Serum vitamin E levels in free-ranging black rhinoceros in the Eastern Cape

I.B. Ndondo, B.S. Wilhelmi*, L.V. Mabinya & J.M. Brand†

Department of Biochemistry and Microbiology, Faculty of Science and Technology, University of Fort Hare, Private Bag X1314, Alice, 5700 South Africa

Received 23 September 2003. Accepted16 March 2004

The serum vitamin E levels in 12 free-ranging black rhinoceros (*Diceros bicornis*) were determined by Reversed Phase High Performance Liquid Chromatography (RP-HPLC). The values obtained ranged from 0.19 to 1.92 µg/ml in nine animals, while three animals had undetectable levels. Generally, females had higher levels than males and the variation observed between individuals is considered to be dependent on the dietary intake of vitamin E, which varies with plant species eaten, and location of the animals.

Key words: vitamin E, α-tocopherol, black rhinoceros, captivity, Great Fish River Reserve, RP-HPLC, serum.

Several nutrient deficiencies have been observed in various animal species in captivity (Dierenfeld & Traber 1992). In some instances, these deficiencies have been associated with pathological conditions that threaten the health and survival of the species in captive situations. This has been a significant limitation in wild animal husbandry, in view of the fact that some of the animals are raised in captivity because their survival in the wild is threatened by factors such as poaching and habitat depletion.

A vitamin E deficiency has been reported in black rhinoceros (*Diceros bicornis*) in captivity (Dierenfeld *et al.* 1988; Dierenfeld & Traber 1992; Dierenfeld 1999; Ghebremeskel *et al.* 1991; Miller 1993). It is characterized by haemolytic anaemia, pathological lesions and neuronal degeneration. In contrast, the captive white rhinoceros (*Ceratotherium simum*) has not been affected by the same disease conditions. Furthermore, these disease conditions in free-ranging animals have a much lower incidence (Miller 1993). These two African rhinoceros have markedly different feeding strategies; the black rhinoceros is a browser while the white rhinoceros is a grazer (Dierenfeld 1994; Grant 2000). In captivity, the diet of the black rhinoceros has been based on that of the Equidae, which are grazers, a factor that may be the cause for some of its nutritional disorders.

This study reports on the serum vitamin E levels of 12 individuals of the black rhinoceros population in the Great Fish River Reserve (GFRR). The reserve is situated in the Eastern Cape Province of South Africa and comprises the former Sam Knott Nature Reserve, Andries Vosloo Kudu Reserve and the Doubledrift Game Reserve. It is 45 000 ha in extent, most of which was formerly farmland for pastural agriculture. The region receives approximately 250–500 mm rainfall annually with peaks in February and October. The vegetation type is mostly succulent thorny shrub about 2–3 m high and species richness is relatively high. Several black rhinoceros of both sexes and of varying ages were introduced into the GFRR, between 1986 and 1997 from KwaZulu-Natal. Since then, the animals have shown very good adaptability as measured by reproductive performance and percentage fatalities, which is an indication of adequate habitat quality. The animals move and browse freely around the reserve and have access to a perennial supply of drinking water (Fike, pers. comm., 2001).

Blood samples were collected from 12 black rhinoceros during an ear-notching exercise in September 2001. The animals were immobilized by darting from a helicopter using etophine hydrochloride to tranquillize them. All the animals scored high in health condition at the time of immobilization, based on physical appearance, rectal temperature, parasite examination and haematological data (performed by South African Institute of Medical Research, Johannesburg). The blood samples were collected in plain glass tubes, stored in an ice-box with minimum light and transported to the laboratory within an hour. Upon arrival, serum was separated from the red cells by centrifugation, aliquoted into smaller Eppendorf tubes and stored at –70°C.

Vitamin E was extracted into hexane using a method described by Alvarez & De Mazancourt (2001). In brief, a 1 ml solution of α-tocopherol acetate (internal standard) in hexane was measured into a test tube and evaporated to dryness under nitrogen. A 1 ml aliquot of serum was then added into the test tube and deproteinized using

*Present address: Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, 6140 South Africa.
†To whom correspondence should be addressed.
E-mail: johnbrand_za@yahoo.com

an equal volume of ethanol. The solution was vortexed for 2 min and extracted into hexane by shaking vigorously. The hexane layer was removed, evaporated to dryness and reconstituted in 50 µl methanol. Analysis was carried out by injecting 20 µl on a RP-HPLC column (Hewlett Packard Agilent Zorbax SB-C18 column; 4.6 mm diameter, 250 mm length and 5 µm particle size) and employing isocratic elution (100% methanol). The HPLC was a Varian 5000 instrument with a Rheodyne injection valve and the run time was 30 min with a flow rate of 0.7 ml/min. Detector settings were 292 nm and 0.001 AUFs using a Linear Instruments UVIS 200 HPLC detector. Peak identification was based on retention times and quantification was based on peak areas using α-tocopherol as external standard.

The results presented in Table 1 show that the serum vitamin E levels ranged from 0.19–1.92 µg/ml in nine of the animals sampled, while three had undetectable levels. Despite the wide variation and low levels detected in some of the animals, all the darted animals were in good condition.

