Animal (2020), **14:S1,** pp s113–s123 © The Animal Consortium 2020
doi:10.1017/S1751731119003161

animal

Review: Comparative methane production in mammalian herbivores

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(Received 1 April 2019; Accepted 2 July 2019)

Methane (CH_4) production is a ubiquitous, apparently unavoidable side effect of fermentative fibre digestion by symbiotic microbiota in mammalian herbivores. Here, a data compilation is presented of in vivo *CH_4 measurements in individuals of 37 mammalian herbivore species fed forage-only diets, from the literature and from hitherto unpublished measurements. In contrast to previous claims, absolute CH_4 emissions scaled linearly to DM intake, and CH_4 yields (per DM or gross energy intake) did not vary significantly with body mass. CH_4 physiology hence cannot be construed to represent an intrinsic ruminant or herbivore body size limitation. The dataset does not support traditional dichotomies of CH_4 emission intensity between ruminants and nonruminants, or between foregut and hindgut fermenters. Several rodent hindgut fermenters and nonruminant foregut fermenters emit CH_4 of a magnitude as high as ruminants of similar size, intake level, digesta retention or gut capacity. By contrast, equids, macropods (kangaroos) and rabbits produce few CH_4 and have low $CH_4 : CO_2$ ratios for their size, intake level, digesta retention or gut capacity, ruling out these factors as explanation for interspecific variation. These findings lead to the conclusion that still unidentified host-specific factors other than digesta retention characteristics, or the presence of rumination or a foregut, influence CH_4 production. Measurements of CH_4 yield per digested fibre indicate that the amount of CH_4 produced during fibre digestion varies not only across but also within species, possibly pointing towards variation in microbiota functionality. Recent findings on the genetic control of microbiome composition, including methanogens, raise the question about the benefits methanogens provide for many (but apparently not to the same extent for all) species, which possibly prevented the evolution of the hosting of low-methanogenic microbiota across mammals.*

Keywords: methanogens, mean retention time, digesta washing, foregut fermentation, hindgut fermentation

Implications

This work reviews existing data on *in vivo* methane emissions in mammalian herbivores, demonstrating no constraint of methane physiology on body size, and no consistent difference between ruminants and nonruminants, or between foregut and hindgut fermenters. However, it singles out three groups – horses, kangaroos and rabbits – as model animals in which to investigate adaptations for low methane emissions.

Introduction

Methanogenesis is ubiquitous

The presence of methanogenic archaea appears to be nearly inevitable in the digestive tracts of animals. Methanogenesis has been reported in a wide array of arthropods (Hackstein and Stumm, 1994) and in the faeces of a large number of vertebrates, including not only herbivores, but also carnivorous reptiles and myrmecophageous mammals (Hackstein and Van Alen, 1996; Lambert and Fellner, 2012). Reports stating that various vertebrate groups do not produce methane (CH_4) or do not harbour methanogens were met by opposite

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evidence. This includes the ostrich (*Struthio camelus*) (Swart *et al.*, 1993; Miramontes-Carrillo *et al.*, 2008; Matsui *et al.*, 2010), kangaroos (Dellow *et al.*, 1988; Vendl *et al.*, 2015), mammalian carnivores (Hackstein and Van Alen, 1996; Middelbos *et al.*, 2008; Tun *et al.*, 2012), sea cows (Marsh *et al.*, 1978; Goto *et al.*, 2004), colobus monkeys (Bauchop and Martucci, 1968; Ohwaki *et al.*, 1974) and arvicoline rodents (Hackstein and Van Alen, 1996; this study). Supplementary Material Table S1 gives an exemplary overview over mammal species in which CH_4 emissions or methanogen presence has been detected. It generally appears prudent to assume that all mammals harbour some methanogens, and produce some CH_4, until consistently proven otherwise. Hence, differences between species are most likely only of a quantitative nature.

Why harbour methanogens?

The likely ubiquitous presence of methanogens in digestive microbiota raises the question about their value for the host. Is their presence the consequence of a convergent adaptation of herbivores in the sense that they provide an adaptive advantage? Or can their presence simply not be avoided because the host animal does not have the means to control the composition of its microbiota? In trying to answer these questions, the loss of ingested energy via CH_4 and the function of methanogens as efficient hydrogen (H_2) removers need to be weighed against each other.

In humans, the presence of methanogens/CH_4 emissions is linked to longer digesta retention and higher human body mass (BM) (Nakamura *et al.*, 2010), suggesting that methanogens improve the digestive efficiency of the gut microbiota. The presence of CH_4 delays peristaltic action in the exenterated dog or guinea pig small intestine (Pimentel *et al.*, 2006; Jahng *et al.*, 2012). For the large intestine – the main site of microbial action in monogastric species – no direct *in vitro* effect of CH_4, but a passage-accelerating effect of H_2, was demonstrated in guinea pigs (Jahng *et al.*, 2012). By removing H_2, methanogens might thus prevent expeditious gut clearance. In mice, the presence of methanogens led to a more efficient carbohydrate digestion and higher body fat stores (but not higher BM) at similar food intakes, compared to animals without methanogens or those inoculated with a sulphate-reducing bacterium as an alternative H_2 sink (Samuel and Gordon, 2006). In rats, reducing methanogens led to a less efficient use of carbohydrates, but no change in BM over 6 weeks (Yang *et al.*, 2016). Reducing methanogens/CH_4 emissions in humans has been associated with a decrease in digesta retention (Ghoshal *et al.*, 2018). Accordingly, the main mode of action of methanogens appears to be H_2 removal that facilitates a higher rate of acetate production than with any other H_2 sinks, and a putative increase in digesta retention. Concerns about human obesity and constipation notwithstanding, the presence of methanogens in monogastric animals could be interpreted as facilitating an efficient resource use.

