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ORIGINAL ARTICLE

Fatty acid status in captive and free-ranging black rhinoceroses (*Diceros bicornis*)*

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Summary

The fatty acid (FA) patterns of plasma/serum triglycerides (TG), phospholipids (PL) and cholesteryl esters (CE) of captive and free-ranging black rhinoceroses (*Diceros bicornis*) were investigated. Free-ranging animals ($n = 28$) stemmed from four different regions. Captive animals sampled included specimens from North American ($n = 11$) and three different European facilities ($n = 6$). The European animals were tested on 1–4 different diets, resulting in a total of 15 blood samples. Regardless of differences between the free-ranging animals from different regions, differences between captive and free-ranging animals were relatively uniform: captive animals had higher overall proportions of polyunsaturated fatty acid (PUFA), due to levels of linoleic acid (LA, 18:2n6) that were drastically increased as compared to free-ranging animals. In contrast, levels of alpha-linolenic acid (ALA, 18:3n3) were consistently lower on conventional zoo diets. n6/n3 ratios for TG, PL and CE were 1.6, 10 and 8 in samples from free-ranging animals, respectively, as compared to 4.1–16.3, 16–148 and 40–277 in samples from captive animals. There was a distinct correlation between the proportion of grain-based products (commercial concentrates, plain grains and bread) in the diets of the European animals and the measured levels of n6 PUFA. An animal from a facility with a very low proportion of grain products in the diet nevertheless had high LA readings, most probably due to the use of sunflower oil as 2% (dry matter basis) of its diet. One animal that received a high proportion of grass meal pellets due to an oral disease had increased ALA contents after the diet change. These results allow conclusions on the suitability of diets fed in captivity: the black rhinoceros is prone to several uncommon diseases that have been suspected to be linked to oxidative damage, possibly due to the disposition of this species to excessive iron storage. An unnatural dietary loading with PUFAs would exacerbate this problem. Additionally, n6 FAs are known as precursors of pro-inflammatory mediators, and their overrepresentation could therefore exacerbate any inflammatory processes. Therefore,

the current practice of using grain-based feeds as major ingredients in captive rhinoceros diets is discouraged. Diet items containing ALA (a precursor of anti-inflammatory mediators) such as, fresh grass, fresh browse, the respective silages should be included at higher levels in diets for captive black rhinoceroses. Grass meal pellets, although a good source of ALA and linked with high levels of ALA in an animal of this study, must be chosen with care for black rhinoceroses due to their particular proneness for high iron contents.

Introduction

Black rhinoceros (*Diceros bicornis*) are strict browsers (reviewed in Clauss et al., 2006a). Like other forages, browse material contains high proportions of polyunsaturated fatty acids (PUFAs), in particular omega(n)-3 PUFAs (mostly alpha-linolenic acid, ALA) (Ghebremeskel et al., 1991b; Grant et al., 2002). This contrasts with the fatty acid (FA) composition reported for diets fed to captive zoo animals (Clauss et al., 2007b). The FA composition of body tissues is correlated to the FA composition of the ingested diet (Hulbert et al., 2005); therefore, it is reasonable to expect that captive black rhinoceroses display, compared to their free-ranging conspecifics, a distinctively different FA composition in different body tissues, due to their different diet.

Historically, such differences in FA status had first been demonstrated for free-ranging vs. captive giraffes (*Giraffa camelopardalis*) (Crawford, 1968) and have subsequently been reported for several other species such as different wild ruminants or elephants (Crawford et al., 1991; Clauss et al., 2003b). Crawford et al. (1991) even mentioned that liver phospholipids (PL) in individual free-ranging vs. captive black rhinoceros showed the same pattern, without giving the data this observation was based on, and Suedmeyer and Dierenfeld (1998) observed a change in the FA status of a recently imported wild-caught individual with increasing time in captivity that followed the same pattern. A review of the available data for mammalian herbivores kept in captivity showed that as compared to their free-ranging conspecifics, captive individuals in general had a lower proportion of PUFAs and a higher n6/n3-ratio in their body tissues (Clauss et al., 2007b), and only two of six adipose tissue samples from captive black rhinoceros in USA, analysed in material stored after necropsy, contained detectable proportions of ALA (Dierenfeld and Frank, 1998).

As an increased n6/n3 ratio has been reported to be associated with alterations of the immune status, inflammatory response, skin health and reproductive potential (reviewed in Clauss et al., 2007b), such a potential difference is of interest for any captive wild animal species. In black rhinoceros, there has been the particular speculation that a potential difference in the FA status of captive as compared to free-ranging animals could be a contributing factor to unusual diseases observed in this species in captivity (Grant et al., 2002) – most importantly, the syndrome of superficial necrolytic dermatopathy (Munson et al., 1998). The concern about the suspected difference in FA status has even sporadically led to diet changes (Suedmeyer and Dierenfeld, 1998). In this contribution, we present data on the FA status of free-ranging and captive black rhinoceros that allow the testing of the general assumption of a distinct difference, and that indicates potential for dietary intervention.

Animals, materials and methods

Serum/plasma samples from adult, free-ranging animals derived from field operations described previously (Clauss et al., 2002; Dierenfeld et al., 2005); twenty-eight animals from four different locations in Zimbabwe were sampled. These samples were analysed, together with samples from 11 adult animals from North American zoos and four additional samples from captive white rhinoceros (*Ceratotherium simum*), at the Texas A & M University, as reported in Bauer et al. (2000). For the analyses, the method as outlined by Bauer et al. (1997) was used, assessing the lipid fractions of the triglycerides (TG), PL and cholesterol esters (CE). The zoo animals had been fed their usual (unknown) zoo diet without any particular diet change prior to the sampling procedure.

Plasma samples in Europe were taken from six adult animals from three zoological institutions (facility 1–3). These animals were tested on one to four different diets, resulting in a total of 15 blood samples. The diet that these animals were exposed to had been kept constant for approximately 3 months prior to blood sampling. As described earlier (Clauss et al., 2006a, 2007a) including a detailed description of the diets used), the food intake was quantified during a 1-week interval close to the

sampling date, by weighing all food items offered and leftovers. Representative samples of all food items were taken and analysed for dry matter (DM) content by drying at 103 °C to constant weight. Blood was taken from the *Vena radialis* on the medial aspect of the carpal joint in trained animals as described by Hatt et al. (2001). Treatment of blood samples [separation into plasma TG, PL and CE as well as and red blood cell phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) fractions] as well as FA analysis was performed at the Institute of Brain Chemistry and Human Nutrition of the University of North London, following the protocol used in Clauss et al. (2003b).

