

Methane production in relation to body mass of ruminants and equids

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ABSTRACT

Background: Mammalian herbivores produce methane during digestion.

Questions: Do ruminants (such as bovids) produce more methane during digestion than do equids (non-ruminants)? What are the effects of allometric scaling of methane production when different species feed on similar diets?

Methods: From the literature, we collected a set of data on methane production (litres · day⁻¹) of ruminants (body mass 26–610 kg) and equids (208–850 kg) fed only roughage. To these data we added our own experimental results from three sheep (*Ovis orientalis aries*, 94 ± 4 kg) and three mini Shetland ponies (*Equus ferus caballus*, 97 ± 6 kg). We gave these six animals *ad libitum* access to the same batch of grass hay. We measured their food intake and methane production (using respiratory chambers), and calculated gut fill and food digestibility.

Results: Daily dry matter intake and dry matter digestibility were 39 ± 10 g · kg^{-0.75} day⁻¹ and 48 ± 2% in sheep and 72 ± 16 g · kg^{-0.75} day⁻¹ and 41 ± 3% in ponies, respectively; the calculated dry matter gut fill was 2.0 ± 0.5% of body mass in sheep and 1.9 ± 0.4% in ponies. Methane production was higher in sheep (30.3 ± 3.0 litres · day⁻¹) than in ponies (13.4 ± 4.6 litres · day⁻¹), representing 6.7 ± 1.7% and 1.5 ± 0.2% of gross energy intake, respectively. The data set revealed a linear increase of methane production with body mass (i.e. $M^{1.0}$) in equids and ruminants. However, a ruminant produces 3.6 times as much methane as does an equid of comparable body mass.

Conclusions: Because energy and food intake scale allometrically with body mass ($M^{0.75}$), our results mean that energetic losses due to methane production (as a proportion of overall energy intake) increase with increasing body mass. The magnitude of the losses is enough to limit the maximum size of a ruminant's body, but not that of an equid.

Keywords: body size, digestive physiology, energetic losses, excretion pattern, herbivory, hindgut fermenter, ruminant.

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INTRODUCTION

Methane production is one of the unavoidable side-effects of vertebrate herbivory (Hackstein & Van Alen, 1996). Methanogenic microorganisms – members of the domain of the *Archaea* – are part of the microbial ecosystems present in the fermentation chambers of the gastrointestinal tracts of herbivores (Stevens & Hume, 1998). *Archaea* act as hydrogen sinks, converting H_2 and CO_2 to methane, thus keeping the partial pressure of hydrogen low; this enhances the activity of fermenting microorganisms in the gut ecosystems (Jensen, 1996). It is generally accepted that methane production in ruminants is higher than in other herbivores such as hindgut fermenters (e.g. equids) or non-ruminant foregut fermenters (e.g. kangaroos) (Crutzen *et al.*, 1986; Clauss *et al.*, 2010). This might be attributed either to higher counts of *Archaea* in the rumen as the major fermentation chamber of ruminants (Morvan *et al.*, 1996), or to a higher prevalence of other hydrogen sinks such as reductive acetogenesis in hindgut fermenters (Prins & Lankhorst, 1977; Fievez *et al.*, 2001).

There has been a shift of the focus in research on methane production in herbivores, from concerns about methane representing a significant feed energy loss in the animal to methane as greenhouse gas thus contributing to global warming (Ellis *et al.*, 2007), resulting in a massive body of agricultural research. Comparative aspects of methane production have so far mostly been of interest for completing the estimation of national or global greenhouse gas inventories with respect to the contribution of non-ruminant domestic and free-ranging herbivores (e.g. Crutzen *et al.*, 1986; Vermorel, 1997). In contrast, evolutionary or ecophysiological aspects of methane production have received little attention (Clauss & Hummel, 2005). This is best exemplified by the fact that the relationship between body mass (M) and methane production has hardly been investigated. Such a relationship has so far been reported as secondary findings in sheep (Pelchen & Peters, 1998) and cattle (Pavao-Zuckerman *et al.*, 1999). Approaches to determine factors of influence on methane production in domestic ruminants have focused on the dietary composition of feed and plant secondary metabolites (Beauchemin *et al.*, 2008), feeding/intake levels (Ellis *et al.*, 2007), pasture management (e.g. DeRamus *et al.*, 2003), genotype and selection (Estermann *et al.*, 2002; Münger & Kreuzer, 2008) – factors that can be influenced by agricultural management practices. To our knowledge, the only comprehensive approach to investigate an effect of body mass on herbivore methane production was recently presented by Smith *et al.* (2010); these authors derived allometric equations for the production of methane from literature data to facilitate extrapolations to fossil herbivores, and to draw conclusions on the relationship between megafaunal extinctions and atmospheric methane.

