



# Effect of pelleted cereal-based feed used in the diet on feed intake, eating behaviour, rumination and nutrient digestibility in antelope sitatunga (*Tragelaphus speki*)

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**ABSTRACT.** The aim of the study was to determine the effect of pelleted cereal-based feed used in the diet for antelope sitatunga (*Tragelaphus speki*) on feed intake, eating behaviour, rumination and nutrient digestibility. Three male sitatunga were fed a basal diet (chopped dehydrated lucerne, ground cereals, soyabean meal, vegetables and fruits) with *ad libitum* access to meadow hay. The animals were allocated to 1 of 3 treatments according to 3 × 3 Latin square design and fed diets where 0, 50 or 100% of dry matter (DM) from basal diet was replaced with a pelleted cereal-based feed. DM intake of the basal diet and meadow hay, as well as overall DM intake and organic matter (OM) intake did not differ between treatments ( $P > 0.05$ ). The eating rate ( $\text{g DM} \cdot \text{min}^{-1}$ ) of the basal diet increased ( $P = 0.03$ ) whereas eating time ( $\text{min} \cdot \text{day}^{-1}$ ) and eating frequency ( $\text{n} \cdot \text{day}^{-1}$ ) tended to decrease ( $P \leq 0.07$ ) linearly as pelleted feed inclusion in the diet increased. On the other hand, a tendency ( $P = 0.07$ ) to longer time of hay intake ( $\text{min} \cdot \text{day}^{-1}$ ) was observed with increasing inclusion of pelleted cereal-based feed in the basal diet. Rumination frequency ( $\text{n} \cdot \text{day}^{-1}$ ), time ( $\text{min} \cdot \text{day}^{-1}$ ) and rate ( $\text{min} \cdot \text{g}^{-1}$  of DM intake) did not differ between treatments ( $P > 0.05$ ). Apparent total tract digestibility of OM decreased linearly ( $P = 0.05$ ) with increasing pellet inclusion in the diet. Pelleted cereal-based supplement used in the diet affects feeding behaviour and thus may affect health and welfare of sitatunga in zoological gardens.

## Introduction

In spite of the general recommendation that herbivores should not receive relevant amounts of fruits or grain-based concentrates (Lintzenich and Ward, 1997; Clauss and Dierenfeld, 2008), typical diets for many wild captive ruminants in zoological gardens still consist of roughages, browse, fruits, vegetables and concentrates. The proportion of those components in the diet, their sources (e.g., lucerne hay or meadow hay) and chemical composition of offered

feed (e.g., fibre or protein content) are widely accepted factors determining health of these animals (Schilcher et al., 2013; Taylor et al., 2013; Gattiker et al., 2014). It is especially accepted that the diet offered in captivity should promote the intake of structured feed (browse and roughages) and limit the intake of unstructured feed easily fermentable in the rumen (cereal-based products, vegetables and fruits), in order to stimulate the normal eating and rumination behaviour and prevent disturbances of rumen function (Hummel et al., 2006; Taylor et al., 2013;

Gattiker et al., 2014). However, unstructured feed intake, namely cereal-based products, often exceeds 50% of dry matter (DM) ingested by captive ruminants, especially browsers (Clauss et al., 2003; Clauss and Dierenfeld, 2008; Schilcher et al., 2013; Taylor et al., 2013). Therefore, the source, chemical composition and structure (e.g., ground or pelleted feed) of concentrates may have a substantial impact on feed intake, eating behaviour, rumination and nutrient digestion, and in consequence welfare and health of captive ruminants in zoological gardens.

When choosing a pelleted food, products that are not cereal-based and have a high fibre content should be preferred (Lintzenich and Ward, 1997; McCusker et al., 2011). Nevertheless, pelleted cereal-based supplements are widely used in diets for wild captive ruminants (Schilcher et al., 2013; Taylor et al., 2013; Gattiker et al., 2014). However, it was shown that pelleted feed increases feeding rate and decreases rumination time as well as decreases digesta retention time in the rumen and ruminal and total tract digestibility of fibre in livestock ruminants (Waghorn and Reid, 1983; Abouheif et al., 2012). These unfavourable effects may have also a negative impact on welfare and health of wild ruminants in captivity, especially when chemical composition of pelleted feed is not within the range recommended for the species. Besides a potential negative impact on forestomachs function, pelleted feed may also influence their natural feeding behaviour. Many ruminants exhibit a specific feeding behaviour, resulting in a selective intake of plant parts (leaves, stems etc.). In general, more digestible parts of the plant (leaves) are consumed rather than less digestible parts (stems). This is an important nutritional strategy which allows the coverage of energy requirement (Van Soest, 1996). When the method of feed provisioning limits opportunity for selective intake, for example when pelleted feed is used, the need of expressing natural feeding behaviour may remain unfulfilled (Van Soest, 1996).

The sitatunga (*Tragelaphus spekii*) is a medium sized, semi-aquatic, tropical antelope with many specific adaptations to wetland areas (Estes, 1991; Tweheyo et al., 2010) for which many different diets in the wild have been reported, ranging from grazing to intermediate feeding (Gagnon and Chew, 2000; Cerling et al., 2003; Sponheimer et al., 2003; Steuer et al., 2014). Most likely, the sitatunga is an intermediate feeder consuming the substantial proportion of grass and herbs in the natural diet (Gagnon and Chew, 2000; Sponheimer et al., 2003). However, herbs and sedges are more preferred by this antelope than climbers, grasses, water weeds, shrubs and

trees (Ndawula et al., 2011). Nevertheless, seasonal changes in the preferred feed occur, and agriculture crops are eaten when the access to natural sources of food is limited (Ndawula et al., 2011).

