



Solute and particle retention in a small grazing antelope, the blackbuck (*Antilope cervicapra*)



Jürgen Hummel^{a,*}, Sven Hammer^{b,1}, Catrin Hammer^{b,1}, Julia Ruf^{c,2}, Monique Lechenne^{c,3}, Marcus Clauss^c

^a Institute of Animal Science, Animal Nutrition, University of Bonn, Germany

^b Al Wabra Wildlife Preservation (AWWP), Doha, Qatar

^c Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland

ARTICLE INFO

Article history:

Received 18 August 2014

Received in revised form 1 December 2014

Accepted 1 December 2014

Available online 7 December 2014

Keywords:

Passage rate

Retention time

Digestive physiology

Saliva

Fluid throughput

Adaptation

Nilgai

Boselaphus tragocamelus

ABSTRACT

Digesta retention patterns have been suggested to play a major role in ruminant diversification. Two major digestion types have been proposed, termed ‘cattle-type’ and ‘moose-type’, that broadly correspond to the feeding categories of grazers and intermediate feeders on the one, and browsers on the other hand. We measured and calculated the mean retention time (MRT) of a solute and a particle (<2 mm) marker in the gastrointestinal tract (GIT) and the reticulorumen (RR) of a small grazer, the Indian blackbuck (*Antilope cervicapra*, $n = 5$, body mass of 26 ± 4 kg) and an intermediate feeder, the nilgai (*Boselaphus tragocamelus*, $n = 5$, body mass of 168 ± 21 kg). MRT_{solute} and MRT_{particle} were 29 ± 4.1 h and 60 ± 6.6 h in blackbuck and 28 ± 2.5 h and 54 ± 8.9 h in the nilgai for the GIT, and 14 ± 1.7 h, 45 ± 5.0 h, 19 ± 2.0 h and 45 ± 8.4 h for the RR, respectively. With a selectivity factor (SF, the ratio of MRT_{particle} to MRT_{solute}) in the RR of 3.2 ± 0.28 for blackbuck and 2.3 ± 0.36 for nilgai, both species are clearly in the category of ‘cattle-type’ ruminants. In particular, the high SF_{RR} of blackbuck, in spite of its small body size, is remarkable, and leads to specific predictions on the RR anatomy of this species (such as a particularly large omasum), which can be tested in further studies. The adaptive value of a high SF_{RR} is mainly considered as an increase in microbial productivity in the RR.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Theory of ruminant feeding types, characterised by their preferred food plants and the correlated morphology of the gastrointestinal tract (GIT) (Hofmann, 1989), has been developed on East African ruminants. However, ruminants also evolved to considerable diversity in other geographic regions, such as India with a prominent fauna of cervids and bovids. Among the most interesting in terms of feeding ecology and morphophysiological adaptations of the GIT is the Indian blackbuck (*Antilope cervicapra*), the only representative of the Antilopinae considered a strict grazer. Grasses represent its major food resource under free-ranging conditions (Schaller, 1967; Chattopadhyay and Bhattacharya, 1986; Goyal et al., 1988; Henke et al., 1988; Jhala, 1997; Solanki and Naik, 1998), but also out of their natural habitat, blackbuck prefer grass if available (Henke et al., 1988; Frisina and Frisina, 1997). The blackbuck is small and with 25–35 kg represents the lower end of

the body mass (BM) continuum of grazing ruminants. Body mass has been discussed as an alternative explanation for patterns of ruminant digestive physiology. The negative correlation of BM and selectivity has been suggested to be a main driver of morpho-physiological adaptations, and typical grazer attributes to be reserved to larger grazing taxa only (Gordon and Illius, 1994; Robbins et al., 1995). However, despite its low BM, the feeding type classification of Indian blackbuck as a grazer (Hofmann, 1991) is supported by a set of morphological characters such as a comparatively large rumen (Henke et al., 1988), small salivary glands (Hofmann et al., 2008), large masseter muscles (Clauss et al., 2008a), distinct ruminal papillation pattern (Clauss et al., 2009c), and prominent reticular crests (Clauss et al., 2010a). However, further evidence from digestive physiology is still missing. Various feeding studies have been performed with blackbuck (Pathak et al., 1992; Garg et al., 2002; Das et al., 2012), but patterns of digesta passage such as the ratio of the mean retention time of small particles and solute/fluid in the gut ($MRT_{\text{particle}}/MRT_{\text{solute}}$, the ‘selectivity factor’ SF) have not been quantified in this species to date. Such traits have been linked to ruminant feeding types (Hummel et al., 2005; Clauss et al., 2006), and more recently have been used to characterise two prototypes of reticulorumen (RR) physiology: the ‘moose-type’ with a comparatively low, and the ‘cattle-type’ with a relatively high ruminal fluid throughput (Clauss et al., 2010b). If the SF_{RR} (selectivity factor for the reticulorumen) is considered to reflect a digestive strategy of certain

* Corresponding author at: Department of Animal Sciences, Ruminant Nutrition, University of Göttingen, Kellnerweg 6, 37077 Göttingen, Germany.