The data are presented as mean values of the respective subpopulations. Before calculating the mean of a subpopulation, the mean values for an individual were calculated in the case of repeated measurements in an individual. Only for the correlation with the actually ingested diet, all measurements from all European animals were used. The unsaturation index (UI, also called double bond index) as a measure of the number of unsaturated (double) bonds per total bonds was calculated according to Pond et al. (1992). Data on food intake and DM content of food items were used to calculate the proportion of grain-based products (including plain grains, bread and commercial concentrates) of the total DM intake (DMI).

Differences between two groups (free ranging vs. captive, US vs. Europe, black rhinoceros vs. white rhinoceros) were tested by exact *U*-test. Potential differences between the animals from the four different free-ranging populations were evaluated by the Kruskal–Wallis test (Monte Carlo approach) without following up regional differences by *post hoc* tests. Correlations between diet and FA status parameters as well as correlations between different FA status parameters were tested by Spearman's correlation coefficient. All analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA). The significance level was set to 0.05.

Results

The FA composition of the three lipid classes of the serum of free-ranging individuals is presented in Tables 1–3 (these data do not include measurements in one captive animal from Europe on a diet supplemented particularly with grass meal pellets). There were significant differences between the four regions, with animals from Matsudona and Midlands having higher proportions of ALA (indicated by asterix and p-value in the tables). The average values from the four regions from the wild are compared with data from captive animals in the same tables (with differences indicated by superscripts and p-values). In general, captive animals had significantly lower proportions of monounsaturated fatty acid (MUFA) and n3 PUFAs, higher proportions of total PUFAs, n6 PUFAs and in particular higher proportions of LA, and lower n3/n6 ratio in all lipid fractions (Tables 1–3); only in the PL fraction was the difference in total PUFAs not significant (Table 2). In TG, the difference in the UI was nearly significant at $p = 0.054$, with captive animals

Table 1 Fatty acid composition of serum/plasma triglycerides (in % of all fatty acids) in free-ranging (FR, from four different regions) and captive (C, from North America and Europe) black rhinoceros (*Diceros bicornis*) and captive white rhinoceros (WR, *Ceratotherium simum*) from North America

	n	16:0*	18:0*	SFA*	18:1 n9*	MUFA*	18:2 n6	20:4 n6	Total n6	18:3 n3*	22:5 n3	22:6 n3	Total n3*	PUFAs*	n3/n6	UI*
FR Chete	11	48.59	7.19	59.45	24.65	32.90	3.19	0.04	3.71	0.82	0.08	0.00	1.09	4.80	0.26a	0.44
FR Matsudona	5	41.91	7.33	54.66	27.76	35.82	3.17	0.04	3.36	3.29	0.00	0.00	3.45	6.81	1.08b	0.53
FR Midlands	8	32.99	9.24	49.04	26.02	33.72	4.05	0.04	4.53	6.27	0.14	0.17	7.93	12.47	1.81c	0.60
FR Hwange	4	41.58	10.06	59.32	20.46	25.97	2.69	0.08	5.83	1.79	0.51	0.00	2.65	8.48	0.46a	0.48
p-value		<0.001	0.008	0.004	0.007	0.003	0.069	0.603	0.198	<0.001	0.372	0.119	<0.001	0.001	<0.001	0.001
C Europe	6	35.64	11.56	50.71	15.03	19.02[a]	20.09	0.53[a]	22.92	1.35[a]	0.26	0.16	1.99[a]	24.91	0.11[a]	0.77
C USA	11	40.22	6.32	49.85	23.64	29.76[b]	17.18	0.06[b]	17.37	0.39[b]	0.00	0.00	0.40[b]	17.77	0.05[b]	0.66
p-value		0.445	0.101	1.000	0.073	0.014	0.445	0.005	0.181	0.014	0.366	0.138	0.005	0.138	0.035	0.445
FR	28	41.27	8.46	55.62	24.72[a]	32.10[a]	3.27[a]	0.05[a]	4.36[a]	3.04[a]	0.18	0.04	3.78[a]	8.14[a]	0.90[a]	0.54
C	13	38.05	8.74	50.20	19.69[b]	24.83[b]	18.52[b]	0.27[b]	19.91[b]	0.85[b]	0.12	0.08	1.15[b]	21.07[b]	0.08[b]	0.71
p-value		0.237	0.195	0.186	0.044	0.003	<0.001	0.031	<0.001	0.004	0.923	0.480	0.021	<0.001	<0.001	0.054
WR	4	43.28	5.46	53.87	22.91	29.22	11.61	0.00	12.60	0.99	0.00	0.00	0.99	13.59	0.06	0.59

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; UI, unsaturation index.

Data represent mean values based on measurements in *n* individuals.

Fatty acids marked by * differ significantly between individual FR regions; values in columns within individual blocks (Europe/USA, FR/C) with different superscript differ significantly (c.f. p-values).

Table 2 Fatty acid composition of serum/plasma phospholipids (in % of all fatty acids) in free-ranging (FR, from four different regions) and captive (C, from North America and Europe) black rhinoceros (*Diceros bicornis*) and captive white rhinoceros (WR, *Ceratotherium simum*) from North America

	n	16:0*	18:0	SFA	18:1 n9	MUFA	18:2 n6	20:4 n6*	Total n6	18:3 n3*	22:5 n3	22:6 n3*	Total n3*	PUFAs	n3/n6*	UI
FR Chete	11	16.33	29.33	52.55	10.21	16.66	17.12	4.54	23.85	1.08	0.16	0.00	1.63	25.48	0.08	0.81
FR Matsudona	5	15.01	30.07	49.97	11.98	16.97	19.49	3.16	23.48	2.85	0.09	0.28	4.01	27.49	0.17	0.86
FR Midlands	8	13.96	29.24	52.28	11.41	17.39	17.50	3.42	22.14	3.83	0.23	0.00	4.67	26.82	0.21	0.85
FR Hwange	4	13.24	26.90	50.46	11.13	18.85	17.19	3.52	22.77	2.17	0.51	0.00	3.05	25.82	0.14	0.84
p-value		0.019	0.271	0.109	0.375	0.345	0.200	0.006	0.622	<0.001	0.055	0.045	<0.001	0.686	0.001	0.596
C Europe	6	14.47^{a}	25.19	42.44^{a}	6.45	11.12^{a}	34.80^{a}	2.97^{a}	40.43^{a}	0.34	0.09	0.24	0.96	41.39^{a}	0.02	1.06^{a}
C USA	11	18.98^{b}	30.64	56.93^{b}	7.66	14.11^{b}	22.94^{b}	1.26^{b}	24.93^{b}	0.38	0.16	0.01	0.61	25.55^{b}	0.04	0.69^{b}
p-value		0.015	0.078	<0.001	0.591	0.048	0.001	0.002	<0.001	0.404	1.000	0.404	0.216	<0.001	0.884	*<0.001*
FR	28	14.64^{a}	28.89	51.31	11.18^{a}	17.47^{a}	17.82^{a}	3.66^{a}	23.06^{a}	2.48^{a}	0.25	0.07	3.34^{a}	26.40	0.15^{a}	0.84
C	17	17.38^{b}	28.74	51.83	7.24^{b}	13.06^{b}	27.10^{b}	1.86^{b}	30.37^{b}	0.37^{b}	0.14	0.09	0.74^{b}	31.10	0.03^{b}	0.82
p-value		0.013	0.371	0.586	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	0.208	0.308	<0.001	0.109	<0.001	0.972
WR	4	18.91	30.75	54.07	9.54	14.49	27.53	1.31	29.90	0.23	0.00	0.00	0.23	30.13	0.01	0.78