We hypothesize that using a pelleted cereal-based feed will affect feed intake, eating behaviour, rumination and nutrient digestibility of sitatunga when compared to a non-pelleted diet of similar nutrient composition. The aim of the study was to determine the effect of pelleted cereal-based feed inclusion in the diet for sitatunga (in exchange for concentrates and chopped dehydrated lucerne) on feed intake, eating frequency, time and rate, as well as rumination frequency, time and rate, and also nutrient digestibility. This study was stimulated by the fact that there are many different commercial feeds available on the market (differing in ingredient and chemical composition) and that there is still little known about how those feeds (not necessarily recommended for particular species) affect feed intake, feeding behaviour and nutrient digestibility in wild captive ruminants.

## Material and methods

The study was conducted in Silesia Zoological Garden (Chorzów) located in southern Poland (50°16'58.6"N, 18°59'42.1"E) between January and April 2014. The experimental procedures were reviewed by the local ethical committee.

### Animals, housing and diets

The study was performed on 3 male sitatunga at the age of 10 months, 2 and 7 years. The animals were kept in individual boxes, in a building equipped with an environmental control system. The temperature was set at 21 °C with a light from 6:00 to 21:00. The floor was bedded with wood shavings that were replaced daily, with exception of faecal collection periods when no bedding was applied. Such individual housing is a standard procedure for male sitatunga in Silesia Zoological Garden during winter season and is well tolerated by the animals.

The experimental design was arranged as 3 × 3 Latin square with 28-day periods: 7 days of gradual diet change, 14 days of diet adaptation and 7 days of data collection. Throughout the study, the animals were fed diet resembling a typical diet used for sitatunga in Silesia Zoological Garden in the winter season (Table 1).

The basal diet consisted of a thoroughly mixed finely ground oat, maize and wheat, oatmeal, soyabean meal, chopped dehydrated lucerne (2–4 cm), vegetables and fruits and was fed at 1.75 kg · day<sup>-1</sup> (as feed); meadow hay was offered *ad libitum*

**Table 1.** Chemical composition of feeds used in the experiment

Feed	Nutrient								
	dry matter	ash	crude protein	crude fat	crude fibre	cell wall components, % DM			NFC <sup>2</sup>
	%		% DM			NDF <sup>1</sup>	hemicellulose	cellulose	% DM
Pelleted feed <sup>3</sup>	90.5 ± 0.6	6.7 ± 0.4	14.6 ± 0.6	2.3 ± 0.3	10.1 ± 0.8	27.4 ± 0.8	13.6 ± 0.8	10.6 ± 0.3	49.0 ± 0.7
Chopped dehydrated lucerne	93.4 ± 2.9	8.4 ± 0.6	18.9 ± 1.2	1.8 ± 0.4	31.8 ± 2.8	46.2 ± 3.5	8.2 ± 1.4	29.3 ± 2.2	24.6 ± 2.0
Ground oat	91.5 ± 0.8	2.7 ± 0.2	10.9 ± 0.4	4.4 ± 1.6	8.9 ± 0.7	29.3 ± 3.8	18.3 ± 3.8	8.9 ± 0.7	52.7 ± 5.6
Ground maize	89.6 ± 1.2	1.2 ± 0.1	9.2 ± 0.5	4.3 ± 0.3	1.4 ± 0.6	7.4 ± 0.7	4.3 ± 1.1	2.4 ± 0.0	77.9 ± 1.3
Ground wheat	89.3 ± 0.8	1.9 ± 0.1	12.7 ± 0.3	2.0 ± 0.6	2.6 ± 1.0	13.6 ± 2.5	9.1 ± 2.3	2.9 ± 0.6	69.8 ± 3.3
Oatmeal	91.2 ± 0.6	2.1 ± 0.1	13.6 ± 0.7	5.7 ± 0.9	1.8 ± 0.8	15.2 ± 5.3	12.2 ± 5.5	1.4 ± 0.5	63.4 ± 6.1
Soyabean meal	92.3 ± 0.5	9.4 ± 0.1	50.2 ± 0.6	2.1 ± 0.4	3.6 ± 0.4	11.4 ± 3.3	4.3 ± 2.2	6.4 ± 1.1	27.0 ± 3.4
Apple	13.1 ± 0.7	3.8 ± 2.4	4.9 ± 3.6	2.4 ± 0.9	8.9 ± 1.3	14.2 ± 2.0	2.9 ± 1.0	8.5 ± 1.3	74.6 ± 6.6
Carrot	11.8 ± 0.6	6.4 ± 0.8	7.7 ± 1.3	1.7 ± 0.4	9.3 ± 0.8	11.6 ± 4.2	2.0 ± 0.7	7.7 ± 2.3	72.6 ± 5.4
Beetroot	12.1 ± 0.9	8.9 ± 0.4	15.1 ± 1.3	1.3 ± 0.1	7.0 ± 0.5	16.1 ± 0.3	6.1 ± 0.8	9.1 ± 0.7	58.6 ± 2.0
Meadow hay	90.2 ± 1.8	7.1 ± 0.1	7.7 ± 0.5	1.3 ± 0.3	33.4 ± 1.6	64.2 ± 2.5	23.1 ± 2.5	34.7 ± 2.5	19.7 ± 2.6

