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NUTRIENT COMPOSITION OF PLANTS MOST FAVOURED BY BLACK RHINOCEROS (*DICEROS BICORNIS*) IN THE WILD

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Abstract—1. The nutrient composition of plants that are most preferred by the black rhinoceros (*Diceros bicornis*) in Laikipia, Kenya, was studied.

2. Mean zinc and selenium concentrations of the plants from Laikipia were higher than those of control (clover and rye, 1:1) material from the U.K.

3. Except in *Tinnea aethiopica*, palmitic (16:0), linoleic (18:2n-6) and linolenic (18:3n-3) were the major fatty acids.

4. The mean RRR-alpha-tocopherol content of the plants was 73.6 μg/g DM, and 2.2 g/d were estimated to be consumed by free-living rhinoceros in the area.

5. The results suggest that the quantitative vitamin E intake of supplemented captive black rhinoceros was comparable with that of their counterparts in the wild.

6. The lack of any obvious relationship between plasma alpha-tocopherol and orally administered racemic alpha-tocopherol and its ester form in captive black rhinos may be due to an insufficiency of emulsifiers.

INTRODUCTION

Advances in nutritional physiology of exotic species (Lehner *et al.*, 1966; Du Boulay and Crawford, 1968; Scott, 1968; Fiennes *et al.*, 1973; Hay and Watson, 1976; and Rivers *et al.*, 1976) have led to improvements of animal management in zoological gardens. Nevertheless, divergence from a natural diet, suited to digestive morphology and metabolism, is manifested by changes in cellular composition, and by subclinical and clinical disorders and mortality in captive and wild animals.

More diverse muscle polyunsaturated fatty acids were found in wild than in captive giraffes, and in woodland buffaloes than in grassland buffaloes or domestic bovids (Crawford, 1968). Habitat destruction is related to arterial disease in East African elephants (Sikes, 1968) and to an accelerated rate of decline and change in population structure in grassland elephants (Laws and Parker, 1968). Moreover, Armstrong (1989) reported failure to feed, undernourishment and death in Namibian desert black rhinos that were translocated away from their natural habitat.

Nutritional stress was suspected to be a primary cause of biochemical and pathological disorders, stillbirths and death in various captive animals (Du Boulay and Crawford, 1968; Rivers *et al.*, 1978; Jones, 1980; Foster, 1981; Van Hoven, 1982; Liu *et al.*, 1983; Wallach and Boever, 1983; Fowler and Boever, 1986; Wixson and Griffith, 1986; Ghebremeskel *et al.*, 1990).

Nutritional problems have often been attributed to a disparity between what animals are fed in captivity, and their qualitative, quantitative and relative nutrient requirements. The discrepancy is mainly due to a lack of baseline information on the feeding habits of exotic species, and on the nutrient composition of their natural foods. Formulation of diets that simulate indigenous foods is not easy even when nutrient composition has been established. The natural forms of some nutrients are unstable. Consequently, these nutrients are provided in a modified form, but the extent of their utilization is not fully understood.

This investigation was undertaken to establish the nutrient composition of plants that are most favoured by black rhinoceros in Laikipia, Kenya. The results are discussed in relation to the observed difference between the concentrations of serum alpha-tocopherol in wild and captive species.

MATERIALS AND METHODS

Sample collection

A year-round study of feeding of a population of 43 free-ranging black rhinoceros on Ol Ari Nyiro ranch, Laikipia, Northern Kenya (Brett and Oloo, in preparation) established 103 plant species from over 37 botanical families as food plants. The diet of the black rhino in the area was found to be at least as diverse as that determined in other bushland areas of East Africa (Goddard, 1968, 1970; Makinya, 1977).

Ten samples of the most favoured food plants were collected during June 1989, at the end of the rainy season. These plants comprised over 70% of the year- round diet of the animals in the study area. The leaf and stem samples collected represented those parts of each plant species commonly consumed by the animals (e.g. black rhinoceros

consumed only the stem of the *umbellifer Ferula communis* and rejected the leaves). The samples were transported at 4°C in light-proof paper bags to the Institute of Zoology, London, where they were deep-frozen within 24 hr of their collection. Control beech (*Fagus sylvatica*), clover (*Trifolium* sp.) and rye grass (*Lolium perenne*) samples were collected in U.K.