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; UI, unsaturation index.
Data represent mean values based on measurements in *n* individuals.
Fatty acids marked by * differ significantly between individual FR regions; values in columns within individual blocks (Europe/USA, FR/C) with different superscript differ significantly (c.f. p-values).

Table 3 Fatty acid composition of serum/plasma cholesterol esters (in % of all fatty acids) in free-ranging (FR, from four different regions) and captive (C, from North America and Europe) black rhinoceros (*Diceros bicornis*) and captive white rhinoceros (WR, *Ceratotherium simum*) from North America

	n	16:0*	18:0*	SFA*	18:1 n9	MUFA	18:2 n6	20:4 n6	Total n6	18:3 n3*	22:5 n3	22:6 n3	Total n3*	PUFAs	n3/n6*	UI*
FR Chete	11	12.09	4.39	17.66	15.08	19.23	53.13	1.51	54.89	3.85	0.00	0.03	4.10	58.99	0.07	1.45
FR Matsudona	5	8.52	1.47	11.20	17.06	21.73	50.31	1.12	51.65	7.80	0.00	0.00	7.92	59.56	0.15	1.51
FR Midlands	8	8.00	1.62	10.91	14.60	19.53	52.56	1.30	54.17	11.95	0.00	0.00	12.03	66.20	0.22	1.67
FR Hwange	4	7.54	1.80	10.64	15.61	19.33	50.66	1.18	52.18	7.45	0.00	0.00	7.52	59.70	0.14	1.49
p-value		0.002	0.020	0.007	0.684	0.750	0.825	0.312	0.779	<0.001	1.000	1.000	<0.001	0.190	<0.001	0.015
C Europe	6	8.22	1.56	10.25^{a}	5.02^{a}	6.75^{a}	79.07^{a}	0.93	80.05^{a}	0.76	0.00	0.00	0.76	80.81^{a}	0.01	1.71^{a}
C USA	11	11.24	1.72	14.06^{b}	9.51^{b}	11.92^{b}	68.70^{b}	0.60	69.53^{b}	0.83	0.00	0.00	0.99	70.52^{b}	0.02	1.56^{b}
p-value		0.122	0.122	0.020	0.003	0.003	0.003	0.180	0.005	1.000	1.000	1.000	0.808	0.003	0.591	0.005
FR	28	9.04	2.32	12.60	15.59^{a}	19.96^{a}	51.67^{a}	1.28^{a}	53.22^{a}	7.76^{a}	0.00	0.01	7.89^{a}	61.11	0.15^{a}	1.53
C	17	10.18	1.67	12.72	7.93^{b}	10.10^{b}	72.35^{b}	0.72^{b}	73.23^{b}	0.81^{b}	0.00	0.00	0.91^{b}	74.14	0.01^{b}	1.61
p-value		0.554	0.164	0.991	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	1.000	1.000	<0.001	<0.001	<0.001	0.269
WR	4	10.92	1.54	13.61	9.54	12.26	67.33	0.76	68.32	0.68	0.00	0.00	0.94	69.25	0.01	1.54

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; UI, unsaturation index.
Data represent mean values based on measurements in *n* individuals.
Fatty acids marked by * differ significantly between individual FR regions; values in columns within individual blocks (Europe/USA, FR/C) with different superscript differ significantly (c.f. p-values).

displaying higher values (Table 1). The average values of the European and the US animals are also compared in the tables (with differences indicated by superscripts and p-values). Compared to captive European animals, captive animals held in the US generally had a significantly lower n3/n6 ratio in the TG serum fraction (Table 1), but this difference was not evident in the PL (Table 2) and CE (Table 3) serum fractions. There were no significant differences in the FA proportions of captive black and white rhinoceros in the US.

There were noticeable differences in the proportions of the different food categories in the diets ingested at the different European facilities. The proportion of grain-based products of the ingested diet ranged from 39% to 60% DM (Fig. 1) in the diets offered at facilities 2 and 3. At facility 1, grain-based products only represented 17% of total DMI; however, at this facility, sunflower oil was mixed into the diet at 2% of total DMI. In facility 1, the roughage:non-roughage ratio of the diet as offered was 77:23 vs. 76:24 in the ingested diet. This ratio was

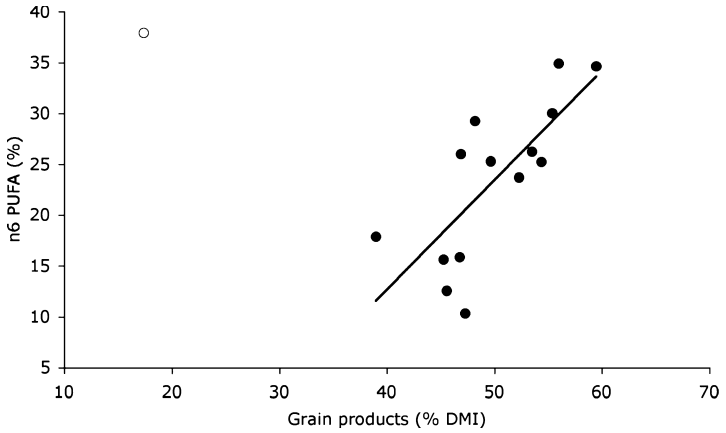

Fig. 1 Correlation between the proportion of grain products [grain-based pelleted compound feeds, plain grains, bread; in % of total dry matter intake (DMI)] and the proportion of n6 polyunsaturated fatty acids (n6 PUFAs, in % of all fatty acids) in the triglyceride fraction of serum lipids in captive black rhinoceroses (*Diceros bicornis*) (Spearman's correlation coefficient = 0.78; p = 0.001). The outlier (open symbol, not included in the correlation) is an animal that received sunflower oil at 2% of its DMI.

