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Research article

# Investigations on rumen health of different wild ruminants in relation to feeding management

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**Abstract**

In this study, the rumen metabolism of four ruminant species – blackbuck (*Antilope cervicapra*), barbary sheep (*Ammotragus lervia*), sika deer (*Cervus nippon dybowskii*) and addax antelope (*Addax nasomaculatus*) – from the Nuremberg Zoo was investigated in relation to their feeding management. Parameters of microbial fermentation in the rumen as well as rumen tissue samples were examined. Additionally, the offered and refused food was weighed, analysed and a dietary intake calculation for these ruminant species was performed. Three of the four ruminant species – blackbuck, barbary sheep and addax antelope – all grazers, showed severe characteristics of subacute ruminal acidosis due to a diet high in fermentable carbohydrates (NfE  $\geq$  55% of DM basis) and low in fibre (XF  $\leq$  18% of DM basis). This was indicated by numerous microabscesses within the rumen mucosa. These results demonstrate that subacute ruminal acidosis is still a severe problem in captive wild ruminants, which are often fed inappropriate diets with a high concentration of easily digestible carbohydrates.

**Introduction**

Ruminants represent a group of highly sophisticated herbivores, which are often specialized for particular groups of feeding plants and cannot be fed with uniform rations (Hofmann 1995). Therefore Hofmann and Stewart (1972) classified them into a flexible system of three overlapping morphophysiological feeding types: concentrate selectors, grass and roughage eaters and intermediate, opportunistic mixed feeders. Today “grass- and roughage eaters” and particularly the contradictory term “concentrate selectors” are replaced by the terms “grazers” and “browsers” according to the natural forage of these ruminants (Claus *et al.* 2003). Numerous studies, for example Ippen and Henne (1985; 1988), Kiupel (1988) and Hatt *et al.* (1995), document the high incidence of gastrointestinal disorders in zoo ruminants due to an inadequate feeding regime. One of the most prominent examples for gastrointestinal disorders in ruminants is ruminal acidosis. This was investigated in detail by Marholdt (1991), who focused on pathological changes of the rumen mucosa of 67 captive wild ruminants. 40% of these investigated animals showed acidotic characters within the rumen mucosa.

In the past few years several indications for subacute ruminal acidosis in certain ruminant species of Nuremberg Zoo were noticed: For example, deformed claws in the barbary sheep (*Ammotragus lervia*), although the claws were regularly trimmed, and poor body condition plus episodes of diarrhoea in the addax antelopes (*Addax nasomaculatus*). The purpose of the study was to investigate the rumen health and feeding management of different ruminant species – blackbuck (*Antilope cervicapra*), barbary sheep (*Ammotragus lervia*), sika deer (*Cervus nippon dybowskii*), addax antelope (*Addax nasomaculatus*) - from the Nuremberg Zoo.

**Methods**

Five individuals of each ruminant species mentioned above were used in this study. All animals were included opportunistically having been selected for removal due to problems in group composition and overcrowding, or in two cases in the blackbuck group due to an acute injury. The sample collection from these animals begun immediately after death and was completed within 30 minutes.

Samples of rumen fluid were taken immediately for measuring the pH with a portable electronic pH meter (SM 102®, Milwaukee, USA). Further rumen fluid was centrifuged (4 min at 6000 rpm) and stored with perchloric acid (0.66N) respectively mercuric chloride (1.25%) for measuring lactic acid (UV-test with Cobas Mira Roche-Autoanalyser®, F. Hoffmann-La Roche Ltd., Basel, Switzerland) respectively short chain fatty acids (SCFA) by gas chromatography (Tangermann and Nagengast 1996).

Furthermore tissue samples of the rumen atrium were taken (1 cm x 2 cm pieces). After fixation in neutral buffered formaldehyde (4%), dehydration in graded ethanol and embedding in paraffin transverse sections of 5 mm thickness were prepared and stained with hematoxylin and eosin to verify the various epithelial layers in the rumen mucosa. The histological evaluation of the rumen mucosa according to the method of Marholdt (1991) where a standardisation of changes caused by acidosis in comparison with normal rumen mucosa, was performed. Additionally, on every microscope slide the number of rumen papillae and micro-abscess was evaluated and the relation of micro-abscess to papillae was calculated.

The diets (roughage, concentrates and fresh food) offered to the animals of the four ruminant species were listed and weighed as well as the refused food. A proximate analysis for dry matter (DM), ash (XA), crude fibre (XF), and crude protein

(N\*6.25) (XP) was carried out by standardized procedures. Data for crude fat (XL) was taken from the literature (Kamphues *et al.* 2009, Souci *et al.* 2008, FAG 1994) respectively the official declaration of the pelleted compound food. Nitrogen free extracts (NfE) were calculated as NfE = 100-(XA+XL+XF+XP). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated by sequential fibre analyses (Van Soest 1967). For each ruminant species the ingested diet composition was calculated.

