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# Identification of 18:3(*n*-3) linolenic acid, 18:3(*n*-6) linolenic acid and 18:2(*n*-6) linoleic acid in Zimbabwean browses preferred by wild black rhinoceroses (*Diceros bicornis*) determined by GC–MS analysis

Jacqualine B. Wright*, Dan L. Brown

*Department of Animal Science, Cornell University, Ithaca, New York, NY 14853, USA*

**Abstract**

Ten browses preferred by black rhinoceroses (*Diceros bicornis*) in Zimbabwe were analyzed for the presence of two isomers of linolenic acid [18:3(*n*-3) and 18:3(*n*-6)] and 18:2(*n*-6) linoleic acid using GC–MS analysis. The 18:3(*n*-3) linolenic acid and 18:2(*n*-6) linoleic acid were found in varying proportions in all of the browses, while 18:3(*n*-6) linolenic acid was found in much smaller amounts in only some of the browses. © 1997 Elsevier Science B.V.

*Keywords:* *Diceros bicornis*; Essential fatty acids; Browses

## 1. Introduction

In zoos in North America black rhinoceroses have been diagnosed with unexplainable mucocutaneous ulcers which may be due to a nutritional deficiency (Miller, 1995). These ulcers are not associated with infestation by *Stephanofilaria dinniki* which is the main cause of skin ulcers in black rhinos in the wild (Miller, 1994). The mucocutaneous ulcerative syndrome found in North American captive

* Corresponding author. Tel.: +1 607 2556615; fax: +1 607 2559829; e-mail: jbw1@cornell.edu


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black rhinos most closely resembles necrolytic migratory erythema in man and superficial necrolytic dermatitis in dogs (Munson et al., 1996). This study was undertaken to determine the range of 18:3(*n*-3) linolenic acid and 18:2(*n*-6) linoleic acid in the diet of wild black rhinos to serve as a comparison for analysis of these fatty acids in captive rhinos' diets. A deficiency of 18:3(*n*-3) linolenic acid is known to cause scaly dermatitis in humans (Jerve et al., 1989). Humans with necrolytic migratory erythema have responded to treatment with intravenous infusions of essential fatty acids and amino acids (Bewley et al., 1996). It is possible that the nutritional deficiency causing the ulcers in captive American black rhinos is caused by a deficiency of 18:3(*n*-3) linolenic acid in their diets compared to levels of 18:3(*n*-3) linolenic acid in diets of wild black rhinos.

Browses are a key part of the black rhinoceros diet in the wild. Black rhinos will not select grasses over browses if given the option (Dierenfeld et al., 1996). In North American zoos black rhino diets consist mainly of alfalfa hay and commercial pelleted feeds (Wright, personal observation). The 10 browses were analyzed to determine if 18:3(*n*-3) linolenic acid is present in measurable amounts in browses preferred by black rhinos as preliminary work to further research in black rhino diet fatty acid composition.

## 2. Materials and methods

### *2.1. Sample collection and handling*

Ten of the black rhino's most preferred browse specimens were collected in the Zambezi Valley, Zimbabwe during the March–April period of 1995. Whole branches were collected. The branches were not divided into separate leaf and twig components. The samples were dried at room temperature, ground to 2-mm mesh size, then put in plastic bags until received for analysis in August of 1995. At this time they were placed in a refrigerator.

### *2.2. Sample preparation for GC–MS*

The fatty acids were prepared for analysis using a micro-extraction method (Browse et al., 1986). Approximately 100 mg of sample was placed in a 5-ml Reactivial with 1 ml of 1-*n*-methanolic HCl (prepared by diluting 3-*n*-methanolic HCl from Supelco [Supelco, Inc. Supelco Park, Bellefonte, PA 16823 USA) to 1 N with HPLC methanol], purged with nitrogen, sealed and heated at 80°C for 1 h to ensure completion of the methylation reaction. The samples were cooled to room temperature. One millilitre of hexane and 1 ml of 0.9% NaCl in distilled water were added. The vials were shaken by hand for 30 s then centrifuged at 1000 × g for 1 min. A 4-$\mu$l sample was taken directly from the hexane phase to be used for GC–MS analysis. The Hewlett Packard GC–MS was fitted with a Supelco SP-2340 capillary column [30 m × 0.32 mm i.d. × 20 $\mu$m film (poly(biscyanopropylsiloxane)]. The mass range used was 15:330 $m/z$ and ultra high purity helium was used as

the carrier gas with a flow rate of 1 ml $min^{-1}$. Program conditions were: inlet, 250°C; detector, 250°C; oven, 50°C; hold, 5.0-min; rate, 20°C $min^{-1}$; final temperature, 200°C; hold, 7.5-min; split injection mode with a split ratio of 87:1. Quantitative standards from Sigma (Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178 USA) were used for positive identification of 18:2(*n*-6) linoleic acid, 18:3(*n*-3) linolenic acid and 18:3(*n*-6) linolenic acid. The following amounts of standard were used to prepare quantitative and qualitative standards: 980 mg of 18:2(*n*-6) linoleic acid standard; 104 mg of 18:3(*n*-3) linolenic acid; and 113 mg of 18:3(*n*-6) linolenic acid were all dissolved in 10 ml of HPLC *n*-hexane. Remaining standards were made from dilutions of these stock standard solutions in HPLC *n*-hexane (see Table 1). The final volume of the hexane phase was 1.0 ml. The identifications were based on a mass spectral library built from the specific conditions for this analysis.

