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Original investigation

Digesta kinetics in two arvicoline rodents, the field vole (*Microtus agrestis*) and the steppe lemming (*Lagurus lagurus*)

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ABSTRACT

A large number of small mammals practice cecotrophy, i.e. ingesting a certain fraction of their own faeces typically termed 'cecotrophs'. This behaviour is generally thought to limit metabolic losses of nitrogen, because a colonic separation mechanism (CSM) selectively accretes fine, nitrogen-rich particles (such as microbes) in the cecotrophs. Two types of CSM have been described, a wash-back CSM (in lagomorphs) where fluids wash through the colonic digesta in a retrograde manner into the caecum, resulting in a selectively prolonged solute marker retention in passage studies; and a mucus-trap CSM (in hystricomorph rodents) where microbes are trapped in mucus-filled anatomical structures in the colon mucosa (grooves/furrows/folds). In the mucus-trap CSM, no selective retention of any passage marker occurs. How the CSM operates in muroid rodents is not well explored, but both mucus-filled anatomical structures and a moderate degree of selective solute marker retention have been reported in the literature. Here we demonstrate selective solute marker retention in two muroid, arvicoline rodent species (field voles *Microtus agrestis*, body mass 28.6 ± 7.4 g, and steppe lemming *Lagurus lagurus*, 19.8 ± 2.0 g) on a pelleted high-fibre diet, where a solute marker was retained 20–55% longer than a particle marker (with mean retention times of 5.6 ± 0.5 vs. 4.8 ± 0.9 and 5.0 ± 1.5 vs. 3.3 ± 0.9 h, respectively). The animals achieved an apparent organic matter digestibility similar to that of much larger herbivores on diets of similar fibre content. In addition to a selective feeding behaviour and a high relative food intake, a digestive physiology that includes coprophagy, and a CSM using both mucus-trap and wash-back effects, are characteristic for herbivorous arvicoline rodents. The relative contributions of the two CSM components – whether they have additive or complementary effects – remain to be explored.

Introduction

The digestive strategy of small herbivorous mammals typically comprises the ingestion of a certain fraction of their own faeces, generally termed 'coprophagy' or 'cecotrophy' (Cork et al., 1999; Karasov and Martínez del Rio, 2007). The latter term is often used to indicate the fact that there is a difference in the physical appearance and chemical composition of the fraction of the faeces that is excreted and not ingested ('hard faeces') and the other fraction of the faeces that is re-ingested ('cecotrophs' or 'soft faeces'). In the following, the term 'coprophagy' will be used for the phenomenon as such, without an intention to suggest an absence of specific cecotrophs. Whether such a difference between 'hard faeces' and 'soft faeces' exists in all species known to re-ingest a part of their own faeces remains to be clarified (Hörnicke and Björnhag, 1980). A separation mechanism in the colon, the 'colonic separation mechanism' (CSM, Björnhag, 1972) is the prerogative for a putative fractionation of digesta into different types of faeces.

Different morphological and physiological aspects of CSM have been described (Björnhag and Snipes, 1999). The definite test for a CSM is a difference in composition between the contents of the caecum and caecotrophs on the one hand, and the contents of the

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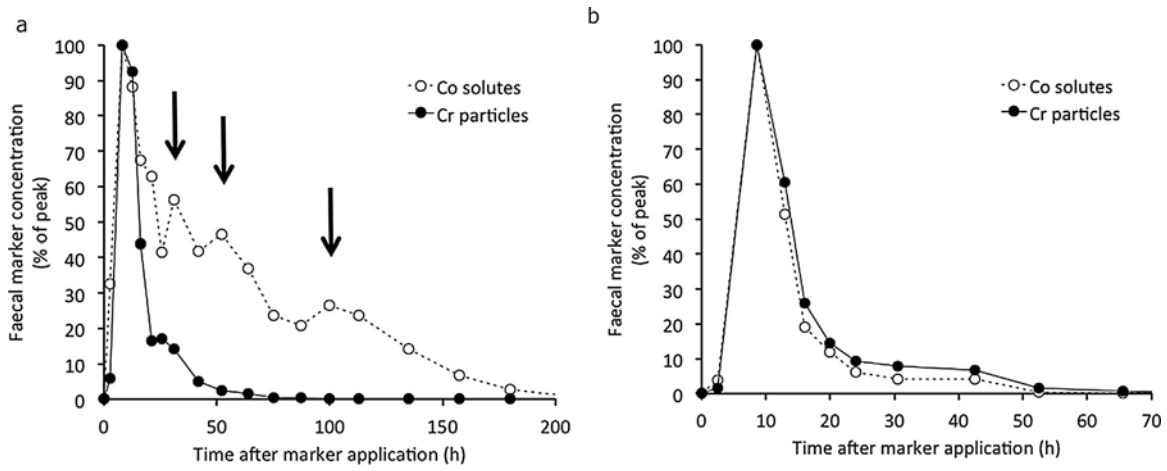

Fig. 1. Typical examples of the excretion patterns for a solute (Co-EDTA) and a particle (Cr-mordanted fibre) marker in (a) rabbit (*Oryctolagus cuniculus*), with a 'wash-back' colonic separation mechanism (CSM) resulting in a distinctively longer solute than particle excretion, and (b) guinea pig (*Cavia porcellus*), with a 'mucus-trap' CSM where the solute and particle marker is excreted in parallel (taken from Franz et al., 2011). Data re-drawn for consistent y-axis scaling. Arrows indicate secondary marker peaks suggestive of coprophagy.

distal colon and hard faeces on the other hand. Typically, caecum contents and caecotrophs contain higher concentrations of protein than colon contents and hard faeces (Björnhag, 1994). Current opinion holds that the main function of coprophagy is the prevention of protein losses that would occur if the symbiotic microbes living in the hindgut of small herbivorous mammals were excreted and lost together with the faeces (Hörnicke and Björnhag, 1980; Cork et al., 1999; Franz et al., 2011).