50:50 in the offered diet vs. 40:60 in the actually consumed diet in facility 2. At facility 3, this ratio varied widely between animals, ranging from 60:40 to 46:54 in the offered and 48:52 to 30:70 in the ingested diet.

The FA composition of the red blood cells of the European animals is shown in Table 4 (these data do not include measurements in one animal on a diet supplemented particularly with grass meal pellets). If the animal from facility 1 (sunflower oil feeding) was excluded, there was a significant correlation between the proportion of grain products in the total diet and the proportion of n6 PUFAs in TG (p = 0.001; Fig. 1); this correlation was also significant for the PL (p = 0.011) but not for the CE fraction (p = 0.27). This correlation approached significance for the red blood cell PE fraction (p = 0.072) but not for the red blood cell PC fraction (p = 0.748). Among the different lipid fractions, there were significant correlations in the proportion of n6 PUFAs between serum TG and red blood cell PE (p = 0.005) and between serum PL and serum CE (p = 0.001), but not between any other fractions.

Due to an oral abscess that impeded roughage mastication, one animal at facility 3 was changed from a roughage/concentrate diet to a diet consisting of concentrates and grass meal pellets (Hatt et al., 2004). As the proportion of grass meal pellets in the diet increased and decreased, the proportion of ALA in the different lipid fractions varied accordingly, with the highest proportions on the highest level of grass meal intake (36% of total dietary DM) (Fig. 2).

Discussion

The results of this study confirm the initial hypothesis that captive black rhinoceros differ systematically in their FA status from their free-ranging conspecifics. Although differences exist among the respective subpopulations of the free-ranging and the captive population sampled in this study (among the different African regions; between North American and European zoos), these differences are small compared to the overriding overall difference between the free-ranging and the captive population. Differences according to age, sex or body condition

Table 4 Fatty acid composition of red blood cell phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) fractions (in % of all fatty acids) in captive European black rhinoceros (*Diceros bicornis*) from three different facilities

Facility	*n*	16:0	18:0	SFA	18:1 n9	MUFA	18:2 n6	20:4 n6	Total n6	18:3 n3	22:5 n3	22:6 n3	Total n3	PUFAs	n3/n6	UI
PC																
1	1	20.31	20.38	41.21	29.38	35.27	14.62	1.16	18.21	0.00	0.00	0.00	0.00	18.21	0.00	0.79
2	2	27.64	27.85	56.35	27.19	31.28	5.33	0.43	7.79	0.00	0.00	0.00	0.05	7.85	0.01	0.54
3	3	15.11	19.26	34.83	33.28	37.61	19.11	1.60	22.66	0.04	0.00	0.00	0.30	22.96	0.01	0.90
PE																
1	1	7.59	14.84	22.76	48.16	56.70	10.26	1.13	12.72	0.20	0.00	0.00	0.20	12.92	0.02	0.85
2	2	13.09	29.68	47.35	27.94	37.81	2.01	1.90	8.37	0.00	0.00	0.00	0.52	8.89	0.04	0.67
3	3	2.94	11.24	14.22	62.37	71.20	7.56	0.69	9.99	0.00	0.00	0.00	0.15	10.14	0.02	0.94

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; UI, unsaturation index.
Data represent mean values based on measurements in *n* individuals.

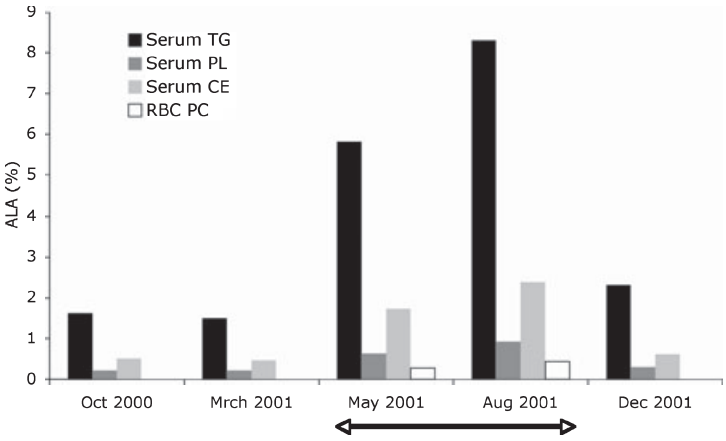

Fig. 2 Proportion of alpha-linolenic acid (ALA, in % of all fatty acids) in plasma triglycerides (TG), phospholipids (PL), cholesteryl esters (CE), and red blood cell phosphatidyl choline (RBC PC) of one individual captive black rhinoceros (*Diceros bicornis*) fed a standard zoo diet which was supplemented for a certain time period (indicated by arrows) with grass meal pellets at 33–36% of total DMI.

between the populations studied were not evaluated; whether discrepancies in these parameters between the different populations might have influenced our results remains debatable.

Diets fed to black rhinoceros in captivity contain, due to the use of grain products, higher levels of n6 PUFAs (mostly LA). The differences in FA composition of the diet of free-ranging and captive black rhinoceros is displayed in Table 5. It should be noted that, due to the perishable nature of PUFAs in stored samples (Ghebremeskel et al., 1991b; Grant et al., 2002), time elapsing between sampling and analysis of the collected material will introduce a bias in the final result; in particular, ALA appears to be particularly affected by storage loss. Therefore, the figures outlined in Table 5 serve as illustration of a general difference, not as exact data on individual browse plants. Nevertheless, these data reflect the FA pattern that can be observed in the serum of free-ranging and captive rhinoceros specimens.

Table 5 Mean proportions of linoleic acid (n6 LA) and alpha-linolenic acid (n3 ALA) in % of all fatty acids in diet items of free-ranging and captive black rhinoceros (*Diceros bicornis*) from different sources

	n	n6 LA	n3 ALA	n6/n3	Source
African	14	8	74*	0.11	(Grant et al., 2002)
browse	10	17	26†	0.65	(Ghebremeskel et al., 1991b)
	3‡	12	51†	0.25	(Ndondo et al., 2004)
Temperate	9	10	64§	0.16	(Grant et al., 2002)
browse	1	13	46†	0.28	(Ghebremeskel et al., 1991b)
Hay¶	31	14	25	0.56	(Grant et al., 2002)
Grain pellets¶	26	47	9	5.22	(Grant et al., 2002)
Zoo diet**	36	22	18	1.22	(Grant et al., 2002)

*Assuming a loss of 70% of original ALA due to storage as determined by these authors.
†Without correction for potential losses.
‡Samples from winter season.
§Analysed immediately after sampling.
¶Sampled as used in zoos.
**Averaging 61% hay and 28% grain pellets.