## 3. Results and discussion

Table 1 shows that all three fatty acids were found in the browse species in varying amounts. 18:3(*n*-3) linolenic acid was higher than 18:2(*n*-6) linoleic acid in

Table 1
Parts per million (g $kg^{-1}$) on a dry matter basis for 18:2(*n*-6), 18:3(*n*-3) and 18:3(*n*-6)

| Species (g $kg^{-1}$) | 18:2(*n*-6) | | 18:3(*n*-3) | | 18:3(*n*-6) | |
|---|---|---|---|---|---|---|
| | Range (g $kg^{-1}$) ppm ± S.E. | Range (ppm) | Range (g $kg^{-1}$) ppm ± S.E. | Range (ppm) | Range (g $kg^{-1}$) ppm ± S.E. | Range (ppm) |
| *Dalbergia melanoxylon* | 1.1 ± 0.2 | 0.9–1.3 | 1.7 ± 0.4 | 1.3–2.0 | 0.3 ± 0.03 | 0.2–0.3 |
| *Elephantorrhiza goetzii* | 2.1 ± 1.7 | 0.9–4.0 | 6.3 ± 5 | 2.4–12.4 | 0.8 ± 0.6 | 0.4–1.2 |
| *Schebra trichoclada* | 0.8 ± 0.07 | 0.8–0.9 | 1.7 ± 0.3 | 1.5–2.0 | 0.6 ± 0.05 | 0.6–0.7 |
| *Diospyros quiloensis* | 1.7 ± 0.2 | 1.4–1.9 | 3.4 ± 0.3 | 3.1–3.7 | nd | nd |
| *Pterocarpus rotundifolius* | 1.8 + 0.4 | 1.4–2.2 | 4.3 ± 0.9 | 3.4–5.3 | 0.5 ± 0.1 | 0.4–0.6 |
| *Vitex petersiana* | 1.6 ± 0.2 | 1.4–1.7 | 2.7 ± 0.06 | 2.7–2.8 | 0.4 ± 0.02 | 0.39–0.42 |
| *Grewia monticola* | 1.8 ± 0.2 | 1.6–2.0 | 0.74 ± 0.1 | 0.6–0.9 | 0.5 ± 0.1 | 0.4–0.6 |
| *Commiphora mossambicensis* | 2.6 ± 0.07 | 2.5–2.6 | 2.0 ± 0.04 | 2.0–2.1 | 1.2 ± 0.1 | 1.0–1.3 |
| *Cassia abreviata* | 1.7 ± 0.2 | 1.5–1.8 | 3.5 ± 0.5 | 3.0–4.0 | nd | nd |
| *Combretum zeyheri* | 1.3 ± 0.2 | 1.2–1.4 | 1.8 ± 0.3 | 1.6–2.2 | nd | nd |

nd, no detection.

all the species analyzed except *Grewia monticola*. This balance is subject to change dependent upon which part of the plant the rhino is feeding on — ingestion of seeds and kernels favors linoleic acid intake, while ingestion of leaves favors linolenic acid (Ghebremeskel et al., 1981). Linoleic acid [18:2(*n*-6)] is the predominant essential fatty acid in corn and soy (Harwood and Russell, 1984) which are ubiquitous in pelleted feeds. Perhaps excess linoleic acid adversely affects the captive rhino? Wild black rhinoceroses ingest 18:3(*n*-3) linolenic acid in their diets. As the availability of seeds and kernels is limited compared to the availability of leaves and bark, they most likely tend to ingest more 18:3(*n*-3) linolenic acid than linoleic acid. Further work needs to be done on a greater range of native browse specimens and on diets fed to captive black rhinos in the United States to determine the difference in composition of fatty acids between the two diets.

The quantitative analysis of these dried samples is affected somewhat by the degradation of fatty acids that occurs upon drying of the plant tissue (Harwood and Russell, 1984). The figures presented here are lower than those which would be found in fresh tissue, but can not be higher than that of fresh foliage on a dry matter basis. Another study (Ghebremeskel et al., 1981) of frozen, fresh forages found higher levels of both 18:3(*n*-3) and 18:2(*n*-6) than were found in this study due mainly to the degradation of 18:3(*n*-3) and 18:2(*n*-6) content upon drying and storage. Because captive black rhino diets consist of a substantial amount of pelleted feed (which is dried and heated) and baled hay which also tends to have undergone drying, an analysis of fatty acids in dried plant material will be of some limited use because the captive animals are ingesting large percentages of dried plant materials in their diets. The dominance of dried plant material in the diet of the captive black rhino also causes suspicion to fall on fatty acid deficiency because of the potential degradation of essential fatty acids during storage. The results of this project indicate that further research (analysis of commercial pelleted feeds and other components of captive diets) and tissue work to determine baseline essential fatty acid levels in healthy black rhinos is warranted.

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