Analytical method

Crude protein, crude fibre, ash, nitrogen-free extract (NFE), moisture and lipid were determined by the Weende method (proximate analysis).

Alpha-tocopherol and fatty acids were determined from lipid extracted by the method of Folch *et al.* (1957). A known weight of each plant sample was homogenized in a chloroform:methanol (2:1 v/v) mixture containing 0.01% butylated hydroxytoluene (BHT) antioxidant, flushed with nitrogen and left overnight at 4°C. The homogenate was filtered and the residue washed with the extracting solvent mixture. Filtrate was transferred to a separatory funnel and left overnight at 4°C following the addition of 25% of its volume of saline (0.85% NaCl). The lower organic phase was evaporated in a Rotavap-R (Buchi) under reduced pressure at 37°C. The resulting lipid was taken up in 10 ml chloroform and stored for the determination of alpha-tocopherol and fatty acids.

Fatty acid determination

Methylation of the fatty acids of part of the lipid extract was carried out under nitrogen at 70°C for 3 hr with 5 ml of 5% sulphuric acid in dry methanol. The resulting methyl esters were extracted into 3 × 2 ml petroleum spirit (b.p. 40–60°C) after the addition of an equal volume of 5% saline. Before injection, the petroleum spirit extract was evaporated to dryness under nitrogen and redissolved in a known volume of heptane. A 0.1 μl of the sample was subsequently injected on to a 25 m × 0.53 mm × 1 μm polyethylene glycol (PEG) capillary column. The operating conditions were: oven temperature 190°C, hydrogen carrier gas flow 20 ml/min and nitrogen make-up gas flow 10 ml/min. The chromatograph used was a Pye model 204 equipped with dual flame ionization detectors. Areas were computed by an LDC/Milton Roy C1-10B electronic integrator. Fatty acid methyl esters were identified by comparison with authentic standards (Sigma Chemical Co.) and by equivalent chain length (Ackman, 1969).

Alpha-tocopherol assay

An aliquot of lipid extract was saponified with 5 ml of 5% ethanolic potassium hydroxide (5% potassium hydroxide/95% ethanol) containing 0.01% BHT at 80°C for 20 min. The saponified material was cooled and extracted by vortexing with 10 ml n-hexane for 5 min. An aliquot of 1 ml of the organic phase (hexane layer) was removed and diluted to 5 ml with hexane. The solution was thoroughly washed with 20 ml water to remove any remaining potassium hydroxide. The washed solution was evaporated to dryness on a water bath at 40°C under a stream of nitrogen. The residue was redissolved in 100 μl of methanol and 25 μl were taken for analysis.

Alpha-tocopherol was separated by the use of a Varian 9010 high performance liquid chromatograph (HPLC) equipped with a variable wavelength UV-9050 and fluorimeter (Fluorichrom II) detectors (Varian Ltd, Palo Alto, CA, USA) in series. Absorption area was integrated by a Varian 4290 integrator. The eluting solvents and column conditions employed have been described by Ghebremeskel and Williams (1988).

Trace element analysis

Aluminium (Al), copper (Cu), magnesium (Mg), Manganese (Mn) and Molybdenum (Mo) were determined by pre-chemical separation neutron activation with hydrated antimony pentoxide; chromium (Cr), iron (Fe) and zinc (Zn) by instrumental neutron activation; selenium (Se) by cyclic neutron activation (Ward and Ryan, 1979).

RESULTS AND DISCUSSION

Tables 1, 2a, b and 3 show the nutrient composition of the ten plants most favoured by black rhinoceroses in Laikipia, Kenya, and of a control beech plant. Because of limited sample size the leaves and twigs were not assayed separately; their proportions in the different samples were not the same.

With the exception of selenium and zinc the mean trace element values of the control and the plants

Table 1. Nitrogen free extract (NFE), ash, moisture (H_2O), crude fibre (C. fib.), crude protein (C. prt.), lipid and ash composition of plants favoured by black rhinoceroses and control beech plant.