In addition to the well-documented fact that the ingested diet is the major determinant of the FA pattern found in body tissues (Hulbert et al., 2005), the evident correlation with grain-based products in the European subsample of the captive population indicates that the general difference between free-ranging and captive black rhinoceros is most certainly caused by a deviation of the feeding regime in captivity from the dietary habits displayed by these animals in the wild. A general shift towards a lower n3/n6 ratio in captive animals, as documented in this study, would be expected on the basis of the difference in FA composition recorded for forages of free-ranging black rhinoceros and the diet these animals receive in North American zoos (Table 5), and is in accord with a general trend in captive wild mammalian herbivore species (Clauss et al., 2007b; Fig. 3).

However, the black rhinoceros of this study deviate from the general trend of not only a lower n3/n6 ratio but also a lower overall PUFA status in captive as compared to free-ranging wild mammalian herbivores (Fig. 4). Actually, the captive animals displayed proportions of PUFAs significantly higher in serum TG, and numerically higher in serum PL and CE, than the respective lipid fractions of the free-ranging individuals (Table 2–4). We believe that this is the first report of such an increase in PUFA status in captive as compared to free-ranging wild herbivores. This increase in captivity can be ascribed to the significantly higher porportions of n6 PUFAs in all serum/plasma lipid fractions (Table 2–4). Given the correlation outlined in Fig. 1, a high proportion of grain products in the captive feeding regime can be made responsible for this elevated n6 PUFA status.

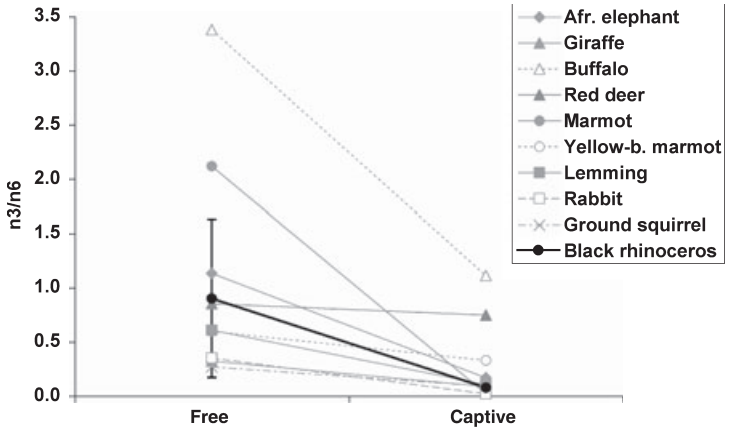

Fig. 3 Comparison of published data on the ratio of n3 vs. n6 polyunsaturated fatty acids (n3/n6) in different body tissues of free-ranging and captive wild animals (data collection from Clauss et al., 2007b) and the mean (±SD) of the plasma triglyceride fraction of black rhinoceros (*Diceros bicornis*) from this study. Note the general trend in a lesser n3/n6 ratio in captive mammalian herbivores.

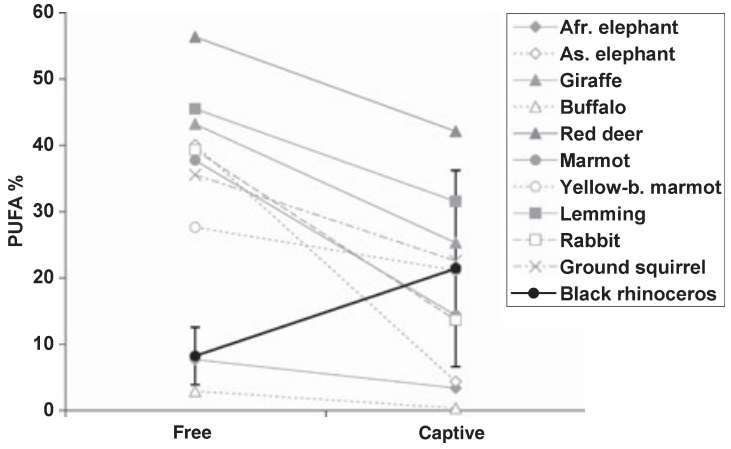

Fig. 4 Comparison of published data on the proportion of polyunsaturated fatty acids (PUFAs, in % of all fatty acids) in different body tissues of free-ranging and captive wild animals (data collection from Clauss et al., 2007b) and the mean (±SD) plasma triglyceride fraction of black rhinoceros (*Diceros bicornis*) from this study. Note that the black rhinoceros does not follow the general trend of lower PUFA proportions in captive mammalian herbivores, due to excessive linoleic acid in zoo diets.

Whether this finding has clinical relevance for the captive black rhinoceros population can only be speculated upon. Captive black rhinoceros are susceptible to several uncommon diseases that have been suspected to be linked to oxidative damage, possibly due to the disposition of this species to excessive iron storage (Paglia and Dennis, 1999). An unnatural dietary loading with unsaturated FA (which is a prime target for oxidants) would exacerbate this problem. As, for example, it has been shown in laboratory animals that the susceptibility of red blood cells to *in vitro* haemolysis is increased by excessive dietary PUFA intake (Bieri and Poukka, 1970), it is tempting to speculate that the idiopathic haemolytic anaemia observed in black rhinoceros (Miller and Boever, 1982; Chaplin et al., 1986) could, at least in part, be contributed to a loading of red blood cells with unnaturally high proportions of PUFAs. Similarly, Ghebremeskel et al. (1991a) reported a case of a captive marmoset (*Callithrix jacchus*) population, whose proneness to *in vitro* red blood cell haemolysis and skin problems could be ameliorated when the unnaturally high dietary PUFA supplementation (due to the use of fish products in the compound feed) was reduced. The potential for oxidative damage of PUFAs is reflected in an increased requirement for antioxidants such as vitamin E with increasing PUFA ingestion (Valk and Hornstra, 2000). After a first report that captive black rhinoceros might have lower circulating vitamin E levels than free-ranging individuals (Dierenfeld et al., 1988), increased awareness of this problem triggered a supplementation regime in North America, and more recently assessed circulating vitamin E levels did not differ significantly between free-ranging and captive North American specimens (Clauss et al., 2002). Given a higher PUFA status in captive than in free-ranging individuals, circulating vitamin E levels of a similar scope as those of free-ranging animals might not be enough to prevent lipid oxidation in captive ones, but this assumption remains to be proven. Other potential antioxidants would include carotenoids and polyphenols or tannins. As tannins are present in the diet of free-ranging browsers,

including the black rhinoceros (Clauss et al., 2007a) but are mostly absent in diets fed in captivity, it has been speculated that captive browsing species might be particularly compromised in terms of their antioxidative supply (Clauss, 2003). The few existing studies on the effect of tannin supplementation on the antioxidant status of captive wildlife (Clauss et al., 2003a, 2006b) seem to support this view, but more evidence is needed before conclusions on the importance of polyphenolic antioxidants in wild animals can be made.