E-mail address: jhummel@gwdg.de (J. Hummel).

¹ Present address: Naturschutz-Tierpark Görlitz e. V., Zittauer Strasse 43, 02826 Görlitz, Germany.

² Present address: Division of Ambulatory Service and Herd Health, Department for Farm Animals, Vetsuisse Faculty, University of Zurich, Switzerland.

³ Present address: Swiss Tropical and Public Health Institute, Basle, Switzerland.

ruminants, it likely bears some physiological/nutritional advantage. A high SF_{RR} ('cattle-type') has been regarded as an indication for a distinct stratification of rumen contents, with a dry 'fibre mat' on top of a more fluid layer and a low-viscosity rumen fluid, as for example in addax (*Addax nasomaculatus*) (Hummel et al., 2008; Clauss et al., 2009b) or cattle (*Bos taurus*) (Hummel et al., 2009; Lechner et al., 2010). In contrast, species with a low SF_{RR} ('moose-type') have been reported to have comparatively homogenous rumen contents with a more viscous rumen fluid, such as roe deer (*Capreolus capreolus*) (Behrend et al., 2004; Clauss et al., 2009a) or moose (*Alces alces*) (Clauss et al., 2009b; Lechner et al., 2010). Furthermore, these digestion types differ in the presence ('cattle-type') or absence ('moose type') of a distinct gas dome in the rumen (Tschuur and Clauss, 2008). A higher microbial output has been shown for fermentation chambers with a high dilution rate (Owens and Goetsch, 1986), which would be increased by a high SF_{RR} in ruminants.

Based on the reported distinct difference in papillation of the dorsal and the atrium rumen mucosa, which is assumed to be directly linked to the difference between the passage of fluids and small particles (Clauss et al., 2009c, 2011b; Hebel et al., 2011), we expected a high SF_{RR} in blackbuck of about 2.0 or higher. Another Asian ruminant, the nilgai (*Boselaphus tragocamelus*; 150–200 kg BM), was also included in the study. As for the blackbuck, to date no data on retention times of solutes and particles exist for this ruminant. It is generally classified as intermediate feeder, seasonally including considerable amounts of browse such as Acacia and other trees in its diet (Schaller, 1967). Its food choice in its Asian habitat is described as including grass, but being clearly dominated by browse (Dinerstein, 1979; Khan, 1994; Sankar et al., 2004); free-ranging animals in Texas display higher proportions of grass but are still clearly identified as intermediate feeders (Sheffield, 1983), a classification that was also confirmed in experimental feeding plots in India (Solanki and Naik, 1998). In consequence, a lower SF_{RR} was expected for nilgai compared to blackbuck.

2. Methods

Five adult blackbuck antelopes (2 males, 3 females) and five adult nilgais (2 males, 3 females) at the Al Wabra Wildlife Preservation (AWWP), Qatar, were used in the study. They had been adapted to a diet of grass hay (Rhodes grass, *Chloris gayana*) and fresh alfalfa (*Medicago sativa*) for three weeks. While grass hay was provided ad libitum, fresh alfalfa was fed restricted to amounts of 0.16 kg (as fed) in blackbucks and 2.9 kg (as fed) in nilgai (dry matter content of fresh alfalfa was 15%; the proportion of grass hay in the ingested diet was 94% for blackbuck and 74% for nilgai on a dry matter basis). Blackbucks were kept individually for an adaptation period of 3 days in climatized indoor shifting pens that allowed feeding and faecal collection without direct contact to the animals, after which the one-week recording of individual food intake and faecal collection began in these pens. Nilgais were kept individually in outdoor enclosures that provided shade as well as shelter against wind and rain, and that were large enough to allow faecal collection without causing nervousness in the animals and having to shift animals. Animals had access to drinking water ad libitum. The food intake of the animals was quantified by weighing the amount of hay and fresh alfalfa offered and the amount of hay left over on a daily basis for six consecutive days (fresh alfalfa was always consumed completely). Animals were weighed at the end of the experiment (Table 1).

Nutrient contents of the forages were for Rhodes grass: ash 13.0% DM; crude protein (CP) 11.8% DM; neutral detergent fibre (ash corrected; NDFom) 68.3% DM; acid detergent fibre (ash corrected; ADFom) 34.0% DM; ether extracts (EE) 2.0% DM and for alfalfa: ash 15.1% DM; CP 22.4% DM; NDFom 38.9% DM; ADFom 26.7% DM; EE 2.7% DM. Blackbucks had an average diet composition of CP 12.4 ± 0.2% DM, NDFom 66.7 ± 0.9% DM and ADFom 33.6 ± 0.1% DM, while it was CP 14.7 ± 0.8% DM, NDFom 60.3 ± 2.2% DM and

Table 1

Body mass, food intake and digesta retention parameters in blackbuck (*Antelope cervicapra*) and nilgai (*Boselaphus tragocamelus*). Significant effects in bold.

