

## ORIGINAL ARTICLE

# The effect of very low food intake on digestive physiology and forage digestibility in horses

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## Summary

Equid digestion is often conceptualized as a high-throughput/low-efficiency system, in particular compared with ruminants. It is commonly assumed that ruminants have an advantage when resources are limited; the effect of low food intake on digestive physiology of horses has, however, not been explored to our knowledge. We used four adult ponies [initial body mass (BM)  $288 \pm 65$  kg] in two subsequent trials with grass hay-only diets [in dry matter (DM): hay1, mid-early cut, crude protein (CP) 10.5%, neutral detergent fibre (NDF) 67.6%; hay2, late cut, CP 5.8%, NDF 69.5%], each fed subsequently at four different dry matter intake (DMI) levels: *ad libitum* and at 75, 55 and 30 g/kg<sup>0.75</sup>/day. We particularly expected digesta mean retention times (MRT) to increase, and hence fibre digestibility to increase, with decreasing DMI. Ponies maintained BM on the first, but lost BM and body condition on DMI55 and DMI30. MRTs were negatively correlated to DMI and ranged (for particles <2 mm) from 23/31 h (hay1/2) on the *ad libitum* to 38/48 h on DMI30. Digestibilities of DM, nutrients and fibre components decreased from DMI75 to DMI30; apparent digestibilities of organic matter and NDF (hay1/2) dropped from 47/43% and 42/37%, respectively, on the *ad libitum* DMI to 35/35% and 30/28% on DMI30. Additional differences evident between the two hays included a higher estimated 'true' protein digestibility for hay1 and finer faecal particles on hay2; there were no differences in faecal particle size between intake levels. The results suggest that below a certain food intake threshold, the major digestive constraint is not fermentation time but nutrient supply to gut bacteria. The threshold for such an effect probably varies between feeds and might differ between ruminants and equids.

**Keywords** equid, ruminant, hindgut fermenter, intake, retention time, chewing, metabolic faecal nitrogen

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## Introduction

In the classification of herbivores, digestive anatomy – hindgut vs. foregut fermentation – and digestive strategy – high throughput/low efficiency vs. low throughput/high efficiency – have traditionally been linked with the dichotomy of horses (equids) and cattle (ruminants) as the prominent example (Bell, 1971; Janis, 1976; Foose, 1982). Corresponding to this concept, free-ranging horses showed a higher food intake and, in spite of lower digestibilities, a higher digestible dry matter intake than sympatric cattle (Menard et al., 2002). However, in contrast to the expectation that horses should have not only a higher food intake than cattle, but also either do not decrease food intake

to the same extent with increasing forage fibre content as ruminants or even maintain or increase food intake as forage becomes more fibrous (Janis, 1976; Duncan et al., 1990), experimental data do not clearly support a difference between cattle and horses in this respect (Cymbaluk, 1990). Actually, in experiments, domestic horses usually ingest lower amounts of forages higher in fibre, responding to forage fibre levels in a similar manner as domestic cattle (Meyer et al., 2010). The general assumption that foregut fermentation and a low-intake/high-efficiency strategy and hindgut fermentation and a high-intake/low-efficiency strategy are linked has recently been modified (Clauss et al., 2010a); in particular, if rumination is added to foregut fermentation, the intake constraints associated

with foregut fermentation appear to be less severe, and some hindgut fermenters may also adopt a low-intake/high-efficiency strategy (Clauss et al., 2010b; Steuer et al., 2010), which may explain the lacking fit of experimental data with the traditional concept.

The horse's digestive strategy is characterized by a high chewing efficiency and comparatively short digesta retention (Clauss et al., 2009; Fritz et al., 2009). Duncan et al. (1990) concluded that the available data – indicating a higher daily nutrient extraction by horses as compared to cattle – do not explain why ruminants so evidently outcompete equids in terms of species diversity. It is suspected that under conditions of food limitation, the ruminant digestive system has an advantage (Duncan et al., 1990; Menard et al., 2002), and resource limitation has been invoked to explain the historical shift from equid- to ruminant-dominated ecosystems in the Miocene fossil record (Janis et al., 1994). However, studies on the effects of low food intake in horses are lacking.

Research in the digestive physiology of horses has repeatedly shown that as in any herbivore, variation in food intake leads to a variation in both digesta retention (higher intakes are linked with shorter retention times) and digestibility (shorter retention times are usually linked with lower digestibilities; Pearson et al., 2001, 2006; Ragnarsson and Lindberg, 2010); these studies, however, never included the very low intake levels used in exploring ruminant digestive physiology (Doreau et al., 2003). In horse trials, the gain in digestible nutrients with increasing intake usually exceeded the disadvantage of reduced digestibility.

In this study, we expanded experiments conducted hitherto in horses insofar as food intake variation included a very low intake level. We predicted that whereas overall digestible energy intake should decrease with decreasing intake level, digesta retention and hence general digestibility, and in particular fibre digestibility, should increase. The quantification of this increase was the major aim of this study. The tests were performed on hays of two different qualities, which allowed to measure whether true protein digestibility varies among forages. Additionally, we tested whether food particle size reduction by chewing might be compromised by low food intake, with hungry horses eating more greedily. Additionally, digesta retention time as well as digestibility of feed under conditions of low feed intake is not only of interest from a comparative point of view. It is extremely important in clinical conditions such as refeeding of horses after colic or colic surgery, feeding in intensive care and horses fed reducing diets.

## Methods

### Animals and husbandry

Four adult ponies [2 mares, 2 geldings, aged 12–18 years, initial body mass (BM)  $284 \pm 65$  kg] were used in two subsequent trials (separated by a 20-week period of their regular maintenance diet of hay and oats) with two grass hay-only diets of different quality (Table 1). Before each feeding trial, animals were dewormed and their teeth were controlled. Animals were kept individually with access to an outdoor enclosure without any plants and with water *ad libitum*. Each hay was fed subsequently at four different dry matter intake (DMI) levels of decreasing food quantity: *ad libitum*, and subsequently at 75, 55 and 30 g/kg<sup>0.75</sup>/day, with a 9-day adaptation and 5-day collection period for each intake level. At the end of each collection period, the next adaptation period started. Animals were weighed at the beginning of the adaptation period and the end of the collection period; at the time of marker feeding, body condition was scored according to Kienzle and Schramme (2004). The food was offered in multiple portions distributed across the whole day to avoid selective feeding and to ensure an even food intake across the whole day on the restricted intake levels. On hay 2, selective feeding was nevertheless observed during *ad libitum* feeding, and leftovers were collected separately and analysed to calculate the composition of the actually ingested diet (Table 1). Hay leftovers, as well as the total daily faecal output, were quantified during the collection period.

**Table 1** Nutrient composition of the hays used in this study

Measurement	Hay 1	Hay 2	Hay 2 <i>ad libitum</i> *
Dry matter (DM, g/kg fresh mass)	877	881	861
Crude ash (g/kg DM)	66	44	43 ± 0
Crude protein (g/kg DM)	105	58	62 ± 1
Crude fat (g/kg DM)	11	12	14 ± 1
Crude fibre (g/kg DM)	318	354	338 ± 5
Nitrogen-free extracts (g/kg DM)	499	532	543 ± 3
Neutral detergent fibre (g/kg DM)	676	695	667 ± 8
Acid detergent fibre (g/kg DM)	360	386	361 ± 7
Acid detergent lignin (g/kg DM)	108	120	115 ± 1
Acid-insoluble ash (g/kg DM)	17.1	12.5	13.9 ± 0.4
Gross energy (MJ/kg DM)	19.0	19.3	19.5 ± 0.1
Digestible energy (MJ/kg DM)†	9.0	–	–

\*Means ± standard deviations, calculated by subtracting leftovers from offered hay for each animal.

†Digestible energy, estimated from crude nutrients according to Zeyner and Kienzle (2002); note that this equation only applies for diets with a crude fibre content below 35% in DM; hay 2 was just above this threshold).

On the evening of the last day of the adaptation period, the horses were fed cobalt(Co)-EDTA (1–2 g per animal dissolved in 15 ml of water and applied to the mouth via syringe) as a solute and chromium (Cr)-mordanted fibre (<2 mm; 10–20 g per animal; Cr content 60 mg/kg dry matter, mixed in a small portion of apple/banana mush and consumed within 5–10 min) as a particle marker (prepared according to Udén et al., 1980). Prior to marker feeding, three faecal samples were taken for analysing the background levels of marker elements. During the first 3 days, each individual defecation was recorded with an accuracy of approximately 1 h; on days 4 and 5, defecations were collected in 3- and 5-h intervals respectively. Representative subsamples of all defecations were collected after weighing the whole sample and later used for marker analysis as well as for the composition of a pool sample representative of the whole collection period for the analysis of faecal nutrient and fibre content.

