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Source: Journal of Wildlife Diseases, 38(1) : 132-142

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.1.132

Journal of Wildlife Diseases, 38(1), 2002, pp. 132–142

ESSENTIAL FATTY ACID PROFILES DIFFER ACROSS DIETS AND BROWSE OF BLACK RHINOCEROS

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ABSTRACT: In captivity, black rhinoceros (*Diceros bicornis*) suffer from idiopathic skin lesions that may be linked to dietary deficiencies, in particular essential fatty acid deficiency (EFAD). Therefore, a study was undertaken from July 1995 to May 1997 to characterize the diet of captive *D. bicornis* in North American zoos and measure fat and fatty acid composition in zoo diet, and African and North American browses. Descriptions of all dietary items offered to black rhinos on a daily basis were compiled from 20 North American zoos; zoo diet contained (mean ± SE) 61 ± 2% hay, 28 ± 2% grain pellets, 6 ± 1% produce, and 5 ± 1% fresh browse, with hay and grain pellets together comprising nearly 90% of items offered. Gas chromatography-mass spectrometry analysis (GC-MS) was used to measure triacylglycerol equivalent (TAG), total fatty acids (TFA), and essential fatty acids (EFA) in zoo diet, and African and North American browses. North American browse contained more TAG and TFA than did zoo diet or African browse. Zoo diet contained more linoleic acid (18:2n6) and less linolenic acid (18:3n3) than either African browse corrected for degradation losses or North American browse, whether measured as weight percentage of dry sample or as weight percentage of TFA. In addition, the ratio of 18:2n6 to 18:3n3 was significantly lower in both browses than in zoo diet. There are significant nutritional differences between the major dietary components of North American captive black rhinoceros diets and native African browses that warrant further exploration given the health problems associated with this animal in captivity.

Key words: Essential fatty acid profile, linoleic acid, linolenic acid, captive animal nutrition, endangered species, *Diceros bicornis*.

INTRODUCTION

The black rhinoceros (*Diceros bicornis*) is one of the five extant rhinoceros species, all of which are currently suffering from considerable declines in their numbers and available habitat (Ashley et al., 1990). Despite recent increases in the overall number of African rhinos, black rhinos have declined more rapidly than any other large terrestrial mammal in recent history (Kelly et al., 1995). Management techniques to effectively prevent poaching and continued habitat encroachment that contribute to decline in black rhinos have not yet been developed. Therefore, much effort has been directed towards maintenance of black rhinos in protected situations such as zoos.

Public zoos are often limited by cost and availability of food items offered to captive animals. For instance, zoos in the USA are not able to afford the costs of importing the huge quantities (>20,000 kg/year/rhino) of native African browses that black rhinos normally consume. Nor are many temperate habitats suitable for growing native African plant species. Instead, most zoos feed black rhinos a diet similar to that fed to white rhinos (*Ceratotherium simum*) in captivity (Ghebremeskel et al., 1991); this diet consists of alfalfa and grass hays supplemented with grain pellets. Although both black and white rhinos are herbivores, the white rhino diet is quite different both physically and chemically from that which the black rhino naturally consumes in the wild (Ghebremeskel et al., 1991). The white rhino is a grazer, preferentially consuming grasses while eschewing browse (trees and shrubs). Conversely, the black rhino is a strict browser and selectively consumes browse to the exclusion of most other plants apart from the occasional blade of grass drawn into its

mouth while browsing on low shrubs (Mukinya, 1977). In North American zoos, black rhinos seem to exhibit a suite of diseases not seen in other captive species of rhinoceratids (Miller, 1996). It is possible that some of these symptoms may be caused by the discrepancy between possible specific nutrient requirements related to the obligate browser status of black rhinos and the nutrients provided by the standard hay/grain pellet diet of captive black rhinos. For example, differences in essential fatty acid composition (among other deficiencies) between browse diets and captive animal diets have been speculated to be a possible cause of the vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis (SND) in captive black rhinos (Munson et al., 1998).

The first step in discovering nutritional differences is to characterize the diets of captive and free-ranging black rhinos. The browse species that make up the majority of the diet of free-ranging black rhino are well documented (Ritchie, 1963; Goddard, 1968, 1970; Mukinya, 1977; Ghebremeskel et al., 1991; Kotze and Zacharias, 1993; Oloo et al., 1994; Dierenfeld, 1995; Dierenfeld et al., 1995; Maddock et al., 1995; Atkinson et al., 1997; Muya and Oguge, 2000). However, similar information for North American zoo diets is lacking; without such data, a comparative study is not possible. Therefore, this project was undertaken to compile a database of North American zoo diets fed to captive black rhinos from which to compare total fatty acids (TFA), triacylglycerol equivalent (TAG—a measure of fat in the diet), and two essential fatty acids (n-6 linoleic acid and n-3 linolenic acid) in those zoo diets with those found in African browses. Given that they are accessible to most zoos, albeit on a seasonal basis, several North American browses were examined to determine if they possessed levels of triacylglycerol and essential fatty acids nutritionally equivalent to those in African browses.