	Blackbuck	Nilgai	p*
n	5	5	
BM (kg)	26 ± 4	168 ± 21	–
Dry matter intake (g/kg BM ^{0.75} /day)	40 ± 8.2	35 ± 8.5	0.421
MRT _{solute} GIT (h)	29 ± 4.1	28 ± 2.5	0.917
MRT _{particle} GIT (h)	60 ± 6.6	54 ± 8.9	0.421
SF _{GIT}	2.1 ± 0.16	1.9 ± 0.25	0.310
MRT _{solute} RR (h)	14 ± 1.7	19 ± 2.0	0.011
MRT _{particle} RR (h)	45 ± 5.0	45 ± 8.4	0.917
SF _{RR}	3.2 ± 0.28	2.3 ± 0.36	0.016

BM body mass, MRT mean retention time, GIT gastrointestinal tract, RR reticulorumen, SF selectivity factor (the ratio of MRT_{particle} to MRT_{solute}).

* Test for differences between the species.

ADFom 32.0 ± 0.5% DM in nilgai. All nutrient analyses were done according to VDLUFA (2012) (ash: method 8.1; NDFom/ADFom: method 6.5.1/6.5.2; CP (Dumas): method 4.1.2; EE: method 5.1.1).

Dissolved cobalt(Co)-EDTA and chromium(Cr)-mordanted fibre (<2 mm) prepared from grass hay according to Udén et al. (1980) were used as markers for the fluid and the particle phase, respectively. A pulse-dose of the markers was fed to each animal and mixed into a handful of wheat bran. The latter was added to increase palatability and to guarantee the ingestion of the markers in a short time period. The marker was fed late in the afternoon and was well accepted. Blackbucks received approximately 0.4 g of Co-EDTA and 5 g of Cr-mordanted fibre and nilgais of 4 g and 30 g, respectively. Prior to marker feeding, three faecal samples were taken to analyse Co and Cr background levels. After marker feeding, faecal samples were taken regularly for 7 days, with intensive faecal sampling during the first two days and increasing time intervals subsequently; in the case of nilgai, sampling only occurred during daylight hours, but blackbucks were sampled also during the night (cf. sampling times indicated on the x-axes in Fig. 1, with evident night intervals missing for nilgai). Note that the equation used to determine mean retention times in this study is not affected by sampling interval (Van Weyenberg et al., 2006). A representative subsample of all defecations was stored frozen until drying at 60 °C and milling with a centrifuge mill (Retsch 2M1, 1 mm sieve; Retsch, Haan, Germany).

Marker analysis followed the procedure outlined by Behrend et al. (2004) and Hummel et al. (2005); a wet ashing with sulfuric acid (72%) was followed by atomic absorption spectroscopy. From the resulting faecal marker concentrations, mean retention time in the GIT was calculated according to Thielemans et al. (1978)

$$MRT = \sum(t_i * dt * c_i) / \sum(dt * c_i)$$

with t_i = time after marker application (h), dt = time interval represented by marker concentration (calculated as $((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2$), and c_i = faecal marker concentration at time i (mg/kg DM). The middle of the sampling intervals was used as t_i .

MRT in the RR was estimated following Lechner-Doll et al. (1990): MRT_{solute}RR is determined by estimating the rate constant of the descending part of the marker excretion curve via an exponential equation:

$$y = A * e^{-k * t}$$

with y = faecal marker concentration at time t (mg/kg DM), A = a constant, k = rate-constant (h^{-1}) and t = time after marker dosing (h); the reciprocal of k represents the MRT for the RR. MRT_{particle}RR is calculated based on the assumption that fluid and particles do not