Samples for marker analysis were treated and analysed as described by Behrend et al. (2004). The MRT for the whole gastrointestinal tract (MRT GIT) was calculated according to Thielemans et al. (1978) as

$$\text{MRT GIT} = \frac{\sum t_i C_i dt_i}{\sum C_i dt_i}$$

with  $C_i$  is the marker concentration in the faecal samples from the interval represented by time  $t_i$  (hours after marker administration, using the midpoint of the sampling interval; for example, if the sampling interval was between 96 and 100 h after marker administration,  $t_i$  was taken as 98 h) and  $dt_i$  is 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 as pre-dose levels.

Hay and faecal samples were analysed for dry matter (DM), crude ash (CA), nitrogen/crude protein (CP), crude fat (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid-insoluble ash (AIA) and gross energy (GE) using standard methods (AOAC, 1997). Detergent fibre data are presented without residual ash. Nitrogen-free extracts (NfE) were calculated as 100-CA-CP-EE-CF. The content of metabolic faecal nitrogen (MFN) in the faeces was calculated as total faecal nitrogen (FN) minus NDF-bound nitrogen

(Mason, 1969) and expressed both per DM and per FN. Apparent digestibility was computed as the fraction not excreted in faeces of the ingested amount (Robbins, 1993, p. 292). Digestible energy (DE) content of hay 1 was calculated according to Zeyner and Kienzle (2002) as  $\text{DE (MJ/kg DM)} = -3.60 + 0.211 \text{ CP} + 0.421 \text{ EE} + 0.015 \text{ CF} + 0.189 \text{ NfE}$  (all nutrients in% DM; Table 1) and compared with the experimentally determined DE on the different intake levels. Note that this equation is valid only for diets with CF <35% DM; hay 2 of this study was just above that threshold.

The indigestible dry matter gut content (indDMC, g) and the total dry matter gut content (DMC, g) were calculated according to Holleman and White (1989):

$$\text{indDMC} = F * \text{MRT}$$

where  $F$  (faeces output, kg DM/h) is the total daily faeces output/24 and MRT is the mean particle retention time through the whole digestive tract (h). To yield DMC, the proportion of digestible DMC must be added to indDMC. This is done making basic assumptions on the occurrence of digestion with MRT:

$$\text{DMC}_{lin} = \text{indDMC} + ((\text{indDMC} * \text{aD DM}/100) / (2(1 - (\text{aD DM}/100))))$$

where aD is the apparent digestibility, assuming linear absorption of ingested food with time spent in the tract (note that this does not mean linear absorption along the digestive tract).

Mean particle size (MPS) of the faeces was determined by wet-sieving using a set of nine sieves with quadrature mesh sizes of 0.063, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 mm followed by calculating the discrete mean particle size according to Fritz et al. (2012; see also for detailed description of the wet-sieving method).

Data were analysed using a mixed effects model (General Linear Models; Statsoft, 2007), with the hay type (two hays) and intake level (four categories) as fixed effects. Individual was included as a random effect to account for non-independence among repeated measures. Interaction terms were omitted because of the relatively small sample size, but intake level was nested within hay type, which serves as a validation check for interaction effects. Where necessary, multiple comparisons were made using Bonferroni *post hoc* tests. Correlations between individual measurements were performed either using correlation analysis or using linear regression; 95%

confidence intervals (95% CI) are given for coefficients of regression equations. Additional general linear models were used to investigate differences between the two hays for relationships of continuous characters.

## Results

Subjectively, it appeared that the ponies ingested high-quality hay 1 faster than low-quality hay 2; this appeared to be due to selective feeding behaviour on hay 2, as evident during the *ad libitum* feeding (Table 1). Even on lower food intake levels, when mostly all the hay offered was consumed, the ponies appeared to first select the finer parts of hay 2, even interrupting their feeding bouts, before consuming the tougher parts.

Ponies lost body mass and body condition with decreasing intake level (Table 2). For the lowest intake level, the equation of Zeyner and Kienzle (2002) overestimated the measured intake of digestible energy on hay 1 (Table 2). A linear regression of the change in body mass on DE intake ( $y = 0.538 [95\% \text{ CI } 0.385\text{--}0.691] x - 0.376 [-0.458 \text{ to } -0.294]$ ,  $R^2 = 0.63$ ,  $p < 0.001$ ) yielded a mean maintenance requirement of  $0.70 \text{ MJ DE/kg}^{0.75}/\text{day}$  in these ponies, which is somewhat higher than the  $0.6 \text{ MJ DE/kg}^{0.75}/\text{day}$  generally observed in horses (Meyer and Coenen, 2002, p. 42).

One pony on intake level 75 on hay 2 had a particularly high dry matter digestibility (50% vs. the mean of the other three animals of 40%) and a long particle retention time (37 h vs. an average of 29 h in the other animals, cf. Table 3), and the excretion curve of the particle marker indicated re-ingestion of faeces.

Therefore, measurements from this animal on this hay and intake level were excluded from further analyses. Additionally, it was noted that all ponies had increasingly negative calculated digestibility coefficients for acid-insoluble ash (Table 4) with decreasing intake level. Although soil consumption was not specifically noted, this most likely indicates allophagic behaviour during food deprivation.

The mean retention time of both the solute and the particle marker generally increased with decreasing food intake level (Table 3). Marker excretion curves showed a later peak and a slower reduction in faecal marker levels with lower food intake levels (Fig. 1). Particle MRT ( $y$ ) decreased with relative dry matter

**Table 3** Average ( $\pm$ SD) mean retention time (MRT in h) of the particle marker (Cr) and the solute marker (Co) in the gastrointestinal tract, the selectivity factor (SF, the ratio of MRT Cr/MRT Co) and the estimated dry matter gut content (DMC, in kg) on two different hays and four different intake levels

Diet	Intake level	MRT Cr	MRT Co	SF	DMC
Hay 1	<i>ad libitum</i>	23 $\pm$ 4 <sup>aA</sup>	21 $\pm$ 4 <sup>aA</sup>	1.37 $\pm$ 0.50 <sup>aA</sup>	4.8 $\pm$ 1.7 <sup>aA</sup>
	75	25 $\pm$ 2 <sup>ab</sup>	22 $\pm$ 2 <sup>ab</sup>	1.16 $\pm$ 0.06 <sup>a</sup>	4.1 $\pm$ 0.9 <sup>ab</sup>
	55	33 $\pm$ 5 <sup>bc</sup>	28 $\pm$ 4 <sup>bc</sup>	1.20 $\pm$ 0.19 <sup>a</sup>	3.9 $\pm$ 1.2 <sup>ab</sup>
	30	38 $\pm$ 5 <sup>c</sup>	31 $\pm$ 2 <sup>c</sup>	1.21 $\pm$ 0.10 <sup>a</sup>	2.6 $\pm$ 0.5 <sup>b</sup>
Hay 2	<i>ad libitum</i>	31 $\pm$ 4 <sup>aB</sup>	23 $\pm$ 3 <sup>abB</sup>	1.38 $\pm$ 0.13 <sup>aA</sup>	5.7 $\pm$ 1.5 <sup>ab</sup>
	75	29 $\pm$ 4 <sup>a</sup>	20 $\pm$ 2 <sup>b</sup>	1.48 $\pm$ 0.31 <sup>a</sup>	5.1 $\pm$ 0.7 <sup>a</sup>
	55	34 $\pm$ 6 <sup>a</sup>	29 $\pm$ 4 <sup>a</sup>	1.18 $\pm$ 0.16 <sup>a</sup>	4.3 $\pm$ 1.0 <sup>ab</sup>
	30	48 $\pm$ 4 <sup>b</sup>	37 $\pm$ 4 <sup>c</sup>	1.29 $\pm$ 0.06 <sup>a</sup>	3.3 $\pm$ 0.4 <sup>b</sup>

Capital superscripts indicate significant differences between hays (main effect: hay); lower case superscripts indicate significant differences within a hay between the different intake levels (Bonferroni *post hoc* tests of the nested term [Intake Level (Hay Type)]).

