

ORIGINAL ARTICLE

The influence of dietary tannin supplementation on digestive performance in captive black rhinoceros (*Diceros bicornis*)

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Summary

Free-ranging browsers such as the black rhinoceros (*Diceros bicornis*) consume a diet that contains tannins, whereas the diets offered to them in captivity consist mostly of items known to contain hardly any such secondary plant compounds. Tannins could have potentially beneficial effects, including the chelation of dietary iron (iron storage disease is a common problem in black rhinos). Here, we tested the acceptance, and the consequences on digestion variables, of a low-dose tannin supplementation in captive animals. Eight black rhinoceroses from three zoological institutions were used. Faecal output was quantified by total faecal collection. Diets fed were regular zoo diets supplemented with either tannic acid (T, hydrolysable tannin) or quebracho (Q, condensed tannins); overall tannin source intake increased at 5–15 g/kg dry matter (DM) in relation to regular zoo diets. Adaptation periods to the new diets were >2 months. Additional data were taken from one hitherto unpublished study. Data were compared to measurements in the same animals on their regular zoo diets. All animals accepted the new diets without hesitation. There was no influence of tannin supplementation on digestion coefficients of DM and its constituents, or faecal concentrations of short-chain fatty acids or lactate. Water intake did not increase during tannin supplementation. Should the inclusion of dietary tannin sources be an objective in the development of diets for captive rhinoceroses, moderate doses such as used in this study are unlikely to cause relevant depressions of digestive efficiency and will not interfere with bacterial fermentation in a relevant way.

Introduction

Black rhinoceros (*Diceros bicornis*) are strict browsers (reviewed in Clauss et al., 2006b). As browse material – the foliage of dicots such as shrubs or trees – contains secondary plant compounds, in particular tannins (Van Soest, 1994), it can be expected that

free-ranging black rhinoceros consistently ingest a certain amount of these substances with their daily diet. Several authors have demonstrated the presence of tannins in natural forage consumed by free-ranging black rhinoceros (Loutit et al., 1987; Atkinson, 1995; Wright, 1998; Muya and Oguge, 2000). Furstenburg and van Hoven (1994) analysed

Table 1 Tannin content in plants consumed by free-ranging black rhinoceros (*Diceros bicornis*)

Analysis	Content (% dry matter)	Source
Total phenols	2.8–7.7	Muya and Ogue (2000)
Soluble tannins	0.2–4.1	Loutit <i>et al.</i> (1987)
Condensed tannins	0.2–5.7	Atkinson (1995)
Condensed tannins	0.2–18.2	Furstenburg and van Hoven (1994)

Assay techniques varied across studies.

African browse consumed by giraffe (*Giraffa camelopardalis*) for condensed tannin (CT) content; many plant species investigated in that study have also been reported to be eaten by the black rhinoceros (Joubert and Eloff, 1971; Ghebremeskel *et al.*, 1991; Kotze and Zacharias, 1993; Atkinson, 1995; Dierenfeld *et al.*, 1995), and invariably contained tannins. Quantified tannin levels in plants consumed by free-ranging black rhinoceros are summarized in Table 1. No selection against secondary plant compounds is evident in black rhinoceros (Atkinson, 1995). Muya and Ogue (2000) describe a negative correlation between total phenol content and the preference of black rhinoceros for the sample of forages they investigated, but the corresponding scatter plot is not compelling.

In contrast, the diet items usually fed to black rhinoceros in captivity – grass and legume hays, pelleted compound feeds – usually contain little or no tannins; only supplemental (temperate) browse will contain levels of secondary plant compounds similar to those found in native African forage. Using a semi-quantitative CT assay (scoring feeds from 0 to 3), Wright (1998) found average CT scores of 2.1 for native African browse consumed by black rhinoceros, 1.9 for temperate browse fed to the species in North American zoos, and an average of 0.2 for total diets fed in captivity. The question whether tannins should be considered important when designing diets and rations for captive browsing species is yet unresolved. Tannins have been traditionally regarded as feeding deterrents due to digestion inhibition or toxic effects; however, positive effects of tannins, such as anthelmintic, antibacterial or antioxidative effects have also been reported (reviewed in Clauss, 2003). As captive black rhinoceros often show signs of excessive iron storage at necropsy (Paglia and Dennis, 1999; Dierenfeld *et al.*, 2005), and tannins chelate iron (Gaffney *et al.*, 2004) but are absent in zoo diets (see above), it has been speculated that the deliberate inclusion of tannins in zoo diets could be beneficial for this species.