Species	Leaf:twig ratio	H_2O %	Lipid %	C. prt. %	C. fib. %	Ash %	NFE %
Acacia brevispica (Mimosaceae)	0.38	23.1	1.4	10.7	36.4	2.7	25.8
Phyllanthus fischeri (Euphorbiaceae)	0.22	26.7	0.9	4.8	37.6	3.5	26.6
Acacia hockii (Mimosaceae)	0.24	37.0	1.0	3.5	25.6	3.4	29.5
Carissa edulis (Apocynaceae)	0.72	16.7	3.1	8.6	14.2	4.3	53.2
Tinnea aethiopica (Labiatae)	0.46	16.5	1.9	3.7	41.1	4.1	32.8
Euclea divinorum (Ebenaceae)	1.19	16.6	3.2	6.7	16.3	8.9	47.8
Ecbolium revolutum (Acanthaceae)	0.68	59.4	2.9	5.6	11.1	2.2	18.1
Ferula communis (Umbelliferae)	Soft stem	88.4	0.6	1.1	3.8	1.5	4.8
Rhus natalensis (Anacardiaceae)	1.48	22.2	2.6	8.3	17.8	4.9	44.9
Lippia javanica (Verbenaceae)	2.92	16.8	3.1	13.1	15.0	8.1	44.0
Beech (control)	Leaf	Dried	11.0	24.0	—	—	—
(*Fagus sylvatica*)	Kernel	Dried	20.0	42.0	—	—	—
	Twig	Dried	1.6	8.4	—	—	—

from Laikipia were similar. The concentrations of the two elements were higher in the latter. The plasma of captive adult black rhinoceros given mineral supplements had 0.9, 0.03, 0.3 and 11.5 mg/l of copper, selenium, zinc and magnesium respectively. For domestic animals the copper and selenium figures would be regarded as deficient and the magnesium as marginal. However, the values do not necessarily indicate low antioxidant-enzyme activity in the animal or any association between the dietary concentration of selenium and the incidence of haemolytic anaemia in the rhinoceros. Indeed, Paglai *et al.* (1986) found no significant difference in erythrocyte enzymes activities between healthy animals and those with haemolytic syndrome. However, they did not assay for catalase or the erythrocyte enzyme (Cu/Zn) superoxide dismutase.

Lipid (1.2–7.1% DM), crude protein (4.4–15.8 DM), crude fibre (17.1–51.3% DM) and nitrogen-free extract (33.6–63.9% DM) values were broadly comparable to those reported by Dougall *et al.* (1964). The lipid and crude protein content of the plants from Laikipia was lower than that of the leaves and kernels of the control beech plant but similar to that of control twigs and buds.

Fatty acid results are expressed as a weight percentage of the total fatty acids. Except in *Tinnea aethiopica* and in the seed of the beech plant, palmitic (16:0), linoleic (18:2n-6), and linolenic (18:3n-3) were the major fatty acids. Oleic acid (18:1n-9)

Table 2(a). Alpha-tocopherol (μg/g) and per cent fatty acid (w/w total fatty acids) composition of plants favoured by black rhinoceroses and control beech plant

Species	Leaf:twig ratio	Alpha-tocopherol μg/g	15:0 %	16:0 %	16:1 *n*-7	17:0 %
Acacia brevispica (Mimosaceae)	0.38	51.8	0.2	15.9	2.2	0.5
Phyllanthus fischeri (Euphorbiaceae)	0.22	69.5	—	22.7	2.2	0.4
Acacia hockii (Mimosaceae)	0.24	14.8	0.5	24.0	2.0	0.7
Carissa edulis (Apocynaceae)	0.72	153.0	0.2	41.4	1.4	1.5
Tinnea aethiopica (Labiatae)	0.46	44.6	0.5	26.5	2.7	—
Euclea divinorum (Ebenaceae)	1.2	17.7	—	33.6	1.1	1.2
Ecbolium revolutum (Acanthaceae)	0.68	22.5	—	31.8	1.9	2.3
Ferula communis (Umbelliferae)	Soft stem	0.4	—	21.2	0.9	5.1
Rhus natalensis (Anacardiaceae)	1.48	67.9	0.1	26.1	1.8	1.3
Lippia javanica (Verbenaceae)	2.92	121.6	0.3	20.3	3.2	0.4
Beech (control)	Leaf	Fresh	—	17.0	1.7	0.7
(*Fagus sylvatica*)	Seed	Fresh	—	9.0	1.4	0.7
	Twigs and bud	Fresh	—	17.0	0.3	1.9