The average serum vitamin E levels reported in this study were comparable to those reported in other studies. Serum vitamin E levels in black rhinoceros have been shown to differ among locations, from 0.23 µg/ml (n = 7) in Kenya (Dierenfeld 1994), 0.77 µg/ml (n = 31) in Zimbabwe (Dierenfeld *et al.* 1988) to 0.80 µg/ml (n = 3) in Namibia (Dierenfeld 1994). Most animals (n = 129) from South Africa and Zimbabwe averaged 0.6 µg/ml (Dierenfeld 1995). This study reported a mean of 0.86 µg/ml and was the first of its kind on the animals in the selected area. The black rhinoceros sample comprised four males and eight females of varying ages, most of which were young adults. Of the animals with detectable levels of vitamin E, the females generally had higher vitamin E levels than the males, 1.39 µg/ml (n = 6) and 0.70 µg/ml (n = 3), respectively. Dierenfeld *et al.,* (1988) reported no sex differences in vitamin E levels in the black rhinoceros.

Vitamin E levels in free-ranging black rhinoceros have been shown to be higher than those found in animals in captivity. Dierenfeld *et al.,* (1988) reported the mean plasma level of vitamin E to be higher (0.77 ± 0.05 µg/ml) in 31 free-ranging rhinoceros than in 11 captive animals (0.18 ± 0.03 µg/ml). As a result, some researchers have recommended vitamin E supplementation in captivity (Dierenfeld 1995; Ghebremeskel *et al.* 1991).

Table 1. Serum vitamin E levels of black rhinoceros in the Great Fish River Reserve.

Rhino identity	Sex	Serum vitamin E (µg/ml)
50	Female	1.92
61	Female	1.79
14	Female	1.55
13	Female	1.49
22	Female	1.44
24	Male	0.93
52	Male	0.66
42	Male	0.53
5	Female	0.19
60	Female	Undetectable
51	Female	Undetectable
15	Male	Undetectable

This study only measured serum vitamin E. However, it has been suggested that vitamin E should be correlated to serum lipids. Vitamin E is fat-soluble and thus carried in lipid components of the blood. Correction for blood lipids has been recommended to standardize and evaluate vitamin E status within species (Dierenfeld *et al.* 1988). A better approach would be to assay for both vitamin E and total lipid and express the two as a ratio (Howitt *et al.* 1972). Furthermore, it is speculated that blood vitamin E levels may be affected by dietary composition over a short period of time, hence a better measure of long-term nutritional adequacy would be tissue vitamin E levels. However, obtaining tissue samples requires more specialized techniques and has practical difficulties for free-ranging animals.

In conclusion, the serum vitamin E levels in this black rhinoceros population were varied and the results obtained showed that serum vitamin E level of animals cannot be used in isolation in the assessment of overall nutritional status. Black rhinoceros populations in other studies in different geographical regions had varying vitamin E levels, and within a population it is speculated that the immediate dietary intake may affect serum levels of the vitamin. In addition, sample handling and preparation can influence the assay of vitamin E. However, our procedure was consistent and was not considered to be the source of variation.

We are grateful to the Management of the GFRR, in particular, Brad Fike, for allowing us access to the animals; the Rhino and Tiger Fund, U.S.A., for funding the black rhino immobilization and blood collection, and the National Research Foundation, Pretoria, for financial support.

REFERENCES

ALVAREZ, J. & DE MAZANCOURT, P. 2001. Rapid and sensitive HPLC method for simultaneous determination of retinol, α-tocopherol, 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 in human plasma with photodiode-array UV detection. *J. Chromatog. B.* 755: 129–135.

AUSLAND, C. & SVEIPE, A.M. 2000. Feeding ecology of the black rhinoceros. Masters thesis, University of Norway, Oslo.

DIERENFELD, E.S., DU TOIT, R. & MILLER, R.E. 1988. Vitamin E in captive and wild black rhinoceros (*Diceros bicornis*). *J. Wildl. Dis.* 24(3): 547–550.

DIERENFELD, E.S. & TRABER M.G. 1992. Vitamin E status in exotic animals compared with livestock and domestics. In: L. Packer & J. Fuchs (Eds), Vitamin E in health and in disease (pp. 345–358). Marcel Dekker, New York.

DIERENFELD, E.S 1994. Vitamin E in exotics: effects, evaluation and ecology. *J. Nutr.* 124: 2579S–2581S.

DIERENFELD, E.S 1995. Rhinoceros nutrition: an overview with special reference to browsers. *Verhandlungsbericht Erkrankungen Zootiere* 37: 7–14.

DIERENFELD, E.S., DU TOIT, R. & BRASELTON, W. 1995. Nutrient composition of selected browses consumed by black rhinoceros (*Diceros bicornis*) in the Zambezi Valley, Zimbabwe. *J. Zoo Wildl. Med.* 26: 220–230.

DIERENFELD, E.S. 1999. Vitamin E: metabolism, sources, unique problems in zoo animals and supplementation. In: M.E. Fowler & R.E. Miller (Eds), Zoo and wildlife animal medicine (pp. 79–82). W.B. Saunders, Philadelphia.

GHEBREMESKEL, K., WILLIAMS, E., BRETT, R.A., BUREK, R. & HARBIGE, L.P. 1991. Nutrient composition of plants most favoured by black rhinoceros (*Diceros bicornis*) in the wild. *Comp. Biochem. Physiol.* 91A: 343–345.

GRANT, J.B. 1999. Essential fatty acids, total lipid, and tannins in the diet of the captive black rhinoceros of North America and in browses native to Zimbabwe, Africa. Masters thesis, Cornell University, Ithaca, NY.

HOWITT, M.K., HARVEY, C.C., DAHM Jr, C.H. & SEARCY, M.T. 1972. Relationship between α-tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann. New York Acad. Sci.* 203: 223–236.

MILLER, R.E. 1993. Haemolytic anaemia in the black rhinoceros (*Diceros bicornis*). In: M.E. Fowler & R.E. Miller (Eds), Zoo and wildlife animal medicine (pp. 32–45). W.B. Saunders, Philadelphia.