<sup>1</sup>NDF – neutral detergent fibre; <sup>2</sup>NFC – non-fibrous carbohydrates = 100 – (ash + crude protein + crude fat + NDF); <sup>3</sup>complete feed for small antelopes ('Morawski', Kcynia, Poland), consisting of (content in descending order according to the manufacturer declaration): ground cereals, high in protein meals, cereal flour and bran, dried lucerne and grass, tree leaves, canary seed, molasses, vitamin-mineral supplement

(diet A). Dietary treatments were applied by replacing 50% (diet B) or 100% (diet C) of dry matter (DM) provided with ground oat, maize and wheat, oatmeal, soyabean meal, chopped dehydrated lucerne in the diet A, with a commercial pelleted cereal-based feed (Complete Feed for Small Antelopes, 'Morawski', Kcynia, Poland). Diets were formulated to be similar for crude protein (CP) and crude fibre (CF) content (Table 2). Due to higher CP and CF contents in the pelleted feed, soyabean meal and dehydrated lucerne inclusions in the diets A and B were balanced to obtain similar CP and CF content for all diets. The basal diet and hay were offered in separate feeders: a plastic cuvette and plastic tub, respectively. The animals were fed once a day (8:30) and had *ad libitum* access to fresh water. The diet change between experimental periods was done gradually during 7 days.

### Sampling, analyses and calculations

The representative samples of feed were collected weekly, pooled by each period of the study and kept dry (concentrates, hay) or frozen (–18 °C; vegetables, fruits) for further analyses. Feed intake was controlled daily by weighting the amount of feed offered and refused next day. Refusals from the last week of each experimental period were collected, weighted and kept frozen (–18 °C; diet A) or dry (hay) for further analyses, and nutrient intake and nutrient digestibility calculations.

Eating and rumination behaviour of animals were recorded for two consecutive days (day 24 and 25 of each experimental period), using a digital video recorder (model BCS-0404LE-AN, Dahua Technology Co., Hangzhou, China) equipped with

3 high resolution colour day/night video cameras (EVA-TV-1200iRW, KAM-TECH, Kraków, Poland; angle lens 2.8 – 12 mm). The recordings were saved on a hard disk (resolution 720 × 576px, D1) with 6 frames per sec speed, continuously for 48 h. The recordings were watched by one person who summarized the number of feeding bouts and the times of eating and rumination for each animal and period. The feeding bout was determined as when the animal put his head into the cuvette or tub with feed and ended when the animal turned back and did not come back for at least 1 minute. On the basis of the episodes of eating, rumination and DM intake, the feeding bout frequency ( $n \cdot \text{day}^{-1}$ ), time ( $\text{min} \cdot \text{day}^{-1}$ ) and rate ( $\text{g DM} \cdot \text{min}^{-1}$ ), as well as rumination bouts ( $n \cdot \text{day}^{-1}$ ), time ( $\text{min} \cdot \text{day}^{-1}$  and  $\text{min} \cdot \text{bout}^{-1}$ ) and rate ( $\text{min} \cdot \text{g}^{-1}$  DM intake) were calculated. The eating and rumination rates were calculated by dividing DM intake by the time spent on eating or ruminating, respectively. Eating time and rate were calculated separately for the basal diet and hay.

The representative floor samples of faeces (not contaminated with urine) were collected (75 g per animal per day) from day 24 to 27 of each experimental period. The samples of each animal and experimental period were pooled together and kept frozen (–18 °C) for further chemical analyses. Apparent total tract digestibility was calculated using acid insoluble ash (AIA) as digestibility marker. Dry matter output in faeces was calculated by dividing the AIA intake ( $\text{g} \cdot \text{day}^{-1}$ ) by AIA content in faeces ( $\text{g} \cdot \text{kg}^{-1}$  of DM), and subsequently DM intake and DM output was used to calculate apparent total tract digestibility.

**Table 2.** Ingredients and chemical composition of diets<sup>1</sup>

Indices	Treatment/diet <sup>2</sup>		
	A	B	C
Ingredient, g · kg <sup>-1</sup>			
pelleted feed	0	585	1184
chopped dehydrated lucerne	233	117	0
ground oat	292	140	0
ground maize	117	59	0
ground wheat	233	117	0
oatmeal	233	117	0
soyabean meal	58	29	0
apple	175	176	175
carrot	175	176	175
beetroot	233	234	233
Ingredient, % dry matter (DM)			
pelleted feed	–	46.8	93.6
chopped dehydrated lucerne	18.7	9.4	–
ground oat	23.7	11.4	–
ground maize	9.3	4.6	–
ground wheat	18.1	9.0	–
oatmeal	18.7	9.4	–
soyabean meal	4.7	2.3	–
apple	2.5	2.5	2.5
carrot	1.9	1.9	1.9
beetroot	2.5	2.5	2.5
Chemical components			
DM, %	64.9 ± 0.9	64.6 ± 0.7	64.5 ± 0.5
ash, % DM	3.9 ± 0.1	5.3 ± 0.2	6.7 ± 0.4
crude protein, % DM	14.9 ± 0.2	14.6 ± 0.3	14.2 ± 0.5
crude fat, % DM	3.4 ± 0.6	2.8 ± 0.4	2.3 ± 0.3
crude fibre, % DM	9.8 ± 1.0	9.9 ± 0.9	10.0 ± 0.7
NDF <sup>3</sup> , % DM	23.2 ± 2.6	24.8 ± 1.5	26.5 ± 0.8
hemicellulose, % DM	10.7 ± 1.9	11.8 ± 1.0	13.0 ± 0.8
cellulose, % DM	9.5 ± 0.1	10.0 ± 0.2	10.5 ± 0.3
lignin, % DM	3.0 ± 0.6	3.1 ± 0.5	3.1 ± 0.4
NFC <sup>4</sup> , % DM	54.6 ± 2.9	52.5 ± 1.8	50.3 ± 0.8