MATERIALS AND METHODS

Zoo diet composition

Between July of 1995 and May of 1997, each zoo participating in this study returned a description of average daily food offerings to their black rhinos, an estimate of how much was eaten per animal, and samples of the two most abundant items: hay and grain pellets. Each of these items was placed into one of four categories: Hay, Pellet, Produce, or Browse. Hay was defined as any combination of grass or alfalfa prepared as hays, i.e., semi-dry bales of plant material; fresh grass is not currently offered in any zoo. Pellet was defined as any grain-based heat-extruded manufactured grain pellets. Produce was defined as any whole fruit or vegetable and Browse was defined as leafy branches of any tree or shrub. Browse and produce samples were not requested due to their perishable nature. Percentage of estimated daily intake was determined for each category, with mean ± SE calculated for each as well.

African Browse

Fourteen African browse species were collected from either the Zambezi Valley region of Zimbabwe or from Harare, Zimbabwe between May and September of 1995. Browses (stems and mature leaves) were received as either dried and ground to 2 mm mesh or whole (10 to 20 cm long) branches which then were ground to 2 mm mesh after receipt. Although black rhinos are known to consume over 100 different species of plants (Goddard, 1968), only fourteen were chosen for analysis because (1) they were among the species preferred by free-ranging Zimbabwean black rhinos (Dierenfeld et al., 1995; Atkinson et al., 1997) and (2) limitations on availability.

North American Browse

Nine North American browses (ten to twenty cm long branch tips including stems and mature leaves) were collected in July of 1995 from the grounds of Cornell University (Ithaca, New York, USA). They were analyzed to allow comparisons between a hypothetical North American browse diet, zoo diet and one diet of African browses that more closely resembled that of wild black rhinos. A diet based solely on North American browses is not currently fed to any captive black rhino and is entirely speculative in nature. It was analyzed to determine its suitability as a substitute for African browses should such a replacement be deemed necessary.

Fat content and makeup

All zoo samples (hays and pellets) and African browses were ground to 2 mm mesh prior to analysis. All samples were analyzed in duplicate. Sub-samples of North American browses (including both stems and leaves) were analyzed whole within 5 minutes of being cut from the tree. Produce and browses were not analyzed because of (1) their perishable nature, (2) their small contribution to total intake, and (3) the high variability among zoos of both species and amounts offered.

A modified microdigestion/methylation procedure (Browse et al., 1986) was used to prepare the samples for gas chromatography/mass spectrometry analysis (GC/MS). Approximately 30 mg of each sample (ground, 2 mm mesh) was weighed into a 5 ml Reactivial with teflon-lined cap (Wheaton). Two hundred μl of internal standard (ISTD: one mg/ml heptadecanoic acid (Sigma Chemical Company, St. Louis, Missouri, USA) in methanol) and one ml of one normal methanolic HCl (Supelco, Incorporated, Bellefonte, Pennsylvania, USA) were added to each vial which was then purged with nitrogen. The vials were heated at 80 C for 1 hr then removed from heat and cooled to room temperature. To each vial, 1 ml of hexane and 1 ml of 0.9% NaCl solution were added. The vials were shaken by hand for 30 sec then centrifuged at 1000 *g* for 1 min. Four μl were taken directly from the upper hexane phase for GC/MS analysis.

All methylated samples were injected onto a 30 m × 0.32 mm ID fused silica capillary column with a 0.20 μm biscyanopropyl polysiloxane film (Supelco Inc.) in a Hewlett-Packard (Hewlett-Packard Co., Fairport, New York, USA) gas chromatograph with a mass spectrometric detector (HP GCD 1800A). The following temperature program was used: initial temperature 50 C, 1 min hold; rate 20 C/min; final temperature 200 C, 7.5 min hold. The split ratio was 87.5:1 and the carrier gas (helium) flow rate was one ml/min. External standards of heptadecanoic acid, n-6 linoleic acid (18:2n6), and n-3 linolenic acid (18:3n3) (Sigma Chemical Company) were used to build a spectral library for secondary verification. Total fatty acids, 18:2n6, 18:3n3, and triacylglycerol equivalent were determined as in Sukhija and Palmquist (1988). Because wild black rhinos eat directly from living plants and the African browses analyzed here were received dry, a study was undertaken to determine approximate losses of 18:3n3 and 18:2n6 (see below). All parameters in African browse were examined both with and without application of a loss factor (69% 18:3n3 after 140 days of storage).

Differences in %TAG and %TFA of sample on a dry matter basis, %18:2n6 and %18:3n3 of TFA, and 18:2n6 to 18:3n3 ratio among zoo diet, African browses, and North American browses were tested using unpaired *t*-tests with alpha = 0.05 (Snedecor and Cochran, 1989).

Zoo diet calculation

Pellet and Hay composition of 36 black rhino zoo diets was determined as a percent of total food offerings. To calculate the amount of TFA derived from Hay and Pellets in an individual diet, the percentage of Hay in that particular diet was multiplied times the mean amount of TFA measured across all items in the category of Hay then added to the percentage of Pellet times the mean amount of TFA measured across all items in the category of Pellet. Approximately ninety percent of captive black rhino daily intake was accounted for by items in the categories of Hay and Pellet, justifying their use as measures of fat intake.