Body size limitations due to methane production have been proposed for large herbivores (Prins & Kreulen, 1991; Van Soest, 1994). These considerations do not refer to the methane production usually observed in herbivores, which is due to a group of fast-growing *Archaea* that use H_2 and CO_2 . They address another group of slow-growing *Archaea* that use acetate – one of the major fermentation products of gut bacteria, and an important energy source for the vertebrate host – and convert it to methane, thus theoretically depriving the herbivore of one of its most important energy resources. These slow-growing *Archaea* have a generation time of approximately 4 days (Van Soest, 1994). If ingesta retention time is assumed to increase systematically with body mass, there should be a body size threshold above which retention times in the fermentation chamber exceed this 4-day limit. Then, energetic losses due to acetate-based methanogenesis would theoretically become prohibitive. Prins and Kreulen (1991) presented a model calculating a maximum possible body mass for ruminants

of 1.0–1.5 metric tons. However, the validity of this concept is doubtful, given the facts that ingesta retention does not increase systematically with body mass in large herbivores, and that ingesta retention times exceeding 4 days have been measured in several vertebrate herbivores such as koalas (*Phascolarctos cinereus*), dugongs (*Dugong dugon*), sloths (*Bradypus tridactylus*) (reviewed in Clauss *et al.*, 2007), and land tortoises (Hatt *et al.*, 2002). In contrast, the question of whether methane production due to faster-growing *Archaea* could impose a body size limit or a digestive disadvantage at increasing body size has so far not been addressed.

We compared methane production in ruminants and horses of similar size on the same diet, and added the results to literature data measured in ruminants and equids fed on roughage-only diets, to test for a scaling of methane production with body mass. In particular, we expected that the resulting scaling relationship would either be similar to that of the volume of gut contents (scaling linearly with body mass, i.e. $M^{1.0}$), or similar to energy/food intake (scaling with $M^{0.75}$), or similar to ingesta retention time (expected to result in no evident scaling with M) (Clauss *et al.*, 2007).

METHODS

Three adult female sheep (*Ovis orientalis aries*, 94 ± 4 kg) and three adult female mini Shetland ponies (*Equus ferus caballus*, 97 ± 6 kg) were housed individually and were offered *ad libitum* access to grass hay originating from one batch exclusively. This hay contained ($\text{g} \cdot \text{kg}^{-1}$ dry matter): organic matter, 803; crude protein, 58; neutral detergent fibre, 582; acid detergent fibre, 326; acid detergent lignin, 46; gross energy $15.9 \text{ (MJ} \cdot \text{kg}^{-1} \text{ dry matter)}$. After an adaptation period of 2 weeks, the hay offered and the refusals were weighed daily, and faeces were collected at regular intervals (from 4 h at the beginning up to 12 h on the last day) for 7 days. Representative subsamples of the hay were analysed for contents of dry matter, nutrients, and gross energy using standard laboratory methods (Association of Official Analytical Chemists, 1997). In the faeces, dry matter, neutral detergent fibre, and combustion energy were determined, and digestibilities of dry matter, neutral detergent fibre, and energy calculated. Mean ingesta retention times were determined as part of a larger comparative study (P. Steuer *et al.*, submitted) by feeding a particle (chromium-mordanted fibre, < 2 mm) marker prepared according to Udén *et al.* (1980); analyses and calculations of mean retention times were performed as described by Behrend *et al.* (2004). Gut dry matter fill was estimated using the exponential model of Holleman and White (1989). Following the 7-day collection period, animals were placed for two consecutive 22.5-h periods into open-circuit respiration chambers constructed and operated as described in Soliva and Hess (2007). The two chambers had a volume of 4.55 m^3 and provided constant humidity (60%), temperature ($20 \pm 1^\circ\text{C}$), airflow ($7.3 \pm 0.1 \text{ m}^3 \cdot \text{h}^{-1}$), and pressure ($987 \pm 8 \text{ hPa}$). Gas analysers were manually calibrated with calibration gases (calibration gas 1: pure nitrogen; calibration gas 2: 19.6% mol oxygen, 1.0% mol carbon dioxide, 0.1% mol CH_4). A possible drift of the analyser was numerically adjusted by performing repeated measurements of the outside air and calibration gases besides measurements of the chamber air composition. Methane concentrations were measured on a Binos 1001 (Fisher-Rosemount, Baar-Walterswil, Switzerland). Gas volumes were corrected for standard conditions (1013 hPa, 0°C , 0% relative humidity). Methane production was expressed in absolute values and in relation to food intake, energy intake, and the intake of digestible energy and digestible neutral detergent fibre (as a measure of fibre).