**Table 2** Average ( $\pm$ SD) body condition score (BCS according to Kienzle and Schramme, 2004), body mass of ponies at the end of each trial period (BM, kg), the daily BM change (in% of the starting BM of the respective trial period), the relative dry matter intake (rDMI in  $\text{g/kg}^{0.75}/\text{day}$ ), the relative digestible energy intake (rDEI in  $\text{MJ/kg}^{0.75}/\text{day}$ ) and the estimated rDEI based on the dietary DE content estimate according to Zeyner and Kienzle (2002)

Diet	Intake level	BCS	BM	BM change	rDMI	rDEI	Estimated rDEI*
Hay 1	<i>ad libitum</i>	7.3 $\pm$ 1.1 <sup>aA</sup>	288 $\pm$ 65 <sup>aA</sup>	0.11 $\pm$ 0.06 <sup>aA</sup>	93 $\pm$ 8 <sup>aA</sup>	0.79 $\pm$ 0.11 <sup>aA</sup>	0.84 $\pm$ 0.07 <sup>a</sup>
	75	6.7 $\pm$ 1.1 <sup>b</sup>	284 $\pm$ 66 <sup>a</sup>	-0.10 $\pm$ 0.04 <sup>ab</sup>	77 $\pm$ 0 <sup>b</sup>	0.69 $\pm$ 0.04 <sup>a</sup>	0.69 $\pm$ 0.00 <sup>a</sup>
	55	6.1 $\pm$ 1.1 <sup>c</sup>	279 $\pm$ 62 <sup>a</sup>	-0.12 $\pm$ 0.09 <sup>b</sup>	54 $\pm$ 1 <sup>c</sup>	0.47 $\pm$ 0.02 <sup>b</sup>	0.48 $\pm$ 0.01 <sup>b</sup>
	30	5.6 $\pm$ 1.2 <sup>d</sup>	268 $\pm$ 59 <sup>b</sup>	-0.29 $\pm$ 0.04 <sup>b</sup>	31 $\pm$ 1 <sup>d</sup>	0.18 $\pm$ 0.02 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>c</sup>
Hay 2	<i>ad libitum</i>	6.7 $\pm$ 1.1 <sup>aB</sup>	284 $\pm$ 64 <sup>aB</sup>	0.01 $\pm$ 0.14 <sup>aA</sup>	80 $\pm$ 7 <sup>aB</sup>	0.64 $\pm$ 0.09 <sup>aB</sup>	-
	75	6.2 $\pm$ 1.1 <sup>a</sup>	283 $\pm$ 63 <sup>a</sup>	-0.02 $\pm$ 0.08 <sup>a</sup>	77 $\pm$ 0 <sup>a</sup>	0.55 $\pm$ 0.02 <sup>a</sup>	-
	55	5.8 $\pm$ 1.0 <sup>b</sup>	273 $\pm$ 59 <sup>b</sup>	-0.24 $\pm$ 0.04 <sup>b</sup>	56 $\pm$ 3 <sup>b</sup>	0.38 $\pm$ 0.02 <sup>b</sup>	-
	30	5.5 $\pm$ 1.1 <sup>b</sup>	264 $\pm$ 57 <sup>b</sup>	-0.25 $\pm$ 0.11 <sup>b</sup>	31 $\pm$ 0 <sup>c</sup>	0.19 $\pm$ 0.01 <sup>c</sup>	-

Capital superscripts indicate significant differences between hays (main effect: hay); lower case superscripts indicate significant differences within a hay between the different intake levels (Bonferroni *post hoc* tests of the nested term [Intake Level (Hay Type)]).

\*Note that this equation only applies for diets with a crude fibre content below 35% in DM; hay 2 was just above this threshold; also note that the equation is recommended for physiologically normal situations and not unusual situations as low intakes.

**Table 4** Average ( $\pm$ SD) apparent digestibilities (in%) of dry matter (DM) and various nutrients in ponies on two different hays and four different intake levels

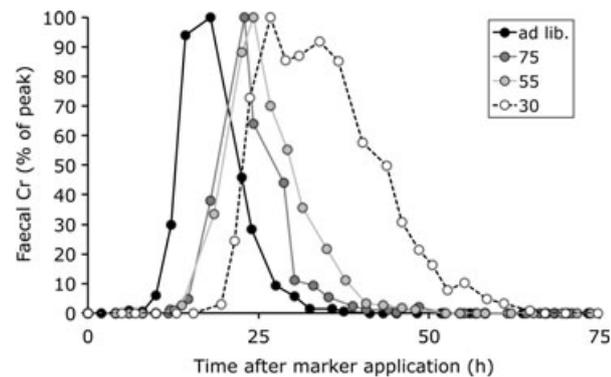
Diet	Intake level	DM	OM	CA	CP	CF	NDF	ADF	AIA	GE
Hay 1	<i>ad libitum</i>	48 $\pm$ 2 <sup>3A</sup>	47 $\pm$ 2 <sup>3A</sup>	54 $\pm$ 2 <sup>3A</sup>	62 $\pm$ 2 <sup>3A</sup>	35 $\pm$ 3 <sup>3A</sup>	42 $\pm$ 3 <sup>3A</sup>	37 $\pm$ 3 <sup>3A</sup>	37 $\pm$ 6 <sup>3A</sup>	44 $\pm$ 3 <sup>3A</sup>
	75	50 $\pm$ 3 <sup>a</sup>	50 $\pm$ 3 <sup>a</sup>	49 $\pm$ 6 <sup>ab</sup>	61 $\pm$ 4 <sup>a</sup>	41 $\pm$ 3 <sup>a</sup>	47 $\pm$ 3 <sup>a</sup>	41 $\pm$ 3 <sup>a</sup>	22 $\pm$ 13 <sup>ab</sup>	47 $\pm$ 3 <sup>a</sup>
	55	49 $\pm$ 2 <sup>a</sup>	50 $\pm$ 2 <sup>a</sup>	40 $\pm$ 5 <sup>b</sup>	62 $\pm$ 2 <sup>a</sup>	40 $\pm$ 3 <sup>a</sup>	47 $\pm$ 3 <sup>a</sup>	41 $\pm$ 3 <sup>a</sup>	2 $\pm$ 11 <sup>bc</sup>	46 $\pm$ 2 <sup>a</sup>
	30	34 $\pm$ 5 <sup>b</sup>	35 $\pm$ 5 <sup>b</sup>	22 $\pm$ 6 <sup>c</sup>	58 $\pm$ 2 <sup>a</sup>	11 $\pm$ 12 <sup>b</sup>	30 $\pm$ 7 <sup>b</sup>	14 $\pm$ 11 <sup>b</sup>	-11 $\pm$ 21 <sup>c</sup>	31 $\pm$ 4 <sup>b</sup>
Hay 2	<i>ad libitum</i>	43 $\pm$ 4 <sup>3B</sup>	43 $\pm$ 4 <sup>3B</sup>	32 $\pm$ 4 <sup>3B</sup>	43 $\pm$ 3 <sup>3B</sup>	34 $\pm$ 5 <sup>3A</sup>	37 $\pm$ 4 <sup>3B</sup>	32 $\pm$ 5 <sup>3B</sup>	10 $\pm$ 8 <sup>3B</sup>	41 $\pm$ 4 <sup>3B</sup>
	75	40 $\pm$ 1 <sup>ab</sup>	41 $\pm$ 1 <sup>ab</sup>	29 $\pm$ 5 <sup>a</sup>	37 $\pm$ 2 <sup>ab</sup>	35 $\pm$ 2 <sup>a</sup>	35 $\pm$ 2 <sup>a</sup>	32 $\pm$ 2 <sup>a</sup>	-9 $\pm$ 4 <sup>ab</sup>	38 $\pm$ 1 <sup>ab</sup>
	55	38 $\pm$ 1 <sup>ab</sup>	39 $\pm$ 1 <sup>ab</sup>	23 $\pm$ 4 <sup>a</sup>	36 $\pm$ 4 <sup>b</sup>	32 $\pm$ 3 <sup>a</sup>	33 $\pm$ 3 <sup>a</sup>	29 $\pm$ 3 <sup>a</sup>	-29 $\pm$ 3 <sup>bc</sup>	35 $\pm$ 1 <sup>ab</sup>
	30	33 $\pm$ 1 <sup>b</sup>	35 $\pm$ 1 <sup>b</sup>	-1 $\pm$ 9 <sup>b</sup>	30 $\pm$ 4 <sup>c</sup>	24 $\pm$ 4 <sup>a</sup>	28 $\pm$ 3 <sup>a</sup>	20 $\pm$ 3 <sup>a</sup>	-55 $\pm$ 12 <sup>c</sup>	32 $\pm$ 1 <sup>b</sup>

OM, organic matter; CA, crude ash; CP, crude protein; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; AIA, acid-insoluble ash; GE, gross energy.

Capital superscripts indicate significant differences between hays (main effect: hay); lower case superscripts indicate significant differences within a hay between the different intake levels (Bonferroni *post hoc* tests of the nested term [Intake Level (Hay Type)]).

intake ( $x$  in  $\text{g/kg}^{0.75}/\text{day}$ ) according to  $y = 198 [97-406] x^{-0.47}$  [ $-0.65$  to  $-0.30$ ] ( $R^2 = 0.70$ ,  $p < 0.001$ ) for hay 1 and  $y = 235 [109-508] x^{-0.47}$  [ $-0.66$  to  $-0.28$ ] ( $R^2 = 0.69$ ,  $p < 0.001$ ) for hay 2 and was mostly in the range of reported literature data (Fig. 2). Using a general linear model to test for a difference between the rDMI-MRT relationship between the two hays, hay as a cofactor was significant ( $F_{2,28} = 11.920$ ,  $p = 0.002$ ). There was no significant change in the selectivity factor, that is, the ratio of particle to solute MRT with food intake ( $p$  for intake level = 0.610; Table 3). The calculated dry matter gut content decreased with decreasing food intake, was generally higher on hay 2 and also matched the pattern found in literature data (Table 3, Fig. 3).