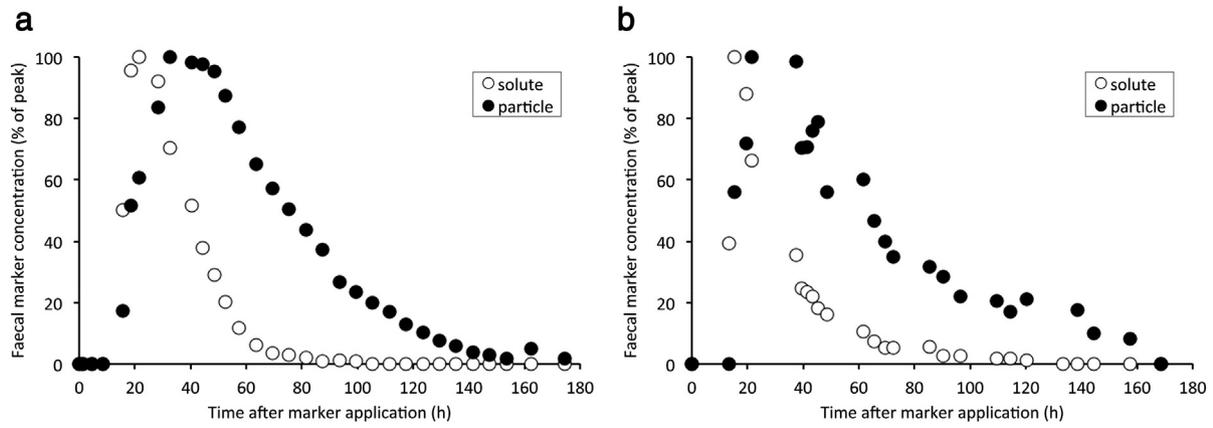


Fig. 1. Faecal excretion patterns for a solute and a particle (<2 mm) marker in a) a blackbuck (*Antelope cervicapra*) and b) a nilgai (*Boselaphus tragocamelus*) of this study. Note the absence of data in night-time intervals in nilgai, where faeces could only be collected during daylight hours.

differ in passage characteristics distal to the RR (Mambrini and Peyraud, 1997):

$$\text{MRT}_{\text{particleRR}} = \text{MRT}_{\text{particleGIT}} - (\text{MRT}_{\text{soluteGIT}} - \text{MRT}_{\text{soluteRR}}).$$

The 'selectivity factor' (SF, the ratio of $\text{MRT}_{\text{particle}}/\text{MRT}_{\text{solute}}$) was calculated for both the total GIT and the RR.

Mann-Whitney U test was used for the comparison of the species. Statistical calculations were done using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

3. Results

Dry matter intake was 0.47 kg/day for blackbuck and 1.6 kg/day for nilgai, resulting in body mass related intakes on a level of 40 g/kg $\text{BM}^{0.75}$ /day for blackbucks and 35 g/kg $\text{BM}^{0.75}$ /day for nilgai during the trial (Table 1). Typical digesta marker excretion curves for blackbuck and nilgai are given in Fig. 1. No differences were found between the species in MRT GIT and $\text{MRT}_{\text{particleRR}}$, but $\text{MRT}_{\text{soluteRR}}$ and in consequence also SF_{RR} differed significantly between the two species (Table 1). Hypotheses on consequences of SF rather focus on the rumen reticulum, since the difference in retention times of solutes and particles are largely due to processes in this part of the GIT. Selectivity factor values for the total GIT are always lower due to the

largely uniform flow of particles and solutes distal to the ruminoreticulum. Values for retention time in the RR are considered to reflect the mechanisms of interest best, therefore.

4. Discussion

Evaluation of the values for the blackbucks of this study arrives at an unequivocal result: a SF_{RR} of 3.2 (Table 1), is at the higher end of values measured for grazing ruminants, and clearly above the level typical for browsing ruminants. It resembles, in its magnitude, values previously reported in cattle (Hummel et al., 2005). When comparing the measurements of this study to a data collection from typical 'cattle-type' and 'moose-type' ruminants (Fig. 2a), it is evident that blackbuck and nilgai fall within the range of 'cattle-type', but with blackbuck being much more extreme in this respect. Comparing the SF_{RR} to the rumen papillae distribution (Clauss et al., 2009c), a similar pattern emerges, with blackbuck displaying even higher SF_{RR} than expected from ruminal papillae distribution, and nilgai close to the expected range (Fig. 2b). The comparison of the two species does not imply a major influence of BM on patterns of retention times of ruminants: A high SF_{RR} is a typical trait of cattle, the prime example of a grazing ruminant. The small grazer blackbuck matches the cattle pattern more than the larger intermediate feeder nilgai (although both can be considered 'cattle-type' ruminants based on their retention time pattern).