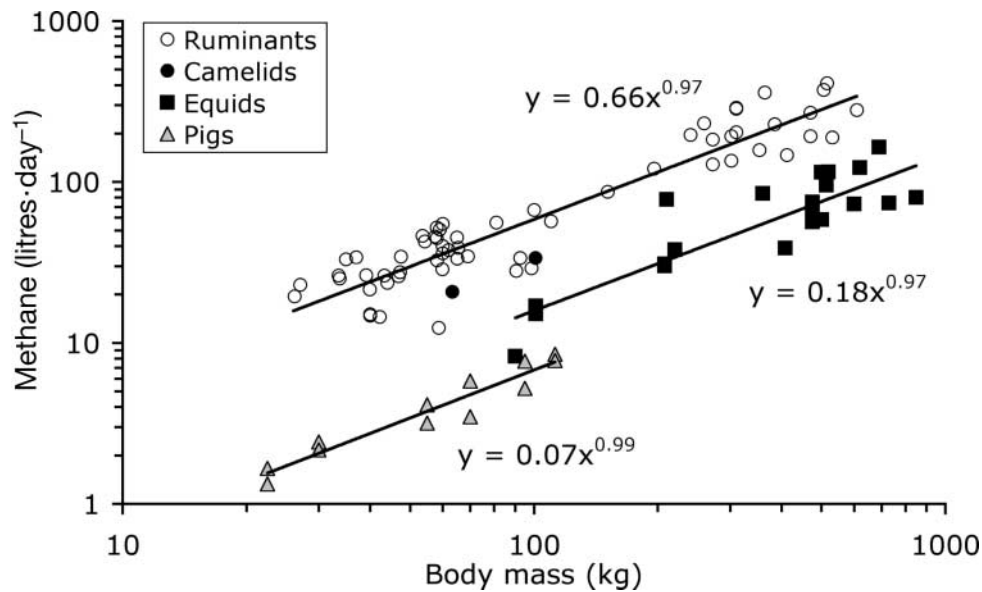


Fig. 1. Methane production in relation to body mass in ruminants (Ritzman and Benedict, 1938; Belyea *et al.*, 1985; Terada *et al.*, 1987; Margan *et al.*, 1988; Okine *et al.*, 1989; Hironaka *et al.*, 1996; Klita *et al.*, 1996; Vermorel, 1997; Vernet *et al.*, 1997; Galbraith *et al.*, 1998; Kurihara *et al.*, 1999; McCaughey *et al.*, 1999; Boadi & Wittenberg, 2002; Ulyatt *et al.*, 2002; Pinares-Patiño *et al.*, 2003a, 2003b; Carulla *et al.*, 2005; Puchala *et al.*, 2005; Swainson *et al.*, 2007; Animut *et al.*, 2008; Tiemann *et al.*, 2008; Hart *et al.*, 2009; and the present study), South American camelids (Vernet *et al.*, 1997; Pinares-Patiño *et al.*, 2003b), horses (Ritzman and Benedict, 1938; Nehring, 1956; Vermorel, 1997; Vermorel *et al.*, 1997a, 1997b; and the present study), and pigs (Christensen and Thorbek, 1987). See Results section for regression equations.