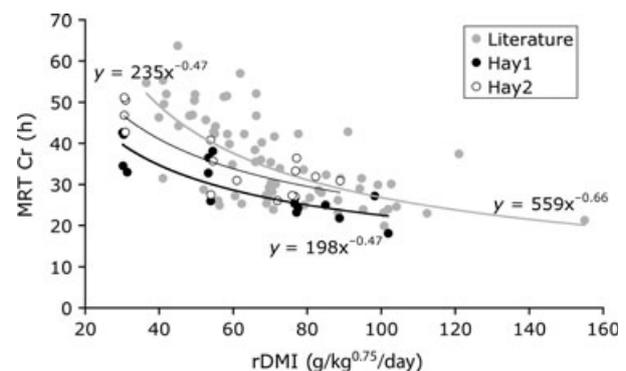
Digestibilities were generally higher for hay 1 than for hay 2 [main effect hay type  $p < 0.0001$ ; except for EE ( $p = 0.179$ ), CF ( $p = 0.662$ ), and ADF ( $p = 0.005$ )]. On hay 1, digestibility decreased from the first three intake levels to intake 30 (Table 4). On hay 2, digestibility decreased more consistently with



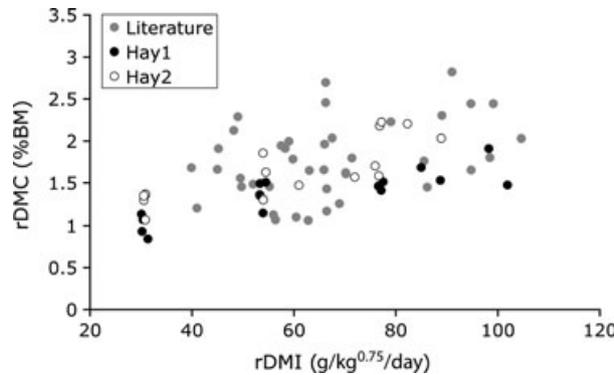
**Fig. 1** Particle marker excretion patterns in one pony on hay 1 and four different intake levels (indicated in  $\text{g dry matter/kg}^{0.75}/\text{day}$ ).

intake level, but – with only three ponies left for evaluation – the effect was often not significant ( $p > 0.05$ ). Notably, on the lowest intake level, fibre digestibilities were lowest (and similar) on both hays. Whereas the differences in protein digestibility were significant between intake levels for hay 2 but not hay 1, the differences in fibre digestibility between intake levels were significant on hay 1 but not hay 2 (Table 4). In contrast, the difference in DM, OM and GE digestibility between the lowest intake level and *ad libitum* feeding was significant for both hays (Table 4).

Faecal dry matter concentration was generally lower for hay 1 than for hay 2 (main effect hay type  $p = 0.0001$ , Table 5), but the numerical increase with



**Fig. 2** Relationship between the relative dry matter intake (rDMI in  $\text{g dry matter/kg}^{0.75}/\text{day}$ ) and the mean retention time (MRT in h) of a particle marker (Cr) in ponies on two different hays and four different intake levels. Comparative data for horses from literature sources (Wolter et al., 1976; Orton et al., 1985a,b; Suhartanto et al., 1992; Cuddeford et al., 1995; Todd et al., 1995; Yoder et al., 1997; Pagan et al., 1998; Drogoul et al., 2000, 2001; Pearson et al., 2001; de Araújo Oliveira et al., 2003; Moore-Colyer et al., 2003; Austbø and Volden, 2006; Pearson et al., 2006; Rosenfeld et al., 2006; Miyaji et al., 2011).



**Fig. 3** Relationship between the relative dry matter intake (rDMI in g dry matter/kg<sup>0.75</sup>/day) and the relative dry matter gut content (DMC in% of body mass) in ponies on two different hays and four different intake levels. Comparative data for horses calculated from literature sources (Orton et al., 1985a,b; Pearson and Merritt, 1991; Cuddeford et al., 1995; Pagan et al., 1998; Drogoul et al., 2000; Pearson et al., 2001; Moore-Colyer et al., 2003; Pearson et al., 2006; Miyaji et al., 2011).

decreasing food intake was not significant. Faecal nitrogen (FN) values on hay 2 were generally lower (main effect hay type  $p < 0.0001$ ). FN was lower on the lowest intake level on hay 1, but there were no differences for hay 2 (Table 5). FN was highly correlated to aD OM ( $R = 0.739$ ,  $p < 0.001$ ), aD NDF ( $R = 0.798$ ,  $p < 0.001$ ) and aD ADF ( $R = 0.618$ ,  $p < 0.001$ ). Metabolic faecal nitrogen (MFN in% DM) was higher for hay 1 (main effect hay type  $p < 0.0001$ ), but did not differ significantly between intake levels; it was also highly correlated with digestibility coefficients (aD OM:  $R = 0.754$ ,  $p < 0.001$ , aD NDF:  $R = 0.790$ ,  $p < 0.001$ , aD ADF:  $R = 0.627$ ,  $p < 0.001$ ). When expressed as a proportion of FN, MFN again was higher on hay 1 (main effect hay type  $p = 0.008$ ) but did not differ between intake levels (Table 5). MFN (% FN) increased with increasing FN ( $R = 0.624$ ,  $p < 0.001$ ).

Hay 2 was masticated into finer particles than hay 1, but there were no differences in faecal particle size between intake levels. A numerical trend of increasing faecal particle size with decreasing food intake level was not significant (Table 5).

## Discussion

Food intake level had the predicted effect on several digestive measurements in this study. In particular, digesta retention closely followed food intake; the relationship between the relative food intake and particle mean retention time in this study and in the horse literature in general (Fig. 2) matches the overall relationship presented by Franz et al. (2011a) of  $MRT_{particle} = 264 [95\% \text{ CI } 94\text{--}739] rDMI^{-0.53}[-0.79 \text{ to } -0.26]$  for mammalian herbivores, which suggests a fundamental principle by which food intake influences digesta passage through the gastrointestinal tract. The magnitude of the effect of intake on digesta passage may well differ between herbivore species, and a flexible gut capacity is considered crucial in this respect (Clauss et al., 2007; Munn et al., 2008); alternatively, because the relationship between MRT and rDMI is best represented by a non-linear regression, different species may predominantly differ in their ranges of rDMI and hence experience different effect sizes on MRT. In the horses of this study, gut capacity estimated as dry matter contents varied between 0.8% and 2.2% of body mass (Fig. 3); this flexibility may help horses to partially compensate the effect of higher food intakes on digestion (Lechner-Doll et al., 1992). The results of this study also indicate that independent of the food intake level, the lower-quality hay led to longer mean retention times, thus indicating an influence of the food itself on digesta independent from the effect of intake level. This finding might link with the observation of impaction colics in horses fed straw, a rough-

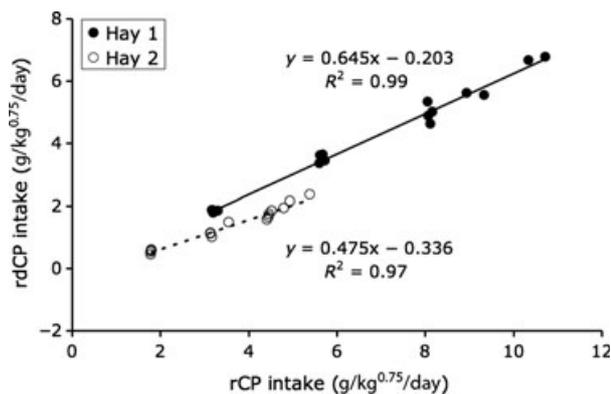
**Table 5** Average ( $\pm$ SD) faecal dry matter concentration (FDM, in% wet weight), faecal nitrogen (FN) and metabolic faecal nitrogen (MFN, both in% DM and in% FN), and the discrete mean particle size (dMPS in mm, Fritz et al., 2012) in ponies on two different hays and four different intake levels