Most studies on CH_4 in mammals stem from domestic ruminants, where CH_4 is, by contrast, mainly considered an unavoidable, unwelcome loss of energy and contribution to greenhouse gas emissions. As in humans, intra-specific variation in CH_4 production in ruminants has been linked to digesta retention: animals with longer digesta retention, either due to more voluminous rumens at similar intake, or due to lower intake at uncontrolled rumen capacity, generally produce more CH_4 per ingested DM (Pinares-Patiño *et al.*, 2003; Goopy *et al.*, 2014; Hammond *et al.*, 2014; Barnett *et al.*, 2015; Cabezas-Garcia *et al.*, 2017). Here, however, it is the retention time that is considered the cause, where more time available is considered responsible for more fermentative digestion with an ensuing increased CH_4 production. When manipulating digesta retention by other means than varying intake, namely by the addition of weights into the rumen, a shorter retention time in the rumen was correspondingly associated with a lower CH_4 production (Okine *et al.*, 1989). On the other hand, when manipulating the CH_4 available in the rumen and accounting for variation in food intake in a cross-over study, a higher presence of CH_4 was associated with shorter retention and increased motility, possibly indicating a mechanism that aims at keeping losses at bay (Dittmann *et al.*, 2016). These findings apparently contradict those made in monogastrics. From settings without cross-over design, no effect of CH_4 inhibition on digesta retention was reported (Nolan *et al.*, 2010; Knight *et al.*, 2011). Evidently, more work is required to understand the effects of CH_4 on peristalsis and digesta kinetics in ruminants.

In ruminants, the absence of methanogens in gnotobiotically raised animals (Fonty *et al.*, 2007), or the chemical inhibition of methanogenesis, do not appear to have evident negative effects. Although on roughage diets food intake may be reduced, this does not translate into BM losses, but may, on the contrary, be linked with higher feed conversion efficiency (McCrabb *et al.*, 1997; Hristov *et al.*, 2015; Dittmann *et al.*, 2016). However, natural variation in residual BM gain or residual feed efficiency was not related to CH_4 production (Freetly *et al.*, 2015; McDonnell *et al.*, 2016; Alemu *et al.*, 2017), and selection for high feeding efficiency might even be associated with increased CH_4 yields (Flay *et al.*, 2019). Yet, CH_4 inhibition has been reported to facilitate higher milk or milk protein yields (Abecia *et al.*, 2012; Hristov *et al.*, 2015). Therefore, the presence of methanogens in ruminants is considered somewhat similar to the presence of protozoa – most likely unavoidable, but with no or only minor disadvantages when lacking. The possibility remains that putatively positive effects of methanogens would only be detectable, for both monogastrics and foregut-fermenting animals, under certain conditions of reduced quality, natural forages.

The comparative approach

With respect to CH_4 physiology, the classification of species and species groups as emitters/non-emitters, or as high *v.* low emitters, has a certain tradition (Crutzen *et al.*, 1986; Hackstein and Van Alen, 1996). Differences in the methanogenic potential of the microbiome of different herbivore species have been long acknowledged (Jensen, 1996;

Fievez *et al.*, 2001; Ouwerkerk *et al.*, 2009), but the causes of these differences remain elusive. An evident outcome of comparative studies are predictive equations related to the scaling of CH_4 emissions with BM (Franz *et al.*, 2011; Pérez-Barbería, 2017), with the intention to reconstruct CH_4 budgets for fossil megafaunas (Smith *et al.*, 2010; Wilkinson *et al.*, 2012; Smith *et al.*, 2016). Additionally, comparative approaches have favoured a dichotomy either between foregut-fermenting and monogastric herbivores (Jensen, 1996; Smith *et al.*, 2015), or between ruminating and non-ruminating herbivores (Franz *et al.*, 2011).

In the present study, data were collated on CH_4 emissions from *in vivo* measurements of herbivores fed diets consisting of forages, such as pasture, or grass or lucerne hay in whole, chopped or pelleted form, without the addition of concentrates, drawing on a collection of literature data and our own measurements. Objectives included a test for scaling of CH_4 emissions with BM, not only using all available emission data, but also those data for which food intake had been recorded in parallel, to assess whether CH_4 emissions actually scale differently than food intake and thus represent a putative disadvantage at increasing herbivore body size (Franz *et al.*, 2010; Franz *et al.*, 2011), or whether CH_4 emissions and food intake scale in parallel. Other objectives were testing differences between foregut and hindgut fermenters, and between ruminating and non-ruminating herbivores. Based on intra-specific findings in domestic ruminants, negative relationships on an inter-specific level between the level of total food intake and CH_4 yield were expected, as well as positive relationships between the digesta mean retention time (MRT) or gut capacity and the CH_4 yield.

