OMEGA-3 FATTY ACIDS IN THE NUTRITION OF THE BLACK RHINOCEROS (DICEROS BICORNIS) IN CAPTIVITY IN THE UNITED STATES

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INTRODUCTION

The black rhinoceros (*Diceros bicornis*) is one of two African rhinoceros species that has been transported to the United States and abroad as a measure of protection from inordinately high rates of poaching and subsequent extinction and to enrich public and private collections. It is important that these animals thrive in captivity, for the survival of the species as well as for the viewing pleasure of the paying public. In captivity in North America, the black rhinoceros population has been subject to several diseases which limit it in both of the previous respects (1). The black rhinoceros in captivity has a high incidence of hemolytic anemia (2), mucocutaneous ulcerative syndrome (3), fungal pneumonia (1), hemosiderosis (4), and encephalomalacia (5). Some of these diseases may be caused by nutritional deficiencies or excesses (1). These diseases are rarely to never seen in the wild (1). Four of nine cases of fungal pneumonia were attributed to a reaction to corticosteroid treatments of mucocutaneous ulcerative syndrome (1) which is one of the diseases most suspected of being nutritionally related. This warrants further study into the composition of black rhinoceros diets in both captivity and the wild.

In the case of mucocutaneous ulcerative syndrome, deficiencies or imbalances of amino acids and/or fatty acids may be a predisposing factor (5). In human nutrition, n-3 linolenic acid deficiency causes clinical symptoms of hemorrhagic dermatitis, hemorrhagic folliculitis, skin atrophy, and scaly dermatitis (6), in addition to reproductive disorders (7). A lack of n-3 linolenic acid in the diet may be a factor in the rhinoceroses' mucocutaneous ulcerative syndrome. A previous study of native Zimbabwean browses that were large constituents of the diets of wild black rhinoceroses found n-6 linolenic acid in 7 out of the 10 browses analyzed (8). The significance of this is not yet known, but might also be a factor if n-6 linolenic acid is not present in the diets of captive black rhinoceroses with mucocutaneous ulcerative syndrome. Another avenue of exploration is that of imbalance between n-3 linolenic acid and n-6 linoleic acid.

This paper focuses on the n-3 linolenic acid, n-6 linolenic acid, and n-6 linoleic acid (linoleic acid) content of diets of black rhinoceroses in captivity in the United States. The other African rhinoceros species, the white rhino (*Ceratotherium simum)*, has adapted to captivity much more readily, displaying a lower frequency of disease with displayed illnesses being more common in nature (1).

MATERIALS AND METHODS

Sixteen dry to semi-dry samples of approximately 1 to 2 kg each of the main components of black rhinoceros diets (hays and preformulated pelleted feed) were collected from 19 black rhino holding facilities over a three month period. The total diets of the rhinos were described and the approximate amount of each component offered was estimated by the primary keeper of the rhinoceros in 18 of the 20 facilities. This information was used to estimate the average makeup of a captive black rhinoceros diet. The samples were stored in a cool, dry cupboard in plastic bags until analysis. Approximately one half of each sample was ground in a Wiley mill through a 2 mm mesh screen. The other half was not ground to prevent undue degradation of the fatty acids. Percent dry matter was determined on 2 to 3 g subsamples taken from each of the ground samples by oven drying to a constant weight at 100°C. The ratio between the weights of original sample and the dried sample was then calculated.

The digestion and methylation method of Browse et al. (9) was modified and used for the determination of the fatty acid composition as follows. Between 30 and 100 mg of each ground sample was weighed into a 5 mL screw capped vial with a Teflon coated rubber liner. Samples were not oven dried prior to extraction and methylation in order to prevent excessive degradation of the fatty acids. One mL of 1 N methanolic HCl was added to each vial which was then purged with nitrogen and sealed. The vials were heated at 80°C for 1 hour to ensure complete digestion and methylation. When the samples had cooled to room temperature 400 µL of 1.0 mg/mL heptadecanoic acid methyl ester in hexane was added, followed by 1 mL of hexane and 1 mL of 0.9% of NaCl. The fatty acid methyl esters (FAMES) were extracted into the hexane by shaking by hand for 30 seconds. The samples were centrifuged (1000 *g* X 30 s) to break any emulsion formed and to completely separate the phases. A 4 µL sample was taken directly from the hexane phase for gas chromatographic analysis. The total sample volume was 1.0 mL.

The gas chromatograph used (Hewlett Packard GCD 1800A) was fitted with a mass spectrometer detector set to a mass range of 15:330 m/z. The column used was a 30 m, 0.32 mm ID, fused silica capillary column with a 0.20 µm biscyanopropyl polysiloxane (very polar) film. The carrier gas (helium) flow rate was 1 mL/min. A 5 min solvent delay was used to prevent detector damage and split injection was used with a split ratio of 87.5:1. The temperature program follows. Initial temperature: 50°C with a 5 min hold, rate: 20°C/min, final temperature 200°C with a 7.5 min hold.