The CSM of lagomorphs and rodents are described as a dichotomy (Hume and Sakaguchi, 1991; Björnhag and Snipes, 1999). In lagomorphs, a so-called 'wash-back' CSM flushes solutes and small particles, including microbes, retrogradely from the colon into the caecum, which results in distinctively longer mean retention times (MRT) of solute as compared to particles markers (Fig. 1a; Sakaguchi et al., 1987; Franz et al., 2011). A similar mechanism has been described in the common ringtail possum (*Pseudocheirus peregrinus*) (Chilcott and Hume, 1985). In contrast, the microbes of rodents are caught in special morphological structures in the colon that are filled with mucus, the so-called 'mucus-trap' CSM. The corresponding morphological structure in hystricomorph rodents is a colonic 'furrow' (Gorgas, 1966; Snipes et al., 1988) that has been studied extensively (Holtenius and Björnhag, 1985; Takahashi and Sakaguchi, 1998, 2000, 2006). In myomorph rodents, the CSM is linked to longitudinal folds and oblique furrows (*Plicae circulares*) in the colon (Behmann, 1973; Sperber et al., 1983), but has not been studied in as much detail as in hystricomorph rodents. In hystricomorph rodents, the MRT of solute and particle markers does not differ markedly; rather, these markers move through the gastrointestinal tract more or less in parallel, as demonstrated in a large number of studies (Fig. 1b; Sakaguchi and Nabata, 1992; Sakaguchi et al., 1992; Sakaguchi and Ohmura, 1992; Franz et al., 2011; Hagen et al., 2015a; Hagen et al., 2016). This indicates that in the mucus-trap CSM, retrograde digesta washing by fluids does not play a relevant role, in contrast to the wash-back CSM. Thus, faecal marker excretion patterns in digesta kinetics studies can help to differentiate between types of CSM (Cork et al., 1999). In addition, they typically reflect coprophagy, and thus confirm its presence, in the form of recurrent (secondary) marker peaks resulting from repeated re-ingestion of markers together with faecal material (Fig. 1a; Lee and Houston, 1993; Clauss et al., 2007).

In their review of CSM, Björnhag and Snipes (1999) stated that the retrograde transport of fluids did not occur in myomorph rodents, which would mean that solute and particle markers should move in parallel in this group, too. Empirical evidence, however, is equivocal. Hörnicke and Björnhag (1980) cite unpublished observations by Björnhag and co-workers indicating that water-soluble substances are selectively delayed in the lower digestive tract of the Norway lemming (*Lemmus lemmus*), but state that it is of lower efficiency than the mechanism found in rabbits. Whereas MRT of solutes and particles did not differ significantly in Mongolian gerbils (*Meriones unguiculatus*) on a high-fibre diet (Fig. 2a; Pei et al., 2001b), the $MRT_{solutes}$ was significantly longer by 1.7 h (13%) than $MRT_{particles}$ in Townsend voles (*Microtus townsendii*) in spite of visually similar excretion patterns in the only individual whose pattern was displayed (Fig. 2b; Hume et al., 1993). In Brandt's vole (*Microtus brandti*), $MRT_{solutes}$ were significantly longer by 1–2 h (20–40%) than $MRT_{particles}$ (Fig. 2c; Pei et al., 2001a). These data suggest that a fluid phase backflow can be part of the CSM in myomorph rodents, in particular in arvicoline rodents. In order to further explore this possibility, we studied the kinetics of solute and particle markers in two arvicoline rodent species. In doing so, we also generated data on selective intake behaviour, digestive efficiency and dry matter gut fill in these species that add empirical evidence to the question how efficiently small herbivorous mammals can digest their diets (Foley and Cork, 1992; Justice and Smith, 1992).