EFA degradation

Because wild black rhinos eat directly from living plants and the African browses analyzed here were received dry, a study was undertaken to determine approximate losses of n-3 linolenic acid and n-6 linoleic acid in a group of nine North American browses (*Liquidambar styraciflua, Lirodendron tulipifera, Malus coronaria, Morus alba, Platanus occidentalis, Populus deltoides, Rhus glabra, Salix babylonica,* and *Vitis labrusca*) collected in July of 1995 from the grounds of Cornell University. All nine browses were reported as acceptable to captive black rhinos (this study). Each sample consisted of three or four 12 to 30 cm terminal branch clippings including leaves (stem diameter <5 mm). This original sample was sub-sampled on day-zero and subjected to microdigestion/methylation within 5 min of removal of the parent sample from the tree. The remaining sample was air-dried and stored in an envelope in the dark at room temperature for 140 days whereupon it was ground to two mm mesh and analyzed. African browse samples were stored dried and whole or ground for >140 days prior to analysis, making this a conservative estimate of 18:3n3 loss.

RESULTS

Zoo diet composition

Between January and December of 1996, seventeen zoos returned diet descriptions and samples from the diets of 36 captive adult black rhinos. On a weight as-

fed basis, the average zoo diet consisted of 61 ± 2% Hay, 28 ± 2% Pellet, 6 ± 1% Produce, and 5 ± 1% Browse (mean ± SE). The participating zoos were Brookfield Zoo, Busch Gardens, Cincinnati Zoo, Dallas Zoo, Denver Zoological Foundation, Detroit Zoological Park, Fossil Rim Wildlife Center, Lee Richardson Zoo, Los Angeles Zoo, Miami Metrozoo, Milwaukee County Zoo, Oklahoma City Zoological Park, Riverbanks Zoological Park and Botanical Gardens, Sedgewick County Zoo, White Oaks Conservation Center, Wildlife Conservation Society, and ZooAtlanta.

The category of Hay consisted of alfalfa (*Medicago* spp.), bermuda grass (*Cynodon* spp.), flowtron (2:1 alfalfa hay:mixed species prairie grass), timothy grass (*Phleum* spp.), red topped cane (*Triodia* spp.), and a variety of unidentified Graminaceae species. Thirty-one samples of Hay were received. The category of Pellet consisted of grain pellets from the following suppliers: Chicago Zoological Society (Brookfield, Illinois, USA; Open formula herbivore grain); Cargill, Inc. (Feed Division, General Office, Minneapolis, Minnesota, USA; Nutrena 9034 ADF 16 Herbivore pellet and Cargill ADF 16 pellet); Denver Zoological Foundation (City Park, Denver, Colorado, USA; Custom-made textured grain); Detroit Zoological Park, Royal Oak, Michigan, USA; Custom mongastric pellets); Land O'Lakes (Seattle, Washington, USA; Herbivore Zoo pellet); PMI Feeds, Incorporated, South Hanley, Saint Louis, Missouri; Mazuri ADF 25 Regional 5629 pellet, Mazuri Moose pellet, Mazuri ADF 16 Regional pellet, Mazuri ADF 16 cube, and Mazuri Elephant pellet; O. H. Kruse Grain and Milling. El Monte, California, USA; (ADF 16 pellet); and Zoo Atlanta (Atlanta, Georgia USA; HMS Low-fiber pellet).

Twenty-six samples of Pellet were received. In the category of Browse, the following 37 species were reported to be offered on an irregular basis to captive black rhinos: *Acacia* spp., *Acer saccharum, Acer saccharinum, Baccharis halmifolia, Bauhinia blakeana, Bauhinia purpurea, Bucida buceras, Celtis* spp., *Ficus* spp., *Hibiscus rosasinensis, Laurus nobilis, Ligustrum japonicum, Liquidambar styraciflua, Lirodendron tulipifera, Lonicera* spp., *Malus coronaria, Morus* alba, *Musa paradisiaca, Myrica cerifera, Nyssa sylvatica, Panicum hemitomon, Phyllostachys* spp., *Pinus* spp., *Platanus occidentalis, Populus deltoides, Prosopsis juliflora, Quercus* spp., *Robinia* spp., *Rhus glabra, Saccharum officinalum, Salix* spp., *Salix babylonica, Sassafras albidum, Scheffelera* spp., *Sorbus* spp., *Ulmus* spp., and *Vitis labrusca.* No Browse samples were received for analysis. In the category of Produce, the following 16 species were reported to be offered on an irregular basis to captive black rhinos: apples (*Malus* spp.), bananas (*Musa* spp.), carrots (*Daucus* spp.), celery (*Apium* spp.), greenbeans (*Phaseolus* spp.), lettuce (*Latuca* spp.), onions (*Allium* spp.), oranges (*Citrus* spp.), parsnips (*Pastinaca* spp.), pears (*Pyrus* spp.), pineapple (*Ananas* spp.), potato (*Solanum* spp.), spinach (*Spinacea* spp.), yams (*Dioscorea* spp.), sweet potato (*Ipomeae* spp.), and wintersquash (*Cucurbita* spp.). Produce was primarily offered as a treat or reward, never as a dietary mainstay.