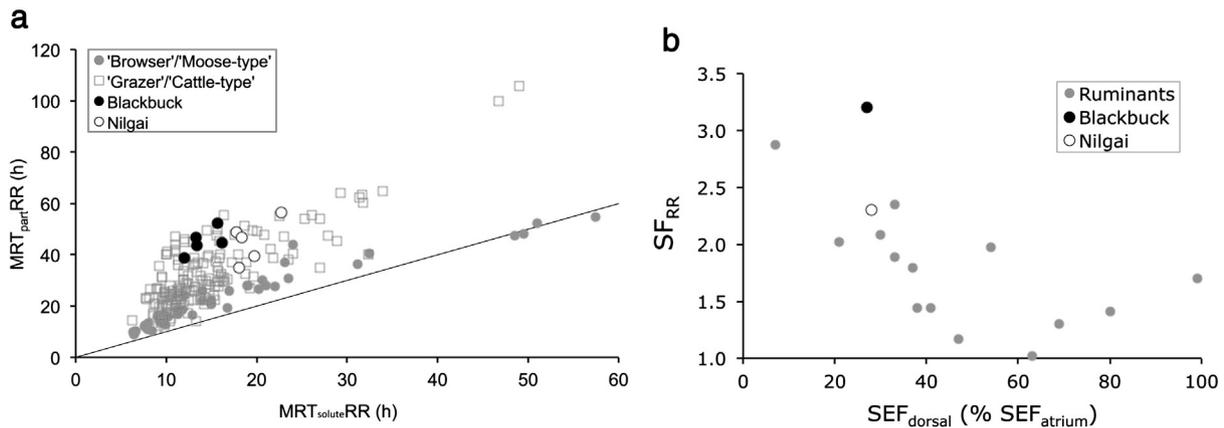


Fig. 2. Comparison of the data on blackbuck (*Antelope cervicapra*) and nilgai (*Boselaphus tragocamelus*) from this study with literature data. a) The relationship of the mean retention time (MRT) of solute and particle (<2 mm) markers in the reticulorumen (RR) as compared to 'moose-type' and 'cattle-type' ruminants from Clauss et al. (2010b). Note that both species plot within the 'cattle-type' ruminants. b) The relationship between the intraruminal papillation pattern, expressed as the surface enlargement factor (SEF) in the dorsal rumen as a percentage of the SEF in the ruminal atrium (a larger value represents a more homogenous intraruminal papillation) from Clauss et al. (2009c) and the average selectivity factor (SF, the ratio of $\text{MRT}_{\text{particle}}/\text{MRT}_{\text{solute}}$) in the RR (from Hebel et al., 2011). Note that while nilgai plot within the expected range, the SF_{RR} of this study indicates that the papillation value reported previously might be too high for blackbuck.

Initial concepts to explain the difference between the digestion types focussed on the potential advantages of rumen content stratification, in particular on the physical effect of the 'fibre mat'. This mat was assumed to retain particles particularly efficiently due to the so-called 'filter-bed effect' (the trapping of small particles in the mat), thus potentially enhancing the efficiency of the sorting mechanism as well as the digestibility of fibre (Clauss et al., 2008b). However, a comparative feeding study indicated that the rumen sorting mechanism is independent of the presence of rumen contents stratification (Lechner et al., 2010) and that the 'filter-bed effect' was caused in a similar way by diet in either digestion type, suggesting that the different physiological adaptations do not markedly influence this process (Clauss et al., 2011a; Lauper et al., 2013). Therefore, other ultimate causes for the difference between 'cattle-type' and 'moose-type' ruminants need to be invoked.

Hummel et al. (2008) for the first time introduced a concept from livestock science into the field of comparative digestive physiology of wild ruminants: the positive influence of an increased ruminal fluid passage (dilution) on the production of microbes from the rumen. The fact that the difference in SF_{RR} between the grazer blackbuck and the intermediate feeder nilgai was due to $MRT_{soluteRR}$ can be interpreted to support the relevance of this concept. In vitro and in vivo evidence on the influence of dilution rate on production of microbial mass is summarized in Owens and Goetsch (1986) and Martínez et al. (2009). Based on in vitro evidence, Hespell and Bryant (1979) (as cited in Owens and Goetsch, 1986) proposed the following equation to estimate the efficiency of microbial production: Efficiency of microbial production (g microbial N/kg Fom) = $(m * D^{-1} + Y_{max}^{-1})^{-1}$, with N = nitrogen (a proxy for protein), Fom = truly fermented organic matter, m = maintenance coefficient (0.000451 kg OM/(g microbial N * h)), D = dilution rate (h^{-1}) and Y_{max} = maximum yield (46.7 g microbial N/kg Fom).

This equation can be used to evaluate dilution rate-mediated differences in rumen microbial production between typical 'moose-' and 'cattle-type' ruminants. Mean retention times for solutes assumed for cattle were 11.4 h and for sheep 9.1 h (Colucci et al., 1990), for giraffe 16 h (Clauss, 1998) and for okapi 17 h (Hummel et al., 2005). Diets of the animals were characterised by an app. 50% concentrate/50% Lucerne hay ratio and intakes of 55–75 g/kg $BM^{0.75}$. The resulting passage (= dilution) rates of 0.088 and 0.110 h^{-1} for 'cattle-type' (cattle and sheep) and 0.063 and 0.059 h^{-1} for 'moose-type' (giraffe and okapi) ruminants result in an app. 10% higher microbial efficiency for 'cattle-type' ruminants (38.5 vs. 34.7 g microbial N/kg Fom). The blackbucks of this trial would arrive at a value of 36.1 g microbial N/kg Fom (retention time RR^{-1} = dilution rate / fractional rate of passage from the $RR = 0.071 h^{-1}$) compared to a value of 33.4 g/kg Fom for the nilgai (dilution rate 0.053 h^{-1}). While overall level of values might be an object of discussion (e.g. the German feeding system for cattle currently assumes a production of 26 g microbial N/kg Fom), the general effect of an increased dilution rate is not questioned. In artificial fermenters, at some point maximising dilution rate is surely limited due to increasing washout of bacterial cells (e.g. Herbert et al., 1956). In ruminants feeding on browse (the common situation among 'moose-type' ruminants), a further factor has been discussed: Ruminal detoxification (e.g. via tannin-binding proteins in saliva) (Robbins et al., 1991; Hagermann and Robbins, 1993; Fickel et al., 1998) could be decreased if fluid passage speeds up (just as digestion of soluble fibre, which is more prominent in browse compared to grass; (Robbins, 1993 p. 248)). Additionally, detoxification may simply be less efficient if larger volumes of saliva are being produced to increase fluid throughput, because the production of tannin-binding salivary proteins is limited (Hofmann et al., 2008; Codron and Clauss, 2010). The optimal relation of $MRT_{particle}$ and MRT_{solute} therefore probably differs between grass and browse.

