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Angela Schwarm, Sylvia Albrecht, Sylvia Ortmann, Christian Wolf, Marcus Clauss. Digesta retention time in roe deer , as measured with cerium-, lanthanum- and chromium-mordanted fibre. European Journal of Wildlife Research, 2010, pp.437-442. 10.1007/s10344-010-0450-5 . hal-00629469

HAL Id: hal-00629469

<https://hal.science/hal-00629469>

Submitted on 6 Oct 2011

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Digesta retention time in roe deer *Capreolus capreolus*, as measured with cerium-, lanthanum- and chromium-mordanted fibre

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Received: 8 July 2010 / Revised: 19 September 2010 / Accepted: 21 September 2010 / Published online: 6 October 2010
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Abstract Studies on particle retention time in ruminants are commonly conducted utilizing chromium (Cr) and/or a lanthanide bound to feeds. Both types of markers have different chemical properties which potentially bias estimates of digesta retention—their combined use is therefore a topic of ongoing discussion. In order to evaluate the suitability of two lanthanides for studies assessing the passage kinetics of different-sized particles, we measured the mean retention time in roe deer with cerium (Ce) and lanthanum (La) labelled to the same particle length and compared it to Cr-mordanted fibre of the same size. We expected a simultaneous excretion of Ce- and La-labelled fibre, but a delayed excretion of Cr-mordanted fibre compared to the other markers. In this study, the mean retention times of Ce- and La-labelled fibre did not differ significantly, and Cr-mordanted fibre was retained on

average 5 h longer than Ce and La. Despite the limitation of the small sample size, Ce and La showed the same excretion pattern and can therefore be recommended as mordants in studies assessing passage kinetics of different particle sizes.

Keywords Ruminant · Browser · Mean retention time · Passage rate · Marker

Introduction

Studies on particle retention time in ruminants are commonly conducted utilizing chromium (Cr) or lanthanides bound to feeds. The lanthanide series consists of 15 elements with similar chemical properties, with atomic numbers 57 through 71, from lanthanum (La) to lutetium (Bernard and Doreau 2000). Cr is the most widely used marker in nutrition studies and has a better reputation than lanthanides like ytterbium (Yb) and cerium (Ce), which have been criticized to potentially dissociate from labelled particles (Beauchemin and Buchanan-Smith 1989; Combs et al. 1992; Huhtanen and Kukkonen 1995); this property has been offered as an explanation for the observed shorter (7–29 h) mean retention time (MRT) of Yb-labelled fibre compared to Cr-mordanted fibre of the same size (Table 1). On the other hand, it has been demonstrated that Cr largely decreases cell wall digestibility (Udén et al. 1980), which in turn could lead to the observed longer retention time compared to a Yb-labelled fibre of the same size (Beauchemin and Buchanan-Smith 1989; Huhtanen and Kukkonen 1995; Table 1). As a consequence, in studies which aim to assess excretion kinetics of large vs. small particles in ruminants, the combined use of Cr and the lanthanide Yb as mordants is not the optimal choice. Looking at studies with cattle fed different-sized particle markers, using Cr and lanthanides as

Communicated by C. Gortázar

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Table 1 MRT of similar-sized and simultaneously fed feed particles—either labelled with Cr or the lanthanide Ce, Dy and Yb—in the gastrointestinal tract of cattle and rabbit from the literature