<sup>1</sup>for each treatment meadow hay was offered *ad libitum*; <sup>2</sup>treatment/diet: A (basal) – without pelleted feed inclusion; B – 50% of concentrates and chopped dehydrated lucerne in the basal diet DM replaced with cereal-based pelleted feed; C – 100% of concentrates and chopped dehydrated lucerne in the basal diet DM replaced with cereal-based pelleted feed; <sup>3</sup>NDF – neutral detergent fibre; <sup>4</sup>NFC – non-fibrous carbohydrates = 100 – (ash + crude protein + crude fat + NDF)

Feeds, refusals and faeces were analysed for DM, ash, CP, crude fat and CF using standard analytical procedures (Nos. 930.15, 923.03, 990.03, 920.39, 962.09 for DM, ash, CP, crude fat and CF, respectively; AOAC International, 2000). Cell wall constituents, including neutral detergent fibre (NDF; Van Soest et al., 1991), acid detergent fibre (ADF) and lignin (Robertson and Van Soest, 1981) were determined using an Ankom<sup>220</sup> Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Cellulose content was calculated as ADF – lignin, where-

as hemicellulose as NDF – ADF. NDF, ADF and lignin results include residual ash. AIA was determined according to Van Keulen and Young (1977). Non-fibrous carbohydrates (NFC) content was calculated as NFC = 100 – (ash + CP + crude fat + NDF).

### Statistical analysis

Data were analysed as 3 × 3 Latin square design using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). The statistical model included treatment and period as fixed effects, and animal as random effect. DM intake was analysed as repeated measurements by including REPEATED statement in the model. The day of the study was considered as repeated measure. For eating behaviour and rumination data, a mean from two days of observation was used for statistical analysis. The best covariance structure was chosen on the basis of the lowest Akaike's Information Criterion. Polynomial contrasts were used to determine linear and quadratic effects of pelleted concentrate inclusion rate in the diet on feed intake, eating and rumination behaviour and nutrient digestibility. Significance was declared at  $P \leq 0.05$  and trends – when  $0.05 < P \leq 0.10$ .

### Results

It should be remembered that age (and thus body weight) of animals used in this study varied greatly (from 10 months to 7 years). As a result, standard deviation for some investigated parameters was high. For example, organic matter (OM) digestibility varied from 69 to 88%. Although the experimental design used in this study (Latin square design) allows for detecting statistical differences between treatments even when a high animal to animal variation is observed, it should be noticed that results presented in this paper are not necessarily representative for particular group of animals (e.g., growing or adult) or whole population.

Due to the fact that a commercial cereal-based pelleted feed for antelopes was used in this study, its exact ingredient and chemical composition could not be provided. The experimental diets were similar for CF, but NDF and hemicellulose contents increased with increasing pelleted feed inclusion in the diet (from 23.2 to 26.5% for NDF and from 10.7 to 13.0% for hemicellulose for diets A and C, respectively). Ash content increased (from 3.9 to 6.7% of DM for diet A and diet C, respectively) and NFC content decreased (from 54.6 to 50.3% of DM for diet A and diet C, respectively) with increasing pelleted feed inclusion in the diet.

Although refusals of both basal diet and hay were collected during the last week of each study period, there were only a few episodes when negligible amounts of the basal diet were refused. A substantial portion of refused hay was scattered around the feeder, so it was difficult to collect them precisely. As a result, chemical composition of refusals was not taken into account in the calculation of nutrient intake and nutrient digestibility. However, several samples of hay refusals were analysed for nutrient content, to determine whether there were any differences in chemical composition between hay offered and refused. This analysis showed that, on average, NDF and cellulose contents were higher by  $9.4 \pm 4.5$  and  $31.5 \pm 6.3\%$ , respectively, and NFC content was lower by  $30.0 \pm 9.3\%$  (mean  $\pm$  SD;  $n = 9$ ) in hay refusals, as compared to hay offered. This indicates that some selection of hay occurred. As a result, the nutrient intake and nutrient digestibility may be over- or underestimated in this study, because DM intake was calculated simply based on feed intake and DM content in feeds. Nevertheless, there was no effect of the diet on DM intake detected in this study, both with basal diet and hay (Table 3). As a result, there was no effect of pelleted feed on nutrient intake, except a tendency towards higher ash intake ( $P = 0.09$ ) with increasing pelleted feed inclusion in the diet. On the other hand, the apparent total tract digestibility of OM decreased linearly ( $P = 0.05$ ) and of CF tended to decrease linearly ( $P = 0.10$ ) with increasing inclusion of pelleted cereal-based feed.