African Browse

Fourteen African browses were chosen for analysis: *Acacia karoo, Cassia abreviata, Combretum zeyheri, Commiphora mossambiscensis, Dalbergia melanoxylon, Dichrostachys cineria, Diospyros quiloensis, Elephantorrhiza goetzii, Grewia monticola, Pterocarpus rotundifolia, Schrebra trichoclada, Securanegra virosa, Vitex petersiana,* and *Ziziphus mucronta.* These browses were a subset of those species preferred by free-ranging Zimbabwean black rhinos (Dierenfeld et al., 1995; Atkinson et al., 1997).

North American Browse

Nine North American browses were chosen for analysis: *Acer saccharum, Liquidambar styraciflua, Lirodendron tulipi-*

TABLE 1. Triacylglycerol equivalent (TAG), total fatty acids (TFA), n-6 linoleic acid (18:2n6), and n-3 linolenic acid (18:3n3) as measured in zoo diet, its major components, and African and North American browses.

Parameter	African browse (n = 14)	North American browse (n = 9)	Zoo diet[a] (n = 36)	Hay (n = 31)	Grain Pellets (n = 26)
Expressed as mg/kg dry sample weight					
TAG (mean ± SE)	8,211 ± 816	16,791 ± 1,845	14,002 ± 472	8,758 ± 445	30,663 ± 1,255
TAG Range	4,292 to 15,316	10,026 to 24,779	8,146 to 21,200	4,882 to 15,031	12,819 to 43,688
TFA (mean ± SE)	7,390 ± 734	15,112 ± 1,660	12,602 ± 425	7,882 ± 401	27,596 ± 1,129
TFA Range	3,863 to 13,784	9,024 to 22,301	7,331 to 19,081	4,394 to 13,528	11,537 to 39,319
18:2n6 (mean ± SE)	561 ± 99	1,548 ± 237	4,360 ± 227	1,129 ± 52	13,031 ± 677
18:2n6 Range	0 to 1,236	664 to 2,541	2,140 to 7,998	602 to 1,708	4,962 to 20,111
18:3n3 (mean ± SE)	1,633 ± 366	9,699 ± 1,094	1,982 ± 33	2,106 ± 195	2,450 ± 190
18:3n3 Range	134 to 3,435	6,326 to 14,373	1,368 to 2,242	0 to 4,704	409 to 4,273
18:3n3[b] (mean ± SE)	2,366 ± 531	—[c]	—	—	—
18:3n3[b] Range	194 to 4,978	—	—	—	—
18:2n6 to 18:3n3 ratio (mean ± SE)	0.43 ± 0.08	0.16 ± 0.02	2.2 ± 0.09:1	0.60 ± 0.04	6.6 ± 0.9
18:2n6 to 18:3n3 ratio Range	0 to 1.1	0.09 to 0.23	1.1:1 to 3.6:1	0.31 to 1.1	1.3 to 13
18:2n6 to 18:3n3[b] ratio (mean ± SE)	0.29 ± 0.06	—	—	—	—
18:2n6 to 18:3n3[b] ratio Range	0 to 0.76	—	—	—	—
Expressed as percentage of dry sample weight					
TAG (mean ± SE)	0.82 ± 0.08	1.7 ± 0.2	1.4 ± 0.05	0.88 ± 0.04	3.1 ± 0.1
TAG Range	0.43 to 1.5	1.0 to 2.5	0.82 to 2.1	0.49 to 1.5	1.2 to 4.4
TFA (mean ± SE)	0.74 ± 0.07	1.5 ± 0.17	1.3 ± 0.04	0.79 ± 0.04	2.8 ± 0.1
TFA Range	0.39 to 1.4	0.90 to 2.2	1.9 to 0.73	0.44 to 1.4	1.2 to 3.9
18:2n6 (mean ± SE)	0.056 ± 0.010	0.16 ± 0.02	0.44 ± 0.02	0.11 ± 0.005	1.3 ± 0.07
18:2n6 Range	0 to 0.12	0.066 to 0.25	0.21 to 0.80	0.060 to 0.17	0.50 to 2.0
18:3n3 (mean ± SE)	0.16 ± 0.04	0.97 ± 0.11	0.20 ± 0.003	0.21 ± 0.02	0.24 ± 0.02
18:3n3 Range	0.013 to 0.34	0.63 to 1.4	0.14 to 0.22	0 to 0.47	0.041 to 0.43
18:3n3[b] (mean ± SE)	0.24 ± 0.5	—	—	—	—
18:3n3[b] Range	0.019 to 0.50	—	—	—	—
Expressed as mg/kg TFA					
18:2n6 (mean ± SE)	74,930 ± 11,805	100,265 ± 9,395	220,883 ± 7,110	145,257 ± 3,870	468,218 ± 10,720
18:2n6 Range	0 to 148,897	57,805 to 146,505	130,267 to 254,455	106,351 to 212,587	352,522 to 648,277
18:3n3 (mean ± SE)	196,296 ± 26,689	644,115 ± 16,644	180,864 ± 3,582	254,273 ± 15,175	88,274 ± 5,598
18:3n3 Range	19,913 to 376,787	539,393 to 714,180	140,272 to 227,608	0 to 387,095	13,343 to 133,871
18:3n3[b] (mean ± SE)	284,487 ± 38,679	—	—	—	—
18:3n3[b] Range	28,859 to 546,068	—	—	—	—

TABLE 1. Continued.