Diet	Intake level	FDM	FN	MFN% DM	MFN% FN	dMPS
Hay 1	<i>ad libitum</i>	18.1 $\pm$ 1.5 <sup>aA</sup>	1.27 $\pm$ 0.08 <sup>abA</sup>	0.56 $\pm$ 0.13 <sup>aA</sup>	43.3 $\pm$ 8.3 <sup>aA</sup>	1.17 $\pm$ 0.17 <sup>aA</sup>
	75	17.5 $\pm$ 1.2 <sup>a</sup>	1.38 $\pm$ 0.06 <sup>b</sup>	0.66 $\pm$ 0.04 <sup>a</sup>	47.7 $\pm$ 3.6 <sup>a</sup>	1.25 $\pm$ 0.36 <sup>a</sup>
	55	19.7 $\pm$ 2.1 <sup>a</sup>	1.33 $\pm$ 0.06 <sup>b</sup>	0.69 $\pm$ 0.04 <sup>a</sup>	51.7 $\pm$ 2.1 <sup>a</sup>	1.55 $\pm$ 0.78 <sup>a</sup>
	30	20.3 $\pm$ 3.0 <sup>a</sup>	1.15 $\pm$ 0.11 <sup>a</sup>	0.49 $\pm$ 0.07 <sup>a</sup>	42.7 $\pm$ 5.7 <sup>a</sup>	1.34 $\pm$ 0.43 <sup>a</sup>
Hay 2	<i>ad libitum</i>	21.7 $\pm$ 1.6 <sup>abB</sup>	1.03 $\pm$ 0.07 <sup>abB</sup>	0.46 $\pm$ 0.11 <sup>abB</sup>	44.1 $\pm$ 8.1 <sup>abB</sup>	0.74 $\pm$ 0.19 <sup>abB</sup>
	75	21.8 $\pm$ 1.7 <sup>a</sup>	1.01 $\pm$ 0.03 <sup>a</sup>	0.42 $\pm$ 0.06 <sup>a</sup>	41.6 $\pm$ 4.8 <sup>a</sup>	0.74 $\pm$ 0.02 <sup>a</sup>
	55	23.1 $\pm$ 1.5 <sup>a</sup>	0.99 $\pm$ 0.08 <sup>a</sup>	0.37 $\pm$ 0.10 <sup>a</sup>	37.1 $\pm$ 7.7 <sup>a</sup>	0.73 $\pm$ 0.21 <sup>a</sup>
	30	23.8 $\pm$ 2.9 <sup>a</sup>	1.01 $\pm$ 0.07 <sup>a</sup>	0.38 $\pm$ 0.06 <sup>a</sup>	37.7 $\pm$ 4.7 <sup>a</sup>	0.85 $\pm$ 0.37 <sup>a</sup>

Capital superscripts indicate significant differences between hays (main effect: hay); lower case superscripts indicate significant differences within a hay between the different intake levels (Bonferroni *post hoc* tests of the nested term [Intake Level (Hay Type)]).

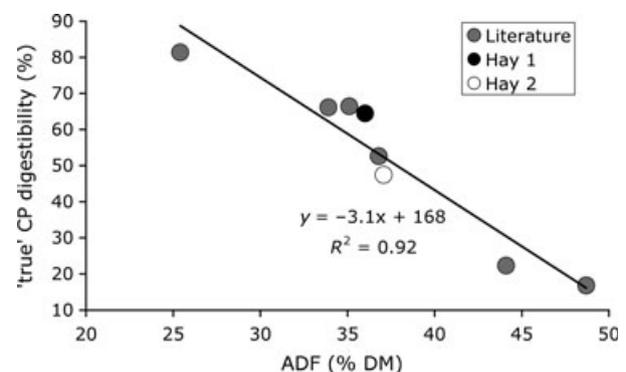
age of very low digestibility (Meyer and Coenen, 2002, p. 93). The higher faecal dry matter concentration on hay 2, and the numerical increase with decreasing intake level, could not only indicate an increased water re-absorption with increasing retention time, but might also be linked to the lower digestibility, with less osmotically active volatile fatty acid production. Whether such osmotic effects are behind the physiological mechanism by which prolonged retention of lower-digestibility roughage occurs remains to be elucidated. If this was the case, the addition of either nitrogen or easily fermentable carbohydrates (Mundt, 1978; Lindemann, 1982) should lead to a reduction of digesta retention on low-quality forages.

According to the regression of digestible CP intake vs. CP intake (the Lucas principle, Van Soest, 1967), the endogenous faecal losses (the intercept of the regression equation) and the 'true' digestibility of CP (the slope of the regression equation) differed between the two hays (Fig. 4). After determining 'true' CP digestibility in a similar fashion for various roughages used at two intake levels from the study by Pearson et al. (2001, 2006; excluding one straw for which protein digestibility was lower on the higher intake level) and Ragnarsson and Lindberg (2010), there was a significant correlation between the ADF content of the roughages and their estimated 'true' protein digestibility (Fig. 5). Usually, an evaluation of the 'true' digestibility using the Lucas principle of



**Fig. 4** Relationship between the daily intake of crude protein (CP, relative to metabolic body weight) and the daily intake of digestible CP (dCP, relative to metabolic body weight) in ponies on two different hays and four different intake levels. Regression equation for hay 1:  $y = 0.645$  [95% CI 0.604–0.685]  $x - 0.203$  [–0.494 to 0.089] ( $R^2 = 0.99$ ,  $p < 0.001$ ); for hay 2:  $y = 0.475$  [0.426–0.523]  $x - 0.336$  [–0.517 to –0.155] ( $R^2 = 0.97$ ,  $p < 0.001$ ); in a general linear model, the hay \* rCP intake interaction was significant ( $F_{3,27} = 17.977$ ,  $p < 0.001$ ), indicating a genuine difference between the slopes.

plotting intake vs. digestible intake (or nutrient concentration vs. digestible nutrient concentration) is performed by combining results from experiments with different diets (Zeyner and Kienzle, 2002; Pearson et al., 2006). In doing so, differences between diets cannot be explored but will be represented by scatter around the regression line. Whether an evaluation of endogenous losses and 'true' nutrient digestibility is better performed on a nutrient concentration basis (which assumes constant endogenous losses per unit of food intake) or on a nutrient intake basis (in absolute or relative terms, which assumes constant endogenous losses per animal or per unit of [metabolic] body mass) is a methodological issue yet unresolved. The results of this study suggest that differences of the diets tested may justify the assumption of constant losses per animal or unit (metabolic) body mass for a given diet. Although this remains to be tested directly, the relationship of 'true' protein digestibility with ADF in the combined data set of this and other studies (Fig. 5) suggests that fibre-bound nitrogen, commonly assumed to be mostly unavailable to the herbivore (Van Soest, 1994, p. 292), is the main influence factor for the calculated protein use in horses (Zeyner et al., 2010): the higher the ADF content, the higher the putative proportion of unavailable ADF-bound nitrogen in the overall analysed protein levels, suggesting that the proportion of indigestible nitrogen in ADF is rather constant across the forages tested. These results match the observation of Zeyner et al. (2010) that the proportion of protein digestible in the small intestine of horses is directly related to the non-fibre-bound protein in the feed; in the case of



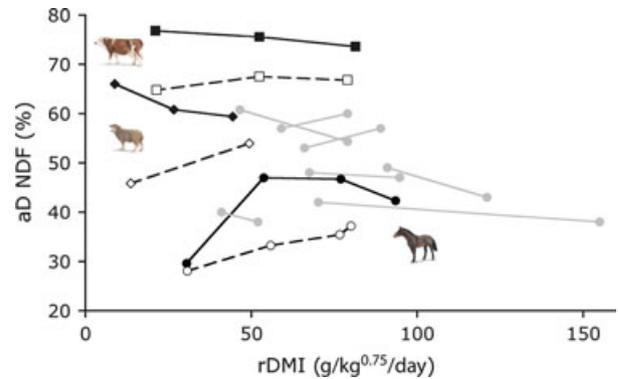
**Fig. 5** Relationship between the acid detergent fibre (ADF, in% dry matter) level of roughages in ponies and the 'true' crude protein digestibility as estimated from applying linear regression to data from digestion trials in which these roughages were fed at different intake levels. Data from this study (hay 1 and 2) and from Pearson et al. (2001, 2006) and Ragnarsson and Lindberg (2010). The regression line is  $y = 168$  [95% CI 132–204]  $- 3.12$  [–4.06 to –2.17]  $x$  ( $R^2 = 0.92$ ,  $p < 0.001$ ).

that study, this protein fraction was quantified as non-NDF-bound protein.

Horses do not appear to compensate for a low amount of food by an increased chewing intensity, and, in contrast to reports in ruminants (Shaver et al., 1988), chewing efficiency does not seem to be compromised at high intake levels. The numerical increase in particle size with decreasing intake level could be an effect of a disproportionately hasty ingestion in hungry animals, as reported in sheep on low food intake levels (Galvani et al., 2010). An increase in chewing intensity with forage fibre content has been reported previously in horses (Janis et al., 2010); the finer mean particle size measured on hay 2 in this study matches this pattern, as does the un-quantified observation of generally longer feeding times on this hay.