Species	Particle type	Length of the particles mordanted (mm)	Metal salt used as mordant	MRT (h)				Source
				Ce	Dy	Yb	Cr	
Cattle	Grass hay	20–50	Na ₂ Cr ₂ O ₇ , Yb-acetate			75	82	1
Cattle	Grass silage	—(coarsely chopped)	Na ₂ Cr ₂ O ₇ , Yb-acetate			39	46	2
Cattle	Grass hay	—(masticated)	Na ₂ Cr ₂ O ₇ , ¹⁶⁹ Yb(NO ₃) ₃			59	61	3
Cattle	Grass hay	60–120	Na ₂ Cr ₂ O ₇ , YbCl ₃			54	72	4
Cattle	Rice straw	1	Na ₂ Cr ₂ O ₇ , Yb-acetate			30	50	5
Cattle	Alfalfa silage	— (large-chopped)	Na ₂ Cr ₂ O ₇ , Yb-acetate			48	57	6
Cattle	Alfalfa hay	25	DyCl ₃ , YbCl ₃		42	42		7
Rabbit	Concentrates	0.05	CeCl ₃ , YbCl ₃		27		28	8

Different lanthanide-labelled fibres had the same MRT, whereas Cr-mordanted fibre retention time exceeds that of lanthanide-labelled fibres

Source: 1 Huhtanen and Kukkonen (1995), 2 Ahvenjärvi et al. (2004), 3 Pond et al. (1989), 4 Mader et al. (1984), 5 Murphy et al. (1989), 6 Beauchemin and Buchanan-Smith (1989), 7 Goetsch and Galyean (1983), 8 Gidenne and Lapanouse (1997)

Ce or La, the observed difference in their respective MRT was greater when Cr was bound to the large particles (Lechner-Doll et al. 1990) rather than to the small particles (Schwartz et al. 2008; Lechner et al. 2010). From this, we concluded that it would be probably advantageous to use two lanthanides bound to different particle lengths rather than the application of Cr and a lanthanide in digesta passage studies in ruminants. So far, only two studies evaluated the combined use of lanthanides, namely Yb combined with dysprosium (Dy) or Ce; these lanthanides were excreted simultaneously from the digestive tract of cattle and rabbit if bound to the same particle length (Goetsch and Galyean 1983; Gidenne and Lapanouse 1997; Table 1). The objective of our trial, as part of a larger study, was to assess the MRT with two lanthanides labelled to the same particle length in roe deer. We chose Ce and La as markers, and a synchronous excretion was expected. Additionally, we intended to feed Cr-mordanted fibre of identical size and expected a delayed excretion compared to the other markers. However, due to the experimental design of the larger study, Cr marker had to be fed 1 day prior to the application of the lanthanide markers. Because food intake was relatively constant throughout the trial, we decided to show and discuss the results nonetheless. Finally, the obtained data are compared to the data of particle retention time in roe deer published so far (Holand 1994; Van Wieren 1996; Behrend et al. 2004).

Materials and methods

The trial, as part of a larger study, was performed with four adult roe deer (17–24 kg) at the field research station of the IZW in June 2006. Details of the animals are recorded in Table 2. Animals 2, 3 and 4 were born in summer 2004 at the field research station. Animal 1 was

bought in 1999; its birth date is unknown. Animals were usually kept in groups in meadow enclosures, but were moved to individual pens 4 weeks before the trial started. On this occasion, animals were weighed. Individual pens measured 25–30 m² and had a carpeted floor. Individuals were offered on average 440 g dry matter per day of a pelleted compound feed for dairy calves (Märka KA-18-3, Märkische Kraftfutter GmbH, Guben, Germany) and 68 g dry matter per day pelleted meadow hay (Pre Alpin Compact, Agrobs GmbH, Münsing, Germany). The chemical composition of the pellets for dairy calves was 23% crude protein (CP), 17% neutral detergent fibre (NDF) and 7% acid detergent fibre (ADF) on a dry matter basis. The pelleted hay contained 15% CP, 50% NDF and 29% ADF on a dry matter basis. Animals had food access from approximately 9 A.M. to 9 P.M. A fifth animal refused to eat and was excluded from the trial. Respective food items offered and leftovers were quantified on a daily basis by weighing. Drinking water was always provided ad libitum. Digesta MRT through the gastrointestinal tract (GIT) was determined with 2-mm particles either labelled with Ce, La or Cr. Markers were prepared according to Udén et al. (1980) and Heller et al. (1986). Preparation and analytical procedures are described in detail elsewhere (Schwartz et al. 2008). In brief, dried grass hay was ground through a 2-mm² perforated screen with a Retsch mill (SM 2000, Retsch GmbH & Co. KG, Haan, Germany). Dust and smaller particles were removed by dry sieving. After neutral detergent treatment, 100 g dried particles each were incubated either with 54 g lanthanum [III] chloride hydrate (LaCl₃·H₂O), with 75 g cerium(III) chloride heptahydrate (CeCl₃·7H₂O) or with 33 g sodium dichromate dihydrate (Na₂Cr₂O₇·2H₂O). After mordanting at 37°C (Ce, La) or 100°C (Cr) and thorough washing with tap water, the fibre was dried at 65°C. We applied 1.5 g of