The eating rate of the basal diet increased linearly ( $P = 0.03$ ) whereas eating time and frequency tended to decrease linearly ( $P \leq 0.07$ ) with increasing pelleted

feed inclusion (Table 4). On the other hand, eating time of hay tended to increase linearly ( $P = 0.07$ ) with increasing pelleted feed inclusion in the diet, and a quadratic tendency of changes for hay eating rate was shown ( $P = 0.10$ ) with the highest eating rate observed for diet B. The overall eating rate was the lowest for diet A and the highest for diet B (quadratic,  $P = 0.03$ ).

**Table 3.** Effect of cereal-based pelleted feed inclusion in diet for sitatunga on nutrient intake and nutrient digestibility

Indices	Treatment/diet <sup>1</sup>			SEM	Polynomial contrasts	
	A	B	C		linear	quadratic
Dry matter intake, kg · day <sup>-1</sup>						
basal diet	1.13	1.13	1.14	0.01	0.87	0.97
hay	0.26	0.29	0.31	0.02	0.68	0.91
total	1.39	1.42	1.44	0.02	0.69	0.91
Nutrient intake, g · day <sup>-1</sup>						
organic matter	1326	1330	1135	21	0.81	0.89
crude protein	187	186	186	2	0.97	0.90
crude fat	42	35	28	1	0.09	0.79
crude fibre	198	210	210	8	0.69	0.90
NDF <sup>2</sup>	424	473	492	16	0.46	0.88
hemicellulose	177	205	225	6	0.25	0.77
cellulose	199	217	219	8	0.64	0.95
NFC <sup>3</sup>	672	637	639	5	0.37	0.92
Apparent digestibility <sup>4</sup> , %						
organic matter	77	73	67	3	0.05	0.62
crude protein	74	71	67	3	0.22	0.87
crude fat	83	79	73	3	0.12	0.90
crude fibre	48	42	32	4	0.10	0.61
NDF	51	35	34	4	0.11	0.53
hemicellulose	59	54	41	4	0.11	0.52
cellulose	50	48	38	4	0.16	0.56
NFC	95	94	92	1	0.14	0.87

<sup>1</sup>see Table 2; <sup>2</sup>NDF – neutral detergent fibre; <sup>3</sup>NFC – non-fibrous carbohydrates; <sup>4</sup>total tract digestibility

**Table 4.** Effect of cereal-based pelleted feed inclusion in diet for sitatunga on eating behaviour and rumination

Indices	Treatment/diet <sup>1</sup>			SEM	Polynomial contrasts	
	A	B	C		linear	quadratic
Basal diet						
eating time, min · day <sup>-1</sup>	67.00	58.00	36.33	8.90	0.06	0.44
eating frequency, n · day <sup>-1</sup>	29.00	30.83	18.50	4.16	0.07	0.11
eating rate, g DM · min <sup>-1</sup>	18.97	24.01	33.38	3.76	0.03	0.43
Hay						
eating time, min · day <sup>-1</sup>	59.50	63.50	75.67	6.62	0.07	0.50
eating frequency, n · day <sup>-1</sup>	24.67	27.83	30.67	2.79	0.43	0.97
eating rate, g DM · min <sup>-1</sup>	3.38	5.68	3.60	1.07	0.82	0.10
Basal diet and hay						
eating time, min · day <sup>-1</sup>	126.50	121.50	112.00	10.78	0.14	0.71
eating frequency, n · day <sup>-1</sup>	53.67	58.67	49.17	5.71	0.37	0.17
eating rate, g DM · day <sup>-1</sup>	11.19	13.59	12.92	1.48	0.03	0.03
Rumination						
bouts, n · day <sup>-1</sup>	26.17	25.83	24.50	2.50	0.45	0.78
time, min · day <sup>-1</sup>	327.50	318.33	292.83	16.26	0.46	0.86
time, min · bout <sup>-1</sup>	14.01	12.57	13.12	1.36	0.39	0.56
time, min · g <sup>-1</sup> DM	0.24	0.22	0.21	0.01	0.12	0.86

<sup>1</sup>see Table 2

Rumination frequency ( $n \cdot \text{kg}^{-1}$ ), rumination time ( $\text{min} \cdot \text{day}^{-1}$ ) and rate ( $\text{min} \cdot \text{g}^{-1}$  of DM consumed) did not differ between treatments ( $P > 0.10$ ).

## Discussion

Feeding rate and time of structured and unstructured feeds were greatly modulated and total tract nutrient digestibility decreased by the use of pelleted feed in this study, which may affect health and welfare of sitatunga in captivity. Although this study possesses some weak points, which were hard to eliminate in the zoological garden conditions, its results may serve a valuable point in the discussion on optimal physical form and/or feeding strategy of concentrates and supplementary feed for wild captive ruminants.

In captivity sitatunga and other ruminants should be fed mostly roughages (browse, legumes or grass, depending on their natural diet) whereas vegetables, fruits and concentrates should be fed in limited amounts, in order to assure normal feeding and rumination behaviour and to avoid disturbances in gastrointestinal tract functioning (Schilcher et al., 2013; Taylor et al., 2013; Gattiker et al., 2014). However, it has been shown that some roughages offered in captivity, particularly grass or meadow hay, are not willingly consumed by some ruminants, especially browsers (Clauss et al., 2003; Taylor et al., 2013). This may lead to a high proportion of unstructured (often cereal-based) feed consumed in the diet by some ruminant species. This may result in a drop of rumen pH below the optimum for rumen function (e.g., rumen acidosis) and other related health problems, such as poor body condition or laminitis (Schilcher et al., 2013; Gattiker et al., 2014). In the present study, the basal diet (consisting mostly of unstructured feeds) was accounted as much as 80% of DM consumed by the animals. Such a high intake of unstructured feed was mainly a result of very low intake of hay by the youngest animal, due to high amount of basal diet offered (as for animal with a low body weight) and a strong preference for the basal diet than for hay. In general, the meadow hay offered in this study did not appear to be very palatable for the animals. Although such a situation could be avoided by high-quality meadow or lucerne hay provision, or by substituting cereal-based pellets with pellets of high fibre content (Lintzenich and Ward, 1997; Clauss and Dierenfeld, 2008; McCusker et al., 2011), diets rich in unstructured carbohydrates are still used for wild ruminants in captivity, leading to unwanted negative health consequences for this group of animals (Clauss and Dierenfeld, 2008; Schilcher et al., 2013; Taylor et al., 2013).