The expected effect of digesta retention on digestibility was only evident on hay 1, in which digestibility decreased at the highest intake level. No such effect was evident for hay 2. Similarly, other studies found that the digestibility of some forages was lower on an *ad libitum* feeding regime than on a moderate intake restriction, but this pattern is not common to all forages (horse literature data in Fig. 6). The increase in digestibility at restricted intake levels is plausibly explained by the additional time available for microbial digestion due to the longer retention times. However, this was not the case for hay 2, where the higher digestibilities were achieved on the shorter digesta retention times, and also not for the most drastic intake restriction on hay 1 where digesta retention was on average 15 h longer than during *ad libitum* feeding, yet OM and NDF digestibility were lower by 12 and 13 percentage points respectively. Clearly, other effects than retention time must play an important role in the digestion of forage material.

The effects of very low food intake on digestion have been studied extensively in domestic ruminants (Doreau et al., 2003, 2004), where a drop in overall and fibre digestibility with very low intakes has been described repeatedly. Doreau et al. (2003) summarize these findings, pointing out the difficulty in tracing the causes for this drop in digestive efficiency: low food intake generally leads to longer digesta retention – as in our study in horses – which should favour (rather than restrict) fibre digestion, and measures of particle size reduction can also not account for the reduced fibre digestibility; therefore, neither digesta retention nor particle size reduction can explain the observed phenomenon. A drop in the digestibility of organic matter or crude protein, as also noticeable in our horses (Table 4), could be explained by the fact



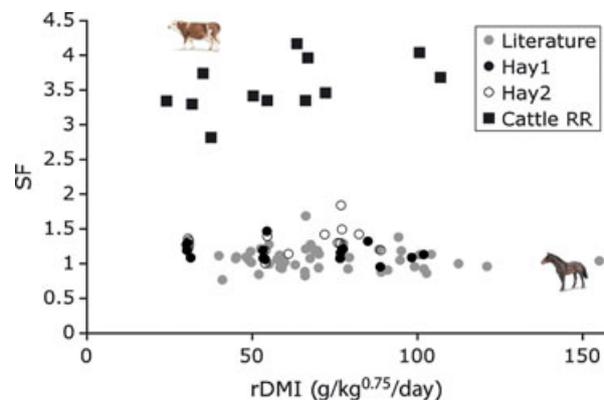
**Fig. 6** Relationship between the level of intake (relative dry matter intake rDMI in  $\text{g/kg}^{0.75}/\text{day}$ ) and the apparent digestibility of neutral detergent fibre (aD NDF in%) in cattle (squares), sheep (diamonds) and horses (circles) on forage only diets. Filled symbols represent higher-quality forage (cattle: CP 12%, NDF 56%; sheep CP 8.8%, NDF 67.5%; horse CP 10.5%, NDF 67.6%, all per dry matter) and open symbols lower-quality forage (cattle: CP 14.1%, NDF 65.3%; sheep CP 7.5%, NDF 80.9%; horse CP 5.8%, NDF 69.5%). Data for cattle and sheep from the literature (Michealet-Doreau and Doreau, 2001; Atti et al., 2002; Doreau and Diawara, 2003) and for horses from this study. Grey symbols indicate results on various forage only diets from studies with two intake levels in horses (Pearson et al., 2001, 2006; Ragnarsson and Lindberg, 2010).

that at lower intakes, inevitable endogenous losses have a proportionately increasing effect on the calculated apparent digestibility (Robbins, 1993, p. 293). On the one hand, this implies a concept of constant endogenous losses irrespective of the level of food intake (i.e. endogenous losses are not a constant fraction of DMI); on the other hand, this cannot apply to fibre measurements, as fibre is not secreted endogenously. Nevertheless, fibre digestibilities were also affected by the low intake levels in this study in horses and in several on domestic ruminants (Doreau et al., 2003). In ruminants, the simultaneous measurement of digestibilities *in vivo* and *in situ* (using fistulated animals) demonstrated that *in situ* digestibility did not follow the *in vivo* decrease at low intakes, suggesting that microbial activity itself is not impaired (Grimaud et al., 1998; Doreau and Diawara, 2003; Doreau et al., 2003, 2004); similarly, these studies also found no evidence for an impairment in the attachment of microorganisms to feed particles (cf. also Michealet-Doreau and Doreau, 2001). The results that the proportion of metabolic faecal nitrogen in faecal dry matter and faecal nitrogen did not vary significantly between intake levels in this study (Table 5) may similarly indicate that microbial biomass as such is not the limiting factor for fibre digestibility. In conclusion, it is suspected that an absolute nutrient shortage at low intakes impairs microbial degradation of fibre. In ruminants, the effect may well be one of dilution,

because rumen contents decrease in dry matter concentration at low intakes (Michealet-Doreau and Doreau, 2001). Such dilution may not operate at the level of *in situ* incubation in nylon bags. Whether the low digestibilities on low intakes in horses are associated with more fluid or more condensed digesta remains to be investigated. For future investigations, the controlled addition of certain nutrients to low-intake feeding regimes could help elucidate the limiting factors at low intakes. Nitrogen and easily fermentable carbohydrates appear as a particularly suitable candidate for such studies, because additions of either nutrient group have been shown to enhance the digestibility of low-quality roughages in horses (Mundt, 1978; Lindemann, 1982). Potentially, differences in the proportions of microbes in maintenance and in growth stages or different metabolic states may also play a role, but these effects also await further investigation.

Doreau et al. (2003) conclude that little difference can be noted between cattle and sheep with respect to the effect of low intake on digestibility; additionally, they suggest that effects of low intake may be more detrimental on low-quality roughages in ruminants. When comparing the reaction of cattle and sheep on roughage-only diets to low food intake with those of the horses of this study (Fig. 6), the reaction to a lower-quality roughage appears to be of a similar direction at least in sheep and horses and may be linked to the low absolute nutrient supply to the gastrointestinal microbes. However, the difference between the ruminants and the horses on the higher-quality hays appears as particularly striking – it is tempting to suggest that such nutrient limitations for microbes still play a role in horses in this case, whereas ruminants do not experience such an effect on the higher-quality roughages. Evidently, studies employing direct comparisons of horses and ruminants on identical forages and intake levels are warranted to really test this hypothesis; if corroborated, the major ecological difference between equids and ruminants might really, as suggested before (Duncan et al., 1990; Janis et al., 1994; Menard et al., 2002), lie in the ruminants' higher tolerance of resource shortages.

It should be noted that this need not imply a general difference between hindgut fermenters and ruminants, but rather represents a peculiarity applicable, until further research is performed, to the ruminant-equid dichotomy only. Other hindgut fermenters have been shown to digest fibre efficiently at the food intake level where the horses of this study were compromised – not only among mammals (reviewed in Müller et al., 2011), but also among reptiles (reviewed



**Fig. 7** Relationship between the 'selectivity factor' (SF, the ratio of particle to solute marker mean retention time) in the reticulo-rumen (RR) of cattle from the literature (Grimaud and Doreau, 1995; Grimaud et al., 1999; Grimaud and Doreau, 2003) and the gastrointestinal tract of horses from this study on two different hays. Grey symbols indicate results on horses from the literature (Orton et al., 1985a,b; Pearson and Merritt, 1991; Cuddeford et al., 1995; Drogoul et al., 2000, 2001; Pearson et al., 2001; de Araújo Oliveira et al., 2003; Pearson et al., 2006; Miyaji et al., 2011).

in Franz et al., 2011a). Given indications for an overall similarity of the microbial population of herbivores (Ley et al., 2008; Franz et al., 2011b), this difference with respect to intake levels is remarkable and clearly warrants corroboration and further investigation. One potential reason for a difference between horses and ruminants could be a fundamental difference in the degree of fluid throughput through the digestive tract and hence through the particulate digesta (Steuer et al., 2010; Müller et al., 2011). Whereas fluids and particles travel more or less simultaneously through the digestive tract of horses, leading to a 'selectivity factor' close to unity (Table 3, Fig. 7), the fluid phase shows a comparatively faster throughput in ruminants (see Fig. 7 for examples). Because most of this higher fluid throughput stems from saliva inflow into the digestive tract, this system has an increased potential to provide gastrointestinal microbes with additional nutrients, such as recycled nitrogen or phosphorus (Lapierre and Lobley, 2001; Bravo et al., 2003), possibly making ruminants somewhat less dependent on the nutrient influx via food intake.

