined by high-performance liquid chromatography. A Series 400 Liquid Chromatograph (Perkin-Elmer, Inc., Norwalk, Connecticut, USA) equipped with a 30-cm C18 reversed-phase column was used for separation; α-tocopherol was analyzed with a Perkin-Elmer Model LS-1 fluorescence detector. Retinol was assessed at 325 nm with a Perkin-Elmer Model LC-95 spectrophotometer, and peak areas on the chromatographs were analyzed against standard concentrations with a Perkin-Elmer Model LCI-100 data processor. Cholesterol was measured on the Technicon RA 1000 (Technicon Instrumentations Corporation, Tarrytown, New York, USA).

All data were analyzed statistically using the SYSTAT computer software package (Wilkinson, 1987). Means were compared by one-way analysis of variance with a Tukey HSD value or a Student's t-test (Snedecor and Cochran, 1980); statistical significance was determined at $P < 0.05$.

RESULTS

Circulating levels of α-tocopherol, retinol, and cholesterol in 15 species of cranes are summarized in Table 1. Captive cranes had a mean ± SE of 6.57 ± 0.82 μg/ml α-tocopherol. Sex and age differences were not significant. Temperate species such as the hooded (*G. monacha*), Siberian (*G. leucogeranus*), and white-naped cranes had significantly higher circulating levels of α-tocopherol than tropical and subtropical species such as the wattled (*Bugeranus carunculatus*), Stanley (*Anthropoides paradisea*), brolga (*G. rubicunda rubicunda*), sarus (*G. antigone antigone*), and black-crowned (*Balearica pavonina*) cranes. Differences between subspecies were not significant; mean ± SE values for greater sandhill cranes from temperate areas (7.05 ± 0.64 μg/ml, n = 30), subtropical Florida (6.08 ± 0.40 μg/ml, n = 56), and Mississippi sandhill cranes (5.01 ± 0.54, n = 9) were similar. Values for eastern sarus cranes (5.27 ± 0.63, n = 29) were similar to Indian sarus cranes (5.39 ± 0.45 μ/ml, n = 3); values for East African (2.74 ± 0.18, n = 3) and West African crowned cranes (2.60 ± 0.19 μg/ml, n = 8) also were similar. The mean α-tocopherol value for free-ranging greater sandhill cranes (n = 5) was 3.71 ± 0.22 μg/ml.

TABLE 1. Plasma α-tocopherol, retinol, and cholesterol in captive Gruiformes.

Species	Number of cranes	α-tocopherol μg/ml	Retinol μg/ml	Cholesterol mg/ml
Common crane (*Grus grus*)	9	6.93 ± 1.24[a]	0.64 ± 0.05	1.51 ± 0.17
Sandhill crane (*G. canadensis canadensis*)	95	6.28 ± 0.49	0.82 ± 0.04	1.57 ± 0.44
Red-crowned crane (*G. japonensis*)	57	6.13 ± 0.46	0.80 ± 0.02	1.29 ± 0.60
Whooping crane (*G. americana*)	28	6.48 ± 0.43	1.08 ± 0.09	1.55 ± 0.12
Siberian crane (*G. leucogeranus*)	51	9.41 ± 0.64	0.84 ± 0.04	1.76 ± 0.05
White-naped crane (*G. vipio*)	77	7.71 ± 1.52	0.61 ± 0.03	1.44 ± 0.03
Brolga crane (*G. rubicunda rubicunda*)	16	7.01 ± 1.17	0.80 ± 0.06	1.55 ± 0.34
Black-necked crane (*G. nigricollis*)	5	4.44 ± 0.64	0.70 ± 0.05	NA[b]
Hooded crane (*G. monacha*)	24	10.46 ± 1.94	0.71 ± 0.05	1.58 ± 0.07
Sarus crane (*G. antigone antigone*)	39	5.53 ± 0.50	0.68 ± 0.03	NA
Wattled crane (*Bugeranus carunculatus*)	37	4.41 ± 0.32	0.75 ± 0.05	1.70 ± 0.03
Demoiselle crane (*Anthropoides virgo*)	4	5.17 ± 1.37	1.05 ± 0.07	1.77 ± 0.15
Stanley crane (*A. paradisea*)	10	2.71 ± 0.42	0.80 ± 0.04	0.90 ± 0.21
Black-crowned crane (*Balearica pavonina*)	23	2.77 ± 0.23	0.57 ± 0.03	1.36 ± 0.43
Gray-crowned crane (*B. pavonia regulorum*)	10	4.54 ± 0.86	0.71 ± 0.04	NA

[a] Mean ± standard error.
[b] NA, Not available.