Material and methods

Six individual field voles (*Microtus agrestis*; 5 females weighing 21–30 g and 1 male weighing 42 g) and six individual steppe lemmings (*Lagurus lagurus*; all females with a body mass of 17–23 g) were available for this study. Animal experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence no. 142/2011). Animals were kept in a room with an ambient temperature of 22–24 °C, in individual enclosures of 40 × 50 cm fitted with a shelter, the roof of which also served as an elevated platform. They had visual, olfactory, acoustic and tactile contact amongst each other via meshed windows in the enclosure walls. Wood wool served as bedding material and enclosure structure. Water was available at ad libitum access from nipple drinkers. The floor consisted of a metal mesh, under which a tray was placed to catch faeces and diet leftovers; multiple trays per enclosure were used in times of intensive faecal collection (see below). Faeces were typically not trapped in the wood wool, and could be shaken out of it easily if they were. Note that a mesh floor does not prevent coprophagy, as animals typically take the part of the faeces they want to re-ingest directly from the anus (Kenagy and Hoyt, 1980). Animals had been familiarized with

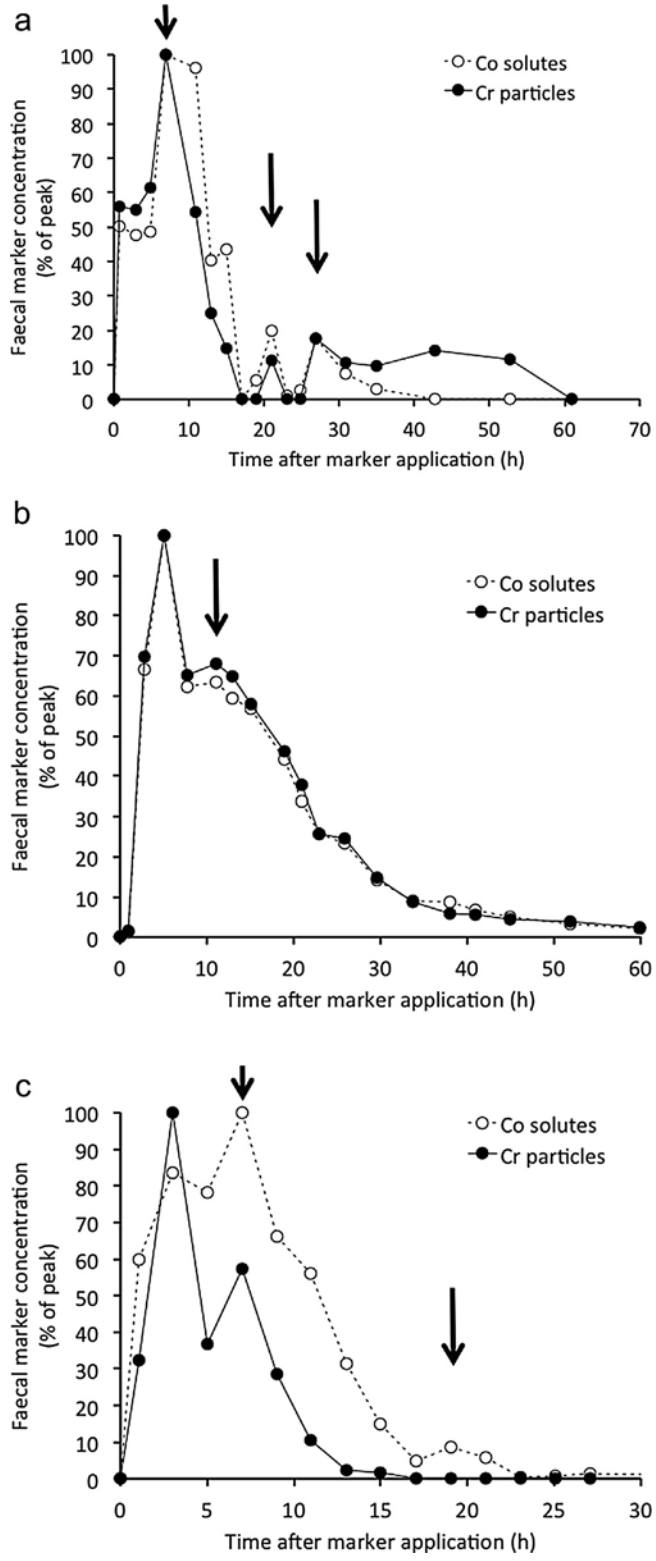

Fig. 2. Excretion patterns for a solute (Co-EDTA) and a particle (Cr-mordanted fibre) marker in (a) Mongolian gerbil (*Meriones unguiculatus*) on a high-fibre diet (Pei et al., 2001b); (b) Townsend vole (*Microtus townsendii*) (Hume et al., 1993); (c) Brandt's vole (*Microtus brandti*) on a high-fibre diet (Pei et al., 2001a). Note that both patterns distinguished in Fig. 1 occur within the *Microtus* genus. Data were read from the graphs of the respective publications and re-drawn for consistent y-axis scaling. Arrows indicate secondary marker peaks suggestive of coprophagy.

Table 1
Nutrient composition (per dry matter) of the lucerne pellets used in the present study, and of the powdered leftovers from 'food grinding' of five out of six field voles (*Microtus agrestis*).

Nutrient		Lucerne pellet	Powdered leftovers
Organic matter	%	87.6	87.1
Crude protein		15.4	15.8
Total ash		12.4	12.9
Crude fibre		30.2	34.6
Neutral detergent fibre		49.0	51.3
Acid detergent fibre		35.7	39.1
Gross energy	kJ/g	18.0	17.9

a diet of pelleted lucerne (*Medicago sativa*, Table 1) for two weeks prior to the experiment, and were provided with the 4 mm pellets *ad libitum*.