Therefore, a first set of feeding trials with captive black rhinoceroses was initiated in which the effect of tannin supplementation was evaluated. The results of these studies indicate that tannin supplementation can trigger the production of tannin-binding salivary proteins in black rhinoceros (Clauss *et al.*, 2005b), increase the faecal antioxidant status (Clauss *et al.*, 2006c) and reduce the number of faecal Enterobacteriaceae (M. Clauss *et al.*, unpubl. data). Here, we report the absence of an effect of this tannin supplementation on digestion parameters in the same animals. In particular, it was tested whether tannin-containing pellets were rejected by the animals, resulted in a lower overall diet intake as observed in many other wild animal species (reviewed in Clauss, 2003), affected both dry matter (DM) (Robbins *et al.*, 1987b) and crude protein (CP) (Robbins *et al.*, 1987a) digestion, or resulted in an increased water intake (Dearing *et al.*, 2001). Additionally, the effect of tannins on the faecal microflora (M. Clauss *et al.*, unpubl. data) raised the question whether parameters of bacterial fermentative activity were influenced by tannins.

Animals, materials and methods

The animals, feeding trial procedures and analytical methods have been outlined in Clauss *et al.* (2005c, 2006b). Eight black rhinoceroses from three zoological institutions were used. The animals were either weighed, or their body weights estimated using the weighed animals as a comparison (Table 2). Animals had regular access to outside enclosures that were cleared of any potential food items. For the trial period, the animals were kept separately to allow individual recording of food intake and faecal excretion. The diets used for these feeding trials were the regular diets used at the respective zoological institution (Clauss *et al.*, 2006b), with the important difference that either 5% tannic acid (a source of hydrolysable tannins, laboratory grade; Merck, Darmstadt, Germany) or 5% quebracho (a source of CTs; Tannin Corporation, Peabody, MA, USA; non-purified, estimated CT content 75% given by Robbins *et al.*, 1991) was included in the pelleted diet compound. This resulted in an additional tannin source intake of 5–15 g/kg DM (Clauss *et al.*, 2005b). The ingredient and chemical composition of the diets ingested by the animals are recorded in Table 3; mainly due to different *ad libitum* intake of roughage during the different dietary periods, diet composition was not completely identical between treatments. Adaptation periods to the new diets lasted more than 2 months.

Table 2 Black rhinoceroses (*Diceros bicornis*) used for digestion trials; animal numbers correspond to those in Clauss et al. (2006a,b)

Animal no.	Studbook no.	Sex	Age (years)	BM (kg)	Diets fed	Study	
1	Emma	451	F	10	1000/1065	(N2), T, Q	This study
2	Quinto	430	M	11	1093/1133	(N2), T, Q	This study
3	Taco	533	M	6	1000*	(N), T, Q	This study
4	Tisa	532	F	7	1000*	(N), T, Q	This study
5†	Mtoto	150	F	31	762	(N), T, Q	This study
6	Sabi	217	F	30‡	1000*	(N), T, Q	This study
7	Parky	318	M	19	1200*	(N), T, (Q)	This study
8	Wanda	662	F	5	900*	(N), T, (Q)	This study
9	Sita	428	F	10	1200*	(N1), T	T. Froeschle and M. Clauss (unpublished data)
10	Ndugu	610	M	4	1200*	(N1), T	T. Froeschle and M. Clauss (unpublished data)
12	Jakob	438	M	9	1200*	(N1), T	T. Froeschle and M. Clauss (unpublished data)
13	Nane	437	F	9	1200*	(N1), T	T. Froeschle and M. Clauss (unpublished data)

The codes N, N1 and N2 refer to the respective diets in Clauss et al. (2006b).

*Body weights represent either actual weights or estimates. Diets used were conventional zoo diets with hay and concentrates (N; these trials are described in Clauss et al., 2006b), and similar diets in which the pelleted compound feed contained 5% tannic acid (T) or quebracho (Q). In animals 7 and 8, diet Q was only fed for the determination of faecal parameters of bacterial activity, but not for a digestion trial.

†Animal had an oral abscess and received a particular diet (Hatt et al., 2004).