Table 2(b). Fatty acid composition of plants favoured by black rhinoceroses and control beech plant

Species	Leaf:twig ratio	18:0 %	18:1 n-9 %	18:2 n-6 %	18:3 n-3 %	20:0 %	22:0 %	24:0 %
Acacia brevispica (Mimosaceae)	0.38	5.7	4.8	16.3	29.9	1.3	—	—
Phyllanthus fischeri (Euphorbiaceae)	0.22	3.1	6.0	8.3	37.0	1.0	5.7	2.2
Acacia hockii (Mimosaceae)	0.24	6.5	8.1	18.7	14.5	4.9	4.3	3.1
Carissa edulis (Apocynaceae)	0.72	4.7	8.3	14.0	15.2	1.6	—	—
Tinnea aethiopica (Labiatae)	0.46	5.4	13.1	12.6	24.2	2.0	—	4.9
Euclea divinorum (Ebenaceae)	1.20	7.5	7.1	13.5	22.2	2.9	—	—
Ecbolium revolutum (Acanthaceae)	0.68	4.7	4.6	25.9	22.4	1.3	1.1	—
Ferula communis (Umbelliferae)	Soft stem	3.0	3.4	37.9	10.1	0.5	1.4	1.2
Rhus natalensis (Anacardiaceae)	1.48	3.0	6.8	15.5	31.2	1.4	1.6	—
Lippia javonica (Verbenaceae)	2.92	2.7	2.5	7.9	55.6	1.2	1.6	0.8
Beech (control)	Leaf	2.4	2.7	13.0	46.0	—	—	—
(*Fagus sylvatica*)	Seed	2.9	35.0	46.0	5.0	—	—	—
	Twigs and buds	1.4	8.0	24.0	23.0	—	—	—

Table 3. Trace elements content of plants favoured by the black rhinoceros and control (clover and rye, 1:1) U.K. material

Species	Material	Se (ng/g)	Cu (μg/g)	Zn (μg/g)	Fe (μg/g)	Mn (μg/g)	Cr (μg/g)	Co (μg/g)	Mo (μg/g)	Al (μg/g)
Acacia brevispica (Mimosaceae)	Leaf	4.5	1.2	2.7	9.1	1.2	0.4	0.4	0.03	2.9
Phyllanthus fischeri (Euphorbiaceae)	Twig + small leaf	6.2	1.0	2.3	11.7	1.8	0.6	1.8	0.04	3.7
Acacia hockii (Mimosaceae)	Twig + thorn + leaf	6.3	1.1	4.6	12.4	1.0	1.1	4.2	0.05	6.7
Carissa edulis (Apocynaceae)	Twig + small leaf	4.1	0.7	3.9	9.8	1.3	0.8	1.7	0.03	2.7
Tinnea aethiopica (Labiatae)	Twig + small leaf	4.5	1.2	4.5	13.6	0.9	0.6	2.2	0.03	3.1
Euclea divinorum (Ebenaceae)	Twig + small leaf	6.7	1.1	6.0	11.9	0.8	0.7	1.6	0.03	4.2
Ecbolium revolutum (Acanthaceae)	Twig + small leaf	4.9	1.5	3.4	12.4	1.3	0.9	2.7	0.04	2.1
Ferula communis (Umbelliferae)	Stem	4.7	1.7	2.7	13.4	4.3	1.0	3.9	0.05	6.9
Rhus natalensis (Anacardiaceae)	Twig + small leaf	3.0	1.0	7.4	9.0	1.7	1.1	2.7	0.04	4.9
Lippia javanica (Verbenaceae)	Twig + small leaf	4.5	0.9	2.6	9.7	0.9	1.4	2.4	0.03	6.7
Control	Broad leaf	1.8	1.0	2.1	11.4	1.4	1.1	2.4	0.03	4.0
	Thin leaf	2.0	1.1	2.7	9.8	1.4	0.7	3.3	0.20	2.1
	Stem	2.7	1.3	3.4	14.6	1.5	1.1	2.6	0.03	8.2