The amount of food offered and leftover as well as the faeces defecated were quantified on a daily basis by weighing, and samples were taken for the analyses mentioned below. 'Food grinding', where experimental rodents grind the offered pelleted diet into crumbs (Cameron and Speakman, 2010), was observed in several field voles but not in any steppe lemming. The non-consumed ground material was collected separately from pelleted leftovers and weighed; in total, it represented sufficient material for one single batch of nutrient analyses.

For digestibility measurements, we used the amount of food ingested and faeces excreted for three consecutive days. Samples of food and leftover were submitted to standard nutrient analyses (AOAC, 1995) for dry matter (DM) and total ash (AOAC no. 942.05), crude protein (AOAC no. 977.02), neutral detergent fibre (NDF, AOAC no. 2002.04), acid detergent fibre (ADF, AOAC no. 973.18), and crude fibre (AOAC no. 930.10). Whereas there is a consensus that the detergent fibre system is better suited to characterise fibre content of plant material than crude fibre and that for an understanding of dietary plant fibre, crude fibre values are unsuitable because of the undefined nature of the components retained in this analysis (Van Soest, 1982; Mertens, 2003), the close association of crude fibre levels with digestibility in herbivores makes this measure suitable for a comparison of the digestive efficiency of different animal species or groups (e.g., Fig. 4 in Demment and Van Soest, 1985). We use the dietary crude fibre value strictly in this latter context and do not imply that it is of value for nutritional evaluation. Gross energy (GE) was determined by bomb calorimetry (IKA-Calorimeter C4000, Ika, Stauffen, Germany). All fibre values were corrected for ash content. Analyses were performed in duplicate. Faecal samples were submitted to analyses for DM, total ash, NDF and GE. Organic matter (OM) was calculated as DM minus total ash. The apparent digestibility (aD) of DM and nutrients was calculated as the percentage of the respective intake not eliminated via faeces (Robbins, 1993).

Cobalt (Co)-EDTA was used as solute marker for the fluid digesta component. Hay particles of 1–2 mm length mordanted with chromium (Cr) were used as particle markers. Co-EDTA and Cr-mordanted fibres were prepared according to Udén et al. (1980). The mordanted fibres contained (as analysed) 36.9 g Cr kg^{-1} DM. Markers were gently applied via syringe, after dissolving the Co-EDTA, as one dosage into the oral cavity of manually restrained animals (0.01 g Co-EDTA and 0.04 g Cr-fibre). Animals were observed to chew on the marker material before swallowing. Prior to marker feeding, faecal samples were taken for assessing the background levels of Co and Cr. After marker application, faeces were collected hourly from 1 h to 13 h after marker application, then every two hours (15–35 h), then every four hours (39–75 h) in the field voles, and 0.5 h later in the steppe lemmings (with an additional sample at 0.5 h after marker application). Sampling

was spaced so closely because voles are known to have very short ultradian rhythms of defecation and coprophagy (Liu et al., 2007).

After drying, faecal samples were submitted to marker analysis as previously described (Frei et al., 2015) by microwave wet ashing followed by analysis of Co and Cr with an inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany). Marker excretion patterns were visualized with plots depicting faecal marker concentrations over time (Clauss et al., 2007) expressing concentrations in% of the peak concentration to compensate for differences in absolute concentrations achieved for the different markers (Matsuda et al., 2015). The mean retention time (MRT) in the whole digestive tract was calculated according to Thielemans et al. (1978) as

$$\mathrm{MRT} = \frac{\Sigma t_i \; C_i \; dt_i}{\Sigma C_i \; dt_i}$$

with C_i = marker concentration in the faecal samples from the interval represented by time t_i (h after marker administration, using the midpoint of the sampling interval) and dt_i = the interval (h) of the respective sample

$$dt_i = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}$$

The marker was assumed to have been excreted completely once the faecal marker concentrations were similar to the background-levels determined in pre-dose faecal samples. The relative dry matter intake was expressed both on the mammalian average basis of $kg^{0.75}$ and the more appropriate basis for small herbivores of $kg^{0.67}$ (Müller et al., 2013). The total DM gut fill was calculated according to Holleman and White (1989) using DM intake, $MRT_{particle}$ and aD DM. Although this method has been applied to animals of various sizes including small rodents (Müller et al., 2013), it has only been truly validated, to our knowledge, for ruminants (Munn et al., 2015), and results must be considered with caution. Comparisons between species were made with independent sample *t*-tests. Statistical analyses were performed with SPSS 23.0 (IBM, Armonk, NY, USA), with the significance level set to $P < 0.05$.

Results

All animals accepted the experimental conditions without evident adverse reactions, approaching the human experimenter every time enclosures were cleaned or food and water were replaced. Female field voles produced powdered leftovers by 'food grinding'; this was not observed in the male field vole, or in any steppe lemming. The material produced by 'food grinding' contained numerically (when analysing the material from the five animals as one sample) more fibre, more ash and less gross energy than the diet offered (Table 1), suggesting that 'food grinding' occurred during a process where the voles selected for certain energy-dense components of the pelleted diet. Ground leftovers represented between 0.3 and 23.2% of all leftovers in these animals and 0.3-13.2% of processed (ingested) dietary DM; however, aD did not differ in the first decimal in calculations that did not or did account for the different composition of ground leftovers, with the exception of aD NDF, which was 0.1 ± 0.1% lower when accounting for ground leftover composition.