Methods

A literature data compilation (Franz *et al.*, 2010; Franz *et al.*, 2011) was expanded to comprise the sources indicated in Supplementary Material Table S2. Only diets made of forages (either fed whole, chopped or pelleted) were accepted, and the minimum information required was BM and CH_4 production. A variety of measurement techniques was included, mainly chamber respirometry, and the SF_6 tracer method where CH_4 production is estimated from the ratio of CH_4 to a tracer gas that is released from the rumen at a known rate. When available, data on DM intake (DMI), diet composition (including NDF), digestibility coefficients, MRTs of solute or particle markers in the gastrointestinal tract and CO_2 production were noted. Whenever possible, missing values were calculated (e.g., if CH_4 yield per DMI and DMI were specified, absolute CH_4 emission was calculated). Transformations of units (grams to litres, or litres to joules) were made using standard conversion factors (Brouwer, 1965).

Hitherto unpublished results derived from experiments (of which in some cases other measures than CH_4 have been published) were included as well. They include CH_4 measurements in various ruminants (gazelles down to a body size of dikdik *Madoqua saltiana* of approximately 1.5 kg), hystricomorph and arvicoline rodents, a giant rabbit breed, and in some cases retention time data for specimens whose intake, digestibility and CH_4 measurements have already been reported (Supplementary Material Table S2). The experimental methods followed those described, for example, in Dittmann *et al.* (2014) or Hagen *et al.* (2019). With data on DMI, DM digestibility and small particle MRT in the total gastrointestinal tract, the DM gut fill of the specimen was calculated according to the occupancy principle (Holleman and White, 1989; linear approach). Species were classified as ruminants or nonruminants, and as foregut or hindgut fermenters. The full dataset is provided as a supplement.

First, all available data for a set of measures (starting from absolute CH_4 emissions and BM) or the corresponding averages per species were used. Subsequently, entries that did not qualify for the next step, for example, did not include data on food intake, were excluded, and again analyses were performed on the total of the remaining data and the corresponding species averages. Results are thus reported for absolute CH_4 emission (the whole dataset), for CH_4 yield (in % of gross energy intake (GEI)), for CH4 : CO2 ratios, for parallel measures of DMI, for parallel measures of intake and fibre digestibility, and for parallel measures of intake and digesta retention.

To assess scaling relationships and their exponents, log-transformed data were submitted to linear regression in R v 3.3.2 (R_Core_Team, 2015) with the package 'nlme' (Pinheiro *et al.*, 2011) in generalised least squares (GLS), for all available data and the species averages, indicating the 95% confidence intervals (CIs) for parameter estimates. To account for phylogeny, species averages were additionally analysed by phylogenetic generalised least squares (PGLS) with package 'caper' (Orme *et al.*, 2013), using a mammalian supertree (Fritz *et al.*, 2009), pruned to include the relevant taxa in our dataset. To retain the two rabbit breeds, the wapiti (contrasted to the red deer) and the alpaca, these groups were linked to the closest relatives of their original species in the tree (*Bunolagus*, *Rucervus* and *Vicugna*, respectively). The strength of the phylogenetic signal (λ, varying from 0 to 1, indicating phylogenetic structure in the dataset) was estimated by maximum likelihood. The analyses were repeated including species classification as ruminant or non-ruminant (Rum) and as foregut or hindgut fermenter (Fore), first including the respective interactions with the independent variable (e.g., when relating absolute CH_4 production to BM, Rum, Fore, and the BM–Rum and BM–Fore interactions were included). If the interactions were not significant, the model was repeated without them. Scaling relationships for four (non-exclusive) herbivore groups (functional ruminants including camelids, taxonomic ruminants without camelids, foregut fermenters including ruminants and hindgut fermenters) are given in the Supplementary Material (for species overlap between groups, see Supplementary Material Table S2). For the dataset that included measurements of MRT, individual relationships were only calculatedfor small (lagomorphs, rodents) and large (all other)

Table 1 *Scaling relationships in mammalian species between CH_4 (in L/day or as % GEI) or the $CH_4 : CO_2$ ratio and body mass (BM, kg) according to $y = a\ BM^b$*