The results from the present experiment were added to a collection of literature data on methane production in ruminants and equids of known body mass fed roughage-only diets (ruminants: $n = 57$ with a range in body mass of 26–610 kg; equids: $n = 20$ with a range in body mass of 208–850 kg; for sources, see legend to Fig. 1), and a data set on pigs from one study where the same diet was used over a range of body masses of 23–113 kg (Christensen & Thorbek, 1987). Experimental data for the sheep and ponies were compared by *t*-test. The complete data set was analysed statistically after transforming the body mass and the methane data by the natural logarithm, using regression analysis and a general linear model (GLM) with methane production as the dependent variable, species group (ruminants, equids, pigs) as a factor, and body mass as covariate (the species $\times$ body mass interaction was not significant) using PSAW 18.0 (SPSS Inc., Chicago, IL). Statistical significance was set to 0.05.

RESULTS

The hay intake of the sheep was little more than half of that of the horses (39 ± 10 vs. 72 ± 16 g $\cdot$ kg $^{-0.75}$ day $^{-1}$; Table 1). In addition, sheep had 1.8 times longer mean particle retention times (54 ± 4 vs. 26 ± 1 h), 1.2 times higher dry matter digestibilities (48 ± 2 vs. $41 \pm 3\%$), and a more than two-fold higher methane production (30.3 ± 3.0 vs. 13.4 ± 4.6 litres $\cdot$ day $^{-1}$). Yet, the calculated total gut fill was similar in sheep (1.9 ± 0.5 kg dry mass or $2.0 \pm 0.5\%$ of body mass) and horses (1.9 ± 0.5 kg dry mass or $1.9 \pm 0.4\%$ of body mass).

Table 1. Feed intake, digestion, and methane production in sheep and horses of similar body mass

	Sheep			Ponies			<i>P</i> *
	1	2	3	1	2	3	
Body mass (kg)	93	91	99	101	101	90	0.488
Dry matter intake ($\text{g} \cdot \text{kg}^{-0.75} \cdot \text{day}^{-1}$)	45	28	43	76	85	54	0.039
Mean retention time (h)	54	58	47	26	22	25	0.001
Dry matter digestibility (%)	47	51	46	41	39	45	0.041
Gut fill (kg dry matter)	2.3	1.4	2.0	2.0	2.0	1.2	0.735
Methane ($\text{litres} \cdot \text{day}^{-1}$)	33.7	28.1	29.1	16.9	15.1	8.3	0.006
Methane ($\text{litres} \cdot \text{kg}^{-1}$ dry mass intake)	24.8	34.4	21.6	7.0	5.6	5.3	0.006
Methane (% of gross energy)	6.2	8.5	5.4	1.7	1.4	1.3	0.005
Methane (% of digestible energy)	10.0	15.8	11.0	4.0	2.9	2.8	0.008
Methane ($\text{litres} \cdot \text{kg}^{-1}$ dNDF)	76	107	93	39	24	23	0.003

Note: dNDF, digestible neutral detergent fibre.

*Independent sample *t*-test comparing sheep and ponies.

In sheep, methane output represented 6.7 ± 1.7 and $12.3 \pm 3.1\%$ of gross energy and digestible energy intake, respectively, whereas it represented 1.5 ± 0.2 and $3.2 \pm 0.7\%$, respectively, in horses. The sheep produced three times more methane per unit of digested fibre (digestible neutral detergent fibre) than the horses (92 ± 15 vs. 28 ± 9 litres $\cdot \text{kg}^{-1}$).

Available data on methane production in ruminants (including domestic cattle, sheep, and goats, as well as bison, red deer, and white-tailed deer) and equids (various breeds of domestic horses) suggest a systematic increase in methane output with body mass (Fig. 1). The few data available for South American camelids suggest a similar methane production as in ruminants. Growing pigs also showed an increase of methane output with body mass in the study included in the data set (Fig. 1).