Whereas absolute DM intake differed significantly between the species, this was not the case when relative DM intake was expressed on a $kg^{0.67}$ basis (range 86–136 g/$kg^{0.67}$/d, $P = 0.051$) or on a $kg^{0.75}$ basis (range 112–185 g/$kg^{0.75}$/d, $P = 0.102$, Table 2). Field voles had significantly higher aD DM and aD OM, with a difference of 5%; numerically similar differences in aD NDF and aD GE were not significant.

Table 2
Body mass, intake, faecal excretion, digestibility, mean retention time and calculated dry matter gut fill of field voles (*Microtus agrestis*) and steppe lemmings (*Lagurus lagurus*) fed lucerne pellets.

		Microtus agrestis	*Lagurus lagurus*
Body mass	g	28.6 ± 7.4[a]	19.8 ± 2.0[b]
Dry matter intake	g/d	9.8 ± 0.8[a]	6.6 ± 0.5[b]
	g/$kg^{0.67}$/d	109 ± 17	91 ± 4
	g/$kg^{0.75}$/d	145 ± 26	124 ± 7
Pelleted leftovers	g/d	6.7 ± 1.6	5.2 ± 1.1
Powdered leftovers	g/d	0.5 ± 0.6	–
Dry matter excretion	g/d	4.5 ± 0.4[a]	3.3 ± 0.2[b]
Apparent digestibility	%		
Dry matter		54 ± 4[a]	49 ± 1[b]
Organic matter		53 ± 4[a]	48 ± 1[b]
Neutral detergent fibre		37 ± 6	33 ± 2
Gross energy		51 ± 5	47 ± 1
Mean retention time	h		
Solutes		5.6 ± 0.5[A]	5.0 ± 1.5[A]
Particles		4.8 ± 0.9[aB]	3.3 ± 0.9[bB]
Dry matter gut fill	g	1.51 ± 0.34[a]	0.71 ± 0.21[b]
	% of body mass	5.4 ± 1.3[a]	3.6 ± 1.1[b]

Lower-case superscript indicate significant differences between the species within lines (t-tests); upper-case superscripts indicate significant differences between mean retention times of the two markers within species (paired *t*-test).

Marker excretion patterns of three individuals of each species are displayed in Fig. 3 (for patterns of the other individuals, see the supplementary material). Secondary marker excretion peaks were visible in four of the field voles and five of the steppe lemmings. In both species, $MRT_{solutes}$ (ranging from 4.8–6.3 h in field voles and 3.7–7.7 h in steppe lemmings) were significantly longer than $MRT_{particles}$ (ranging from 3.3–5.8 h in field voles and 2.3–4.4 h in steppe lemmings; Table 2), with a mean difference of 0.8 ± 0.6 h (21 ± 21%) in field voles and 1.7 ± 1.0 h (56 ± 30%) in steppe lemmings. The ratio of $MRT_{solutes}/MRT_{particles}$ was 1.20 ± 0.20 (range 1.04–1.61) in field voles and 1.56 ± 0.30 (range 1.08–1.91) in steppe lemmings.

The calculated DM gut fill represented 4.1–7.6% of body mass in field voles and 2.4–5.0% in steppe lemmings (Table 2).

Discussion

The present study provides evidence that a wash-back mechanism is involved in the colonic separation mechanism (CSM) of arvicoline rodents as determined in field voles and steppe lemmings. However, the degree of fluid wash-back, resulting in prolonged solute marker excretion, was not as pronounced in the two species investigated as that observed in lagomorphs (cf. Figs. 1a, 2c and 3). Nevertheless, it represents a typical feature of the digestive physiology of the two species, as also in the case of Brandt's voles (Pei et al., 2001a). Additionally, although determined in a different way, it appears also evident in Norway lemmings (Hörnicke and Björnhag, 1980; Sperber et al., 1983). The $MRT_{particle}$ of field voles measured in the present study (3.3–5.8 h) was within the range previously measured in this species by Lee and Houston (1993) of 3.9–10.7 h. These authors found different MRT on a seed (longer) or a leaf (shorter) diet, which can be most parsimoniously explained by the different intake levels on these diets (not reported in their publication) (Levey and Martínez del Rio, 1999). Voles have been shown to increase intake on lower-quality diets (Cranford and Johnson, 1989; Young Owl and Batzli, 1998). Typically, across and within mammal species, lower food intake (as possible on a higher energy diet such as seeds) will lead to longer MRT (e.g., Clauss et al., 2014), as also shown in voles (Young Owl and Batzli, 1998).