										P Factors (direction)		*P* Interactions	
Data	λ	*a*	95% CI		*P*	*b*	95% CI		*P*	Rum	Fore	BM × Rum	BM × Fore
CH_4 (L/day) ($n = 693$, 37 species)													
all		**0.538**	**0.486**	**0.595**	**<0.001**	**0.96**	**0.94**	**0.98**	**<0.001**	**<0.001 (+)**	**<0.001 (–)**	**0.004**	**<0.001**
av		**0.609**	**0.451**	**0.822**	**0.003**	**0.88**	**0.81**	**0.96**	**<0.001**	**<0.001 (+)**	**0.030 (–)**	0.166	0.080
av	**0.97**	0.447	0.202	0.989	0.055	**0.84**	**0.77**	**0.92**	**<0.001**	0.401	0.994	0.648	0.489
CH_4 (% GEI) ($n = 463$, 34 species)													
all		**3.014**	**2.734**	**3.322**	**<0.001**	**0.14**	**0.12**	**0.16**	**<0.001**	**<0.001 (+)**	**0.037 (+)**	0.400	0.385
av		**2.727**	**2.070**	**3.591**	**<0.001**	**0.11**	**0.04**	**0.18**	**0.005**	**0.042 (+)**	0.826	0.402	0.612
av	**0.98**	2.287	0.939	5.569	0.078	0.02	–0.06	0.11	0.599	0.488	0.287	0.678	0.922
$CH_4 : CO_2$ ratio (L/L) ($n = 168$, 26 species)													
all		**0.031**	**0.027**	**0.034**	**<0.001**	**0.12**	**0.09**	**0.15**	**<0.001**	**<0.001 (+)**	**<0.001 (–)**	**0.001**	**0.024**
av		**0.035**	**0.028**	**0.043**	**<0.001**	**0.09**	**0.03**	**0.15**	**0.007**	**0.003 (+)**	0.451	0.150	0.194
av	**0.82**	**0.028**	**0.016**	**0.048**	**<0.001**	0.06	0.00	0.13	0.080	**0.038 (+)**	0.798	0.198	0.325

GEI = gross energy intake; BM = body mass; 95% CI = 95% confidence interval.
Using either all individual values (all) in generalised least squares (GLS), or species averages (av) in GLS or in phylogenetic generalised least squares (PGLS, indicating the phylogenetic signal λ). Significant parameter estimates as well as λ significantly different from 0 are set in bold. Results of additional models that include whether a species is a ruminant or nonruminant (Rum) or a foregut or hindgut fermenter (Fore) are indicated in their direction (if interaction terms were nonsignificant, models were repeated without them). Scaling relationships for individual species groups are given in Supplementary Material Tables S3 to S5.

herbivores. The significance level was set to 0.05. Additional explanations on the statistical approach are given in the Supplementary Material S1.

Results

Complete dataset (absolute CH_4 emission and body mass)

Using the complete dataset and species averages, absolute CH_4 emission (L/day) had a significant phylogenetic signal and scaled to $BM^{0.84}$, with the 95% CI for the exponent ranging from 0.77 to 0.92 (Table 1). Using all individual data instead of species averages led to a slightly steeper scaling ($BM^{0.96}$) that did not include linearity in the 95% CI. In GLS, when using species averages, being a ruminant had a positive, and being a foregut fermenter a negative effect on CH_4 emissions. Nevertheless, the 95% CI of the parameter estimates for the scaling relationships of ruminants, nonruminants and hindgut fermenters overlapped (Supplementary Material Table S3). The 95% CI of the scaling exponent included linearity only in taxonomic ruminants. The single elephant measurement, horses, macropods and rabbits were on a generally lower level than ruminants, whereas many hystricomorph rodents as well as the nonruminant foregut fermenters peccary and pygmy hippo had levels similar to those of similar-sized ruminants (Figure 1a).

CH_4 yield in % gross energy intake

Whereas CH_4 (in % of GEI) showed some scaling with BM when all individual data or species averages were used in GLS, indicating that ruminants were on a generally higher level, this was not the case when accounting for phylogeny, indicating no scaling within related groups (Table 1). Correspondingly, there was also no scaling of CH_4 (in % GEI) in any of the four individual herbivore groups (Supplementary Material Table S4). Generally, CH_4 losses (in %GEI) appear to be constrained to a maximum of 10% (Figure 1B).

$CH_4 : CO_2$ ratio

The $CH_4 : CO_2$ ratio showed some BM scaling when phylogeny was not controlled for. There were significant interactions between BM and digestion types when all individual data were used, and indication that ruminants generally have higher ratios using species averages, even when accounting for phylogeny (Table 1). Within herbivore groups, this measure again showed no scaling (Supplementary Material Table S5). Ruminants, camelids and hystricomorph rodents as well as hippos can achieve high ratios of 0.06 and higher, whereas equids, macropods and rabbits appear limited to lower ratios; therefore, average estimates for nonruminants or hindgut fermenters are lower than those of ruminants (Supplementary Material Table S5). At BM below 1 kg, measured ratios appear limited to below 0.04 (Figure 1C).