The results of this study lead to specific predictions on blackbuck and nilgai digestive anatomy that can be corroborated in future studies: given the prominent SF_{RR} of both species, they can be expected to

display a distinct difference in the fluid content of the dorsal and ventral rumen contents (cf. Codron and Clauss, 2010), indicative of a clear rumen contents stratification. While the data on the intraruminal papillation pattern of the three nilgai from (Clauss et al., 2009c) are in good agreement with the SF_{RR} values (Fig. 2b), that of the one blackbuck from the same publication appears questionable, and it is expected that further measurements suggest a larger difference between the dorsal papillation and that of the atrium ruminis. Additionally, blackbucks are expected to display a particularly large omasum for the reabsorption of the high fluid throughput through the reticulorumen.

Acknowledgements

We thank H.E. Sheikh Saoud bin Mohammed bin Ali Al-Thani for his support of scientific investigations. We also thank the whole team of AWWP for their contributions in the realisation of this study, especially Dr. Abdi Arif, Dr. Amrita Deb, Muthu Krishna Chellapandi Thevar, Herbert Paje and Durga Bahadur Sarki. The experimental work was part of the Zoo Research Camp 2008 organized by Al Wabra Wildlife Preservation and the Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich. Nadja Wahl and Petra Jacquemien (Institute of Animal Science, Friedrich-Wilhelms-Universität Bonn) are thanked for sample analyses.

References

- Behrend, A., Lechner-Doll, M., Streich, W.J., Clauss, M., 2004. Seasonal faecal excretion, gut fill, liquid and particle marker retention in mouflon (*Ovis ammon musimon*), and a comparison with roe deer (*Capreolus capreolus*). *Acta Theriol.* 49, 503–515.
- Chattopadhyay, B., Bhattacharya, T., 1986. Food habits of blackbuck (*Antelope cervicapra*) of Ballavpur Wildlife Sanctuary, West Bengal, India. *Trop. Ecol.* 27, 93–100.
- Clauss, M., 1998. Feeding giraffe (*Giraffa camelopardalis*). (MSc. thesis). The Zoological Society of London, London.
- Clauss, M., Hummel, J., Streich, W.J., 2006. The dissociation of the fluid and particle phase in the forestomach as a physiological characteristic of large grazing ruminants: an evaluation of available, comparable ruminant passage data. *Eur. J. Wildl. Res.* 52, 88–98.
- Clauss, M., Hofmann, R.R., Streich, W.J., Fickel, J., Hummel, J., 2008a. Higher masseter mass in grazing than in browsing ruminants. *Oecologia* 157, 377–385.
- Clauss, M., Kaiser, T., Hummel, J., 2008b. The morphophysiological adaptations of browsing and grazing mammals. In: Gordon, I.J., Prins, H.H.T. (Eds.), *The Ecology of Browsing and Grazing*. Springer, Heidelberg, pp. 47–88.
- Clauss, M., Fritz, J., Bayer, D., Hummel, J., Streich, W.J., Südekum, K.-H., Hatt, J.-M., 2009a. Physical characteristics of rumen contents in two small ruminants of different feeding type, the mouflon (*Ovis ammon musimon*) and the roe deer (*Capreolus capreolus*). *Zoology* 112, 195–205.
- Clauss, M., Fritz, J., Bayer, D., Nygren, K., Hammer, S., Hatt, J.-M., Südekum, K.-H., Hummel, J., 2009b. Physical characteristics of rumen contents in four large ruminants of different feeding type, the addax (*Addax nasomaculatus*), bison (*Bison bison*), red deer (*Cervus elaphus*) and moose (*Alces alces*). *Comp. Biochem. Physiol. A* 152, 398–406.
- Clauss, M., Hofmann, R.R., Fickel, J., Streich, W.J., Hummel, J., 2009c. The intraruminal papillation gradient in wild ruminants of different feeding types: implications for rumen physiology. *J. Morphol.* 270, 929–942.
- Clauss, M., Hofmann, R.R., Streich, W.J., Fickel, J., Hummel, J., 2010a. Convergence in the macroscopic anatomy of the reticulum in wild ruminant species of different feeding types and a new resulting hypothesis on reticular function. *J. Zool.* 281, 26–38.
- Clauss, M., Hume, I.D., Hummel, J., 2010b. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. *Animal* 4, 979–992.
- Clauss, M., Lechner, I., Barboza, P., Collins, W., Tervoort, T., Südekum, K.-H., Codron, D., Hummel, J., 2011a. The effect of size and density on the mean retention time of particles in the reticulorumen of cattle (*Bos primigenius f. taurus*), muskoxen (*Ovibos moschatus*) and moose (*Alces alces*). *Br. J. Nutr.* 105, 634–644.
- Clauss, M., Lunt, N., Ortmann, S., Plovmann, A., Codron, D., Hummel, J., 2011b. Fluid and particle passage in three duiker species. *Eur. J. Wildl. Res.* 57, 143–148.
- Codron, D., Clauss, M., 2010. Rumen physiology constrains diet niche: linking digestive physiology and food selection across wild ruminant species. *Can. J. Zool.* 88, 1129–1138.
- Colucci, P.E., Macleod, G.K., Grovum, W.L., McMillan, I., Barney, D.J., 1990. Digesta kinetics in shetle feed diets with different forage to concentrate ratios at high and low intakes. *J. Dairy Sci.* 73, 2143–2156.
- Das, A., Katole, S., Kumar, A., Gupta, S.P., Saini, M., Swarup, D., 2012. Feed consumption, nutrient utilization and serum metabolite profile of captive blackbucks (*Antelope cervicapra*) fed diets varying in crude protein content. *J. Anim. Physiol. Anim. Nutr.* 96, 442–449.
- Dinerstein, E., 1979. An ecological survey of the Royal Karnali-Bardia Wildlife Refuge, Nepal. Part II: habitat/animal interactions. *Biol. Conserv.* 16, 265–300.

