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## Vitamin E in Captive and Wild Black Rhinoceros (*Diceros bicornis*)

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**ABSTRACT:** The mean plasma level of  $\alpha$ -tocopherol (vitamin E) measured in 31 free-ranging black rhinoceros (*Diceros bicornis*) was significantly higher ( $P < 0.001$ ) than that in 11 captive animals ( $\bar{x} \pm SE = 0.77 \pm 0.05$  and  $0.18 \pm 0.03 \mu\text{g/ml}$ , respectively). Vitamin E status may influence the health of captive black rhinoceros; in particular, it may be linked to hemolytic anemia commonly observed in these animals in captivity.

**Key words:** Vitamin E, nutrition, herbivore, black rhinoceros, *Diceros bicornis*, hemolytic anemia, field study.

Deficiency of  $\alpha$ -tocopherol (vitamin E) has been reported in a number of captive herbivores (Dolensek and Combs, 1985). Lesions observed in captive hoofstock and primates include muscular dystrophy (Liu et al., 1982), neuronal degeneration (Liu et al., 1983) cardiomyopathy (Liu et al., 1984) and anemia (Dinning and Day, 1957). Pathology consistent with vitamin E deficiency also has been reported in cases of capture myopathy in wild populations (Basson and Hofmeyr, 1973). However, capture myopathy has been recorded in species with apparently normal vitamin E levels (Spraker, 1980) and several factors including muscular exertion, stress and nutrient deficiencies are probably involved in the etiology of this disease. Nonetheless, comparisons between vitamin E levels in captive species and their free-ranging counterparts are rare, making it difficult to assess vitamin E status in either population even when circulating levels are known. This report documents preliminary data concerning the vitamin E status of black rhinoceros (*Diceros bicornis*) and discusses the possible link of vitamin E with

hemolytic anemia in these large herbivores.

Blood samples from 31 black rhinoceros were obtained between 3 June and 28 July 1986 during a translocation operation centered in the Zambezi Valley (16°00'S, 29°30'E), Zimbabwe. An additional 16 blood samples were taken from 12 captive animals at various zoos in the United States between 1984 and 1987 (St. Louis Zoological Park, Forest Park, St. Louis, Missouri 63110, USA;  $n = 7$ , Denver Zoological Gardens, City Park, Denver, Colorado 80205, USA;  $n = 3$ , Lee Richardson Zoo, Box 499, Garden City, Kansas 67846, USA;  $n = 2$ , Zoo Atlanta, 800 Cherokee Avenue SE, Atlanta, Georgia 30315, USA;  $n = 1$ , Cheyenne Mountain Zoological Park, Box 158, Colorado Springs, Colorado 80901, USA;  $n = 1$ , Detroit Zoological Park, Box 39, Royal Oak, Michigan 48068, USA;  $n = 1$ , and Los Angeles Zoo, 5333 Zoo Drive, Los Angeles, California 90027, USA;  $n = 1$ ). Although no special precautions were taken to protect blood samples from sunlight, potential exposure times were usually  $< 5$  min.

Plasma was separated by centrifugation and frozen immediately. Following ethanol precipitation and hexane extraction by the procedures described in Dierenfeld and Dolensek (1988),  $\alpha$ -tocopherol (vitamin E) content was determined using high performance liquid chromatography. A Series 400 chromatograph (Perkin-Elmer, Inc., Norwalk, Connecticut 06859, USA) equipped with a 30-cm C-18 column was used for separation, the vitamin E peak was monitored by a Perkin-Elmer fluores-

cence detector (Model LS-1), and peak areas on the chromatograph were analyzed with a Perkin-Elmer Model LCI-100 data processor. Serum cholesterol was measured on the Technicon RA 1000 (Technicon Instrumentations Corporation, Tarrytown, New York 10591, USA). Means were compared using Student's *t*-test (Snedecor and Cochran, 1967); statistical significance was determined at  $P \leq 0.05$ .

Circulating levels of vitamin E in the wild black rhinoceros ( $\bar{x} = 0.77 \pm 0.05 \mu\text{g/ml}$ ) were significantly ( $P < 0.001$ ) higher than those seen in 11 captive animals ( $\bar{x} = 0.18 \pm 0.03 \mu\text{g/ml}$ ) fed diets unsupplemented with vitamin E. One 212-kg 7-month-old female, experimentally supplemented with 1000 IU of d,l  $\alpha$ -tocopheryl acetate orally since 1 mo of age, was not included in the mean for the zoo animals. Her plasma vitamin E level was  $3.87 \pm 0.05 \mu\text{g/ml}$ . Differences between sexes were not significant ( $P > 0.05$ ).

Cholesterol levels measured in zoo ( $70.10 \pm 10.47 \text{ mg/dl}$ ,  $n = 10$ ) and free-ranging ( $71.71 \pm 6.84 \text{ mg/dl}$ ,  $n = 17$ ) black rhinoceros were not statistically different, but cholesterol was positively correlated with vitamin E ( $r = 0.92$ ,  $P < 0.05$ ). Vitamin E is a fat soluble vitamin carried in lipid components of the blood; correction for blood lipids (either cholesterol or total lipids) has been suggested to standardize and evaluate vitamin E status within species (Horwitt et al., 1972; Mayhew et al., 1987) and provide a basis of comparison among species. Vitamin E/cholesterol ratios (per ml plasma) ranged from 0.1 to 0.4 in captive black rhinoceros, and 0.8 to 2.0 in free-ranging animals.