‡Exact age unknown as animal was caught from the wild.

One animal (no. 5) had difficulties in ingesting roughage material due to an oral abscess (Hatt et al., 2004) and received a special diet with a high proportion of grass meal pellets.

The measurement of food intake and total faecal excretion, as well as the sampling procedure, the laboratory analyses and the calculations followed the same protocol as outlined for Indian rhinoceroses (*Rhinoceros unicornis*) in Clauss et al. (2005c). Analyses included DM, CP, crude ash, crude fibre (CF) and ether extract (without preceding acid hydrolysis) according to Naumann and Bassler (1988), neutral detergent fibre (NDF, including amylase incubation and expressed on an ash-free basis), acid detergent fibre (ash-free basis) and acid detergent lignin (ash-free basis) according to Van Soest (1967), gross energy and non-dietary faecal nitrogen (NDFN) according to the protocol developed by Mason and Frederiksen (1979) for domestic sheep, using freeze-dried material for all analyses except for DM. NDFN is the faecal N not recovered in the faecal NDF residue. For all diets (those reported here and in Clauss et al., 2006b), a subsample of fresh faecal water (gained by centrifugation of fresh faeces mixed with tridistilled water) preserved in orthophosphoric acid was used for the determination of faecal short-chain fatty acid (SCFA) content; another subsample preserved in perchloric acid was used for the determination of L-lactate. L-lactate was determined by enzymatic reaction and photometric measuring of the resulting NADH/H⁺. The total SCFA concentration was determined by gas chromatography (glass

column length 30 m, 25 mm diameter, column 150 °C, detector and injection block 300 °C, filling 'Crossbond Carbowax-PEG', carrier gas pressure 160 kPa; Perkin Elmer Auto System; Überlingen, Germany). Results for L-lactate and SCFA represent the average of 10 individual measurements per animal and diet. Drinking water intake could be recorded in five animals (1, 3, 4, 5 and 6) on the regular and the two experimental diets by measuring the water volume consumed from water troughs until the next feeding time; relevant water losses due to evaporation did not occur as assessed by weighing filled water buckets positioned adjacent to the enclosures after a similar timespan as that between water measurements inside the enclosures; however, differences in spillage between animals or trials could not be accounted for. Data from animals in which major spillage losses were observed to occur (such as animal 2) were excluded.

In one facility (animals 5–8), a misunderstanding led to the feeding of the regular pelleted feed during the quebracho trial period (after samples for lactate and SCFA, salivary tannin-binding proteins, faecal antioxidant status and Enterobacteriaceae count had been taken, but before the digestibility study had started). In animals 5 and 6, the quebracho-feeding period, including the lengthy adaptation period, was repeated and digestibility data were generated. However, for animals 7 and 8 only the lactate and SCFA data on the quebracho diet is available here.

Additional data were available from a hitherto unpublished study by T. Froeschle and M. Clauss in

Table 3 Ingredient and nutrient compositions of rations fed to black rhinoceroses (*Diceros bicornis*) in digestion trials