- Fickel, J., Göritz, F., Joest, B.A., Hildebrandt, T., Hofmann, R.R., Breves, G., 1998. Analysis of parotid and mixed saliva in roe deer (*Capreolus capreolus*). *J. Comp. Physiol. B* 168, 257–264.
- Frisina, M.R., Frisina, R.M., 1997. Fall diet of red and fallow deer, blackbuck, and mouflon sheep on Argentina's Patagonian steppe. *Rangel. Arch.* 19, 26–28.
- Garg, A.K., Sahoo, A., Arora, B.M., Pathak, N.N., 2002. Utilisation of cereal green fodder in blackbuck and different species of deer under semi-captivity. *Anim. Nutr. Feed Technol.* 2, 75–81.
- Gordon, I.J., Illius, A.W., 1994. The functional significance of the browser-grazer dichotomy in African ruminants. *Oecologia* 98, 167–175.
- Goyal, S.P., Bohra, H.C., Ghosh, P.K., Prakash, I., 1988. Role of *Prosopis cineraria* pods in the diet of two Indian desert antelopes. *J. Arid Environ.* 14, 285–290.
- Hagermann, A.E., Robbins, C.T., 1993. Specificity of tannin-binding salivary proteins relative to diet selection by mammals. *Can. J. Zool.* 71, 628–633.
- Hebel, C., Ortmann, S., Hammer, S., Hammer, C., Fritz, J., Hummel, J., Clauss, M., 2011. Solute and particle retention in the digestive tract of the Phillip's dikdik (*Madoqua saltiana phillipsi*), a very small browsing ruminant: biological and methodological implications. *Comp. Biochem. Physiol. A* 159, 284–290.
- Henke, S.C., Demarais, S., Pfister, J.A., 1988. Digestive capacity and diets of white-tailed deer and exotic ruminants. *J. Wildl. Manag.* 52, 595–598.
- Herbert, D., Elsworth, R., Telling, R.C., 1956. The continuous culture of bacteria: a theoretical and experimental study. *J. Gen. Microbiol.* 14, 601–622.
- Hespeel, R.B., Bryant, M.P., 1979. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on Y_{ATP} . *J. Anim. Sci.* 49, 1640–1659.
- Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia* 78, 443–457.
- Hofmann, R.R., 1991. Endangered tropical herbivores – their nutritional requirements and habitat demands. In: Ho, Y.W., Wong, H.K., Abdullah, N., Tajuddin, Z.A. (Eds.), *Recent Advances on the Nutrition of Herbivores*. Malaysia Society of Animal Production, UPM Serdang, pp. 27–34.
- Hofmann, R.R., Streich, W.J., Fickel, J., Hummel, J., Clauss, M., 2008. Convergent evolution in feeding types: salivary gland mass differences in wild ruminant species. *J. Morphol.* 269, 240–257.
- Hummel, J., Clauss, M., Zimmermann, W., Johanson, K., Norgaard, C., Pfeffer, E., 2005. Fluid and particle retention in captive okapi (*Okapia johnstoni*). *Comp. Biochem. Physiol. A* 140, 436–444.
- Hummel, J., Steuer, P., Südekum, K.-H., Hammer, S., Hammer, C., Streich, W.J., Clauss, M., 2008. Fluid and particle retention in the digestive tract of the addax antelope (*Addax nasomaculatus*) – adaptations of a grazing desert ruminant. *Comp. Biochem. Physiol. A* 149, 142–149.
- Hummel, J., Südekum, K.-H., Bayer, D., Ortmann, S., Hatt, J.-M., Streich, W.J., Clauss, M., 2009. Physical characteristics of reticuloruminal contents of cattle in relation to forage type and time after feeding. *J. Anim. Physiol. Anim. Nutr.* 93, 209–220.
- Jhala, Y.V., 1997. Seasonal effects on the nutritional ecology of blackbuck (*Antelope cervicapra*). *J. Appl. Ecol.* 34, 1348–1358.
- Khan, J.A., 1994. Food habits of ungulates in dry tropical forest of Gir Lion Sanctuary, Gujarat, India. *Acta Theriol.* 39, 185–193.
- Lauper, M., Lechner, I., Barboza, P., Collins, W., Hummel, J., Codron, D., Clauss, M., 2013. Rumination of different-sized particles in muskoxen (*Ovibos moschatus*) and moose (*Alces alces*) on grass and browse diets, and implications for rumination in different ruminant feeding types. *Mamm. Biol.* 78, 142–152.