Values were based on standardized fresh weight conditions.
Control—clover (*Trifolium* sp.) and rye grass (*Lolium perenne*) 1:1 mixture.

comprised 13.1% in *Tinnea aethiopica*, 35% in the beech seed and less than 8.5% in the rest of the samples. Stearic acid (18:0) was not present in high concentrations; palmitoleic (16:1n-7) accounted for 0.3–3%. There was more linolenic acid than linoleic acid in *Lippia javanica*, *Rhus natalensis* and *Euclea divinorum* reflecting a higher proportion of leaves relative to twigs. It was interesting that *Ferula communis*, which has a soft stem but whose leaves are not eaten by the rhinoceros, had 37.9% linoleate and only 10.1% linoleneate. C20 polyunsaturated fatty acids were not detected in any of the samples. However, the long chain saturates (C20, C22 and C24) and the odd carbon chain fatty acids (C15 and C17) were present in variable amounts. The fatty acid profiles of leaves, and twigs and buds, of acacia (*Acacia senegal*), balanities (*Balanities aegyptiaca*), and oak (*Quercus robur*) found by Williams and Crawford (unpublished data) were similar to those reported in this communication; they also observed that leaves were rich in linolenic acid, seeds and kernels in linoleic acid, and that bark, twigs and buds had equivalent amounts of both acids.

It is evident that black rhinos in the wild obtain appreciable quantities of the essential parent fatty acids linoleic and linolenic; the proportion must vary, however, according to the relative amounts of leaves, seeds and kernels, and bark ingested. An increased consumption of seeds and kernels will provide more linoleic than linolenic acid. Conversely, a higher intake of leafy material will favour linolenic acid. Because of the absence of a fermenting chamber in the front gut of the black rhinoceros these essential fatty acids would not be expected to undergo hydrogenation before absorption. Captive rhinoceros have access to pony and browser cubes, potatoes, carrots, hay and browse branches. These foods are unable to provide the necessary essential fatty acids that black rhinos in the wild ingest from a wide variety of trees and bushes. Moreover, both linoleic and linolenic are lost during storage and drying. The linoleic and linolenic content of an oak leaf dropped from 14% to 8% and 45% to 4% respectively after 2 weeks of drying; storage was found to have a similar effect (Williams and Crawford, unpublished data).

Reduced intake of the essential fatty acids would be reflected in lower tissue composition of these nutrients and their long chain derivatives. Crawford (1968) and Crawford, Gale, Woodford and Casperd (1970) reported that the composition of muscle tissue lipids of wild mammals free to select their own food was different from that of domestic animals or wild mammals maintained in captivity. Our preliminary investigations show that the erythrocyte phospholipid of captive black rhinoceros contained reduced amounts of linoleic, linolenic, arachidonic (20:4n-6), eicosapentaenoic (20:5n-3) and docosapentaenoic (22:5n-3) acids. Moreover, the cells on exposure to hydrogen peroxide underwent oxidation rather than lysis and haemoglobin release as observed with erythrocytes of other animals. Chaplin *et al.* (1986), however, did not observe osmotic fragility consistent with an intrinsic membrane abnormality in the red blood cells of black rhinoceros. The dry scaly skin seen in some captive black rhinoceros may indicate essential fatty acid insufficiency.

Alpha-tocopherol concentrations ranged 0.4–153.0 μg/g sample fresh weight (3.5–183.7 μg/g DM). These values were similar to those reported by Booth (1963, 1964) and Booth and Hobson-Frohock (1961); the mean value of the ten plants, however, was higher than that of straw, silage, hay (Bieber-Wlaschny, 1981), wheat, barley, soyabean meal, fish meal and oats (Cort *et al.*, 1983). *Ferula communis* had the lowest and *Carissa edulis* the highest alpha-tocopherol concentrations. The disparity in the content of the nutrient between the samples may have been due to the difference in the relative proportions of leaves and twigs, to the age of the leaves and to species variability. Booth and Hobson-Frohock (1961) reported that the concentrations of alpha-tocopherol were high in old, in dormant and in dying

leaves or parts of leaves, and low in young and actively growing leaves and near the base of long leaves.