In addition to the problem of a higher PUFA status, the n3/n6 ratio is of concern. n6 FA are known as precursors of pro-inflammatory mediators, and their overrepresentation could therefore exacerbate any inflammatory processes. With particular respect to the dermopathies observed in black rhinoceros, Grant et al. (2002) collated publications on the treatment of skin conditions with essential FAs but had to conclude that to date, no undebated dietary protocol can be derived from the literature.

The observed difference in FA status between black rhinoceros from North American and European zoos, with numerically higher proportions of n6 PUFAs in the TG and significantly higher n6 PUFAs in the PL and CE fractions in Europe, could be explained by the difference in diet composition suggested by the rations fed at European (with a roughage:non-roughage ratios of 46:54 to 77:23) and the North American facilities (with a roughage:nonroughage ratio of 66:34; Grant et al., 2002), with higher non-roughage proportions fed in Europe. Nevertheless, the fact that even the North American animals have a distinctively higher n6 PUFA and overall PUFA but lower n3 PUFA status indicates that even these diets do not mimick the natural forage in its FA composition. The results on the food intake of the European animals monitored during this study indicate that in designing zoo diets, the proportion of the offered food that is actually consumed must always be taken into account; just as the diets offered at European facilities 2 and 3 differed from the diets actually ingested by the animals, the offered diets recorded by Grant et al. (2002) might actually overestimate the proportion of roughage ingested by the North American animals. The feeding regime of European facility 1 could serve as a positive example here, where non-roughage items were fed in such a restricted way that the offered roughage was ingested nearly completely. In contrast, the feeding regime of the other European facilities evidently offered non-roughage items in amounts that precluded the intake of the intended roughage proportion by the animals.

The lack of difference in FA status between captive black and white rhinoceros from North America can be explained by the observation that both species generally receive similar diets in this part of the world. In contrast, studies on European black, white and Indian rhinoceros (*Rhinoceros unicornis*) suggest that among the animals investigated in Europe, black rhinoceros ingest a diet particularly high in non-roughage (i.e. mostly grain-based) items (Kiefer, 2002; Clauss et al., 2005, 2006a).

At present, it cannot be decided whether a change of the zoo feeding regime that would lead to a FA status similar to that of free-ranging animals would actually be beneficial for captive animals; however, given the general assumption that animals have adapted during evolution to a particular set of nutrients – an assumption pronounced especially in connection with FAs (Eaton et al., 1997, 1998; Broadhurst et al., 1998; Simopoulos, 1998; Crawford et al., 2001) –, such a measure would appear reasonable. The scope of the observed difference, – n6/n3 ratios for TG, PL and CE were 1.6, 10 and 8 in free-ranging animals, respectively, as compared to 4.1–16.3, 16–148 and 40–277 in captive animals – could in itself be considered an incentive to change zoo diets. Clauss et al. (2007b) reviewed dietary measures to that effect: a reduction in the use of conventional, grain-based pelleted feeds, grains, and bread, and a corresponding increase in the proportion of forages – either dried (hays), ensilaged or fresh – has been shown to effectively enhance the n3/n6 FA ratio in numerous studies in domestic animals. Compared to grain-based pelleted feeds, pelleted feeds based on forage meals (grass or alfalfa/lucerne meal pellets) also increase the n3/n6 FA ratio in domestic ruminants (Daniel et al., 2004). These findings are reflected in the repeated measurements of one black rhinoceros of this study that received grass meal pellets as a significant component of its diet during a certain time period (Fig. 2). However, due to the high iron content that is often observed in grass meal pellets (Clauss, 2003; Hatt et al., 2004), such diet items should be chosen with particular care for a species known to be susceptible to excessive iron storage, like the black rhinoceros (Paglia and Dennis, 1999; Dierenfeld et al., 2005).

Flaxseed/linseed products are another source of ALA; an inclusion of linseed into pelleted feeds will also enhance the n3/n6 ratio in domestic ruminants (Raes et al., 2004); and linseed product supplementation has been documented to increase the n3 PUFA status in three out of six captive black rhinoceroses (Suedmeyer and Dierenfeld, 1998) and in giraffe

(*Giraffa camelopardalis*) (Clauss et al., 2000). Nevertheless, it may be debatable whether the n3/n6 ratio of a grain-based product or diet should be adjusted by adding an extra source of n3 PUFAs. Such an addition will increase the overall PUFA proportion, and hence the general susceptibility to oxidative damage, even more. In a feeding trial with domestic horses, the addition of flaxseed oil to a control diet led to an increased lipid peroxidation, measured as whole blood malondialdehyde, in the treatment as compared to the control group (Hansen et al., 2002). Additionally, the authors of that study pointed out that flaxseed/linseed oil also contains relevant proportions of LA; in their study, the n3/n6 ratio of an alfalfa-based pelleted diet was not altered by the addition of flaxseed oil, indicating that a forage-based diet will not be improved by such an addition in terms of its FA pattern. Of the six black rhinoceros in which the serum FA status was assessed before and after the feeding of a flaxseed supplement by Suedmeyer and Dierenfeld (1998), three animals also had elevated proportions of LA. Therefore, a general reduction or exclusion of the grain-based ingredients appears to be a more rational approach than the quest for an additional supplement. Although a reduction of grain-based ingredients alone will result in an increased n3/n6 ratio, studies performed with horses so far have only assessed the consequence of an additional n3 PUFA supplementation (Morris et al., 1989, 1991; Henry et al., 1990, 1991; O'Neill et al., 2002), mostly in the form of a linseed product. Unless the control group is given a comparable load of n6 PUFAs, and the additional PUFA load is balanced by a corresponding increase in antioxidants, results of such studies will remain difficult to interpret.

In humans, clinically relevant physiological effects of an alteration of the FA pattern are usually assessed by controlled, double blind studies that require a large sample size (e. g. GISSI, 1999). Evidently, such studies are unlikely to occur in zoo animals. Reports such as the spontaneous healing of a chronic skin problem after the introduction of linseed extraction chips in a giraffe (Clauss et al., 2000) or the absence of clinical problems in a black rhinoceros after dietary supplementation with a flaxseed product (Suedmeyer and Dierenfeld, 1998) must remain anecdotal. Changes in dietary FA composition are most likely to be reflected quickly in the serum/plasma TG fraction, which also showed a good correlation to diet composition. However, although the CE fraction of captive animals was significantly different from that of free-ranging animals, no correlation to the diet fed for approximately 3 months prior to blood sampling could be demonstrated in this study; this discrepancy could be explained by a slower responsiveness of this lipid fraction to FA changes that may be linked to fatty acyl specificity of circulating lecithin cholesterol acyl transferase activity. These findings underline that clinical benefits of dietary FA manipulation can most likely not be expected in short-term studies. If the concept of adjusting diets in captivity to the characteristics of diets in the wild is not considered convincing as such, long-term studies with some model animal species appear warranted.