n	Diet	Lucerne		Grass		Fruits and vegetables		CA	CP	EE	CF	NDF	ADF	ADL	Source
		hay	hay	Conc.	Conc.	Conc.	Conc.								
1	T	42	-	-	52	6	7.3	14.0	2.4	27.6	49.1	33.6	7.0	This study	
1	Q	41	-	-	53	6	7.7	13.7	2.0	30.0	50.3	34.7	7.4	This study	
2	T	46	-	-	52	2	7.4	14.4	2.3	29.3	52.0	35.5	7.1	This study	
2	Q	50	-	-	48	3	7.7	13.8	1.8	32.6	53.5	37.7	8.0	This study	
3	T	-	71	5	19	5	7.6	10.6	3.8	22.1	49.6	26.4	1.5	This study	
3	Q	-	68	5	22	5	6.3	8.5	4.9	24.0	49.0	26.3	1.9	This study	
4	T	-	70	4	20	5	7.6	10.6	3.9	21.9	49.2	26.1	1.5	This study	
4	Q	-	58	4	31	7	6.8	8.8	5.9	22.2	45.6	24.5	2.0	This study	
5	T	22	9	-	60	10	6.7	12.8	1.6	17.0	32.6	19.5	3.1	This study	
5	Q	32	10	-	48	10	7.9	13.1	1.9	19.6	36.2	21.7	3.8	This study	
6	T	8	27	-	56	9	6.3	11.7	1.6	17.5	35.9	20.0	2.4	This study	
6	Q	13	30	-	48	9	6.4	10.7	1.8	19.0	40.3	23.1	2.9	This study	
7	T	20	28	-	45	7	6.6	12.0	1.5	20.7	39.9	23.6	3.2	This study	
7	(Q)	28	27	-	39	5	7.0	11.7	1.5	24.1	46.8	28.0	4.8	This study	
8	T	12	20	-	58	9	6.5	12.0	1.7	17.5	35.3	20.1	2.7	This study	
8	(Q)	28	17	-	48	7	7.1	12.3	1.7	21.9	42.4	25.5	4.7	This study	
9	T	19	45	-	32	4	5.5	14.5	NA	23.7	45.4	23.7	4.0	T. Froeschle and M. Clauss (unpublished data)	
10	T	17	54	-	27	3	5.6	14.4	NA	24.5	47.2	24.4	3.9	T. Froeschle and M. Clauss (unpublished data)	
12	T	-	64	-	16	20	9.7	10.4	NA	23.6	44.6	25.6	6.2	T. Froeschle and M. Clauss (unpublished data)	
13	T	-	64	-	16	20	9.7	10.4	NA	23.7	44.7	25.6	6.2	T. Froeschle and M. Clauss (unpublished data)	
1-6	N						7.7 ± 1.0	13.1 ± 2.3	3.1 ± 1.4	22.2 ± 4.6	44.2 ± 7.1	26.0 ± 5.8	3.8 ± 2.1	Clauss <i>et al.</i> (2006b)	
1-6	T						7.2 ± 0.5	12.4 ± 1.7	2.6 ± 1.0	22.6 ± 5.1	44.7 ± 8.3	26.9 ± 6.7	3.8 ± 2.6	This study	
1-6	Q						7.1 ± 0.7	11.4 ± 2.4	3.1 ± 1.8	24.6 ± 5.6	45.8 ± 6.5	28.0 ± 6.6	4.3 ± 2.7	This study	

Values are in % dry matter. Conc., concentrates (including pelleted compound feeds, grains and bread); CA, crude ash; CP, crude protein; EE, crude fat; CF, crude fibre; NDF, neutral detergent fibre/cell walls; ADF, acid detergent fibre; ADL, acid detergent lignin; for coding of diets see Table 2.

*Dried temperate browse.

which four animals from two other zoos that had been studied on their regular diet (Clauss *et al.*, 2006b) were also fed the same diet with the inclusion of 5% tannic acid in the pelleted diet item. The setup of these studies and the analytical procedures were identical to this study; however, the chemical analyses were performed by another laboratory, and the adaptation to the new diet only lasted 14 days. These additional data are added to the tables and the calculations where appropriate.

The digestibility data generated in this study were compared to the data for the regular feeding period in the same animals (Clauss *et al.*, 2006b) by Friedman test with subsequent *post hoc* tests (Bonferroni adjustment); in the same way, water intake was compared between the three feeding periods. The Wilcoxon test was used to compare the DM intake between periods N (regular zoo diet) and T (12 animals) and between periods N and Q (six animals).

Results

With the exception of animal 5, the general health of the animals during the study period did not seem to be compromised. Judged by external appearance, no animal seemed to lose weight during the study period. All animals accepted the tannin-containing pellets without hesitation.

Overall daily food intake did differ statistically between animals fed the regular zoo diet and the one with tannic acid-containing pellets (79.1 ± 19.2 g DM/kg^{0.75} body weight on the regular diet vs. 84.3 ± 20.9 g/kg^{0.75} body weight on the tannic acid diet, $n = 12$, $p = 0.034$), with a slightly higher intake on diet T. There was no difference in overall food intake between animals fed the regular zoo diet and the one with quebracho-containing pellets (82.2 ± 23.3 g/kg^{0.75} body weight on the regular diet vs. 83.1 ± 21.5 g/kg^{0.75} body weight on the quebracho diet, $n = 6$, $p = 0.753$).