Data are presented as mean ± standard deviation (SD) for each ruminant species. A one-way analysis of variance (ANOVA) was performed with the factor “group”. This was followed by the one way Kruskal-Wallis test. The analyses were calculated by use of a statistical software program (Systat for Windows® 11.0, SPSS Inc. Chicago, USA) For all tests the level of significance was set at p = 0.05, so if the p-value was less than 0.05, differences were considered statistically significant.

**Results**

**Parameters of rumen fermentation**

The mean rumen pH of all investigated species was between 6.3 and 6.6 (Table 1). There was a significant difference (p < 0.05) between the mean rumen pH of sika deer and addax antelope.

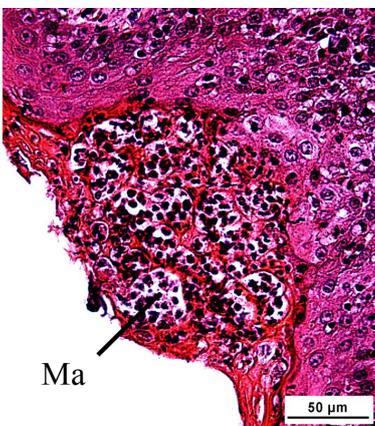
Neither d-lactate nor l-lactate were detectable in the rumen fluid of any animal (detection limit: 0.3 mg/l). The SCFA concentrations in the rumen fluid of the four investigated species were between 50 and 81 mmol/l (Table 1)

Species	BW (kg)	pH	SCFA (mmol/l)
<i>Antilope cervicapra</i>	27 ± 8	6.3 ± 0.4	71 ± 20
<i>Ammotragus lervia</i>	64 ± 42	6.5 ± 0.4	81 ± 29
<i>Addax nasomaculatus</i>	69 ± 28	6.3 ± 0.2*	80 ± 13
<i>Cervus nippon dybowskii</i>	69 ± 11	6.6 ± 0.2*	50 ± 13

**Table 1.** Mean body weight (BW) ± standard deviation (SD) of the investigated animals and mean pH and concentration of short chain fatty acids (SCFA) ± SD in the rumen fluid. \* p<0.05

**Rumen epithelium**

The rumen mucosa of all five investigated sika deer showed a multi-layered epithelium. Particularly the Stratum corneum with ballooned and low keratinised cells indicates active resorption due to a large amount of easily digestible carbohydrates. In contrast, severe characteristics of ruminitis were present in the rumen mucosa of blackbuck, barbary sheep and addax antelopes. There were numerous aggregations of leukocytes, interpreted as micro abscesses (Figure 1), as well as diffuse spread leukocytes within the rumen epithelium in these species.



**Figure 1:** Rumen epithelium of a barbary sheep (BS 4) with cumulation of leukocytes, interpreted as a micro abscess (Ma).

concentrations of keratin.  
**Ingested diet composition**

Animals	Ma / papillae	Diffuse spread leukocytes
BB 1	-	No
BB 2	0.03	No
BB 3	-	Yes
BB 4	-	Yes
BB 5	-	No
BS 1	1.42	Yes
BS 2	0.15	Yes
BS 3	0.57	Yes
BS 4	1.14	Yes
BS 5	0.16	-
AA 1	0.25	Yes
AA 2	0.79	Yes
AA 3	0.29	Yes
AA 4	-	No
AA 5	-	Yes

**Table 2.** Ratio of micro abscesses (Ma) in the rumen mucosa to rumen papillae on one microscopical slice and the presence of diffuse spread leukocytes in the rumen mucosa of the investigated grazer species. BB = Blackbuck; BS = Barbary sheep; AA = Addax antelope.

Species	Roughage	Concentrates	Vegetables/ Fruits	R:C:V/F in % on DMB
<i>Antilope cervicapra</i>	Hay	Savannenfutter** Kofu® Oats Bran	Apples Carrots Beets	16:77: 7
<i>Ammotragus lervia</i>	Hay	Ziegenpellets** Kofu® Bran Oat flakes	Beets	37:49:14
<i>Addax nasomaculatus</i>	Hay	Kofu® Oat flakes	Apples Carrots	39:57: 4
<i>Cervus nippon dybowskii</i>	Hay	Kofu® Corn pellets Oats Bran	Beets	18:57:25

**Table 3.** Ingested diet composition and the ratio of roughage (R) to concentrates (C) to vegetables and fruits (V/F) on dry matter basis (DMB) in the ingested diets. \* Savannenfutter®, Kofu® and Ziegenpellets® are pelleted compound feeds special compounded for the Nuremberg Zoo.

Species	DM %	XP %	XF %	XL %	NfE %	NDF %	ADF %	ADL %
<i>Antilope cervicapra</i>	63	17	14	3	58	40	18	4
<i>Ammotragus lervia</i>	50	14	18	3	55	44	23	4
<i>Addax nasomaculatus</i>	74	16	18	3	55	44	22	4
<i>Cervus nippon dybowskii</i>	37	11	15	3	65	38	20	4

**Table 4.** Proximate analysis of the ingested diets (in winter) of the four ruminant species; DM = dry matter, XP = crude protein, XF = crude fibre, XL = crude fat, NfE = nitrogen free extracts, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin. XP, XF, XL, NfE, NDF, ADF and ADL are given in % of dry matter basis.