## Conclusion

This study underlines the fundamental influence of low food intake on digestion parameters in horses and thereby suggests that horses – and herbivores in general – may be adapted to a certain range of intake levels below which their digestive tract does not

operate optimally any more. In spite of the well-documented positive correlation between fermentation time and digestibility of forage *in vitro* (Hummel et al., 2006), the longest digesta retention did not lead to high digestibility *in vivo*, indicating that below a certain food intake threshold, the major digestive constraint is not fermentation time but absolute nutrient supply to gut bacteria. Horses evidently need a food intake level above 30 g/kg<sup>0.75</sup>/day to maintain proper gut function. Estimations of dietary energy content using crude nutrient composition will only

apply for intakes above this level. Potential differences between herbivores regarding such an intake threshold remain to be investigated. For feeding practice under clinical conditions, the results support the recommendation that intensive care patients, whose food intake might still be compromised, should be offered roughage of moderate fibre and high protein content (Ralston, 2002), to possibly avoid the negative effects of low food intake on digestibility observed in this study.

## References

- AOAC, 1997: *Official Methods of Analysis*. Association of Official Analytical Chemists, Arlington, VA.
- de Araújo Oliveira, C. A.; de Almeida, F. Q.; Vieira, A. A.; Quintão Lana, A. M.; Macedo, R.; Adese Lopes, B.; Corassa, A., 2003: Kinetics of passage of digesta and water and nitrogen balance in horses fed diets with different ratios of roughage and concentrate. *Revista da Sociedade Brasileira de Zootecnia* **32**, 140–149.
- Atti, N.; Kayouli, C.; Mahouachi, M.; Guesmi, A.; Doreau, M., 2002: Effect of a drastic and extended underfeeding on digestion in Barbary ewe. *Animal Feed Science and Technology* **100**, 1–14.
- Austbø, D.; Volden, H., 2006: Influence of passage model and caecal cannulation on estimated passage kinetics of roughage and concentrate in the gastrointestinal tract of horses. *Livestock Science* **100**, 33–43.
- Behrend, A.; Lechner-Doll, M.; Streich, W. J.; Clauss, M., 2004: Seasonal faecal excretion, gut fill, liquid and particle marker retention in mouflon (*Ovis ammon musimon*), and a comparison with roe deer (*Capreolus capreolus*). *Acta Theriologica* **49**, 503–515.
- Bell, R. H. V., 1971: A grazing ecosystem in the Serengeti. *Scientific American* **225**, 86–93.
- Bravo, D.; Sauvant, D.; Bogaert, C.; Meschy, F., 2003: Quantitative aspects of phosphorus excretion in ruminants. *Reproduction, Nutrition, Development* **43**, 285–300.
- Clauss, M.; Streich, W. J.; Schwarm, A.; Ortmann, S.; Hummel, J., 2007: The relationship of food intake and ingesta passage predicts feeding ecology in two different megaherbivore groups. *Oikos* **116**, 209–216.
- Clauss, M.; Nunn, C.; Fritz, J.; Hummel, J., 2009: Evidence for a tradeoff between retention time and chewing efficiency in large mammalian herbivores. *Comparative Biochemistry and Physiology, Series A* **154**, 376–382.
- Clauss, M.; Hume, I. D.; Hummel, J., 2010a: Evolutionary adaptations of ruminants and their potential relevance for modern production systems. *Animal* **4**, 979–992.
- Clauss, M.; Lang-Deuerling, S.; Müller, D. W. H.; Kienzle, E.; Steuer, P.; Hummel, J., 2010b: Retention of fluid and particles in captive tapirs (*Tapirus* spp.). *Comparative Biochemistry and Physiology, Series A* **157**, 95–101.
- Cuddeford, D.; Pearson, R. A.; Archibald, R. F.; Muirhead, R. H., 1995: Digestibility and gastro-intestinal transit time of diets containing different proportions of alfalfa and oat straw given to thoroughbreds, Shetland ponies, Highland ponies and donkeys. *Animal Science* **61**, 407–417.
- Cymbaluk, N. F., 1990: Comparison of forage digestion by cattle and horses. *Canadian Journal of Animal Science* **70**, 601–610.
- Doreau, M.; Diawara, A., 2003: Effect of level of intake on digestion in cows: influence of animal genotype and nature of hay. *Livestock Production Science* **81**, 33–45.
- Doreau, M.; Michalet-Doreau, B.; Grimaud, P.; Atti, N.; Nozière, P., 2003: Consequences of underfeeding on digestion and absorption in sheep. *Small Ruminant Research* **49**, 289–301.
- Doreau, M.; Michalet-Doreau, B.; Béchet, G., 2004: Effect of underfeeding on digestion in cows. Interaction with rumen degradable N supply. *Livestock Production Science* **88**, 33–41.
- Drogoul, C.; Poncet, C.; Tisserand, J. L., 2000: Feeding ground and pelleted hay rather than chopped hay to ponies. I. Consequences for *in vivo* digestibility and rate of passage of digesta. *Animal Feed Science and Technology* **87**, 117–130.
- Drogoul, C.; de Fombelle, A.; Julliard, V., 2001: Feeding and microbial disorders in horses. II. Effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. *Journal of Equine Veterinary Science* **21**, 487–491.
- Duncan, P.; Foote, T. J.; Gordon, I. J.; Gakahu, C. G.; Lloyd, M., 1990: Comparative nutrient extraction from forages by grazing bovids and equids: a test of the nutritional model of equid/bovid competition and coexistence. *Oecologia* **84**, 411–418.
- Foote, T. J., 1982: *Trophic Strategies of Ruminant Versus Nonruminant Ungulates*. University of Chicago, Chicago.
- Franz, R.; Hummel, J.; Müller, D. W. H.; Bauert, M.; Hatt, J.-M.; Clauss, M., 2011a: Herbivorous reptiles and body mass: effects on food intake, digesta retention, digestibility and gut capacity, and a comparison with mammals. *Comparative Biochemistry and Physiology, Series A* **158**, 94–101.
- Franz, R.; Soliva, C. R.; Kreuzer, M.; Hatt, J.-M.; Furrer, S.; Hummel, J.; Clauss, M., 2011b: Methane output of tortoises: its contribution to energy loss related to herbivore body mass. *PLoS ONE* **6**, e17628.
- Fritz, J.; Hummel, J.; Kienzle, E.; Arnold, C.; Nunn, C.; Clauss, M., 2009: Comparative chewing efficiency in mammalian herbivores. *Oikos* **118**, 1623–1632.
- Fritz, J.; Streich, W. J.; Schwarm, A.; Clauss, M., 2012: Condensing results of wet sieving analyses into a single data: a comparison of methods for particle