The digestion coefficients achieved by the black rhinoceroses, as well as true digestibility of dietary CP and NDFN as derived by analysing CP content in the faecal NDF residue according to Mason and Frederiksen (1979), are recorded in Table 4. In general, values were in relation to those recorded on the regular zoo diets (Clauss *et al.*, 2006b). In particular, there was no discernible influence of tannin feeding on DM digestibility (Fig. 1). The Friedman test indicated no difference in CP digestibility between the different diets ($p = 0.676$) or any other digestibility coefficients. There was a significant difference in the percentage of NDFN of the total faecal nitrogen

(Friedman $p = 0.030$; pair-wise tests: N-T $p = 0.167$, N-Q $p = 0.044$, T-Q $p = 0.024$), but the pair-wise differences were no longer significant after Bonferroni adjustment. A plot of the data suggests that this perceived difference was rather an effect of the different levels of dietary CP than different dietary regimes (Fig. 2).

Drinking water intake of animals 1, 3, 4, 5 and 6 was numerically higher during the regular zoo diet than during either tannin diet period (5.5 ± 1.1 l/100 kg body weight on the regular zoo diet vs. 4.9 ± 1.0 l/100 kg on the tannic acid and 4.9 ± 0.7 l/100 kg on the quebracho diet), but the difference was not significant (Friedman $p = 0.076$). The total SCFA content of faecal water did not differ between the tannin treatments (Fig. 3). It averaged 74 ± 15 mmol/l faecal water (range 42–109) over all diets, with no discernible influence of dietary fibre levels; instead, a pattern according to the roughage sources fed was evident (Fig. 4). Lactate content (1.7 ± 0.4 mmol/l faecal water, range 0.7–2.5) did not show any difference due to tannin supplementation.

Discussion

To our knowledge, this is the first report of a digestion study in a perissodactyl including a deliberate tannin supplementation. The results indicate that, at the level of supplementation used in this study, neither tannic acid nor quebracho had a measurable effect on digestive parameters in black rhinoceros.

An interesting finding of this study was the seeming absence of inter-individual variation in the acceptance of the tannin-containing pelleted feeds. None of the animals used in this study or in the unpublished study by Froeschle and Clauss, rejected the tannin-containing pellets, although these were very different in smell and taste from the regular pellets (as assessed by humans), and although the animals could have compensated for a reduced pellet intake by an increase in the intake of the *ad libitum*-offered roughage sources. In feeding trials with captive roe deer (*Capreolus capreolus*), a large inter-individual variation in the acceptance of tannin-containing pellets was noted; yet, some individual roe deer consistently ingested pellets supplemented with 6% quebracho (Clauss *et al.*, 2003b). In a feeding trial with three captive giraffes, again inter-individual differences in the acceptance of a pellet mixture containing 3% tannic acid was noted; however, in this case, all three animals unanimously refused to ingest a pellet containing 5% tannic acid (Clauss

Table 4 Apparent digestibility coefficients for nutrients and gross energy (GE), 'true protein digestibility' (TPD) and non-dietary faecal nitrogen (NDFN, in % of total faecal nitrogen) in black rhinoceroses (*Diceros bicornis*)

No.	Diet	apparent digestibility (%)										Source
		DM	OM	CP	EE	CF	NDF	ADF	GE	TPD	NDFN	
1	T	54	55	53	47	36	40	41	50	90	79	This study
1	Q	53	53	54	46	37	38	38	48	91	81	This study
2	T	55	55	66	47	36	41	41	52	93	78	This study
2	Q	50	50	61	34	34	36	38	46	92	79	This study
3	T	51	52	52	44	27	32	28	47	90	79	This study
3	Q	50	52	44	47	34	33	31	47	89	80	This study
4	T	53	54	49	42	33	39	36	49	90	80	This study
4	Q	57	58	40	56	45	43	41	52	91	84	This study
5	T	73	74	71	9	48	51	48	70	93	77	This study
5	Q	66	66	67	19	37	43	36	61	93	78	This study
6	T	65	67	59	3	37	42	37	62	92	81	This study
6	Q	62	63	60	37	29	38	33	60	93	82	This study
7	T	64	65	70	0	37	40	36	61	93	76	This study
8	T	69	70	70	44	40	44	39	66	95	82	This study
9	T	56	58	62	–	30	39	33	54	–	–	T. Froeschle and M. Clauss (unpublished data)
10	T	50	57	61	–	32	37	31	52	–	–	T. Froeschle and M. Clauss (unpublished data)
12	T	56	58	60	–	36	39	38	55	–	–	T. Froeschle and M. Clauss (unpublished data)
13	T	56	58	61	–	33	39	38	55	–	–	T. Froeschle and M. Clauss (unpublished data)
1–6	N	57 ± 9	58 ± 10	58 ± 6	48 ± 10	35 ± 11	38 ± 11	34 ± 10	54 ± 9	91 ± 2	78 ± 3	Clauss et al. (2006a,b)
1–6	T	59 ± 9	60 ± 9	58 ± 9	32 ± 20	36 ± 7	41 ± 6	39 ± 7	55 ± 9	91 ± 2	79 ± 1	This study
1–6	Q	56 ± 7	57 ± 6	54 ± 11	40 ± 13	36 ± 5	39 ± 4	36 ± 4	52 ± 7	92 ± 2	81 ± 2	This study