CH_4 in relation to DM intake

Dry matter intake scaled to an exponent close to metabolic BW, with scaling exponents for ruminants being higher than those of nonruminants or hindgut fermenters (Tables 2 and Supplementary Material Table S6). Apart from the comparatively high intake in the smallest species, arvicoline rodents, no deviation from the overall pattern was apparent (Supplementary Material Figure S1A). The absolute CH_4 emission scaled very similarly to DMI with overlapping 95% CI for the scaling exponents when using species averages, when controlling for phylogeny or for individual herbivore groups (Tables 2 and Supplementary Material Table S6). Correspondingly, the scaling of absolute CH_4 emissions to DMI included linearity in the 95% CI for the scaling exponent

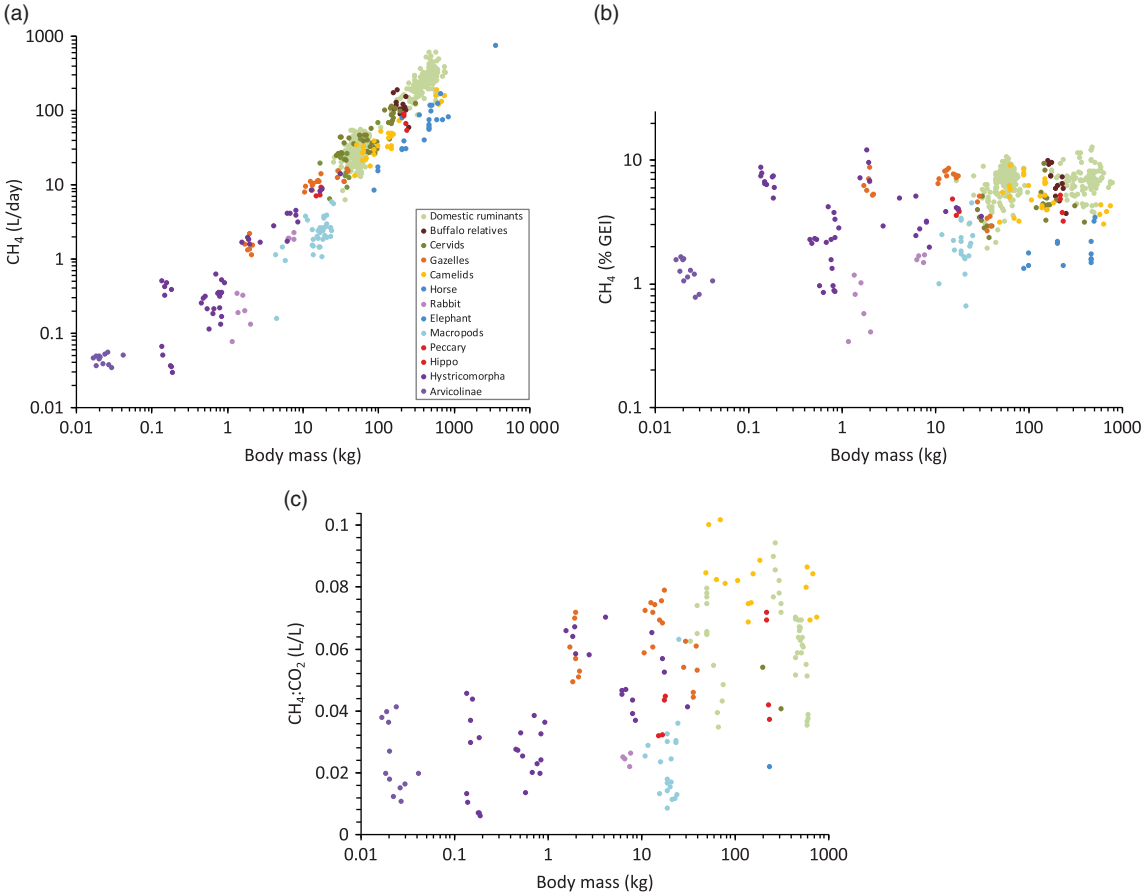

Figure 1 Relationship of body mass (BM) and (a) absolute daily CH_4 emission, (b) CH4 yield (in % gross energy intake), (c) the $CH_4 : CO_2$ ratio in the data collection of the present study. Domestic ruminants comprise goat, sheep and cattle. For a complete list of species, cf. Supplementary Material Table S2; for statistics, Table 1. Note that while horses, macropods (kangaroos) and rabbits generally have lower values than ruminants, hystricomorph rodents as well as the nonruminant foregut fermenters peccary and hippo are in the ruminant range. GEI = gross energy intake.

in all groups (Tables 2 and Supplementary Material Table S6). Only when using all individual data or species averages without controlling for phylogeny, this scaling exceeded linearity, due to an effect of ruminants in these datasets. Similar to the CH_4 yield (in % gross energy), the CH_4 yield (per DMI) did not scale with BM, again except when using all individuals or uncontrolled averages, because of the ruminants (Supplementary Material Figure S1B). When using all individuals, and when controlling averages for phylogeny, there was a negative relationship between the relative DMI and CH_4 yield (per DMI), with ruminants on a higher level than nonruminants (Table 2). In datasets using all individuals, this effect was driven by the ruminants, and in datasets using species averages, by the arvicoline rodents (Supplementary Material Figure S1C).