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References

Bauer, J. E.; McAllister, K. G.; Rawlings, J. M.; Markwell, P., 1997: Molecular species of cholesteryl esters formed via plasma lecithin: cholesterol acyltransferase in fish oil supplemented dogs. *Nutrition Research* **17**, 861–872.

Bauer, J. E.; Bigley, K. E.; Dierenfeld, E. S., 2000: Fatty acid metabolism in free-ranging and captive rhinoceros: evidence for low delta-6 desaturase activities. *Journal of the American College of Veterinary Internal Medicine* **14**, 388 (abstract).

Bieri, J. G.; Poukka, K. H., 1970: In vitro hemolysis as related to rat erythrocyte content of alpha-tocopherol and polyunsaturated fatty acids. *Journal of Nutrition* **100**, 557–564.

Broadhurst, C. L.; Cunnane, S. C.; Crawford, M. A., 1998: Rift valley lake fish and shellfish provided brain-specific nutrition for early homo. *British Journal of Nutrition* **79**, 3–21.

Chaplin, H.; Malecek, A. C.; Miller, R. E.; Bell, C. E.; Gray, L. S.; Hunter, V. L., 1986: Acute intravascular hemolytic anemia in the black rhinoceros: hematologic and immunohematologic observations. *American Journal of Vetarinary Research* **47**, 1313–1320.

Clauss, M., 2003: Tannins in the nutrition of captive wild animals. In: A. Fidgett, M. Clauss, U. Ganslosser, J. M. Hatt, J. Nijboer (eds), *Zoo Animal Nutrition*, **Vol. 2**. Filander, Fuerth, Germany, pp. 53–89.

Clauss, M.; Flach, E.; Ghebremeskel, K.; Tack, C.; Hatt, J., 2000: Supplementing the diet of captive giraffe (*Giraffa camelopardalis*) with linseed extraction chips. In: J. Nijboer, J. Hatt, W. Kaumanns, A. Beijnen, U. Ganslosser (eds), *Zoo Animal Nutrition*. Filander, Fürth, Germany, pp. 271–279.

Clauss, M.; Jessup, D. A.; Norkus, E. B.; Holick, M. F.; Streich, W. J.; Dierenfeld, E. S., 2002: Fat soluble vitamins in blood and tissues of free-ranging and captive rhinoceros species. *Journal of Wildlife Diseases* **38**, 402–413.

Clauss, M.; Lason, K.; Gehrke, J.; Lechner-Doll, M.; Fickel, J.; Grune, T.; Streich, W. J., 2003a: Captive roe deer (*Capreolus capreolus*) select for low amounts of tannic acid but not quebracho: fluctuation of preferences and potential benefits. *Comparative Biochemistry and Physiology B* **136**, 369–382.

Clauss, M.; Wang, Y.; Ghebremeskel, K.; Lendl, C. E.; Streich, W. J., 2003b: Plasma and erythrocyte fatty acids in captive Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *Veterinary Record* **153**, 54–58.

Clauss, M.; Polster, C.; Kienzle, E.; Wiesner, H.; Baumgartner, K.; von Houwald, F.; Ortmann, S.; Streich, W. J.; Dierenfeld, E. S., 2005: Studies on digestive physiology and feed digestibilities in captive Indian rhinoceros (*Rhinoceros unicornis*). *Journal of Animal Physiology and Animal Nutrition* **89**, 229–237.

Clauss, M.; Castell, J. C.; Kienzle, E.; Dierenfeld, E. S.; Flach, E. J.; Behlert, O.; Ortmann, S.; Streich, W. J.; Hummel, J.; Hatt, J. M., 2006a: Digestion coefficients achieved by the black rhinoceros (*Diceros bicornis*), a large browsing hindgut fermenter. *Journal of Animal Physiology and Animal Nutrition* **90**, 325–334.

Clauss, M.; Pellegrini, N.; Castell, J. C.; Kienzle, E.; Dierenfeld, E. S.; Hummel, J.; Flach, E. J.; Streich, W. J.; Hatt, J. M., 2006b: Antioxidant status of feces of captive black rhinoceros (*Diceros bicornis*) in relation to dietary tannin supplementation. *Journal of Veterinary Medicine Series A* **53**, 319–322.

Clauss, M.; Castell, J. C.; Kienzle, E.; Dierenfeld, E. S.; Flach, E. J.; Behlert, O.; Ortmann, S.; Streich, W. J.; Hummel, J.; Hatt, J. M., 2007a: The influence of tannin supplementation on digestive performance in captive black rhinoceros (*Diceros bicornis*). *Journal of Animal Physiology and Animal Nutrition*. doi: 10.1111/j.1439-0396.2006.00673.x.

Clauss, M.; Grum, C.; Hatt, J. M., 2007b: Fatty acid status of captive wild animals: a review. *Der Zoologische Garten NF* **76**, 382–401.

Crawford, M., 1968: Fatty-acid ratios in free-living and domestic animals. *Lancet* **i**, 1329–1333.

Crawford, M. A.; Budowski, P.; Drury, P.; Ghebremeskel, K.; Harbige, L.; Leighfield, M.; Phylactos, A.; Williams, G., 1991: The nutritional contribution to bovine spongiform encephalopathy. *Nutrition and Health* **7**, 61–68.

Crawford, M. A.; Bloom, M.; Cunnane, S.; Holmsen, H.; Ghebremeskel, K.; Parkington, J.; Schmidt, W.; Sinclair, A. J.; Broadhurst, C. L., 2001: Docosahexaenoic acid and cerebral evolution. *World Review of Nutrition and Dietetics* **88**, 6–17.

Daniel, Z. C. T. R.; Wynn, R. J.; Salter, A. M.; Buttery, P. J., 2004: Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissue: the role of stearoyl-CoA desaturase. *Journal of Animal Science* **82**, 747–758.

Dierenfeld, E. S.; Frank, C. L., 1998: Fatty acid composition of white adipose tissue in captive rhinoceros. *Proceedings of the American Association of Zoo- and Wildlife Veterinarians*, pp. 508–509.