Parameter	African browse (n = 14)	North American browse (n = 9)	Zoo diet[a] (n = 36)	Hay (n = 31)	Grain Pellets (n = 26)
Expressed as weight percentage TFA					
18:2n6 (mean ± SE)	7.5 ± 1.1	10 ± 0.9	22 ± 0.7	14 ± 0.4	47 ± 1
18:2n6 Range	0 to 15	5.8 to 15	13 to 28	11 to 21	35 to 65
18:3n3 (mean ± SE)	20 ± 2.7	64 ± 1.7	18 ± 0.4	25 ± 2	8.8 ± 0.6
18:3n3 Range	2.0 to 38	54 to 71	14 to 23	0.0 to 35	1.3 to 13
18:3n3[b] (mean ± SE)	28 ± 3.9				
18:3n3[b] Range	2.9 to 55	—	—	—	—
18:2n6 to 18:3n3 ratio (mean ± SE)	0.43 ± 0.08	0.16 ± 0.02	1.2 ± 0.05:1	0.60 ± 0.04	6.6 ± 0.9
18:2n6 to 18:3n3 ratio Range	0 to 1.1	0.09 to 0.23	0.8:1 to 2.2:1	0.31 to 1.1	1.3 to 13
18:2n6 to 18:3n3[b] ratio (mean ± SE)	0.29 ± 0.06	—	—	—	—
18:2n6 to 18:3n3[b] ratio Range	0.0 to 0.76	—	—	—	—

[a] Zoo diet equals 61 percent hay plus 28 percent grain pellets.
[b] A degradation factor of 69% 18:3n3 loss was applied to these samples.
[c] Degradation factor only applied to African browse as all other samples were received and analyzed in the same condition as normally ingested by black rhinos.

fera, Malus coronaria, Morus alba, Platanus occidentalis, Populus deltoides, Rhus glabra, and *Vitis labrusca.* These browses were a subset of those reported as offered to captive black rhinos in North American zoos.

Fat content in zoo diets

The twenty-six items in the category of Pellet contained (mean ± SE followed by range enclosed by parentheses) 27,596 ± 1,129 mg total fatty acids (TFA)/kg dry matter (11,537 to 39,319 mg/kg) that accounted for 2.8 ± 0.1% (1.2 to 3.6%) of the sample weight on a dry matter basis and 30,663 ± 1,255 mg triacylglycerol equivalent (TAG)/kg (12,819 to 43,688 mg/kg) that accounted for 3.1 ± 0.1% (2.2 to 4.4%) of the sample weight on a dry matter basis. The mean amount of 18:2n6 was 1,3031 ± 677 mg/kg dry matter (4,962 to 20,111 mg/kg) or 1.3 ± 0.07% of dry matter (0.5 to 2.0%). When measured as a component of TFA, the mean amount of 18:2n6 was 46,8218 ± 10,720 mg/kg TFA (352,522 to 648,277 mg/kg) or 47 ± 1% (35 to 65%) of TFA. The mean amount of 18:3n3 was 2,450 ± 190 mg/kg dry matter (409 to 4273 mg/kg) or 0.25 ± 0.02% of dry matter (0.04 to 0.43%). When measured as a component of TFA, the mean amount of 18:3n3 was 8,8274 ± 5,598 mg/kg TFA (13,343 to 13,3871 mg/kg) or 8.8 ± 0.6% of TFA (1.3 to 13%). The ratio of 18:2n6 to 18:3n3 was 6.6 ± 0.9:1 (1.3:1 to 13:1) (Table 1).

Thirty-one items in the category of Hay contained (mean ± SE followed by range enclosed by parentheses) 7,882 ± 401 mg/kg dry matter (4,394 to 13,528 mg/kg) TFA that accounted for 0.79 ± 0.04% (0.44 to 1.4%) of the sample weight on a dry matter basis and 8,758 ± 445 mg/kg (4,882 to 15,031 kg/kg) TAG that accounted for 0.88 ± 0.04% (0.49 to 1.5%) of the sample weight on a dry matter basis. The mean amount of 18:2n6 was 1129 ± 52 mg/kg dry matter (602 to 1,708 mg/kg) or 0.11 ± 0.01% of dry matter (0.06 to 0.17%). When measured as a component of TFA,

TABLE 2. Percentage loss of n-6 linoleic acid and n-3 linolenic acid from nine North American browse species after 140 days in storage.