The scaling of methane production (in litres $\cdot \text{day}^{-1}$) was $0.66 \times M^{0.97}$ ($r^2 = 0.87$; $P < 0.001$; $n = 62$; 95% confidence interval (CI) for exponent: 0.88–1.07) in ruminants, $0.18 \times M^{0.97}$ ($r^2 = 0.76$; $P < 0.001$; $n = 23$; 95% CI for exponent: 0.72–1.22) in horses, and $0.07 \times M^{0.99}$ ($r^2 = 0.93$; $P < 0.001$; $n = 12$; 95% CI for exponent: 0.79–1.19) in pigs. In the general linear model, where methane production was considered as the dependent variable, both body mass ($F = 205.2$, $P < 0.001$) and species group ($F = 6.06$, $P = 0.003$) were significant.

When expressed per unit of food intake (Fig. 2a) or as a proportion of gross energy intake (Fig. 2b), the data indicate a slight but significant increase with body mass in ruminants: methane production in litres $\cdot \text{kg}^{-1}$ dry matter intake was $16.6 \times M^{0.12}$ ($r^2 = 0.25$; $P < 0.001$; $n = 45$; 95% CI for exponent: 0.06–0.18); methane production as a percentage of gross energy intake was $3.5 \times M^{0.13}$ ($r^2 = 0.25$; $P < 0.001$; $n = 44$; 95% CI for exponent: 0.06–0.20). For horses, the resulting exponents for the scaling of methane production per unit of dry matter and gross energy intake were 0.26 and 0.17, respectively; the 95% CI of these exponents, however, included 0 in both cases, i.e. the regressions were not significant: methane production in litres $\cdot \text{kg}^{-1}$ dry matter intake was $2.0 \times M^{0.26}$ ($r^2 = 0.29$; $P = 0.056$; $n = 13$; 95% CI for exponent: –0.01 to 0.53); methane production as a percentage of gross energy intake was $0.7 \times M^{0.17}$ ($r^2 = 0.16$; $P = 0.171$; $n = 13$; 95% CI for exponent: –0.09 to 0.42).