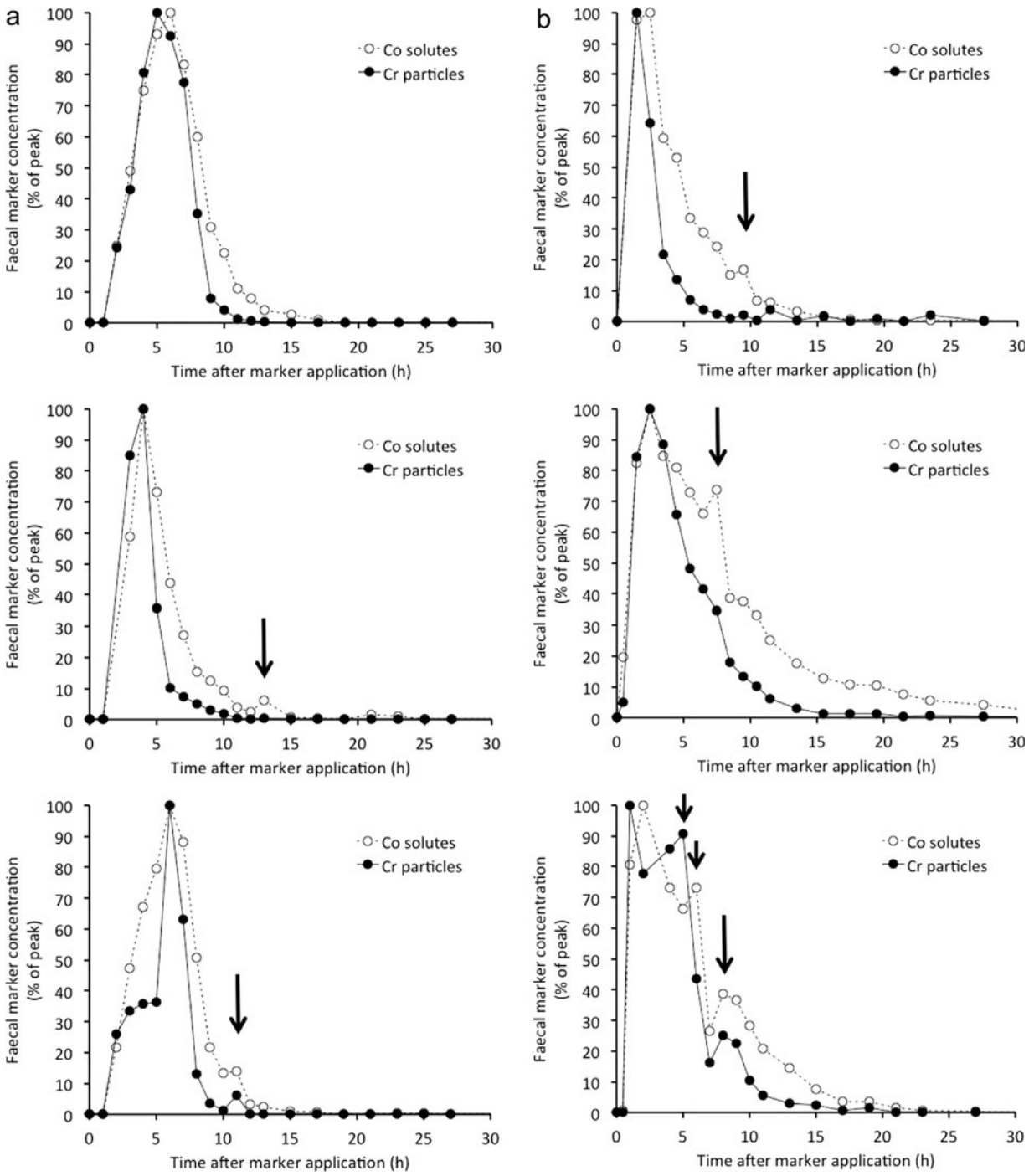

Fig. 3. Excretion patterns for a solute (Co-EDTA) and a particle (Cr-mordanted fibre) marker in (a) three individual field voles (*Microtus agrestis*) and (b) three individual steppe lemming (*Lagurus lagurus*). Arrows indicate secondary marker peaks suggestive of coprophagy. For the excretion patterns in the other individuals, see the electronic supplement.

Secondary marker excretion peaks have been documented in various digesta kinetics studies in lagomorphs and rodents, and are typically interpreted to be the consequence of coprophagic behaviour, which leads to a re-ingestion of the passage markers (Lee and Houston, 1993; Pei et al., 2001a; Clauss et al., 2007; Franz et al., 2011; Hagen et al., 2015a; Hagen et al., 2016). Although one can assume that herbivorous arvicoline rodents practice coprophagy continuously at multiple bouts throughout the day (Liu et al., 2007), individual differences may exist. Coprophagy was shown to vary with diet in rabbits (*Oryctolagus cuniculus*) (Fekete and Bokori, 1985; Carabaño et al., 1988; García et al., 1995), tuco-tucos (*Ctenomys talarum*) (Martino et al., 2007), capybaras (*Hydrochoerus hydrochaeris*) (Nogueira-Filho et al., 2013) or viscachas (*Lagostomus maximus*) (Hagen et al., 2015a), and also in voles (Cranford and Johnson, 1989), typically with an increased use of coprophagy on lower-quality diets. In viscachas, coprophagy was not evident on a pelleted lucerne diet originating from the same production batch as the food used in the present study (Hagen et al., 2015a). The observation that most individual field voles and steppe lemmings displayed evidence consistent with coprophagy on the same diet