Species[a]	EFA—Day Zero[b] (mg/kg DM[c])		EFA—Day 140 (mg/kg DM)		Percent loss[d]
Linoleic acid					
Liquidambar styraciflua	1,029 ± 290[e]	(227–1,760)	723 ± 35	(687–758)	30
Lirodendron tulipifera	808 ± 291	(268–1,265)	1,326 ± 108	(1,218–1,434)	−64
Malus coronaria	491 ± 206	(146–858)	562 ± 3	(556–559)	−14
Morus alba	1,332 ± 493	(346–1,857)	1,993 ± 675	(1,319–2,668)	−50
Platanus occidentalis	564 ± 138	(332–811)	1,065 ± 415	(649–1,480)	−89
Populus deltoides	861 ± 424	(202–1,653)	629 ± 8	(621–637)	27
Rhus glabra	1,437 ± 751	(199–2,792)	723 ± 138	(585–861)	50
Salix babylonica	1,783 ± 722	(420–2,876)	863 ± 75	(789–938)	52
Vitis labrusca	1,815 ± 746	(363–2,241)	1,947 ± 765	(1,182–2,712)	−7.2
Linolenic acid					
Liquidambar styraciflua	6,159 ± 1,197	(2,510–8,691)	2,179 ± 269	(1,910–2,448)	65
Lirodendron tulipifera	6,202 ± 2,334	(1,542–8,760)	1,969 ± 168	(1,801–2,137)	68
Malus coronaria	5,829 ± 2,036	(2,601–9,593)	1,781 ± 63	(1,718–1,844)	69
Morus alba	10,435 ± 3,986	(2,559–15,441)	2,650 ± 177	(2,473–2,828)	75
Platanus occidentalis	4,818 ± 1,747	(1,565–7,550)	484 ± 484	(0–969)	90
Populus deltoides	4,722 ± 1,604	(1,514–6,383)	1,521 ± 21	(1,500–1,542)	68
Rhus glabra	10,943 ± 4,253	(2,602–16,555)	5,372 ± 659	(4,713–6,030)	51
Salix babylonica	7,394 ± 4,278	(1,108–15,565)	760 ± 760	(0–1,519)	90
Vitis labrusca	7,786 ± 3,696	(1,507–14,303)	3,387 ± 781	(2,605–4,168)	57

[a] All species were collected on the Cornell University campus as fully leaved branches.
[b] Day zero subsamples were analyzed fresh within five minutes of being cut from the tree or vine on 3 July 1996.
[c] DM = dry matter.
[d] Mean percent loss of n-6 linoleic acid was −7.4 ± 17% and 70 ± 4.4% of n-3 linolenic acid.
[e] Data are reported as mean ± SE followed by range enclosed in parentheses.

the mean amount of 18:2n6 was 14,5256 ± 3,870 mg/kg TFA (10,6351 to 21,2587 mg/kg) or 14 ± 0.4% (11 to 21%) of TFA. The mean amount of 18:3n3 was 2,106 ± 195 mg/kg dry matter (0 to 4,704 mg/kg) or 0.21 ± 0.02% (0.0 to 0.47%) of dry matter. When measured as a component of TFA, the mean amount of 18:3n3 was 254,273 ± 15,175 mg/kg TFA (0 to 387,095 mg/kg) or 25 ± 1% (0.0 to 39%) of TFA. The ratio of 18:2n6 to 18:3n3 was 0.6 ± 0.04:1 (0.3:1 to 1.1:1) (Table 1).

When the categories of Pellet and Hay were combined in thirty-six diets (Pellet + Hay = Zoo diet), it was found that this zoo diet contained (mean ± SE followed by range enclosed by parentheses) 12,602 ± 425 mg/kg dry matter (7,331 to 19,081 mg/kg) TFA that accounted for 1.3 ± 0.04% (0.73 to 1.9%) of the sample weight on a dry matter basis and 14,002 ± 472 mg/kg (8,146 to 18,221 mg/kg) TAG that accounted for 1.4 ± 0.05% (0.8 to 1.8%) of the sample weight on a dry matter basis. The mean amount of 18:2n6 was 2,346 ± 219 mg/kg dry matter (2,346 to 6,367 mg/kg) or 0.44 ± 0.02% of dry matter (0.22 to 0.63%). When measured as a component of TFA, the mean amount of 18:2n6 was 22,0883 ± 7110 mg/kg TFA (16,4001 to 328,217 mg/kg) or 22 ± 0.7% (16 to 33%) of TFA. The mean amount of 18:3n3 was 1,982 ± 33 mg/kg dry matter (1368 to 2,242 mg/kg) or 0.20 ± 0.003% (0.14 to 0.22%) of dry matter. When measured as a component of TFA, the mean amount of 18:3n3 was 180,864 ± 3,582 mg/kg TFA (140,272 to 227,608 mg/kg) or 18 ± 0.4% (14 to 23%) of TFA. The ratio of 18:2n6 to 18:3n3 was 2.2 ± 0.1:1 (1.1:1 to 3.6:1) (Table 1).

No degradation factor was applied to any category of zoo diet because these items were received and analyzed in the

same state as in which they were normally ingested.