Although high concentrate intake is the main factor contributing to rumen acidosis, rumen pH is also affected by eating time and rate. Both eating time and rate affect saliva production, an important buffer of rumen pH (Beauchemin et al., 2008). It is estimated that in cattle, 30 to 40% of fermentation acid produced in the rumen is neutralized by saliva and saliva production during eating may be even two times higher than during resting (Allen, 1997; Beauchemin et al., 2008). In this study the use of pelleted feed decreased the eating time of the basal diet by up to 54% and substantially increased its eating rate. Both shorter eating time (and also numerically shorter rumination time) observed when pelleted feed was offered can be explained by the associated lower inclusion or lack of chopped dehydrated lucerne in the basal diet. The particle size of this component of the diet ranged from 2 to 4 cm so it can be classified as a structural feed. It has been shown that the eating rate of ungrounded roughages is lower in comparison to ground and pelleted roughages (Waghorn and Reid, 1983), which is a most likely result of easier consumption of pelleted feed as opposed to longer particles. Furthermore, both eating time and rumination time increased as proportion of structured feed in the diet increased, too (Zebeli et al., 2007; Yang and Beauchemin, 2009). Nevertheless, it cannot be excluded that the inclusion of a cereal-based pelleted feed had substantial impact on feeding behaviour of sitatunga in this study.

Because in the most cases feeds are finely ground prior to pelleting, it can be speculated that pelleting has a little impact on eating and ruminating behaviour, as compared to feeding ground but not-pelleted feeds. As a result, eating and ruminating behaviour of ruminants should be affected predominantly by structured feed intake, as it is suggested by the results of this study. This observations are in line with the findings of Castrillo et al. (2013) who showed no differences in eating pattern of ruminants fed ground or ground and pelleted concentrates, but are contrary to the study of Gimeno et al. (2015). For example, the size or hardness of pellets were shown to affect feeding pattern in cattle (Gimeno et al., 2015). It may also affect feed disintegration in the rumen and in consequence nutrient digestion (Bertipaglia et al., 2010; Gimeno et al., 2015), and indirectly eating and ruminating behaviour. Furthermore, pelleting of cereal-based feeds, as it was a case in this study, affects starch gelatinization and its fermentability in the rumen (and also protein and cell wall constituents degradation in the rumen; Bertipaglia et al., 2010; Razzaghi et al., 2016), and in consequence the rumen

environment and nutrient digestion, at least shortly after feed intake, due to a higher fermentation rate of processed cereals (Bertipaglia et al., 2010; Razzaghi et al., 2016). Because rumen fermentability of cereal-based feed may vary greatly depending on its final composition and processing prior (e.g., extrusion) or during pelleting (e.g., grinding intensity) (Castrillo et al., 2013; Górka et al., 2015; Razzaghi et al., 2016), the different pelleted compound feeds may differently affect the feed intake, and also eating and ruminating behaviour of ruminants (Castrillo et al., 2013; Gimeno et al., 2015). In this study diets differed both in lack or presence of pelleted cereal-based feed and chopped lucerne, which could affect rumen fermentation, so the interpretation of nutrient digestion and eating and ruminating pattern was not easy. Also, the composition of pelleted cereal-based feed was different from the composition of basal diet routinely used in the sitatunga diet (diet A) in Silesian Zoological Garden. Specifically, pelleted feed used in this study contained tree leaves, canary seed and molasses, which were not present in the diet A. This could also affect the digestive tract functioning or feed palatability, and in consequence eating and ruminating behaviour. Therefore, results of this study do not give an unequivocal answer whether observed differences between treatments were a result of the presence or absence of pelleted feed and/or chopped dehydrated lucerne in the basal diet. However, taking into account that a large variety of commercial pelleted feeds for zoo ruminants is available on the market, and that accessibility of good quality roughages is often limited, this study increases our knowledge on potential pros and cons for using cereal-based diets in zoo ruminants.

A faster eating rate when pelleted feed was used in the diet could be also a result of limited possibility of selective intake of the basal diet components. Although after mixing ground concentrates, chopped dehydrated lucerne, fruits and vegetables (diet A) a quite homogenous mixture was obtained, selective intake of some component of the diet was possible (e.g., particles of dehydrated lucerne or oatmeal against finely ground concentrates), which could be responsible for longer eating time. This possibility was greatly reduced when the pelleted feed was replaced by cereal grains and chopped dehydrated lucerne in the diets B and C. However, the intensity and pattern of selection of each ingredient of the basal diet after feed allocation were not analysed in this study. Furthermore, the animals could select between diet components that are currently not recommended for zoo ruminants, like cereals, fruits and vegetables, and in this situation the possibility

of selecting feed components may be especially unfavourable. Nevertheless, our results confirm that higher inclusion of forage in the diet, for example by chopped dehydrated lucerne provision, is a simple and effective method to decrease the feeding rate and obtain a more homogenous daily eating pattern, which should decrease probability of gastrointestinal tract related diseases in sitatunga. As a result, when high in starch and simple sugars feeds are fed to wild captive ruminants, high quality meadow or grass hay, browse or lucerne hay should be provided in order to promote the normal feeding behaviour and high forage intake.