Free-ranging rhinoceros were immobilized using etorphine hydrochloride (M-99 etorphine, D-M Pharmaceuticals, Rockville, Maryland 20850, USA). All, with the exception of one female with a prolapsed rectum, were assessed to be in excellent physical condition at the time of capture based upon veterinary clinical, hematological and parasite examinations. Medical history of the zoo animals (also immobi-

lized with etorphine hydrochloride) indicates that one rhinoceros (Studbook #161), whose vitamin E level was undetectable ( $<0.05 \mu\text{g/ml}$ ) in this sample, had previously experienced recurrent bouts of mild hemolytic anemia. Another rhinoceros (Studbook #328), with a plasma  $\alpha$ -tocopherol level of  $0.22 \mu\text{g/ml}$ , was in the midst of a hemolytic crisis when this sample was collected. Others appeared clinically normal.

Circulating levels  $<1.5 \mu\text{g/ml}$   $\alpha$ -tocopherol are considered deficient in both cattle and horses (Stowe, 1968; Stuart, 1987); normal levels of the vitamin are generally  $>3.0 \mu\text{g/ml}$ . By comparison with domestic livestock, both free-ranging and captive black rhinoceros may be vitamin E deficient.

Vitamin E deficiency is known to influence membrane integrity and cause erythrocyte hemolysis in primates, rats and horses (Stowe, 1968; Bieri and Poukka, 1970; Ausman and Hayes, 1974). Vitamin E deficiency in the black rhinoceros should be considered as one possible etiology for hemolytic anemia in this species. One can speculate that vitamin E deficiency may act primarily as a factor in increased erythrocyte (RBC) membrane fragility with subsequent rupture (hemolysis), and/or increase the susceptibility of the RBC to a number of oxidant stresses (Horwitt, 1968; Stowe, 1968; Tappel, 1972; Tudhope and Hopkins, 1975). Although other factors may be involved in the development of hemolytic anemia, further research is needed to demonstrate these relationships in the black rhinoceros.

Hemolytic anemia has been reported in 15 captive black rhinoceros in zoos in North America, Japan and Europe (Miller and Boever, 1982) and observed in an additional 14 animals (R. E. Miller, unpubl. data). Twenty of these animals died during hemolytic crises. Diets of the zoo animals typically contained large amounts of produce and dried forages, with little or no access to fresh pasture.

By comparison, all wild black rhinoceros sampled in this study were found in prime

habitat, with high availability of herbaceous and woody browse species growing on a fertile mix of alluvial and colluvial soils at the base of the Zambezi escarpment. Although levels of vitamin E were not determined in either captive or free-ranging animal diets, domestic livestock fed commercially processed diets and/or hays typically consume less than one-third the amount of  $\alpha$ -tocopherol they would if allowed to graze fresh pasture; they often develop vitamin E deficiencies (Mayhew et al., 1987; Stuart, 1987). The lack of information on dietary vitamin E levels is a serious limitation of this study, but it is currently under investigation.

The magnitude of the differences in plasma  $\alpha$ -tocopherol levels seen between free-ranging and captive black rhinoceros is almost identical to that found between wild and unsupplemented captive elephants ( $\bar{x} = 0.79 \pm 0.05$  and  $0.17 \pm 0.03$   $\mu\text{g}/\text{ml}$   $\alpha$ -tocopherol, respectively; U. S. Seal, pers. comm.). Again, information is lacking, but evidence suggests that dietary vitamin E requirements are not being met for many herbivores fed typical zoo diets (Liu et al., 1982, 1983, 1984). Elephants that have been supplemented daily with vitamin E at a level of approximately 2 IU/kg body mass show a mean circulating vitamin E value of  $0.6 \pm 0.1$   $\mu\text{g}/\text{ml}$  (Dierenfeld and Dolensek, 1988).

One Indian rhinoceros (*Rhinoceros unicornis*) supplemented with vitamin E at 2 IU/kg body mass daily for 2 yr showed a single plasma  $\alpha$ -tocopherol value of  $0.5$   $\mu\text{g}/\text{ml}$  (E. S. Dierenfeld, unpubl. data). The heavily supplemented black rhinoceros described earlier was fed a minimum of 4.5 IU vitamin E per kg body mass per day for 6 mo prior to blood sampling.

White (*Ceratotherium simum*) and Indian rhinoceros are primarily grazers, rather than browsers like the black rhinoceros (Hoppe, 1984). Hemolytic anemia has not been reported for either species in captivity. Dietary requirements for vitamin E may vary between these grazers and the browsing black rhinoceros. Nonethe-

less, it is suggested that dietary supplementation of  $\alpha$ -tocopherol at a minimum of 2.0 to 2.5 IU/kg body mass is a prudent management practice for feeding captive black rhinoceros.

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