Dierenfeld, E. S.; du Toit, R.; Miller, R. E., 1988: Vitamin E in captive and wild black rhinoceros (*Diceros bicornis*). *Journal of Wildlife Diseases* **24**, 547–550.

Dierenfeld, E. S.; Atkinson, S.; Craig, M.; Walker, K. C.; Streich, W. J.; Clauss, M., 2005: Mineral concentrations in serum/plasma and liver tissue of captive and free-ranging rhinoceros species. *Zoo Biology* **24**, 51–72.

Eaton, S. B.; Eaton, S. B. I.; Konner, M. J., 1997: Paleolithic nutrition revisited: a twelve-year retrospective on its nature and implications. *European Journal of Clinical Nutrition* **51**, 207–216.

Eaton, S. B.; Eaton, S. B. I.; Sinclair, A. J.; Cordain, L.; Mann, N. J., 1998: Dietary intake of long-chain polyunsaturated fatty acids in the paleolithic. *World Reviews of Nutrition and Diet* **83**, 12–23.

Ghebremeskel, K.; Harbige, L. S.; Williams, G.; Crawford, M. A.; Hawkey, C., 1991a: The effect of dietary change on in vitro erythrocyte haemolysis, skin lesions and alopecia in common marmosets (*Callithrix jacchus*). *Comparative Biochemistry and Physiology A* **100**, 891–896.

Ghebremeskel, K.; Williams, G.; Brett, R. A.; Burek, R.; Harbige, L. S., 1991b: Nutrient composition of plants most favoured by black rhinoceros (*Diceros bicornis*) in the wild. *Comparative Biochemistry and Physiology A* **98**, 529–534.

GISSI, 1999: Dietary supplementation with n3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* **354**, 447–455.

Grant, J. B.; Brown, D. L.; Dierenfeld, E. S., 2002: Essential fatty acid profiles differ across diets and browse of black rhinoceros. *Journal of Wildlife Diseases* **38**, 132–142.

Hansen, R. A.; Savage, C. J.; Reidlinger, K.; Traub-Dargatz, J. L.; Ogilvie, G. K.; Mitchell, D.; Fettman, M. J., 2002: Effects of dietary flaxseed oil supplementation on

equine plasma fatty acid concentrations and whole blood platelet aggregation. *Journal of Veterinary Internal Medicine* **16**, 457–463.

Hatt, J. M.; Casares, M.; Wenker, C.; Clauss, M. 2001: *Blutgewinnung beim Spitzmaulnashorn (Diceros bicornis)*. Arbeitstagung der Zootierärzte im deutschsprachigen Raum Halle, Germany, 126–130.

Hatt, J. M.; Wenker, C.; Castell, J.; Clauss, M., 2004: Dietary and veterinary management of a lingual abscess in a geriatric captive black rhino (Diceros bicornis) with iron storage disease. *Proceedings of the European Association of Zoo- and Wildlife Veterinarians* **5**, 339–340.

Henry, M. M.; Moore, J. N.; Feldman, E. B., 1990: Effect of dietary alpha-linolenic acid on equine monocyte procoagulant activity and eicosanoid synthesis. *Circulation and Shock* **32**, 173–188.

Henry, M. M.; Moore, J. N.; Fischer, J. K., 1991: Influence of an n-3 fatty acid-enriched ration on in vivo responses of horses to endotoxin. *American Journal of Veterinary Research* **52**, 523–527.

Hulbert, A.; Turner, N.; Storlien, L. H.; Else, P. L., 2005: Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews* **80**, 155–169.

Kiefer, B., 2002: *Qualität und Verdaulichkeit der vom Breitmaulnashorn aufgenommenen Nahrung*, Dissertation. LMU München, München.

Miller, R. E.; Boever, W. J., 1982: Fatal hemolytic anemia in the black rhinoceros: case report and a survey. *Journal of the American Veterinary Medical Association* **181**, 1228–1231.

Morris, D. D.; Henry, M. M.; Moore, J. N.; Fischer, J. K., 1989: Effect of dietary linolenic acid on endotoxin-induced thromboxane and prostacyclin production by equine peritoneal macrophages. *Circulation and Shock* **29**, 311–318.

Morris, D. D.; Henry, M. M.; Moore, J. N.; Fischer, J. K., 1991: Effect of dietary alpha-linolenic acid on endotoxin-induced production of tumor necrosis factor by peritoneal macrophages in horses. *American Journal of Veterinary Research* **52**, 528–532.

Munson, L.; Koehler, J. W.; Wilkinson, J. E.; Miller, R. E., 1998: Vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis in captive black rhinoceroses (*Diceros bicornis*). *Veterinary Pathology* **35**, 31–42.

Ndondo, I. B.; Wilhelmi, B. S.; Mabinya, L. V.; Brand, J. M., 2004: Alpha-tocopherol and fatty acids of major browse plant species of black rhinoceros in the Great Fish River Reserve. *South African Journal of Wildlife Research* **34**, 87–93.

O'Neill, W.; McKee, S.; Clarke, A. F., 2002: Flaxseed (*Linum usitatissimum*) supplementation associated with reduced skin test lesional area in horses with *Culicoides* hypersensitivity. *Canadian Journal of Veterinary Research* **66**, 272–277.

Paglia, D. E.; Dennis, P., 1999: Role of chronic iron overload in mutliple disorders of captive black rhinoceroses (*Diceros bicornis*). *Proceedings of the American Association of Zoo Veterinarians*, pp. 163–170.

Pond, C. M.; Mattacks, C. A.; Colby, R. H.; Ramsey, M. A., 1992: The anatomy, chemical composition, and metabolism of adipose tissue in wild polar bears (*Ursus maritimus*). *Canadian Journal of Zoology* **70**, 326–341.

Raes, K.; Fievez, V.; Chow, T. T.; Ansorena, D.; Demeyer, D.; Smet, S. D., 2004: Effects of diet and dietary fatty acids on the transformation and incorporation of C18 fatty acids in double-muscled Belgian Blue young bulls. *Journal of Agricultural Food Chemistry* **52**, 6035–6041.

Simopoulos, A. P., 1998: Overview of evolutionary aspects of n3 fatty acids in the diet. *World Reviews of Nutrition and Diet* **83**, 1–11.

Suedmeyer, W. K.; Dierenfeld, E. S., 1998: Clinical experience with fatty acid supplementation in a group of black rhinoceros (*Diceros bicornis*). *Proceedings of the American Association of Zoo Veterinarians*, pp. 113–115.

Valk, E. E.; Hornstra, G., 2000: Relationship between vitamin E requirement and polyunsaturated fatty acid intake in man: a review. *International Journal for Vitamin and Nutrition Research* **70**, 31–42.