It is worth noting that as the eating time of the basal diet decreased, the eating time of hay tended to increase. This observation may be interpreted as compensation for a need of expressing natural feeding behaviour and/or some protective mechanism against rumen pH drop below the optimum for rumen function in animals rapidly consuming concentrates.

The chemical analysis of hay refusals demonstrated that the sitatunga showed a selective food intake. So, further studies are needed to describe the importance of expressing selective intake of diet components by sitatunga. It has been shown that ruminants try to overcome the negative consequences of a sudden drop in rumen pH by selective consumption of structured components and increased saliva production shortly after an episode of rumen acidosis (Schwaiger et al., 2013; DeVries et al., 2014), which may also indicate a higher probability of rumen acidosis when pelleted feed was used in the basal diet. On the one hand, faster eating rate of pelleted diet may increase the probability of rumen acidosis, but on the other, depending on feed processing prior to pelleting and feed composition, pelleted feed may decrease the ruminal OM digestibility, due to faster rumen escape (Górka et al., 2015), and in consequence decreases short-chain fatty acids production in the rumen. The probability of rumen acidosis can be especially decreased by feeding pelleted feeds with high fibre content, which was also shown to increase the forage intake in medium size browsing ruminants (McCusker et al., 2001).

Although nutrient digestibility may be over- or underestimated in this study, a negative effect of pelleted feed on total tract digestibility is in line with the results of Abouheif et al. (2012). A negative impact of pelleting on nutrient digestibility can be expected especially for pelleted roughages because pelleting and the associated grinding of forages prior to pelleting may decrease the feed retention time in the rumen, and thus decrease the fibre digestibility (Abouheif et al., 2012). However, in this study pelleted feed inclusion resulted in higher

hemicellulose content, although diets were formulated to be similar for CF. This inevitably was responsible for lower OM digestibility when pelleted feed was used to replace components of basal diet in this study (Van Soest, 1996). Nevertheless, as opposed to aims of livestock ruminant nutrition, high digestibility of the diet is not a priority in zoo ruminant nutrition (Lintzenich and Ward, 1997) and, actually, feeds with high fibre content are recommended in order to assure high feed intake and normal feeding behaviour (Lintzenich and Ward, 1997; Clauss and Dierenfeld, 2008; McCusker et al., 2011), which inevitable leads to lower nutrient digestibility.

## Conclusions

The pelleted cereal-based feed in the diet for sitatunga increased the feeding rate of the basal diet (unstructured feeds) and decreased the feeding rate of meadow hay (structured feed) without an impact on overall dry matter intake. Furthermore, the total tract nutrient digestibility was decreased. As a result, a physical structure of supplemental feed (concentrates) may affect the eating behaviour and gastrointestinal tract functioning, and thus welfare and health of sitatunga and also other ruminants in zoological gardens.