Table 2 DMI (mean \pm SD) and MRT of 2-mm hay particles in the gastrointestinal tract of four roe deer (*C. capreolus*) studied in June 2006 at the field research station of the Leibniz Institute for Zoo and Wildlife Research

Animal	Age (years)	Sex	Body mass (kg)	DMI of the respective food item ^a (June 10–15)	DMI (June 10–15)			Relative DMI (June 9, the day prior to Cr-marker feeding)		
					Pelleted compound feed for dairy calves (g day ⁻¹)	Pelleted hay (g day ⁻¹)	Sum of both food items (g day ⁻¹)	Sum of both food items (g kg ^{-0.75} day ⁻¹)	Sum of both food items (g kg ^{-0.75} day ⁻¹)	Cr
1	>7	Female	17.1	420 \pm 39	42 \pm 31	462 \pm 36	55 \pm 4	42	43	45
2	2	Female	23.8	420 \pm 39	60 \pm 13	480 \pm 30	45 \pm 3	39	42	46
3	2	Male	22.9	465 \pm 55	28 \pm 22	492 \pm 65	47 \pm 6	25	33	33
4	2	Female	23.1	412 \pm 20	68 \pm 0	480 \pm 20	46 \pm 2	42	31	40
Mean \pm SD			21.7 \pm 3.1	429 \pm 24	49 \pm 18	478 \pm 12	48 \pm 5	37 \pm 8	37 \pm 6	38 \pm 7

Particles were labelled either with Ce, La or Cr. Ce and La markers were ingested simultaneously. Cr marker was ingested 21 h (animals 1–2) and 25 h (animals 3–4) prior to Ce and La

^a Individuals were offered on average 440 g DM day⁻¹ pelleted compound feed for dairy calves and 68 g DM day⁻¹ pelleted hay

each marker per animal. As outlined in “Introduction”, only the Ce and La markers could be fed simultaneously due to the experimental design of the larger study. Ce- and La-labelled fibres were mixed with 15 g ground compound feed for dairy calves, pressed to pellets and fed in the morning of trial day 1 (June 11) prior to the daily ration. The average time between start (6 A.M.) and end (9 A.M.) of marker feeding was taken as the time of marker uptake. Faeces were collected in toto the following 5 days (June 12–16). Cr marker had to be fed 1 day prior to the application of the other markers. At day 0 of the trial (June 10), Cr-mordanted fibre (with 15 g concentrate compound) was offered at 6 A.M. The pens were checked at 7 A.M., 9 A.M. and 12 A.M. for marker uptake, after which the daily ration was fed. Marker uptake in animals 1 and 2 was between 9 A.M. and 12 A.M. (the marker was not touched by these animals before 9 A.M.). Animals 3 and 4 totally consumed the marker between 6 A.M. and 7 A.M.. The middle of the recorded time period was used as the starting time for MRT calculations (10:30 A.M. in animals 1 and 2 and 6:30 A.M. in animals 3 and 4). Faeces were collected in toto the following 6 days (June 11–16). Individual enclosures were checked for defaecations seven times per day on days 1 to 5 of the trial (1:30 A.M., 6 A.M., 9 A.M., 12 A.M., 3 P.M., 6 P.M. and 9 P.M.) and two times at day 6 of the trial (9 A.M., 9 P.M.). The average time between collections was taken as the time of defaecation. Individual defaecations were cleaned from hair, weighed, and the whole sample or an aliquot (after thoroughly mixing; max. 300 g) was stored frozen until drying at 60°C. Dry matter (DM) content of food and faecal subsamples was determined by drying an aliquot at 103°C to constant weight. Ground faecal samples were microwave-digested and analysed for Ce, La and Cr