CH_4 in relation to digested fibre

In the dataset for which NDF intake and digestibility were available, DMI and absolute CH_4 emissions again scaled closely to each other, and the scaling of NDF intake was similar as that of DMI, as was the scaling of digestible NDF intake (Supplementary Material Tables S7 and S8). Neither NDF digestibility (Supplementary Material Figure S2A) nor CH_4 yield (per kg digested NDF) scaled with BM (Supplementary Material Tables S7 and S8), with the exception of the functional ruminants, in which this scaling was negative (due to the inclusion of Bactrian camels in this subset). Absolute CH_4 emissions scaled to digestible NDF intake below linearity when controlling for phylogeny (Supplementary Material Table S7), also in ruminants but not in nonruminants or hindgut fermenters (Supplementary Material Table S8). There was a negative relationship between NDF digestibility and CH_4 yield (per digested NDF) when accounting for phylogeny (Supplementary Material Table S7), and the effect occurred also within the nonruminants and hindgut fermenters (Supplementary Material Table S8). In all groups, there was a negative relationship between the relative intake of digestible NDF and CH_4 yield (per digested NDF) (Supplementary Material Figure S2B, Tables S7 and S8).

CH_4 in relation to digesta retention and gut capacity

In the dataset of experiments in which the MRT of small particles (and solute markers) had been measured in parallel to CH_4 measurements, DMI and absolute CH_4 emissions again scaled nearly identically when controlling for phylogeny (Supplementary Material Table S9). The scaling of small

Table 2 *Scaling relationships in mammalian species between DMI or CH_4 (in L/day or as L/kg DMI) and BM or absolute or relative DMI according to $y = a\ BM^b$*

										P Factors (direction)		*P* Interactions	
Data	λ	*a*	95% CI		*P*	*b*	95% CI		*P*	Rum	Fore	BM × Rum	BM × Fore
DMI (kg/day) ~ BM (kg)													
all		**0.046**	**0.043**	**0.050**	**<0.001**	**0.78**	**0.76**	**0.80**	**<0.001**	0.938	**<0.001 (–)**	0.718	**0.016**
av		**0.056**	**0.046**	**0.068**	**<0.001**	**0.76**	**0.71**	**0.81**	**<0.001**	0.196	0.226	0.840	0.144
av	**1.0**	**0.043**	**0.023**	**0.080**	**<0.001**	**0.83**	**0.77**	**0.88**	**<0.001**	0.990	0.263	0.784	0.634
CH_4 (L/day) ~ BM (kg)													
all		**0.578**	**0.524**	**0.638**	**<0.001**	**0.94**	**0.92**	**0.97**	**<0.001**	**<0.001 (+)**	**<0.001 (–)**	0.064	**<0.001**
av		**0.656**	**0.493**	**0.873**	**0.007**	**0.88**	**0.80**	**0.95**	**<0.001**	**0.002 (+)**	0.471	0.273	0.124
av	**1.0**	0.439	0.185	1.044	0.072	**0.84**	**0.76**	**0.92**	**<0.001**	0.490	0.777	0.700	0.747
CH_4 (L/day) ~ DMI (kg/day)													
all		**23.598**	**22.584**	**24.657**	**<0.001**	**1.16**	**1.13**	**1.19**	**<0.001**	**<0.001 (+)**	**0.003 (+)**	0.060	0.274
av		**18.450**	**15.166**	**22.445**	**<0.001**	**1.14**	**1.05**	**1.24**	**<0.001**	**0.024 (+)**	0.627	0.256	0.450
av	**0.96**	**10.742**	**4.770**	**24.193**	**<0.001**	**1.01**	**0.91**	**1.11**	**<0.001**	0.388	0.209	0.557	0.928
CH_4 (L/kg DMI) ~ BM (kg)													
all		**12.48**	**11.41**	**13.64**	**<0.001**	**0.16**	**0.14**	**0.18**	**<0.001**	**<0.001 (+)**	0.119	**0.015**	**0.032**
av		**11.80**	**9.12**	**15.26**	**<0.001**	**0.12**	**0.06**	**0.19**	**0.001**	**0.049 (+)**	0.646	0.460	0.725
av	**0.95**	**10.09**	**4.51**	**22.60**	**<0.001**	0.04	–0.04	0.13	0.317	0.438	0.223	0.695	0.985
CH_4 (L/kg DMI) ~ relative DMI (g/kg$^{0.75}$ per day)													
all		**69.4**	**46.0**	**104.7**	**<0.001**	**–0.28**	**–0.38**	**–0.17**	**<0.001**	**0.035 (+)**	0.057 (–)	0.629	**0.043**
av		**83.4**	**9.1**	**768.2**	**<0.001**	–0.40	–0.94	0.15	0.163	0.115	**0.039 (–)**	0.158	**0.035**
av	**1.0**	**115.5**	**17.0**	**786.3**	**<0.001**	**–0.59**	**–1.03**	**–0.16**	**0.012**	0.387	0.259	0.524	0.409

DMI = dry matter intake; BM = body mass; 95% CI = 95% confidence interval.
From experiments where CH_4 and intake were measured in parallel ($n = 573$, 34 species), using either all individual values (all) in generalised least squares (GLS), or species averages (av) in GLS or in phylogenetic generalised least squares (PGLS, indicating the phylogenetic signal λ). Significant parameter estimates as well as λ significantly different from 0 are set in bold. Results of additional models that include whether a species is a ruminant or nonruminant (Rum) or a foregut or hindgut fermenter (Fore) are indicated in their direction if they were significant. Scaling relationships for individual species groups of this dataset are given in Supplementary Material Table S6.