There were no significant differences after Bonferroni adjustment between the three feeding periods in animals 1–6.

DM, dry matter; OM, organic matter; CP, crude protein; EE, crude fat; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; for coding of diets see Table 2.

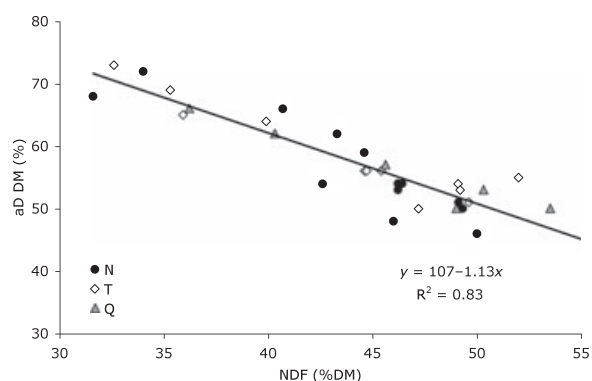


Fig. 1 Relationship between dietary neutral detergent fibre content (NDF in % dry matter) and the apparent digestibility of dry matter (aD DM) for regular zoo diets (N) and the same diets with an addition of either tannic acid (T) or quebracho (Q) at 5% of the pelleted compound feed fed to the same animals. Note the absence of a distinct effect of tannin supplementation. Data from Tables 3 and 4; and Clauss et al. (2006b).

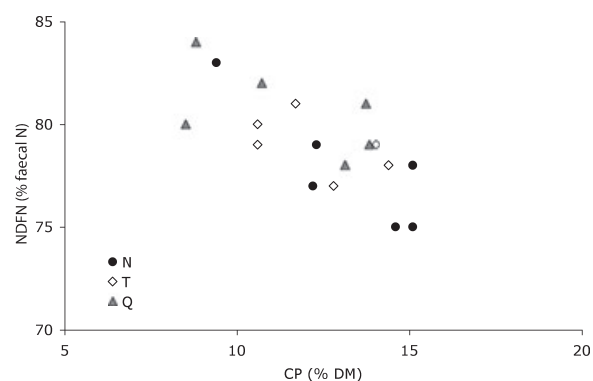


Fig. 2 Relationship between dietary crude protein content (CP in % dry matter) and the percentage of non-dietary faecal nitrogen (in % of total faecal nitrogen) for regular zoo diets (N) and the same diets with an addition of either tannic acid (T) or quebracho (Q) at 5% of the pelleted compound feed fed to the same animals. Note the absence of a distinct effect of tannin supplementation. Data from Tables 3 and 4; and Clauss et al. (2006b).

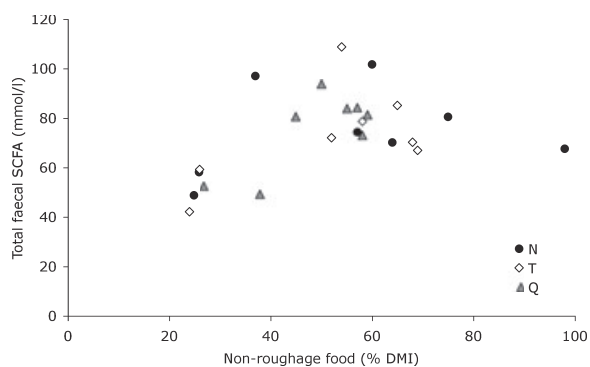


Fig. 3 Relationship between the proportion of non-roughage diet items (in % of total dry matter intake) and the total faecal content of short-chain fatty acids (SCFA in mmol/l faecal water) for regular zoo diets (N) and the same diets with an addition of either tannic acid (T) or quebracho (Q) at 5% of the pelleted compound feed fed to the same animals. Note the absence of a distinct effect of tannin supplementation.