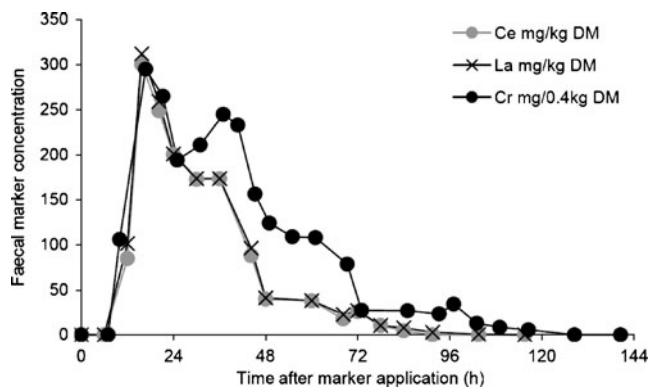


Fig. 1 Excretion pattern for 2-mm hay particles (labelled either with cerium (Ce), lanthanum (La) or chromium (Cr)) in roe deer (animal 4). Marker concentrations adjusted for visual comparability. Note: Ce and La marker were ingested simultaneously as a pulse dose within 3 h. Cr marker uptake was 25 h prior to Ce and La; it was ingested within 1 h. The animal had a dry matter intake during the 6 days of the trial of 46 \pm 2 g kg^{-0.75} day⁻¹. Intake the day prior to Cr marker feeding was 42 g kg^{-0.75} day⁻¹

Table 3 MRT of feed particles in the gastrointestinal tract and relative DMI of roe deer (*C. capreolus*) from the literature and details of the animals and the methods used

n	Body mass (kg)	Age (years)	Breeding	Size of enclosure (m ²)	Staple food	Relative DMI (g kg ^{-0.75} day ⁻¹)	Season	Fibre mordanted length (mm)	Administration	MRT (h)	Source
2	21.0	1–2	Hand-reared (tame)	600	Roughage (ad libitum), pelleted compound feed for dairy calves (0.1 kg day ⁻¹ each), oats (0.1 kg day ⁻¹ each), carrot (1 kg day ⁻¹ each)	—	Autumn	Grass hay	1.5	Rumen cannula, frozen cube	—
3	24.8	1.5–3	Hand-reared (tame)	4	Roughage (blueberry stems)	37	Winter	Oat grain	—	Orally	31 — —
24.4	25.2					24	Winter				36
25.0						34	Summer				31
3	24.3	1.5–3	Hand-reared (tame)	4	Pelleted compound feed for dairy calves	24	Summer				36
24.4	24.8					48	Winter	Oat grain	—	Orally	24 — —
24.9						34	Winter				19
5	17.0	—	—	6	Willow Alfalfa Hay	58	Summer				20
					Grass pellet	34	Summer				19
4	21.7	2–7	Mother-reared	25–30	Pelleted compound feed for dairy calves	76	Autumn	Willow Alfalfa Hay	—	Orally	— — —
						73					21
						54					20
						69					18
						48	Summer	Grass hay	2	Orally	37 38 43 —
											This study

Source: 1 Behrend (1999); Behrend et al. (2004), 2 Holland (1994), 3 Van Wieren (1996)