Mean ± SE retinol values were 0.69 ± 0.05 μg/ml; there were no significant variations by species, subspecies, age, or sex. Free-ranging cranes had a mean circulating retinol level of 0.66 ± 0.08 μg/ml. These values are within ranges cited as normal (0.2 to 0.6 μg/ml) for various mammalian and avian species (Schweigert et al., 1991), and provide evidence that there was no excess dietary vitamin A to interfere with absorption of vitamin E (Frigg and Broz, 1984). Vitamin A requirements for domestic poultry range from 2,000 to 11,000 IU/kg (Robbins, 1983); diets containing up to 16,500 IU vitamin A/kg have been demonstrated to be suitable for sandhill cranes (Serafin, 1982).

The mean ± SE cholesterol value was 1.50 ± 0.31 mg/ml among all cranes examined; mean values did not differ substantially from previously reported values of 1.60 ± 0.23 or 1.63 ± 0.41 mg/ml for East African crowned cranes (Chappell and Brannian, 1984) or demoiselle cranes (*Anthropoides virgo*) (International Species Inventory System, 1989), respectively. Vitamin E/cholesterol ratios, a comparative measure of vitamin E status, varied from 2.6 (wattled cranes) to 6.6 (hooded cranes); all were within ranges (2 to 5 μg/mg) predicted for healthy herbivorous or omnivorous avian species (Dierenfeld and Traber, 1992).

Plasma α-tocopherol levels were logarithmically related to dietary vitamin E levels, indicating an apparent saturation at

lipid carrying capacity of the blood. Using the wide range in dietary vitamin E concentrations in this study (22 to 660 IU/kg dry matter), plasma α-tocopherol concentration of cranes could be predicted from the equation $y = 4.333x - 0.1296$, where y = plasma concentration in μg/ml and x = natural log of the dietary vitamin E concentration ($R^2 = 0.999$). Based on this equation, one would predict from the α-tocopherol measured in the migrating sandhill cranes (3.71 μg/ml) that their natural diet contained <22 IU vitamin E/kg dry matter at the time of blood sampling; no data are available to verify this. Dietary types and levels of fat, as well as lipid mobilization during migration, with a concurrent elevation in plasma α-tocopherol, also may affect the relation between feed and circulating levels of vitamin E.

DISCUSSION

All cranes (>100 individuals) at ICF were fed pellets from the same manufacturing lots 1986 through 1989. Plasma α-tocopherol levels decreased to less than one-half the original value following increased Se supplementation in 18 birds sampled consecutively over this 4-yr period (Diet B, Table 2). Similar patterns of plasma α-tocopherol depletion/repletion were noted in the ICF population as a whole. Although no negative health effects were observed clinically and Se concentrations in tissues were not measured, we suggest an overall change in oxidative status of the cranes due to the addition of Se.

Although Se often is associated with vitamin E nutrition, and their cooperative functions in prevention of tissue peroxidative damage have been documented (Combs and Scott, 1977), these two nutrients should not be considered interchangeable. Selenium, as an integral component of the enzyme glutathione peroxidase, is essential for the prevention of hydroxyl radical formation and attack on unsaturated membrane lipids whereas vitamin E functions as a more general biological antioxidant (Combs and Scott, 1977). Potentially negative effects of dietary Se at 0.5 mg/kg when vitamin E concentrations are 55 IU/kg (as reported here) may have relevance in the development of selenosis in various aquatic avifauna inhabiting polluted environments (Ohlendorf et al., 1988, 1989), but requires further study.

TABLE 2. Mean (±SE) plasma α-tocopherol concentrations (μg/ml) in eighteen cranes fed pelleted rations containing varying levels of vitamin E and selenium (Se) at the International Crane Foundation, Baraboo, Wisconsin, USA. Diets contained: A) 55 IU/kg vitamin E, 0.1 mg/kg Se; B) 55 IU/kg vitamin E, 0.5 mg/kg Se; C) 200 IU/kg vitamin E, 0.3 mg/kg Se.

Diet	Year	Plasma α-tocopherol (μg/ml)
A	1986	7.35 ± 1.00[a]
B	1987	2.55 ± 0.48[b]
C	1988	6.28 ± 0.52[a]
C	1989	6.65 ± 0.58[a]

[a,b] Means (±SE) with the same superscripts in columns do not differ significantly ($P > 0.05$).

None of the cranes described in this survey had any clinical signs of vitamin E deficiency or capture myopathy; thus, both plasma and dietary concentrations may be considered to reflect at least short-term adequacy of this nutrient. However, specific dietary requirements for vitamin E have not been established for cranes or other exotic avian species, and the relations between diet and physiological status, or circulating levels and overall body status of vitamin E, are not clearly defined.

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