External standards of n-3 linolenic acid methyl ester and n-6 linolenic acid methyl ester were used to differentiate between the two isomers. An external standard of linoleic acid methyl ester was used to qualify that methyl ester. An internal standard of heptadecanoic acid

methyl ester was used to quantify the methyl esters. The conversion ratios of n-3 linolenic acid: n-3 linolenic acid methyl ester, n-6 linolenic acid: n-6 linolenic acid methyl ester, and linoleic acid: linoleic acid methyl ester were all 0.95.

RESULTS AND DISCUSSION

The estimated average diet of the black rhinoceros population in the United States was found to be predominantly alfalfa hay and preformulated pellets (see Figure 1). Fresh browse was estimated at approximately 3% of the diet which is in direct contrast with the wild diet of almost 100% browse (10). Black rhinos in captivity in the Port Lympne Zoo in the UK which have been fed primarily large quantities of freshly cut browse have not experienced any mucocutaneous ulcerative syndrome disorders (11). Although this may not be related to the fatty acid question, it points to nutrition and diet composition as a cause of this disease in black rhinos. The black rhinoceros is a non-selective, heavy browser of woody trees, shrubs, and succulent plants (10) as opposed to the grasses favored by the white rhinoceros. This is quite evident by the differing mouth parts of both species. The white rhinoceros has a wide flat mouth and broad lips well adapted to mowing down wide swaths of grass while eating and the black rhinoceros (also known as the "hooked lipped" rhinoceros) has a prehensile upper lip more suited to the grasping of small branches. The white rhinoceros may be more successful at adapting to captivity due to its different eating habits (12).

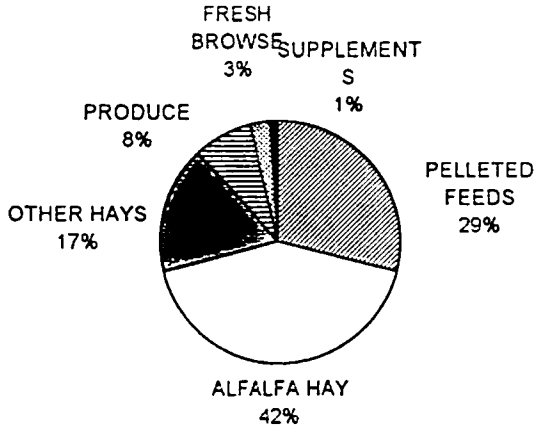

Figure 1. Estimated Average Diet Composition of Black Rhinos in Captivity in the U.S.

The fatty acid information is contained in Table 1. The linoleic acid and n-3 linolenic acid content of the categories of alfalfa hay and other hay are comparable to the levels found in the selected african browse species. It appears that levels of n-3 linolenic acid are slightly higher than those of linoleic acid in the hays and African browse species. The striking difference is found in the fatty acid content of the pellets. The ratio in pellets is heavily skewed towards linoleic acid (20.5% 18:2 versus 4.7% 18:3n3). The pellets also have a higher total lipid

content than the rest of the diet items. The average American black rhinoceros is being fed a diet higher in fat and with more linoleic acid than it would receive in the wild. Of course, this may not be the cause of the skin problems because the levels of n-3 linolenic acid appear to be adequate. No n-6 linolenic acid was detected in any of the feed categories except the African browse, and there only in low levels and not in all species. This may or may not be significant. It has not been determined whether the n-6 linolenic acid isomer is an artifact of degradation or if it is actually present in the browses. It also is not known whether or not n-6 linolenic acid plays any metabolic role in the health of the black rhinoceros.

Table 1. Ppm on a dry matter basis ± standard deviation, range of fatty acid content, average % total lipid composition of feed and approximate % fatty acid of total lipids for linoleic acid, n-3 and n-6 linolenic acids in each feed category.

FEED CATEGORY	*n*	*ppm (mg/g DMB ± SD)*		*RANGE*	*TOTAL LIPID (mg/g)*	*% OF TOTAL LIPIDS*
				n-6 Linoleic acid		
ALFALFA HAY	5	1.65 ± 0.99		0.68-3.3	2.8	5.9
OTHER HAY	4	1.21 ± 0.58		0.63-2.0	2.8	4.3
PELLETS	7	8.62 ± 1.67		6.5 - 11	4.5	20.5
AFRICAN BROWSE	5	1.67 ± 0.20	(8)	1.4 - 1.8	3.8	4.4
				n-3 Linolenic acid		
ALFALFA HAY	5	2.62 ± 1.39		1.5 - 4.9	2.8	9.4
OTHER HAY	4	2.26 ± 1.95		0.43 - 5.0	2.8	8.1
PELLETS	7	1.96 ± 0.68		1.1 - 3.3	4.5	4.7
AFRICAN BROWSE	5	2.43 ± 0.93	(8)	1.3 - 3.9	3.8	6.4
				n-6 Linolenic acid		
ALFALFA HAY	5	ND*		N/A**	2.8	N/A
OTHER HAY	4	ND		N/A	2.8	N/A
PELLETS	7	ND		N/A	4.5	N/A
AFRICAN BROWSE	5	0.20 ± 0.22	(8)	0 - 0.54	3.8	0.53

*No Detection
**Not Applicable

This project is in progress and will include analysis of fresh browse fed in the United States and a greater range of samples to determine their influence on the fatty acid composition of the black rhinoceros in captivity in the U.S.

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