Comparative Biochemistry and Physiology, Part A 150 (2008) 32–39

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Excretion patterns of fluid and different sized particle passage markers in banteng (*Bos javanicus*) and pygmy hippopotamus (*Hexaprotodon liberiensis*): Two functionally different foregut fermenters

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ARTICLE INFO

Article history:
Received 16 November 2007
Received in revised form 22 February 2008
Accepted 22 February 2008
Available online 29 February 2008

Keywords:
Mean retention time
Forestomach
Foregut fermentation
Pygmy hippopotamus
Cattle
Herbivore

ABSTRACT

Processing of ingesta particles plays a crucial role in the digestive physiology of herbivores. In the ruminant forestomach different sized particles are stratified into a small and a large particle fraction and only the latter is regurgitated and remasticated to smaller, easier-to-digest particles. In contrast, it has been suggested that in non-ruminating foregut fermenters, such as hippopotamuses, larger particles should be selectively excreted since they tend to be digested at a slower rate and hence can be considered intake-limiting bulk. In our study we determined the mean retention time (MRT) of fluids and different sized particles (2 mm and 10 mm) in six pygmy hippos (*Hexaprotodon liberiensis*) and six banteng (*Bos javanicus*) on a diet of fresh grass at two intake levels. We used cobalt ethylendiamintetraacetate (Co-EDTA) as fluid and chromium (Cr)-mordanted fibre (2 mm) and cerium (Ce)-mordanted fibre (10 mm) as particle markers, mixed in the food. Average total tract MRT for fluid, small and large particles at the high intake level was 32, 76 and 73 h in pygmy hippos and 25, 56 and 60 h in banteng, and at the low intake level 39, 109, and 105 h in pygmy hippos and 22, 51 and 58 h in banteng, respectively. In accordance with the prediction, large particles moved faster than, or as fast as the small particles, through the gut of pygmy hippos. In contrast, large particles were excreted slower than the small particles in the ruminant of this study, the banteng. Pygmy hippos had longer retention times than the banteng, which probably compensate for the less efficient particle size reduction. Although the results were not as distinct as expected, most likely due to the fact that ingestive mastication of the larger particle marker could not be prevented, they confirm our hypothesis of a functional difference in selective particle retention between ruminating and non-ruminating foregut fermenters.

1. Introduction

Among the variety of gastrointestinal tract designs that can be found in herbivorous mammals, several different foregut fermentation systems have evolved — e.g. in ruminants, camelids, sloths, peccaries, hippopotamuses, colobine monkeys, or macropod marsupials (Langer 1988; Stevens and Hume 1998). Among the different foregut fermenters, two taxonomic groups, the camelids and the ruminants (taxonomic definition: Ruminantia, the 'true' ruminants), evolved a mechanism by which the ingested food is regurgitated and submitted to repeated mastication (i.e., rumination; the functional definition "ruminants" therefore includes camelids). Although a similar behaviour, termed 'merycism', has been observed repeatedly in macropods (Home 1814; Owen 1834; Moir et al., 1956; Calaby 1958; Mollison 1960; Barker et al., 1963; Hendrichs 1965), the actual occurrence, circumstances and physiology of this behaviour have not been quantified; the seemingly low prevalence of it, as indicated by the absence of reference to it in many experimental studies on macropod digestion, suggests that it is an occasional, facultative, but not obligatory strategy (Hume 1999).

Rumination has an important effect on the digestive physiology that sets functional ruminants apart: the particle size to which the ingesta is finally reduced to is distinctively smaller than in other similar-sized herbivores (Udén and Van Soest 1982; Grenet et al., 1984; Okamoto 1997; comparing data from Clauss et al., 2002; Clauss et al., 2004a, b). Given the effect of particle size on the fermentation kinetics of plant material (reviewed in Clauss and Hummel 2005), this smaller ingesta particle size represents an important digestive advantage.