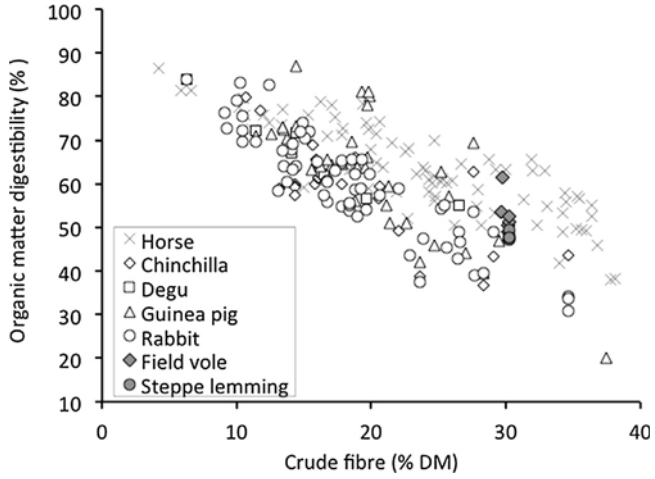

Fig. 4. Relationship of dietary crude fibre levels (in% dry matter) and measured apparent digestibility of organic matter (in%) in horses, rabbits and rodents on various diets including pelleted feeds, forages, and mixed diets (data collection from Hagen et al., 2015b) compared to the digestibility measured on pelleted lucerne in field voles (*Microtus agrestis*) and steppe lemmings (*Lagurus lagurus*) of the present study.

could be an indication that a prevention of metabolic faecal nitrogen losses is particluarly important for animals of very small body size (0.02–0.03 kg vs. 4.5 kg in viscachas), low fibre digestibility (aD NDF 30–40% vs. 55% in viscachas) and high relative DM intake (112–185 g/kg$^{0.75}$/d vs. 45 g/kg$^{0.75}$/d in viscachas).

A limitation of our experimental design was the use of a particle marker (of dimensions of 1–2 mm) that was larger than the size of particles typically recovered in the faeces or from the gastrointestinal tract of arvicoline rodents (Lee, 1993; Fritz et al., 2009). Although we consider it likely that the chewing movements observed during marker application resulted in a particle size reduction of the marker material, this could not be verified. It is a general feature of retention studies that it is rarely verified whether the particle size of a marker employed remains constant during the passage through the gastrointestinal tract, or whether its size is modified. Ideally, particle marker experiments should be conducted either using faecal material of the same species or individual as the particle marker basis, ensuring that the particle marker is representative for the material actually excreted (Udén et al., 1980), or whole, marked forages (i.e., letting the animal itself perform the particle size reduction), combined with marker analysis of the different particle size fractions of the faeces (Hummel et al., 2017); both options are particularly challenging in very small animals. In the case of the present study, the uncertainty of the size at which the marked particles actually reached the colon (chewed or unchewed) represents a serious limitation of our ability to draw conclusions from the data.

Another limitation is the use of a pelleted diet. On the one hand, most studies with small herbivores have used pelleted diets (reviewed in Bezzobs and Sanson, 1997), including many studies on digesta passage (e.g. Pei et al., 2001a). On the other hand, it has been shown that a lower *ad libitum* food intake occurred when the same plant material was offered whole to *Rattus lutreolus*, a small herbivore, than when it was offered in ground form (Bezzobs and Sanson, 1997). In the same study, another small herbivore, *Mastacomys fuscus*, was less limited in the food intake on the whole plant diet. Such differences may influence whether a species is able to subsist on a natural high-fibre forage by obtaining the necessary high intake. Although chewing behaviour was observed in the animals of the present study when ingesting the pelleted diet, we could not assess whether the provision of whole plant material would have led to a different digesta particle size than the ingestion of the ground and pelleted lucerne, and whether this would have an effect on the retention characteristics measures.

Yet another limitation of our study was that we were restricted by our experiment licence to non-invasive measurements only. Additional analyses that would support the presence of a CSM in general would be a demonstration of particularly fine particles (Foley and Hume, 1987; Naumova et al., 2017) or of a concentration of nitrogen or bacteria (Holtenius and Björnhag, 1985; Takahashi and Sakaguchi, 1998, 2006) in the caecum or the cecotrophs. The presence of a wash-back CSM would additionally include the demonstration of increased moisture content or water secretion in the proximal colon (Staaland, 1975; Rübsamen et al., 1983). A comparative investigation of these factors across a larger number of small herbivores of different CSM in conjunction with measures of passage kinetics could corroborate the presumed correlations of these different characteristics and put the classification of species into different CSM types on a more solid basis.