^a MRT was measured after offering a single amount of 10% of the diet stained with basic fuchsin at the start of the measuring period during 4 h. Faecal grab samples were taken

concentration by inductively coupled plasma mass spectroscopy (ELAN 6,000, PerkinElmer Life and Analytical Sciences, Milano, Italy) following Schwarm et al. (2008). The MRT in the total GIT was calculated according to Thielemans et al. (1978). MRT of different markers were compared with paired Student's *t* test and with general linear model repeated measurements (GLM RM). Comparison with literature data was performed by the use of an unpaired Student's *t* test. Monotonous associations between pairs of variables were measured by calculating Spearman's correlation coefficient. The significance level was generally set to $\alpha=0.05$. Statistical calculations were performed with the SPSS 15 software (SPSS, Chicago, IL, USA).

Results

Without exception, the resulting marker excretion curves for the simultaneous fed Ce- and La-labelled particles (both 2 mm) show a simultaneous processing of the two mordants (Fig. 1). Accordingly, the MRT for Ce- and La-labelled fibres did not differ significantly (paired *t* test, $n=4$; mean \pm SD, 37 ± 6 h vs. 38 ± 7 h, $p=0.391$). Individual MRT measurements, as well as the dry matter intake (DMI), are recorded in Table 2.

The excretion curve for Cr deviated from the Ce and La excretion curves (Fig. 1). Cr-mordanted fibre was retained on average 5 h longer than Ce and La (MRT, 43 ± 9 h; Table 2) despite the same particle size of 2 mm; however, this difference was not significant (GLM RM, $p=0.383$). Only in one animal (no. 3) were Cr-mordanted particles excreted as fast as Ce- and La-labelled particles. In this animal, food intake was not constant over time (Table 2), being lower the day prior to Cr marker feeding compared to the subsequent days.

The relative DMI (mean \pm SD) in roe deer was 48 ± 5 g (food) kilogram (body mass) $^{-0.75}$ per day (Table 2).

Discussion

Lanthanides

Despite the limitation of the small sample size, the results of this study support the hypothesis of a simultaneous excretion of the lanthanide markers Ce and La labelled to the same-sized particles from the ruminant digestive tract. This result is in accordance with a study using two other lanthanides, namely Dy vs. Yb, bound to same-sized particles as passage markers in cattle (Goetsch and Galyean 1983). Also in a study with rabbits, same-sized particles labelled either with the lanthanide Ce or the lanthanide Yb were excreted simultaneously (Gidenne and Lapanouse

1997; Table 1). Since we did not find a systematic difference in the excretion of Ce- and La-labelled fibres of the same length, conclusions on animal physiology as those of Schwarm et al. (2009) and Lechner et al. (2010) based on lanthanide marker excretion of different lengths can be supported by our study. These authors found in different ruminants that particle passage kinetics and retention of 10-mm particles labelled with Ce and 20-mm particles labelled with La did not differ. They concluded that particle retention in the ruminant forestomach is not a continuous function of particle size (i.e. the larger a particle, the longer it is retained) but rather threshold-dependent (i.e. all particles above a certain size, e.g. 10 mm, are retained for the same period of time).

Chromium vs. lanthanides

Conclusions concerning Cr marker excretion are limited by the fact that Cr-mordanted fibre could not be fed simultaneously with Ce and La, but was ingested on average 21 h (animals 1 and 2) and 25 h (animals 3 and 4) prior to Ce- and La-labelled fibres. MRT of Cr-mordanted fibre did not differ significantly from fibres labelled with lanthanides (Ce, La), which may be due to the low sample size and the fact that in one animal (no. 3) relative DMI prior to Cr marker feeding was 50% lower than the average intake of the following 6 days (Table 2). The animals with a relatively constant intake (nos. 1, 2 and 4) excreted Cr-mordanted fibre 4–9 h after the Ce- and La-labelled fibres (Table 2). It seems that irrespective of the type of lanthanide used (Yb, see data collection in Table 1; Ce/La, this study), Cr has a comparably longer MRT. Therefore, results from studies where small, Cr-mordanted fibres were shown to be