A prerequisite for the efficient functioning of the rumination process is that, in the forestomach, those ingesta particles that need to be remasticated are separated from those that do not require further mastication. In both camelids and true ruminants, this is achieved by a mechanism of stratification, whereby the ingesta particles separate according to their specific gravity into more buoyant and more

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doi:10.1016/j.cbpa.2008.02.022

sedimenting particles, with buoyant particles being the larger ones and sedimenting particles the smaller ones (Lechner-Doll et al., 1991). The anatomical positions of the cardia (the opening of the oesophagus into the forestomach) and the orifice through which particles leave the main chamber of the forestomach (the 'ostium reticuloomasale' in true ruminants/the 'ostium ruminoreticularis' in camelids, Langer 1988), ensure that larger particles are regurgitated for rumination and only smaller ones leave the forestomach.

In parallel to what is known in ruminants, it has been suspected that larger particles are also excreted slower from the forestomach of non-ruminating foregut fermenters (Langer 1988; Foley et al., 1995). However, it appears that in non-ruminating foregut fermenters, the slower excretion of larger particles would not make as much sense as in ruminants, due to the fact that large particles tend to be digested at a slower rate and hence can be considered potentially intake-limiting bulk. In hindgut fermenters it has been found that larger particles are selectively excreted, presumably in order to clear the gut and to maintain high food intake (Björnhag et al., 1984; Björnhag, 1987, 1989; Hume and Sakaguchi, 1991; Cork et al., 1999). In parallel to the observations in hindgut fermenters, Clauss (2004) suggested that larger particles should be selectively excreted in non-ruminant foregut fermenters and reviewed literature reports that supported such an interpretation for sloths. Similarly, Hume (1999) speculated, based on data from Dellow (1982) and Forbes and Tribe (1970), that large particles might be excreted faster than small particles from the forestomach of macropods. In feeding trials with two common hippopotamuses (*Hippopotamus amphibius*) and two pygmy hippopotamuses (*Hexaprotodon liberiensis*), Clauss et al. (2004a) observed that in one common and in one pygmy hippo large particles (2–10 mm) passed the gastrointestinal tract in parallel to small particles (<2 mm), whereas they were excreted faster in both other respective individuals. Those observations were, to our knowledge, the first direct experimental indication that large particles are not excreted slower than smaller particles from the forestomach of a non-ruminant foregut fermenter. These findings are in contrast to domestic ruminants and camelids who selectively retain large particles (Lechner-Doll et al., 1990). However the sample size in the study of Clauss et al. (2004a) was small, the markers had not been simultaneously validated in a ruminant, and the particle marker length was not exactly defined. Therefore, we performed passage experiments in pygmy hippopotamuses and a wild "cattle-type" ruminant, the banteng (*Bos javanicus*), using particle markers of different length (2 mm and 10 mm) that originated from the same marker batch. We predicted that in the non-ruminant foregut fermenter (pygmy hippo), larger particles are excreted faster than smaller particles or move together with the smaller particles, in contrast to the ruminant where the larger particles should be excreted slower than the smaller ones.

2. Materials and methods

The trials were performed with six pygmy hippos and six banteng at the Zoological Gardens of Berlin (ZGB) and Halle (ZGH) in summer 2005 and 2006. Body mass (BM) of the pygmy hippos was measured at the beginning and the end of each trial period, whereas BM of the banteng were estimated by the keepers by visual judgement (height and width) and age and sex as reference parameters. Details of the animals are summarized in Table 1.

The animals were fed with fresh grass only, the staple diet during summer. The dry matter fraction of the offered grass at Berlin and Halle zoo contained on average 93±3 and 92±1% organic matter, 63±2 and 60±1% neutral detergent fibre (aNDFom), 34±2 and 33±1% acid detergent fibre (ADFom) and 3±1 and 5±1% Lignin (sa), respectively (for fibre terminology see Udén et al., 2005). Since the grass diet was usually supplemented with fruits and vegetables in hippos and with sugar beet pulp in banteng, an adaptation period of 14 days was allowed to pass before the trial started. It was planned to study each animal in two trials on different intake levels (with a second adaptation period of 5 days in between) — *ad libitum* (high intake, HI), and, subsequently, at approximately 75% of the individual *ad libitum* intake (low intake, LI). Each trial lasted 7 days. Due to a shortage of grass, one pygmy hippo (animal 6) and three banteng (animals 10–12) could only be assessed at one intake level (HI) and some animals had to be fed grass hay (soaked in water); this never exceeded one individual day per animal (animal 2: at day 1 after marker feeding; animal 4: day 6; animal 7: day 1; animal 8 and 9: day 2).