- Lechner, I., Barboza, P., Collins, W., Fritz, J., Günther, D., Hattendorf, B., Hummel, J., Südekum, K.-H., Clauss, M., 2010. Differential passage of fluids and different-sized particles in fistulated oxen (*Bos primigenius f. taurus*), muskoxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*) and moose (*Alces alces*): rumen particle size discrimination is independent from contents stratification. *Comp. Biochem. Physiol. A* 155, 211–222.
- Lechner-Doll, M., Rutagwenda, T., Schwartz, H.J., Schultka, W., von Engelhardt, W., 1990. Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing in a thornbush savanna pasture in Kenya. *J. Agric. Sci.* 115, 409–420.
- Mambri, M., Peyraud, J.L., 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculation based on fecal collection. *Reprod. Nutr. Dev.* 37, 427–442.
- Martínez, M.E., Ranilla, M.J., Ramos, S., Tejido, M.L., Carro, M.D., 2009. Effects of dilution rate and retention time of concentrate on efficiency of microbial growth, methane production, and ruminal fermentation in RUSITEC fermenters. *J. Dairy Sci.* 92, 3930–3938.
- Owens, F.N., Goetsch, A.L., 1986. Digesta passage and microbial protein synthesis. In: Milligan, L.P., Grovum, W.L., Dobson, A. (Eds.), *Control of Digestion and Metabolism in Ruminants*. Reston Book, Englewood Cliffs, pp. 196–223.
- Pathak, N.N., Kewalramani, N., Kamra, D.N., 1992. Intake and digestibility of oats (*Avena sativa*) and berseem (*Trifolium alexandrinum*) in adult blackbuck (*Antelope cervicapra*). *Small Rumin. Res.* 9, 265–268.
- Robbins, C.T., Hagerman, A.E., Austin, P.J., McArthur, C., Hanley, T.A., 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *J. Mammal.* 72, 480–486.
- Robbins, C.T., 1993. *Wildlife Feeding and Nutrition*. Academic Press, San Diego.
- Robbins, C.T., Spalinger, D.E., Van Hoven, W., 1995. Adaptations of ruminants to browse and grass diets: are anatomical-based browser-grazer interpretations valid? *Oecologia* 103, 208–213.
- Sankar, K., Johnsingh, A.J.T., Acharya, B., 2004. Blue bull or nilgai (*Boselaphus tragocamelus*, Pallas, 1766). In: Sankar, K., Goyal, S.P. (Eds.), *Ungulates of India*. ENVIS bulletin: wildlife and protected areas vol. 71. Wildlife Institute of India, Dehradun, India, pp. 120–143.
- Schaller, G.B., 1967. *The deer and the tiger. A study of wildlife in India*. University of Chicago Press, Chicago IL.
- Sheffield, W.J., 1983. Food habits of nilgai antelope in Texas. *J. Range Manag.* 36, 316–322.
- Solanki, G.S., Naik, R.M., 1998. Grazing interactions between wild and domestic herbivores. *Small Rumin. Res.* 27, 231–235.
- Thielemann, M.-F., Francois, E., Bodart, C., Thewis, A., 1978. Mesure du transit gastro-intestinal chez le porc à l'aide des radiolanthanides. Comparaison avec le mouton. *Ann. Biol. Anim. Biochim. Biophys.* 18, 237–247.
- Tschur, A., Clauss, M., 2008. Investigations on the stratification of forestomach contents in ruminants: an ultrasonographic approach. *Eur. J. Wildl. Res.* 54, 627–633.
- Udén, P., Colucci, P.E., Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31, 625–632.
- Van Weyenberg, S., Sales, J., Janssens, G.P.J., 2006. Passage rate of digesta through the equine gastrointestinal tract: a review. *Livest. Sci.* 99, 3–12.
- VDLUFA, 2012. *Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. und Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch) Die Chemische Untersuchung von Futtermitteln Bd. III. VDLUFA-Verlag, Darmstadt, Germany.*