particle MRT with BM differed distinctively between models that did not and did account for phylogeny (Supplementary Material Table S9), because MRT increased particularly with BM among rodents but less so across larger herbivores (Supplementary Material Figure S3A and Table S10). Dry matter gut fill scaled slightly below linearity (Supplementary Material Table S9 and Figure S3B). While absolute CH_4 emissions scaled positively with MRT in all datasets, MRT was less clearly related to CH_4 yield (per DMI) (Supplementary Material Tables S9 and S10). Although there was a positive relationship between the two measures (Supplementary Material Table S9), rabbits, macropods and horses had lower CH_4 yields at similar MRT than other species, and in particularly hystricomorph rodents were very variable in this relationship (Supplementary Material Figure S3C). The relative DM gut fill did not scale with CH_4 yield (Supplementary Material Tables S9 and S10), and again, rabbits, macropods and horses had lower CH_4 yields at similar relative gut fills than other species (Supplementary Material Figure S3D).

In an expanded model, using CH_4 yield (per DMI) as the dependent variable and not only small particle MRT but also the ratio of MRTparticles:MRTsolute as a covariable, both MRT and the ratio had a positive relationship when using all individuals ($P < 0.001$ and 0.014, respectively), but only MRT was significant when using species averages ($P = 0.049$ and $P = 0.635$), and there was no significance when accounting for phylogeny ($P = 0.168$ and $P = 0.975$). The effect in the dataset with individual data was considered due to the presence of rodents and in particular lagomorphs, that have very small MRTparticles:MRTsolute ratios due to their wash-back colonic separation mechanism. When repeating the analyses for large herbivores only, neither MRT nor the ratio were significant in any model (individual data, species averages, phylogeny control; *P* always >0.233).

Discussion

The present study represents a compilation of *in vivo* measurements of CH_4 emissions in mammals and shows up scaling relationships with BM, food intake, digesta retention times and gut capacity that lead to modifications of existing concepts. This holds in particular for the presumed dichotomy between foregut and hindgut fermenters, or between ruminant and nonruminant herbivores, which is not consistent in the dataset. Rather, individual herbivore species – in particular horses, macropods (kangaroos) and rabbits stand out as peculiar with respect to their low CH_4 emissions.

Limitations

Typical limitations of comparative data compilations occur, where measurements from different researchers are compiled

in one catalogue. Because of the well-known effect that diets with concentrates lead to lower CH_4 emissions, the present data compilation included only experiments in which animals were fed diets without concentrates, excluding for example the hyrax or the sloth (cf. Supplementary Material Table S1). The case of the hyrax data (von Engelhardt *et al.*, 1978) provides an instructive example: the animals of that study had not only been fed mixed diets, but also been fasted prior to respiration measurements; nevertheless, the data had been used prominently by ourselves (Franz *et al.*, 2011) and others (Smith *et al.*, 2010; Smith *et al.*, 2015) for the establishment of hindgut fermenter-specific regression equations.

There was evident variation in the fibre content of the roughage diets used in the different experiments. Across experiments, such variation may contribute to reductions of CH_4 yield per digestible fibre, when fibre digestibility varied as a function of diet. Potentially, additional analyses could account for variation in dietary fibre levels, or in variation between CH_4 measuring methods. Both would have made the approach in the present study additionally complex, but the information is retained in the supplementary datafile and can be used in further approaches.

Scaling relationships may not be appropriately captured in simple allometric power functions, as in the log–log regressions performed in the present study. Various examples of 'quadratic' scaling exist (Müller *et al.*, 2012), including measures of food intake and digesta retention in mammalian herbivores (Müller *et al.*, 2013). The data from the present study, which are largely independent from those used by Müller *et al.* (2013), indicate a similar pattern with a particularly high intake, and short retention, in arvicoline rodents (Supplementary Material Figures S1A and S3A), and in the different scaling exponents for several measurements between small and large herbivores (Supplementary Material Table S10). While our approach of simple allometries, using various measurements taken simultaneously for the same animals, can be used to compare species groups, a more detailed model using quadratic scaling may be more appropriate to extrapolate data for species not measured, but within the BM range of our data compilation. Actually, Smith *et al.* (2015) developed a complex regression equation for the extrapolation of CH_4 emissions of hindgut-fermenting mammals. However, that regression estimation should be considered with caution, because the hyrax data mentioned above (taken from fasted animals) represent the data entry of the lowest BM in their collection. In a similar way, predictive equations for carbon isotope signature related to CH_4 physiology for hindgut and foregut fermenters presented by Tejada-Lara *et al.* (2018) depend critically on individual data points at the low end of the body size range.