Fat content in African browses

The fourteen African browses contained (mean ± SE followed by range enclosed by parentheses) 7,390 ± 734 mg/kg dry matter (3,900 to 14,000 mg/kg) TFA that accounted for 0.74 ± 0.07% (0.39 to 1.4%) of the sample weight on a dry matter basis and 8,211 ± 816 mg/kg (4,300 to 15,000 mg/kg) TAG that accounted for 0.82 ± 0.08% (0.43 to 1.5%) of the sample weight on a dry matter basis. The mean amount of 18:2n6 was 561 ± 99 mg/kg dry matter (0 to 1236 mg/kg) or 0.06 ± 0.01% of dry matter (0.0 to 0.12%). When measured as a component of TFA, the mean amount of 18:2n6 was 74,930 ± 11,805 mg/kg TFA (0 to 148,897 mg/kg) or 7.5 ± 1.2% (0 to 15%) of TFA. The mean amount of 18:3n3 was 1,633 ± 366 mg/kg dry matter (134 to 3,435 mg/kg) or 0.16 ± 0.04% (0.01 to 0.34%) of dry matter. When corrected for an approximate loss of 69% due to 18:3n3 degradation during storage (see below), the mean amount 18:3n3 was 2,366 ± 531 mg/kg dry matter (194 to 4,978 mg/kg) or 0.24 ± 0.05% (0.02 to 0.50%) of dry matter. When measured as a component of TFA, the mean amount of 18:3n3 was 196,296 ± 26,689 mg/kg TFA (19,913 to 37,6787 mg/kg) or 20 ± 3% (2 to 38%) of TFA. Corrected for 69% n-3 linolenic acid loss, the mean amount of 18:3n3 was 284,487 ± 38,679 mg/kg TFA (28,859 to 546,068 mg/kg) or 28 ± 4% (2.9 to 55%) of TFA. The ratio of 18:2n6 to 18:3n3 was 0.43 ± 0.08:1 (0.0:1 to 1.1:1) uncorrected for 18:3n3 loss and 0.29 ± 0.06:1 (0.0:1 to 0.76:1) when corrected for loss (Table 1).

EFA degradation

There was no loss of 18:2n6 in the nine North American browses analyzed. However, the average loss of 18:2n6 in nine North American browses was 69 ± 6% (mean ± SE) over a period of 140 days (Table 2). This was a conservative estimate of loss because the air-dried North American browses were not ground until just prior to analysis, unlike the African browse samples that either were ground before or upon receipt then remained in storage for up to 200 days before analysis, increasing their susceptibility to oxidative degradation.

Fat content in North American browses

The nine North American browses (Table 1) contained (mean ± SE followed by range enclosed by parentheses) 15,112 ± 1,660 mg/kg dry matter (9,024 to 22,301 mg/kg) TFA that accounted for 1.5 ± 0.2% (0.9 to 2.2%) of the sample weight on a dry matter basis and 16,790 ± 1,844 mg/kg (10,026 to 24,779) TAG that accounted for 1.7 ± 0.2% (1.0 to 2.5%) of the sample weight on a dry matter basis. The mean amount of 18:2n6 was 1,548 ± 237 mg/kg dry matter (664 to 2,541 mg/kg) or 0.15 ± 0.02% of dry matter (0.07 to 0.25%). When measured as a component of TFA, the mean amount of 18:2n6 was 10,0265 ± 9,394 mg/kg TFA (57,805 to 146,505 mg/kg) or 10 ± 0.9% (5.8 to 15%) of TFA. The mean amount of 18:3n3 was 9,699 ± 1,094 mg/kg dry matter (6,326 to 14,373 mg/kg) or 0.97 ± 0.11% (0.63 to 1.4%) of dry matter. When measured as a component of TFA, the mean amount of 18:3n3 was 644,115 ± 16,644 mg/kg TFA (539,393 to 714,180 mg/kg) or 64 ± 1.7% (54 to 71%) of TFA. The ratio of 18:2n6 to 18:3n3 was 0.16 ± 0.02:1 (0.11:1 to 0.23:1) (Table 1).

Zoo/Browse comparison

African browse contained lower mean percentages on a dry sample weight basis of TFA, TAG, and percent 18:2n6 (whether measured as % dry sample weight or % TFA) than did zoo diet (unpaired *t*-tests, $\alpha = 0.05$, $P < 0.0001$). African browse and zoo diet contained the same mean amount of 18:3n3 whether measured as percentage TFA or dry sample weight (unpaired *t*-test, $\alpha = 0.05$, $P > 0.10$) and the mean ratio of 18:2n6 to 18:3n3 was significantly lower in African browse than in zoo diet (unpaired

t-test, $\alpha = 0.05$, $P < 0.0001$). When a 69% 18:3n3 loss factor was applied to African browses, the mean percent 18:3n3 of TFA became significantly greater in African browse than in Zoo diet; results of all other above statistical tests remained equivalent.

Compared to North American browse, African browse contained lower mean percentages on a dry sample weight basis of TFA, TAG, 18:3n3 (whether measured as percentage dry sample weight or TFA), and 18:2n6 (unpaired *t*-tests, $\alpha = 0.05$, $P < 0.0005$). On a TFA basis, the percent 18:2n6 did not differ between the two browse groups (unpaired *t*-tests, $\alpha = 0.05$, $P > 0.10$). The ratio of 18:2n6 to 18:3n3 was significantly higher in African browse than in North American (unpaired *t*-test, $\alpha = 0.05$, $P = 0.02$). However, once corrected for 18:3n3 degradation, the ratio of 18:2n6 to 18:3n3 in African browse was not significantly different from that in North American browse (unpaired *t*-test, $\alpha = 0.05$, $P > 0.05$); results of all other above statistical tests remained equivalent.