Table 1
Details of the pygmy hippos (*Hexaprotodon liberiensis*) and banteng (*Bos javanicus*) studied at the Zoological Gardens of Berlin (ZGB) and Halle (ZGH)

Species	Animal	Born	Sex	BM (kg)	Facility
Pygmy hippo	1	1985	Male	248	ZGB
	2	1983	Female	225	ZGB
	3	1997	Female	238	ZGB
	4	1998	Female	203	ZGH
	5	1976	Female	202	ZGH
	6	2000	Male	196	ZGH
Banteng	7	2002	Male	550[a]	ZGB
	8	2004	Female	220[a]	ZGB
	9	2004	Male	200[a]	ZGB
	10	1996	Female	700[a]	ZGB
	11	2001	Female	600[a]	ZGB
	12	1997	Female	650[a]	ZGB

BM = body mass.
[a] Estimated.

All animals were fed separately. Due to the husbandry techniques at the respective zoos, not all animals could be kept separately at all times, and feeding regimes differed. Three banteng (animals 10–12) were kept together; two of these animals received a coloring agent in their food ration (animal 10: titanium dioxide 40 g/day; animal 11: brilliant blue food color, Sensient Food Colors Germany GmbH, Geesthacht, 2 g/day; both fed twice daily), so that faeces could be ascribed to the individual animals.

Three pygmy hippos (animals 1–3) received food once daily, in the afternoon; the other pygmy hippos (animals 4–6) as well as the banteng received food twice daily, in the morning and in the afternoon. Food items offered and leftovers were quantified on a daily basis by weighing. During the day (approximately 08:00–18:00 h), the pygmy hippos were kept on land with no access to a water pool. During the night (approximately 18:00–08:00 h), the pygmy hippos had free access to a water pool, except for animal 1 on the high intake level. Drinking water was provided at all times to all animals.

Markers for ingesta retention, cobalt ethylendiamintetraacetate (Co-EDTA; fluid marker), chromium (Cr)-mordanted fibre (2 mm particle marker) and cerium (Ce)-mordanted fibre (10 mm particle marker), were prepared according to Udén et al. (1980) and Heller et al. (1986). Before mordanting, grass hay was dried (40 °C) and ground through 2 and 10 mm square perforated screens (Retsch catalogue no. 03.647.0167 and -0024) with a retsch mill (SM 2000, Retsch GmbH & CoKg, Haan, Germany). After removing dust and smaller than the desired particles by dry sieving (by hand), small as well as large particles were incubated with 33 g and 75 g mordant per 100 g particles (sodium dichromate dihydrate, $Na_2Cr_2O_7{\cdot}2H_2O$, and cerium (III) chloride heptahydrate, $CeCl_3{\cdot}7H_2O$), respectively. After mordanting and washing, the particles were dried at 65 °C. The marker dose applied was 0.2 g/kg BM of each mordanted fibre marker, and 0.03 g/kg BM of Co-EDTA, respectively. Markers were fed in the afternoon shortly before the regular feeding. Co-EDTA was dissolved in little water, mixed with particle markers and offered at time zero (t_0) in pygmy hippos with a portion of fruits or grass, and in banteng with soaked sugar beet pulp for better acceptance. The markers were mostly consumed completely within 5 to 60 min; the middle of the recorded time period was used as t_0 in subsequent calculations. After 60 min, any marker leftovers were removed if present, and the regular food was provided.