The mean alpha-tocopherol value of the 10 plants from Laikipia was 73.6 μg/g sample DM. Analysis of the food consumption of captive male and female rhinoceroses for 14 days showed that the daily dry matter intake of an adult animal was about 30 kg. Assuming that the food consumption of captive and wild rhinos was comparable, the RRR-alpha-tocopherol intake in the wild amounts to about 2.2 g/d. This estimate would be subject to daily and seasonal variations.

Vitamin E supplementation in captivity ranges from 1 to 5 IU/kg body weight (0.67–3.36 mg RRR-alpha-tocopherol). Allowing for qualitative and quantitative fluctuations in food intake in the wild, and loss during storage, diet mixing and feeding in captivity, it appears that free-living and captive black rhinoceros ingest similar amounts of vitamin E. This observation suggests that the significant difference in serum alpha-tocopherol concentration between wild and captive groups (Dierenfeld *et al.*, 1988; Ghebremeskel *et al.*, 1988) cannot be attributed to a difference in the amount of vitamin E ingested. It is possible that the widely used synthetic all-rac-alpha-tocopheryl acetate may have low bioavailability in the rhinoceros. Hidiroglou *et al.* (1988) reported that sheep fed RRR-alpha-tocopherol had higher tissue concentrations of the nutrient than those receiving other forms of alpha-tocopherol. Supplementation with natural RRR-alpha-tocopherol and RRR-alpha-tocopheryl acetate was more effective than the corresponding synthetic forms in raising the blood vitamin E concentrations in steers (Hidiroglou *et al.*, 1988). Machlin and Gabriel (1982) found consistently higher alpha-tocopherol concentrations in human blood when the free tocopherol was administered instead of tocopheryl acetate.

Hydrolysis of alpha-tocopheryl acetate and optimal absorption of alpha-tocopherol depends on the presence of bile and pancreatic juice (Forsgren, 1969; Gallo-Torres, 1970). The black rhinoceros lacks a gall bladder and may therefore be unable to produce sufficient bile to break ester bonds and emulsify the released alpha-tocopherol. This may be significant under conditions of captivity in which bolus feeding would be followed by a sudden demand for a large amount of bile. The presence and size of the gall bladder is related to the rate of bile secretion, intermittence of feeding and fat content of the diet (Hilderbrand, 1974). Sokol *et al.* (1983) did not detect an increase in plasma vitamin E when racemic alpha-tocopherol was given orally to patients with severe cholestatic liver disease; there was, however, an increase in plasma level concentrations when it was administered with bile salts. The low bioavailability of racemic alpha-tocopherol (Ghebremeskel *et al.*, unpublished data) and its esterified form may be partly due to the nature and quantity of fatty acids. Linoleic and linolenic make up a major proportion of dietary fatty acids in the wild; whereas in captivity the saturates stearic and palmitic dominate. Weber *et al.* (1964) reported that the nature of associated oil was critical for the absorption of alpha-tocopherol since it interacts with polyunsaturates within the intestinal lumen. Feed for captive rhinoceros which is essentially high in concentrates and low in fibre, would have an increased rate of passage along the digestive tract, thereby reducing the absorption of alpha-tocopherol and other nutrients.

The results indicate that the alpha-tocopherol intakes of wild and supplemented captive black rhinoceroses were quantitatively comparable. The failure of plasma alpha-tocopherol concentrations to respond to increased intake of the latter group may have resulted from an insufficiency of emulsifiers. Low consumption of essential fatty acids by the captive animals may also reduce the bioavailability of vitamin E supplements and the proportions of linoleic, linolenic, arachidonic, eicosapentaenoic and docosapentaenoic acids of the erythrocyte membrane. Alteration of membrane composition would make it potentially unstable and susceptible to external insults.

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