DISCUSSION

The survey and sampling of U.S. zoos indicated that nearly ninety percent of offerings to black rhinos in North American zoos consist of hays and grain-based grain pellets, neither of which play major roles in the browse-based diet of wild black rhinos (Goddard, 1968; Goddard, 1970; Mukinya, 1977; Hall-Martin et al., 1982; Ghebremeskel et al., 1991; Kotze and Zacharias, 1993; Oloo et al., 1994; Dierenfeld, 1995; Dierenfeld et al., 1995; Maddock et al., 1995; Atkinson et al., 1997; Muya and Oguge, 2000). In sharp contrast to diets of wild black rhinos, browse is a very minor component in black rhino zoo diets (approximately 5%); this lack of browse may be responsible for the nutritional discrepancies seen in these two diets.

On the surface, zoo diet appears to contain sufficient to surfeit amounts of both EFA when compared to African browse. However, when distribution of EFA is examined as the ratio of 18:2n6 to 18:3n3, it becomes apparent that EFA composition in zoo diet is quite different from that in African or North American browses. The competition that exists between these two fatty acids may cause a deficiency in the long-chain, desaturation products of either one, even when intake is apparently sufficient (Holman and Johnson, 1983). Given the high 18:2n6 to 18:3n3 ratio seen in zoo diet, the possibility exists that the long-chain 18:3n3 desaturation products are deficient in captive members of this species. Essential fatty acid requirements of black rhinos are not known, so it is not possible to say whether zoo diet is sufficient or deficient in either EFA. Essential fatty acid deficiency has not been documented in another perissodactylid, the horse (National Research Council, 1989), but the NRC-NAS recommends a minimum dietary dry matter intake of 0.5 percent linoleic acid for horses and has made no recommendation for linolenic acid (National Research Council, 1989). Lower than expected levels of 18:3n3 in African browse, despite the application of a conservative loss factor, may possibly be attributed to the dried, degraded condition in which they were analyzed.

If an EFA deficiency in black rhinos does exist, these data may support the hypothesis that certain SND-like signs seen in captive black rhinos may be linked to EFA and/or nutritional discrepancies between browse and zoo diet as speculated by other researchers (Munson, 1993; Miller, 1995, 1996; Munson et al., 1998). Similar skin lesions described in humans such as (necrolytic migratory erythema) and in dogs (superficial necrolytic dermatitis and ulcerative dermatosis) have been alleviated with treatments of one or both EFA, essential amino acids, and/or zinc, but no consensus has been made as to which treatment is best (Doyle et al., 1979; Blackford et al., 1991; Thorisdottir et al., 1994; Marinkovich et al., 1995; Nyland et al., 1996; Wermers et al., 1996). The signs of EFA deficiency in perissodactylids, much less black rhinos, are not known

(National Research Council, 1989) leaving 18:3n3 and/or its metabolites as possible factors in the SND-like skin disease seen in captive black rhinos in North America. Essential fatty acid deficiency is also suspect due to evidence of low delta-6 desaturase activity in black rhinos (Bauer et al., 2000).

Currently a large variety of browse species native to North America are fed to captive black rhinos as treats (when available), indicating that they seem to find these browses palatable. This is important because although North American browse as a diet remains hypothetical, such browse is readily available. If EFA ratios in North American zoo diets are found to be related to disease, then supplementation with North American browses might be used to correct EFA ratios. However, further research is required before the supplementation of zoo diets with browse or 18:3n3 can be recommended. Although the nutrient composition of some African browses has been determined (Dierenfeld, 1995), it is not currently feasible to supplement captive black rhinos in North America with such expensive browse. Examination of the nutrient differences between the captive North American diet and African browse diets and possible similarities between African browse and North American browse may prove beneficial in the nutritional care of black rhinos.

Because their digestive tract is similar to that of other related perissodactylids (Clemens and Maloiy, 1982), rhinocerotids are commonly fed grazer diets. This approach to captive black rhinoceros nutrition fails to account for specific (and as yet, largely unquantified) differences in digestive physiology between grazing equids and browsing rhinoceratids (Ghebremeskel et al., 1988). Several studies have postulated that nutritional inadequacies may be responsible for poor health conditions of captive black rhinos (Dierenfeld et al., 1995; Miller, 1995, 1996). The macro- and micro- nutrient differences between diets of wild and captive black rhinos have not been completely determined, leaving one with the question of their nutritional compatibility. Significant nutritional differences between the diets of free-ranging and captive animals, such as those documented in this study may contribute to a species-threatening disease and should be further evaluated.

ACKNOWLEDGEMENTS

We are indebted to S. J. Atkinson and M. Duncan for providing samples of African browse and to all the zoological facilities participating in the study. This project was supported by a teaching assistantship from the Department of Animal Science, Cornell University.

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Received for publication 8 January 2000.