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## References

- AOAC International, 2000. Official Methods of Analysis of AOAC International. 17<sup>th</sup> Edition. Gaithersburg, MD (USA)
- Abouheif M.A., Al-Saiady M.Y., Al-Mufarrej S.I., Makkawi A., Ibrahim H.A., Aljumaah R.S., 2012. Effect of physical of diet and frequency of feeding on digesta retention time and digestion in Najdi lambs. *J. Anim. Vet. Adv.* 11, 1774–1779
- Allen M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80, 1447–1462
- Beauchemin K.A., Eriksen L., Nørgaard P., Rode L.M., 2008. Short communication: Salivary secretion during meals in lactating dairy cattle. *J. Dairy Sci.* 91, 2077–2081
- Bertipaglia L.M.A., Fondevila M., van Laar H., Castrillo C., 2010. Effect of pelleting and pellet size of a concentrate for intensively reared beef cattle on *in vitro* fermentation by two different approaches. *Anim. Feed Sci. Tech.* 159, 88–95
- Castrillo C., Mota M., Van Laar H., Martín-Tereso J., Gimeno A., Fondevila M., Guada J.A., 2013. Effect of compound feed pelleting and die diameter on rumen fermentation in beef cattle fed high concentrate diets. *Anim. Feed Sci. Tech.* 180, 34–43
- Cerling T.E., Harris J.M., Passey B.H., 2003. Diets of East African Bovidae based on stable isotope analysis. *J. Mammal.* 84, 456–470
- Clauss M., Dierenfeld E.S., 2008. The nutrition of browsers. In: M.E. Fowler, R.F. Miller (Editors). *Fowler's Zoo and Wild Animal Medicine. Current Therapy.* Vol. 6. Saunders Elsevier, St. Louis, MO (USA), pp. 444–454
- Clauss M., Kienzle E., Hatt J.M., 2003. Feeding practice in captive wild ruminants: peculiarities in the nutrition of browsers/concentrate selectors and intermediate feeders. A review. In: A. Fidgett, M. Clauss, U. Ganslosser, J.M. Hatt, J. Nijboer (Editors). *Zoo Animal Nutrition.* Vol. 2. Filander Verlag, Fürth (Germany), pp. 27–52
- DeVries T.J., Schwaiger T., Beauchemin K.A., Penner G.B., 2014. The duration of time that beef cattle are fed a high-grain diet affects feed sorting behavior both before and after acute ruminal acidosis. *J. Anim. Sci.* 92, 1728–1737
- Estes D.R., 1991. *The Behaviour Guide to African Mammals: Including Hoofed Mammals, Carnivores, Primates.* University of California Press, Berkeley and Los Angeles (USA)
- Gagnon M., Chew A.E., 2000. Dietary preferences in extant African Bovidae. *J. Mammal.* 81, 490–511
- Gattiker C., Espie I., Kotze A., Lane E.P., Codron D., Clauss M., 2014. Diet and diet-related disorders in captive ruminants at the national zoological gardens of South Africa. *Zoo Biol.* 33, 426–432
- Gimeno A., Al Alami A., Toral P.G., Frutos P., Abecia L., Fondevila M., Castrillo C., 2015. Effect of grinding or pelleting high grain maize- or barley-based concentrates on rumen environment and microbiota of beef cattle. *Anim. Feed Sci. Tech.* 203, 67–78
- Górka P., Castillo-Lopez E., Joy F., Chibisa G.E., McKinnon J.J., Penner G.B., 2015. Effect of including high-lipid by-product pellets in substitution for barley grain and canola meal in finishing diets for beef cattle on ruminal fermentation and nutrient digestibility. *J. Anim. Sci.* 93, 4891–4902
- Hummel J., Nogge G., Clauss M., Nørgaard C., Johanson K., Nijboer J., Pfeffer E., 2006. Energy supply of the okapi in captivity: fermentation characteristics of feedstuffs. *Zoo Biol.* 25, 251–266
- Lintzenich B.A., Ward A.M., 1997. Hay and pellet ratios: considerations in feeding ungulates. *Nutrition Advisory Group Handbook Fact Sheet 006*, pp. 12
- McCusker S., Shipley L.A., Tollefson T.N., Griffin M., Koutsos E.A., 2011. Effects of starch and fibre in pelleted diets on nutritional status of mule deer (*Odocoileus hemionus*) fawns. *J. Anim. Physiol. Anim. Nutr.* 95, 489–498
- Ndawula J., Tweheyo M., Tumusiime D.M., Eilu G., 2011. Understanding sitatunga (*Tragelaphus spekii*) habitats through diet analysis in Rushebeya-Kanyabaha wetland, Uganda. *Afr. J. Ecol.* 49, 481–489
- Razzaghi A., Larsen M., Lund P., Weisbjerg M.R., 2016. Effect of conventional and extrusion pelleting on *in situ* ruminal degradability of starch, protein, and fibre in cattle. *Livest. Sci.* 185, 97–105
- Robertson J.B., Van Soest P., 1981. The detergent system of analysis and its application to human foods. In: W.P.T. James, O. Theander (Editors). *The Analysis of Dietary Fiber in Food.* Marcel Dekker, New York, NY (USA), pp. 123–158
- Schilcher B., Baumgartner K., Geyer H., Liesegang A., 2013. Investigations on rumen health of different wild ruminants in relation to feeding management. *J. Zoo Aquar. Res.* 1, 28–30
- Schwaiger T., Beauchemin K.A., Penner G.B., 2013. Duration of time that beef cattle are fed a high-grain diet affects the recovery from a bout of ruminal acidosis: Short-chain fatty acid and lactate absorption, saliva production, and blood metabolites. *J. Anim. Sci.* 91, 5743–5753

- Sponheimer M., Lee-Thorp J.A., DeRuiter D.J. et al., 2003. Diets of Southern African Bovidae: stable isotope evidence. *J. Mammal.* 84, 471–479
- Steuer P., Südekum K.-H., Tütken T., Müller D.W.H., Kaandorp J., Bucher M., Clauss M., Hummel J., 2014. Does body mass convey a digestive advantage for large herbivores? *Funct. Ecol.* 28, 1127–1134
- Taylor L.A., Schwitzer C., Owen-Smith N., Kreuzer M., Clauss M., 2013. Feeding practices for captive greater kudu (*Tragelaphus strepsiceros*) in UK collections as compared to diets of free-ranging specimens. *J. Zoo Aquar. Res.* 1, 7–13
- Tweheyo M., Amanyá B.K., Turyahabwe N., 2010. Feeding patterns of sitatunga (*Tragelaphus Speki*) in the Rushebeya-Kanyabaha wetland, south western Uganda. *Afr. J. Ecol.* 48, 1045–1052
- Van Keulen J., Young B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44, 282–287
- Van Soest P.J., 1996. Allometry and ecology of feeding behavior and digestive capacity in herbivores: A review. *Zoo Biol.* 15, 455–479
- Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597
- Waghorn G.C., Reid C.S.W., 1983. Rumen motility in sheep and cattle given different diets. *New Zeal. J. Agr. Res.* 26, 289–295
- Yang W.Z., Beauchemin K.A., 2009. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: chewing and ruminal pH. *J. Dairy Sci.* 92, 1603–1615
- Zebeli Q., Tafaj M., Weber I., Dijkstra J., Steingass H., Drochner W., 2007. Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. *J. Dairy Sci.* 90, 1929–1942