- size description. *Journal of Animal Physiology and Animal Nutrition* **96**, 783–797.
- Galvani, D. B.; Pires, C. C.; Wommer, T. P.; Oliveira, F.; Santos, M. F., 2010: Chewing patterns and digestion in sheep submitted to feed restriction. *Journal of Animal Physiology and Animal Nutrition* **94**, e366–e373.
- Grimaud, P.; Doreau, M., 1995: Effect of extended underfeeding on digestion and nitrogen balance in nonlactating cows. *Journal of Animal Science* **73**, 211–219.
- Grimaud, P.; Doreau, M., 2003: Effects of level of intake and nitrogen supplementation on digestion by cows in a tropical environment. *Animal Research* **52**, 103–118.
- Grimaud, P.; Richard, D.; Kanwé, A.; Durier, C.; M, D., 1998: Effect of undernutrition and refeeding on digestion in *Bos taurus* and *Bos indicus* in a tropical environment. *Animal Science* **67**, 49–58.
- Grimaud, P.; Richard, D.; Vergeron, M. P.; Guilleret, J. R.; Doreau, M., 1999: Effect of drastic undernutrition on digestion in zebu cattle receiving a diet based on rice straw. *Journal of Animal Science* **82**, 974–981.
- Holleman, D. F.; White, R. G., 1989: Determination of digesta fill and passage rate from non absorbed particulate phase markers using the single dosing method. *Canadian Journal of Zoology* **67**, 488–494.
- Hummel, J.; Südekum, K.-H.; Streich, W. J.; Clauss, M., 2006: Forage fermentation patterns and their implications for herbivore ingesta retention times. *Functional Ecology* **20**, 989–1002.
- Janis, C., 1976: The evolutionary strategy of the Equidae and the origins of rumen and caecal digestion. *Evolution* **30**, 757–774.
- Janis, C. M.; Gordon, I. J.; Illius, A. W., 1994: Modelling equid/ruminant competition in the fossil record. *Historical Biology* **8**, 15–29.
- Janis, C. M.; Constable, E. C.; Houpt, K. A.; Streich, W. J.; Clauss, M., 2010: Comparative ingestive mastication in domestic horses and cattle: a pilot investigation. *Journal of Animal Physiology and Animal Nutrition* **94**, e402–e409.
- Kienzle, E.; Schramme, S., 2004: Body condition scoring and prediction of bodyweight in adult warm-blooded horses. *Pferdeheilkd* **20**, 517–524.
- Lapierre, H.; Lobley, G. E., 2001: Nitrogen recycling in the ruminant: a review. *Journal of Dairy Science* **84**, E223–E236.
- Lechner-Doll, M.; Becker, G.; von Engelhardt, W., 1992: Comparative aspects of digesta passage in equines. *Pferdeheilkd, First European Conference on Equine Nutrition*, pp. 36–38.
- Ley, R. E.; Hamady, M.; Lozupone, C.; Turnbaugh, P. J.; Ramey, R. R.; Bircher, J. S.; Schlegel, M. L.; Tucker, T. A.; Schrenzel, M. D.; Knight, R.; Gordon, J. L., 2008: Evolution of mammals and their gut microbes. *Science* **320**, 1647–1651.
- Lindemann, G., 1982: *Untersuchungen über den Einfluss von Lactose- und Stärkezulagen auf die Verdaulichkeit von NH<sub>3</sub>-aufgeschlossenem Stroh beim Pferd*. TiHo Hannover, Hannover.
- Mason, V. C., 1969: Some observations on the distribution and origin of nitrogen in sheep faeces. *Journal of Agricultural Science* **73**, 99–111.
- Menard, C.; Duncan, P.; Fleurance, G.; Georges, J. Y.; Lila, M., 2002: Comparative foraging and nutrition of horses and cattle in European wetlands. *Journal of Applied Ecology* **39**, 120–133.
- Meyer, H.; Coenen, M., 2002: *Pferdefütterung*. Parey Verlag, Berlin.
- Meyer, K.; Hummel, J.; Clauss, M., 2010: The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. *Mammal Review* **40**, 221–245.
- Michealet-Doreau, B.; Doreau, M., 2001: Influence of drastic underfeeding on ruminal digestion in sheep. *Animal Research* **50**, 451–462.
- Miyaji, M.; Ueda, K.; Hata, H.; Kondo, S., 2011: Effects of quality and physical form of hay on mean retention time of digesta and total tract digestibility in horses. *Animal Feed Science and Technology* **165**, 61–67.
- Moore-Colyer, M. J. S.; Morrow, H. J.; Longland, A. C., 2003: Mathematical modelling of digesta passage rate, mean retention time and in vivo apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. *British Journal of Nutrition* **90**, 109–118.
- Müller, D. W. H.; Caton, J.; Codron, D.; Schwarm, A.; Lentle, R.; Streich, W. J.; Hummel, J.; Clauss, M., 2011: Phylogenetic constraints on digesta separation: variation in fluid throughput in the digestive tract in mammalian herbivores. *Comparative Biochemistry and Physiology, Series A* **160**, 207–220.
- Mundt, H. C., 1978: *Untersuchungen über die Verdaulichkeit von aufgeschlossenem Stroh beim Pferd*. TiHo Hannover, Hannover.
- Munn, A. J.; Streich, W. J.; Hummel, J.; Clauss, M., 2008: Modelling digestive constraints in non-ruminant and ruminant foregut-fermenting mammals. *Comparative Biochemistry and Physiology, Series A* **151**, 78–84.
- Orton, R. K.; Hume, I. D.; Leng, R. A., 1985a: Effect of level of dietary protein and exercise on growth rates of horses. *Equine Veterinary Journal* **17**, 381–385.
- Orton, R. K.; Hume, I. D.; Leng, R. A., 1985b: Effect of exercise and level of dietary protein on digestive function in horses. *Equine Veterinary Journal* **17**, 386–390.
- Pagan, J. D.; Harris, P.; Brewster-Barnes, T.; Duren, S. E.; Jackson, S. G., 1998: Exercise affects digestibility and rate of passage of all-forage and mixed diets in thoroughbred horses. *Journal of Nutrition* **128**, 2704S–2707S.
- Pearson, R. A.; Merritt, J. B., 1991: Intake, digestion and gastrointestinal transit time in resting donkeys and ponies and exercised donkeys given *ad libitum* hay and straw diets. *Equine Veterinary Journal* **23**, 339–343.
- Pearson, R. A.; Archibald, R. F.; Muirhead, R. H., 2001: The effect of forage quality and level of feeding on digestibility and gastrointestinal transit time of oat straw and alfalfa given to ponies and donkeys. *British Journal of Nutrition* **85**, 599–606.
- Pearson, R. A.; Archibald, R. F.; Muirhead, R. H., 2006: A comparison of the effect of forage type and level of feeding on the digestibility and gastrointestinal mean retention time of dry forages given to cattle, sheep, ponies and donkeys. *British Journal of Nutrition* **95**, 88–98.
- Ragnarsson, S.; Lindberg, J. E., 2010: Impact of feeding level on digestibility of haylage-only diet in Icelandic horses. *Journal of Animal Physiology and Animal Nutrition* **94**, 623–627.
- Ralston, S. K., 2002: Nutritional support after alimentary tract surgery. In: T. S. Mair, T. Divers, N. Ducharme (eds), *Manual of Equine Gastroenterology*. WB Saunders, Philadelphia, PA, pp. 196–198.
- Robbins, C. T., 1993: *Wildlife Feeding and Nutrition*. Academic Press, San Diego, CA.
- Rosenfeld, I.; Austbø, D.; Volden, H., 2006: Models for estimating digesta pas-

- sage kinetics in the gastrointestinal tract of the horse. *Journal of Animal Science* **84**, 3321–3328.
- Shaver, R. D.; Nytes, A. J.; Satter, L. D.; Jorgensen, N. A., 1988: Influence of feed intake, forage physical form, and forage fiber content on particle size of masticated forage, ruminal digesta, and feces of dairy cows. *Journal of Dairy Science* **71**, 1566–1572.
- Statsoft, 2007: *STATISTICA Version 8.0 (data analysis software system)*. Statsoft, Tulsa, OK.
- Steuer, P.; Clauss, M.; Südekum, K.-H.; Hatt, J.-M.; Silinski, S.; Klomburg, S.; Zimmermann, W.; Hummel, J., 2010: Comparative investigations on digestion in grazing (*Ceratotherium simum*) and browsing (*Diceros bicornis*) rhinoceroses. *Comparative Biochemistry and Physiology, Series A* **156**, 380–388.
- Suhartanto, B.; Julliard, V.; Faurie, F.; Tisserand, J. L., 1992: Comparison of digestion in donkeys and ponies. *Pferdeheilkd, First European Conference on Equine Nutrition* pp. 158–161.
- Thielemans, M. F.; Francois, E.; Bodart, C.; Thewis, A., 1978: Mesure du transit gastrointestinal chez le porc à l'aide des radiolanthanides. Comparaison avec le mouton. *Annales de Biologie Animale, Biochimie, Biophysique* **18**, 237–247.
- Todd, L. K.; Sauer, W. C.; Christopherson, R. J.; Coleman, R. J.; Caine, W. R., 1995: The effect of level of feed intake on nutrient and energy digestibilities and rate of feed passage in horses. *Journal of Animal Physiology and Animal Nutrition* **73**, 140–148.
- Udén, P.; Colucci, P. E.; Van Soest, P. J., 1980: Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *Journal of the Science of Food and Agriculture* **31**, 625–632.
- Van Soest, P. J., 1967: Development of a comprehensive system of feed analyses and its applications to forages. *Journal of Animal Science* **26**, 119–128.
- Van Soest, P. J., 1994: *Nutritional Ecology of the Ruminant*, 2nd edn. Cornell University Press, Ithaca.
- Wolter, R.; Durix, A.; Letourneau, J. C., 1976: Influence du mode de présentation d'un aliment complet sur la vitesse du transit digestif et la digestibilité chez le poney. *Annales Zootechnica* **25**, 181–188.
- Yoder, M. J.; Miller, E. H.; Rook, J.; Shelle, J. E.; Ullrey, D. E., 1997: Fiber level and form: effects on digestibility, digesta flow and incidence of gastrointestinal disorders. *Proceedings of the 15th Equine Nutrition and Physiology Symposium, Fort Worth, Texas* pp. 122–127.
- Zeyner, A.; Kienzle, E., 2002: A method to estimate digestible energy in horse feed. *Journal of Nutrition* **132**, 1771S–1773S.
- Zeyner, A.; Kirchhof, S.; Susenbeth, A.; Südekum, K.-H.; Kienzle, E., 2010: Protein evaluation of horse feed: a novel concept. In: A. D. Ellis, A. C. Longland, M. Coenen, N. Miraglia (eds), *The impact of nutrition on the health and welfare of horses. 5th European Workshop on Equine Nutrition*. Publications No. 128. EAAP, Cirencester, UK, 19–22 September, pp. 40–42.