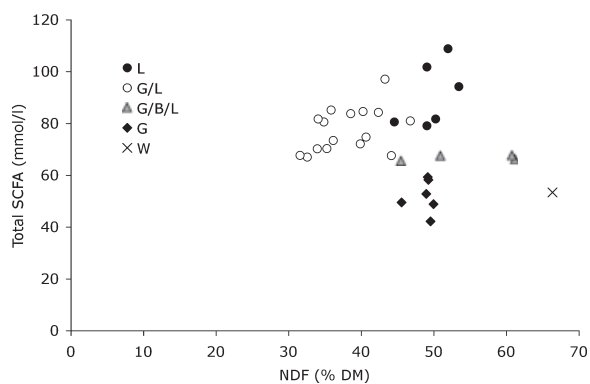


Fig. 4 Relationship between the proportion of dietary neutral detergent fibre (in % dry matter intake) and the total faecal content of short-chain fatty acids (SCFA in mmol/l faecal water) in black rhinoceros for diets with different roughage sources (L, lucerne hay; G/L, grass hay and lucerne hay; G/B/L, grass hay, browse and lucerne hay; G, grass hay; W, animals from the wild). Note the grouping according to the roughage sources used. Data for animals from the wild from Clemens and Maloiy (1982).

et al., 2003a). Nevertheless, the findings in the black rhinoceros of this study indicate that the inclusion of tannins up to 5% in pelleted compound feeds is unlikely to result in rejection. Our data also indicate that offering such a pelleted food in a diet based on one or several roughage sources, as is usual in the feeding of large zoo herbivores (Lintzenich and Ward, 1997), will not lead to a reduction in overall food intake.

In large zoo herbivores such as the black rhinoceros, it is extremely difficult to achieve an identical diet composition in subsequent trials if the diet is

composed of more than one food item; in the case of this study, differences in the *ad libitum* intake of the roughage component within individuals in the subsequent trial periods invariably resulted in differences in nutrient composition of the overall ration. Therefore, digestion coefficients measured must always be compared against the actual composition of the respective diets (Figs 1 and 2). In ruminants, natural tannins and quebracho at dietary levels of 3–6% DM, were shown to reduce the apparent digestibility of both DM and protein, with a less drastic effect in browsing deer species in relation to domestic sheep (Robbins et al., 1991; Hanley et al., 1992). Robbins et al. (1991) demonstrated that species that produce tannin-binding salivary proteins, such as mule deer (*Odocoileus hemionus*) or black bears (*Ursus americanus*) were less affected in their digestive efficiency when fed quebracho tannins. In contrast, tannic acid did not reduce the digestibility of protein in either species at the same supplementation level (Hagerman et al., 1992), and the ingestion of tannic acid-containing pellets did not result in a detectable influence on apparent digestibilities measured in three captive giraffe (Clauss et al., 2003a). Potential modes of action in reducing DM digestion and increasing faecal protein excretion are an adverse effect on the symbiotic fermentative microflora and the complex formation with both feed protein (increasing its excretion in the faeces) and endogenous and/or bacterial protein (thus increasing the endogenous/metabolic losses and decreasing overall apparent digestibility) (reviewed by Foley et al., 1999). However, the results of Hagerman et al. (1992) emphasize that differences in the effects of different tannins and tannin sources must be expected, and that some tannins may lack an effect observed in others. Regardless of the negative effects of tannins such as quebracho on the digestive performance of animals not naturally adapted to tannin-containing diets such as sheep, Śliwiński et al. (2002) did not find a reduction in apparent DM or protein digestibility in sheep when fed a diet with a CT at 1–2% DM. Therefore, the absence of an effect of even the quebracho-containing pellet in the black rhinoceros in this study could indicate both an adaptation of black rhinoceros to tannin diets and the absence of a measurable effect due to the low dose used in this study.