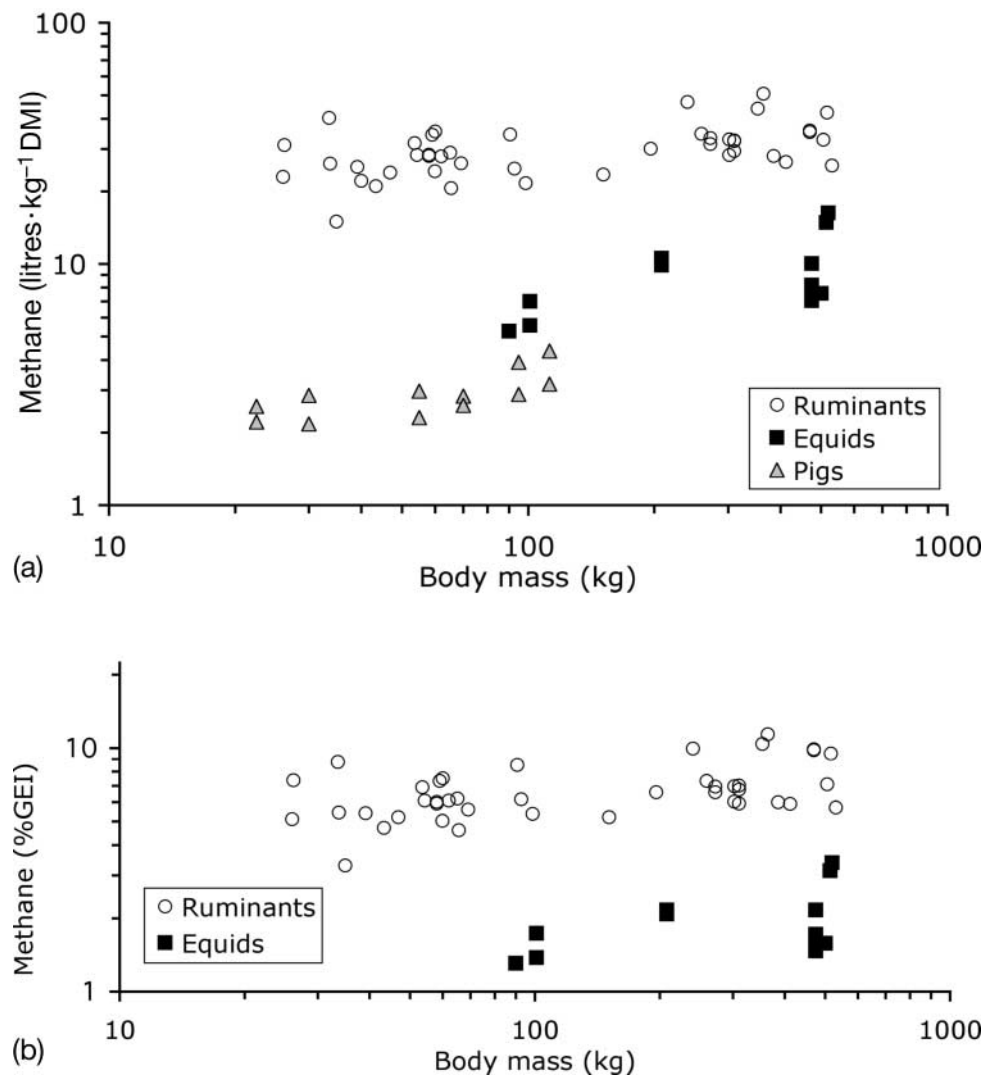


Fig. 2. Methane production (a) per unit of dry matter intake (DMI) and (b) per unit of gross energy intake (GEI) in relation to body mass in ruminants, horses, and pigs. Same data sources as Fig. 1 (excluding those from which the respective measures could not be derived). See Results section for regression equations.

DISCUSSION

Our results are consistent with the knowledge that methane losses constitute about 6–10% of the gross energy intake in forage-fed ruminants (Immig, 1996), with the average being very close to the default value of 6.5% assumed by the Intergovernmental Panel on Climate Change (IPCC, 2006), and that horses produce less methane than ruminants (Crutzen *et al.*, 1986). Ideally, all comparisons should be made on the basis of digested plant cell wall or at least digestible energy, to rule out the possibility that differences in methane production

are simply an effect of the amount of digested material taken up by the animal. The comparisons of methane production as a proportion of digestible energy or per unit of digested plant fibre in Table 1 indicate that even when comparing data on such a basis, systematic differences between ruminants and horses remain. These are strong indications for systematic differences in the microbial ecosystem between the species.

The collection of literature data demonstrates that a 100-kg ruminant may have a similar methane output as a 400-kg horse. The horses were comparatively poorer utilizers of the nutrients in the roughage and therefore had to ingest higher amounts than the sheep. This was associated with a shorter ingesta retention time in the horses but at a similar calculated dry matter gut fill in the two species. This means that ruminants have a significantly higher methane production than equids even under the condition of a similar gut fill, and could be due to several factors (Vermorel *et al.*, 1997a). Ingesta retention in ruminants is longer than in horses (Foose, 1982; Pearson *et al.*, 2006) (cf. Table 1) and hence gives the *Archaea* more time to produce methane. Accordingly, methane production was shown to be related to ingesta retention time in ruminants (Okine *et al.*, 1989; Pinares-Patiño *et al.*, 2003a). Thus, assuming that retention time is an important factor, methane production should be high as well in hindgut fermenters, such as rhinoceros, and non-ruminant foregut fermenters, such as hippopotamus, which have ingesta retention times of the same magnitude as ruminants (Clauss *et al.*, 2004, 2005; Steuer *et al.*, 2010). This assumption remains to be investigated.

The microbial profile in the fermentation chambers of the digestive tract differs between ruminants and horses. Horses have lower concentrations of *Protozoa* (Kern *et al.*, 1974) and *Archaea* (Morvan *et al.*, 1996) in the hindgut than ruminants have in their main fermentation chamber, the rumen. The putative effect of these differences is evident in the higher methane production of ruminants; the actual causes for the differences in the microbial gut ecosystem – the reason why rumination is apparently linked to such a high methane output – remain to be elucidated. Again, if it is hypothesized that the well-known differences in retention time and in the amount of non-microbial digestion of non-fibre carbohydrates suffice to explain the observed differences in methane production between ruminants and equids, then similar high levels of methane production as in ruminants should be observed in other non-ruminant foregut fermenters such as hippopotamuses, peccaries, sloths, macropods or even colobine monkeys. At least in the case of the macropods, the limited evidence to date suggests that this is not the case (Kempton *et al.*, 1976; von Engelhardt *et al.*, 1978; Dellow *et al.*, 1988).