The literature on digestive physiology in small herbivores generally contrasts wash-back and mucus-trap CSM as a dichotomy (Hume and Sakaguchi, 1991; Björnhag and Snipes, 1999; Pei et al., 2001a; Franz et al., 2011). The mucus-trap CSM has so far only been demonstrated in mammals, and it appears obligatorily linked to coprophagy. A wash-back CSM, which occurs in coprophagic mammals as well, has also been demonstrated in birds (that do not practice coprophagy) (Gasaway et al., 1975; Björnhag and Sperber, 1977; Frei et al., 2017), and in the koala (*Phascolarctos cinereus*) (Cork and Warner, 1983; Krockenberger and Hume, 2007) which does not practice coprophagy but uses caecum contents for feeding its young (Smith, 1979). We suggest that rather than allocating arvicoline (or other muroid) rodents by default to one of the two extreme CSM as represented by the lagomorphs and hystricomorph rodents investigated so far (Fig. 1), CSM might be considered as a continuum between these extremes, with arvicoline rodents placed at various positions on that continuum. Following Pei et al. (2001a), species can be ranked by the ratio of their $MRT_{solute}/MRT_{particle}$. These authors suggest a range of 1.3–4.8 for wash-back and values up to 1.2 in mucus-trap CSM species. But they also note that microtine species, which they ascribe to the mucus-trap CSM, can cross the borderline. Ranging from 1.04 to 1.91, the arvicolines of the present study also cross this threshold. Given that the longitudinal folds and oblique furrows are filled with mucus that traps microbes (Sperber et al., 1983), these animals evidently use a mucus-trap CSM. However, given the selective retention of a solute marker, they most likely additionally also employ a wash-back mechanism to various degrees. With the data existing at present, it is impossible to decide which of the two mechanisms is quantitatively more important in an arvicoline species, and whether the two components of the CSM have different, possibly complementary, functions.

Other clear-cut differences ascribed to the CSM dichotomy might also be not as strict as previously assumed. For example, it is often stated that in contrast to rabbits, no two types of faeces (hard faeces and caecotrophs) can be identified in rodents (Björnhag and Snipes, 1999). However, in fact the occurrence of different kinds of faeces have been either described or documented in various rodent species such as rats (*Rattus norwegicus*) (Sperber et al., 1983), guinea pigs (*Cavia porcellus*) and chinchillas (*Chinchilla laniger*) (Holtenius and Björnhag, 1985), nutrias (*Myocastor coipus*) (Takahashi and Sakaguchi, 1998), capybaras (*Hydrochaeris hydrochaeris*) (Lord, 1991), or tuco-tucos (Martino et al., 2007).

Franz et al. (2011) suggested that one possible effect of the wash-back CSM might be the facilitation of both, a CSM and a reduced DM gut fill. This was based on their own observations comparing rabbits and guinea pigs, as well as the calculation of DM gut fill using data from Sakaguchi et al. (1987). Similar to these results, the

species with the higher $MRT_{solute}/MRT_{particle}$ ratio in the present study, the steppe lemming, also had the lower calculated DM gut fill. It could therefore be assumed that a wash-back mechanism might, in certain species, help alleviate constraints placed on gut capacity. However, this hypothesis remains to be tested conclusively as gut capacity is related to wet and not dry gut contents, and wet gut contents cannot be calculated from data gained in digestion and passage rate studies.

An important component in the digestive strategy of small herbivorous mammals is a selective feeding behaviour. For example, Justice and Smith (1992) and Smith (1995) found that woodrats (*Neotoma* spp.) selectively avoided fibre when feeding on a compressed lucerne diet, although the increase in selectivity with decreasing body mass found in the former study was equivocal in the latter study. Similar to the results of the present study for field voles, with an increase in NDF levels from 49 to 52% in DM from offered food to leftovers, the *Neotoma* spp. left orts of 42–43% of a food that contained 40% NDF. As a note of caution, it should be noted that these measured differences are relatively small. Why a similar discrimination was not observed in our steppe lemmings is unclear. A systematic investigation of selective feeding behaviour in small mammals is warranted. In laboratory mice, a large individual variation in the degree of food grinding is observed (Koteja et al., 2003). One factor among many, explaining the degree of food grinding, seems to be the level of difficult-to-digest fibre in the diet (Cameron and Speakman, 2010). A self-evident yet important factor to consider when investigating selective feeding behaviour is the amount of food actually offered. When offering food at a level below requirements, food selectivity may not be expressed (Cameron and Speakman, 2010), but the degree to which selective feeding behaviour is influenced by the level of over-supplementation of a diet is unknown. In the present study, where pelleted food was offered at about 70% in excess of consumption, most likely no selectivity-reducing shortage was perceived by the animals.

The level of aD NDF (33–37%) found in the arvicolines of the present study is comparable to levels reported for other small herbivorous mammals (Smith, 1995). However, aD DM (49–54%) was lower, and intake level was higher, than typically reported from digestion studies of small herbivorous mammals (Müller et al., 2013), most likely due to the comparatively higher fibre levels in the diet used in the present study. When comparing the aD of total OM from the animals of the present study to those of other herbivores in relation to dietary fibre levels (Fig. 4), the small arvicolinae do not represent outliers but match the overall pattern, adding to the evidence that body mass is not a good predictor of digestive efficiency (Justice and Smith, 1992; Müller et al., 2013; Steuer et al., 2014). Small herbivores that rely on a CSM may actually be able to increase food intake on diets of higher fibre (i.e., lower quality) – something typically not observed in other herbivores, especially when fed forages of different fibre content (Cork, 1994; Meyer et al., 2010). The results confirm that small herbivores are not necessarily constrained to dietary niches of low fibre content, but that some species can sustain themselves on fibrous diets (Foley and Cork, 1992; Clauss et al., 2013). One factor enhancing the digestive efficiency of very small herbivores is their capacity to grind their food into extremely fine particles (Lee, 1993). In addition, individual species may achieve this with the help of a wash-back CSM, a mucus-trap CSM, or variable combinations of both.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.mambio.2018.01.003.

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