Studies with ruminants have yielded conflicting results on the effect of the feeding of natural or artificial sources of tannins on the intake of drinking water. Several of such studies are limited in their conclusions because they used a hay as the tannin-free

control diet and fresh tannin-containing forage as the tannin source; in these cases, the animals drank more water on the drier control diet (Aganga et al., 1998; Aganga and Monyatsiwa, 1999; Shetaewi et al., 2001). In a similar trial in which both the control lucerne hay and the tannin-containing browse species were offered dried, calves had a higher water intake on the lucerne diet (Coppock and Reed, 1992), similar to the findings in this study. Salem et al. (2004) found that when sheep were fed lucerne hay treated with 5% quebracho, their water intake increased; in contrast, the same diet did not lead to an increase in water intake in goats. Śliwiński et al. (2002) did not find an increased water intake in lambs when fed diets with tannins added at 1–2% DM. A literature survey and experimental study by Dearing et al. (2001) reaches the conclusion that one potential mechanism by which tannins negatively affect herbivores is by a diuretic action, which would translate into both higher water and sodium requirements. Increased urine output, and the corresponding increased sodium losses, would be particularly dramatic in black rhinoceros as this species already has higher endogenous faecal sodium losses than domestic horses (Clauss et al., 2006a). The results in the black rhinoceros indicate that the tannins as used in this study did not increase water intake, and therefore unlikely increased urine output.

Free-ranging black rhinoceros had a lactate level of 0.9 mmol/l faecal water (Clemens and Maloiy, 1982). In horses, faecal lactate levels can rise from 0.1–0.9 mmol/l on roughage-only diets (Alexander and Davies, 1963) up to 4.0 mmol/l on rations with concentrates (Argenzio et al., 1974). As in horses, the average faecal lactate level in the black rhinoceros on zoo rations that invariably included concentrate feeds (1.7 mmol/l) was higher than the values measured in free-ranging individuals on forage-only diets. Similarly, the values measured for total faecal SCFA in the wild (53.3 mmol/l, Clemens and Maloiy, 1982) was in the lower range of the values measured in the captive animals (Fig. 4). The fact that grass hay-based diets achieved lower total faecal SCFA contents than lucerne hay-based diets is in accord with a faster degradability of lucerne in relation to grass in *in vitro* fermentation (Hummel et al., 2006) – a fact of particular relevance in animals with comparatively short ingesta retention times such as black rhinoceros (Clauss et al., 2005a). In spite of an effect of quebracho supplementation on the faecal microflora (M. Clauss et al., unpubl. data.), there was no evident influence of tannin supplementation on the parameters of microbial fermentation activity in the faeces.

On similar doses of dietary tannins, Śliwiński et al. (2002) found a reducing effect on rumen microbes in lambs but no associated decrease in the levels of SCFA in rumen contents. Therefore, although tannins have been shown to affect forage digestion *in vitro* (Makkar et al., 1995) and at higher doses *in vivo* (Zimmer and Cordesse, 1996), it can be assumed that the inclusion of tannins at dietary tannin levels of 1–2% DM does not affect microbial digestion in a relevant way (Śliwiński et al., 2002; this study).

The reason for a lack of effect of the tannin supplementation in this study, even the addition of quebracho, could be that the dose of supplementation was too low to produce detectable results; another possible explanation is a particular adaptation of black rhinoceros to tannin-containing diets. The tannin-binding salivary proteins produced by the black rhinoceros in response to the tannin feeding in this study (Clauss et al., 2005b) might have neutralized any potential digestion-reducing potential. To date, the black rhinoceros remains the only ungulate species in which an induction of tannin-binding salivary proteins in response to tannin intake has been demonstrated. Historically, the black rhinoceros has been regarded as a species particularly apt at handling adverse chemical substances: in the wild, black rhinoceros are known for feeding on plants, such as *Euphorbia ingens*, *Euphorbia virosa*, *Lopholeana corrifolia*, *Phytolacca dodecandra* or *Datura stramonium*, which are toxic to other herbivore species (Goddard, 1968; Loutit et al., 1987; Emslie and Adcock, 1994). Should the assumption that an addition of tannins to diets for captive black rhinoceros is beneficial (Paglia and Dennis, 1999) lead to a deliberate inclusion of tannin substances in diet formulas, tannin levels comparable with those in this study are unlikely to have particularly negative effects that need to be accounted for.

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