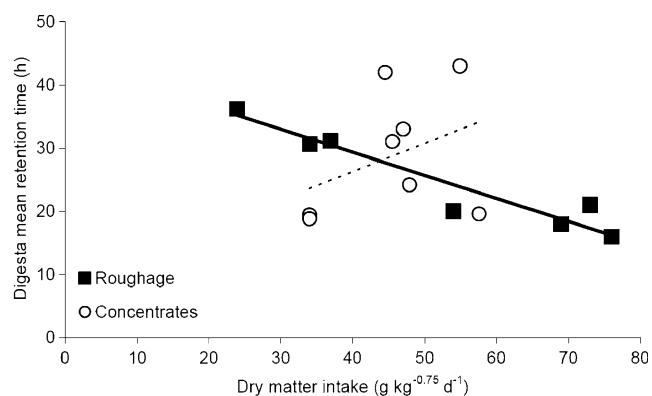


Fig. 2 Relationship between relative dry matter intake ($\text{g kg}^{-0.75} \text{ day}^{-1}$) and mean retention time (h) of feed particles in the gastrointestinal tract of roe deer fed either roughage (solid line) or concentrates (dashed line). Spearman correlation coefficient (SCC): roughage: $N=8$, SCC=-0.904, $p=0.002$; concentrates: $N=8$, SCC=0.419, $p=0.301$. Source: roughage: Holand (1994), Van Wieren (1996); concentrates: Holand (1994), this study

retained shorter than large, Ce- or La-labelled fibres (Schwartz et al. 2008; Lechner et al. 2010) can be regarded as very reliable because they demonstrate a difference opposite to the one expected based on the type of mordant alone.

Effect of dry matter intake on mean retention time—comparison with literature data

Animals ingested on average $48 \pm 5 \text{ g kg}^{-0.75} \text{ d}^{-1}$, which is in agreement with the intake of a similar pelleted food in the study of Holand (1994) ($43 \pm 12 \text{ g kg}^{-0.75} \text{ d}^{-1}$, $n=3$; Table 3). However, the MRT of Ce-labelled fibre in roe deer from our study was significantly longer (1.8-fold) than in the study of Holand (1994) ($37 \pm 6 \text{ h}$ vs. $21 \pm 2 \text{ h}$, Student's *t*-test, $p=0.008$, $N=4$ each), although relative DMI did not differ (Student's *t* test, $p=0.493$, $N=4$ each; Table 3). Usually, MRT decreases with increasing relative DMI, as seen in roe deer fed a roughage-only diet (Fig. 2, data from Holand 1994; van Wieren 1996). However, this is not the case on a pelleted concentrate diet (for statistics, see Fig. 2 legend). Possible explanations for the shorter MRT in the study of Holand 1994 compared to our study are the chemical composition of the diet (ADF, 5% vs. >7%), the environmental conditions (4- vs. 28-m² enclosure size) and the type of fibre labelled (oat grain vs. grass hay).

In the study of Behrend et al. (2004), the same type of fibre was mordanted as in our study (grass hay), with resulting comparatively short MRT of 24 h (Table 3). DMI was not assessed by Behrend et al. (2004); however, the faster excretion of Cr-mordanted particle markers in their study may be attributed to the considerable amount of available roughage (natural vegetation of the 600-m² enclosure supplemented with fresh and dried browse) with correspondingly accelerated digesta passage (cf. Fig. 2).

To conclude, Ce and La showed the same excretion pattern and can therefore be recommended as mordants in studies assessing the passage kinetics of different particle sizes.

Acknowledgements This study was supported by grants from the Freie Universität Berlin (NaFoG) to AS and the German Science Foundation (DFG) to SO (OR 86/1-1). We thank A. Brandenburger, H. Barleben, J. Mahr, B. Baumann and U. Baum for their engaged support.

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