With an even lower contribution of microbial fermentation to the overall energy gain from feed compared with the horses, pigs potentially have an even lower methane output at the same body mass and gut fill, but this remains to be investigated on roughage-only diets or diets resembling the natural diet of suids. Existing data in domestic pigs suggest that high-fibre diets lead to an increase in methane production compared with the commonly fed low-fibre diets (Kirchgessner *et al.*, 1991).

Whether methane production increases systematically with body mass has been suggested (Clauss & Hummel, 2005), observed incidentally in individual species (Christensen & Thorbek, 1987; Pelchen & Peters, 1998; Pavao-Zuckerman *et al.*, 1999), and only once been demonstrated (in an unpublished data collection; Smith *et al.*, 2010) to date. In itself, this finding is unsurprising – since larger animals consume more food, greater methane production would be expected because of the increase in absolute amount of processed food. Rather, it is the pattern of the increase – the scaling with body mass – that is of particular interest. A linear scaling of methane production with body mass, as suggested by the regression equations from our data collection [in contrast

with the scaling with $M^{0.75}$ as assumed by IPCC (2006, p. 10.28); but in accord with the scaling with $M^{1.057}$ reported by Smith *et al.* (2010) for ruminants], has important consequences for general herbivore physiology and evolution. If this linear scaling can be confirmed in further studies, it would suggest that methane production might be, across body mass ranges within a digestion type (e.g. ruminant or equid), mainly a factor of gut capacity, as found within sheep (Pinares-Patiño *et al.*, 2003a). Gut capacity (measured as wet contents) has been shown repeatedly to scale linearly with body mass (reviewed in Clauss *et al.*, 2007). Because food intake scales with $M^{0.75}$ (reviewed in Clauss *et al.*, 2007), a linear scaling of methane production with body mass would translate into increasing energetic losses due to methane per unit of food intake with increasing body mass. This is illustrated by the relationship of relative methane production – either per unit food intake or per unit of energy intake – in Fig. 2. For horses, these relationships were not significant, potentially due to the comparatively low sample size. The magnitude of proportionate methane production in ruminants per unit of energy intake is so large that a limit in body size increase in this group can be expected by the action of fast-growing, H_2 - and CO_2 -using *Archaea* alone (with methane losses approaching on average 9% of gross energy intake at a body mass of one metric ton). A digestive system like that of the equids, in contrast, would not reach the same limitation even when scaled up to body sizes of the largest presumed hindgut fermenters, the mammalian Indricotheres [15 tons (Fortelius & Kappelman, 1993)] or the dinosaur sauropods [up to 100 tons (Sander *et al.*, 2010)], with methane losses estimated at about 3.3–4.4% of gross energy intake. Whatever the causes of the increased methane production in ruminants are, its scaling with body mass may be responsible for the different body size ranges achieved by ruminant and non-ruminant herbivores (Clauss *et al.*, 2003) and thus represents an intriguing example of a physiological constraint on the evolutionary history of a particular animal group. It also suggests, following Smith *et al.* (2010), that the composition of any given fauna, in terms of its body size range and in terms of the digestive strategy of its main players (ruminant vs. non-ruminant), will influence the magnitude by which this fauna contributes to the composition of Earth's atmosphere.

The present paper and the recent publication by Smith *et al.* (2010) independently compile, analyse, and interpret information on scaling of methane production with body size in herbivorous mammals. Interestingly, they both come up with scaling relations that differ from 3/4-power scaling, indicating that methane losses in herbivores increase disproportionately with increasing body mass. This will be an important topic for future research, also given the recent interest in the contribution of mammalian herbivores to greenhouse gas emissions.

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