The composition of the ingested diets of the four ruminant species and the ratio of roughage to concentrates in the winter diets are listed in Table 3. In summer all species additionally get grass but no beets. The addax antelopes were separated indoor over night and were also fed separately in their boxes. In contrast there was a group feeding situation with the blackbuck, barbary sheep and sika deer. The animals of the investigated ruminant species were fed with the concentrates, vegetables and fruits once daily but hay was available all day ad libitum. The proximate analysis of the chemical components of the ingested diets are shown in Table 4.

**Discussion**

The parameters of rumen fluid (pH, SCFA, lactate) are closely related with feeding regime (Steger *et al.* 1970). In this study these values must be interpreted with caution because due to zoo management reasons (opening times for visitors), the sample collection had to be done early in the morning before the animals were fed. Therefore

samples were taken long after the last meal when the pH is upregulated and SCFA concentrations lowest. This would explain why the parameters like pH and SCFA concentration, which are usually changed during subacute acidosis are not changed in the present study. After the single daily feeding of concentrates, vegetables and fruits however, the pH is decreased which leads to lesions in the long term. The concentration of lactate in the rumen fluid is decreased during acute ruminal acidosis but not during subacute ruminal acidosis (Nocek 1997).

The severe lesions of the rumen mucosa of the blackbucks, barbary sheep and addax antelopes are fundamental characteristics of subacute ruminal acidosis (Krause and Oetzel 2006). The reasons for developing subacute ruminal acidosis in these cases were probably the diets, which consisted of high amounts of concentrates and fresh food. A high level of NfE (55-65% of DM basis) and a low level of NDF (38-44% of DM basis) combined with high amounts of concentrates, fruits and vegetables leads to the assumption that a big part of the NfE-fraction consisted of starch and sugars. Additionally, farmed fruits like apples have a very fast fermenting time and lead to a fast production of high amounts of SCFA (Hummel *et al.* 2006). Furthermore, all four diets had a deficiency of fibre since crude fibre contents of the diets (14-18% of DM basis) reached the critical threshold (18%) of beef cattle (Kamphues *et al.* 2009). Diets with high amounts of easily digestible carbohydrates combined with a deficiency of fibre causes a temporarily altered state of the rumen (low pH after feeding) which may have lead in the cases of blackbuck, barbary sheep and addax antelopes to the lesions of rumen epithelium. Additionally the feeding regime, like the number of meals and the sequence of roughage and concentrates during feeding is important to prevent subacute ruminal acidosis (Nordlund *et al.* 1995). In the case of Nuremberg Zoo the investigated animals were usually fed once a day, normally in the morning, with a mixture of concentrates, vegetables and fruits. At this time hay also was offered in amounts which passed for the whole day. The problem with this feeding situation is that the animals usually eat the concentrates, vegetables and fruits in preference so hay intake is initially low. This might have influenced the rumen milieu in a negative way, since synchrony (the synchronic ingestion of protein and energy, which is important for the ruminant microbial flora), was not guaranteed with this feeding regime.

In the group of sika deer there were no indications of subacute ruminal acidosis since there were no pathological changes in the ruminal mucosa. Furthermore in this species the rumen pH was highest (6.6) and the concentration of SCFA lowest (50 mmol/l). This could lead to the conclusion that the sika deer, classified as an intermediate feeder, seemed to tolerate increased amounts of highly fermentable carbohydrates since no pathological changes were present. This is in contrast to the findings of Kuipel (1988), Marholdt (1991), and Clauss *et al.* (2003) who showed that intermediate feeders and browsers are even more prone than grazers to GIT disorders, like subacute ruminal acidosis, due to a high amount of concentrates in the diets. Apart from the classification in different feeding-types the age of the investigated animals could be of relevance in this case. Whereas the investigated groups of blackbuck, barbary sheep and addax antelopes were heterogenous (age ranged from 8 month to 15 years) in the group of sika deer the animals were aged between 12 and 20 months. Additionally, younger animals are normally more subdominant and consequently were not able to get high amounts of concentrates in a group feeding situation compared to the dominant animals. Apart from the diet with high amounts of concentrates the sika deer used in this study were probably too young to develop long term effects in the rumen mucosa.

## Conclusions

The blackbuck, barbary sheep and addax antelopes, all grazers, showed severe characteristics of subacute ruminal acidosis due to a diet high in fermentable carbohydrates and low in fibre.

The feeding management, the ingested diet composition and the feeding regime (frequency and sequence), of those ruminant species from Nuremberg Zoo must be improved. The diets should consist mainly of roughage (hay of good quality) and only that amount of low energy pelleted compound food to cover specific demands on vitamins and minerals like vitamin E and selenium. Fresh food such as apples and carrots should not be fed since they are not adequate for a physiological rumen milieu even in a grazing ruminant species. The single daily meal of concentrates should be divided at least into two meals a day. Changing the sequence of food provision so that some roughage is consumed before the concentrate meal, particularly in the morning, would also be very important.

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