One important limitation in the current dataset is the derivation of gut capacity as DM gut fill from data on intake, digestibility and retention following Holleman and White (1989). Although the method has been validated, and deviation from measurements by dissection can be explained by variable food intake prior to slaughter (Munn *et al.*, 2012), there is a systematic underestimation of the real DM gut fill in ruminants if retention time is measured (as in the current dataset) by a small particle marker (Munn *et al.*, 2015). However, Supplementary Material Figure S3D indicates that even if data for ruminants would be increased (following Munn *et al.*, 2015, by a factor of 1.2), the explanatory power of gut fill for differences in CH_4 physiology would not increase.

Scaling with body mass: no size constraint

In previous work of our group (Franz *et al.*, 2010; Franz *et al.*, 2011) and of others (Smith *et al.*, 2010), linear scaling relationships between CH_4 emissions and BM had been suggested. Given that energy intake by herbivores is generally not assumed to scale linearly with BM but to a lower (allometric) exponent, the logic implication was to construe a CH_4-driven body size limit for herbivores, because at some point, energetic losses as CH_4 would become prohibitive (Clauss and Hummel, 2005; Clauss *et al.*, 2013). Correspondingly, positive scaling relationships of CH_4 yield (per unit of energy intake) were detected (Franz *et al.*, 2011), with the attractive side effect that the models indicated that ruminants would reach any putative threshold at lower BM than hindgut fermenters, thus offering an explanation for an apparent, intrinsic body size limitation in ruminants (Clauss *et al.*, 2003).

This concept needs to be revised. An analysis of ruminant data by Pérez-Barbería (2017) already questioned whether CH_4 emissions really scaled higher than food intake. Consistent with those findings, our dataset not only suggests that CH_4 yield is not related to BM, but that in subsets where food intake was measured in parallel to CH_4 emissions, both measures show a more or less identical scaling (Tables 2, Supplementary Material Tables S6, S7, S9 and S10). Correspondingly, when scaling CH_4 emissions against intake, a linear relationship results. Current evidence suggests that the process of methanogenesis resulting from fermentative digestion does not represent a body size limitation.

Digestive strategy: no clear dichotomies

In contrast to a seemingly clear dichotomy between ruminant and nonruminant herbivores (Crutzen *et al.*, 1986; Franz *et al.*, 2011) or between ruminants/foregut fermenters and hindgut fermenters (Smith *et al.*, 2010; Smith *et al.*, 2015; Tejada-Lara *et al.*, 2018), the current data indicate that no simple categories might apply to classify herbivores in relation to CH_4. The historical intermingling of the terms 'ruminants' and 'foregut fermenters' notwithstanding (Clauss *et al.*, 2010), the CH_4 literature does not treat these categories consistently, for example, when the hippopotamus (a nonruminant foregut fermenter) CH_4 emission is extrapolated based on 'nonruminants' by Crutzen *et al.* (1986) and based on 'ruminants' by Smith *et al.* (2015). No simple rule can be established: Among the nonruminant foregut fermenters, macropods have particularly low comparative CH_4 emissions, peccaries and hippos are at the lower range of ruminants, and the sloth – excluded from the present data analysis to remain consistent as to the diets used – is in the upper range of ruminants even on a low-fibre diet (Vendl

et al., 2016b). Similarly, no clear pattern seems to apply for the hindgut fermenters. Although the dichotomy between horses and ruminants is evident, additional measurements of other hindgut fermenters, mainly by our group (Table 2), do not yield a simple pattern, but indicate that several hystricomorph rodent species can have CH_4 emissions of a magnitude expected for ruminants of similar size. In Figure 1A, the absolute CH_4 emissions of the smallest gazelle, the dikdik, and the hystricomorph rodent nutria, are identical. While a focus on ruminants with respect to mitigation strategies follows logically from their great relevance as food producing production animals with an enormous greenhouse gas footprint (Steinfeld *et al.*, 2006), these findings indicate that one should not consider the ruminant digestive tract as the only one capable of harbouring intensely productive methanogenic microbiota.

One possible approach to determine the CH_4 strategy of a larger number of species could be the diet–bioapatite carbon isotope enrichment offset. Studies in which such data have been applied to herbivores have implied a difference in CH_4 emissions between herbivore digestion types (Codron *et al.*, 2018; Tejada-Lara *et al.*, 2018). However, the data from Codron *et al.* (2018) suggest little differences between the hindgut fermenters rock hyrax, black and white rhinoceros (*Diceros bicornis*, *Ceratotherium simum*), warthog (*Phacochoerus africanus*) or even a zebra species (*Equus quagga*) and ruminants. The data from Tejada-Lara *et al.* (2018) potentially corroborate the finding of CH_4 emission in arvicoline rodents (voles) of the present study and indicate little difference between the hindgut fermenters African elephant (*Loxodonta africana*), the black rhinoceros, the horse, a zebra species (*Equus burchelli*) and the pig and the ruminants giraffe (*Giraffa camelopardalis*), Bactrian camel and guanaco (*Lama guanicoe*). At other places in the body size range of that study, little differences appear evident in foregut-fermenting sloth species (*Choloepus hoffmanni* and *Bradypus variegatus*) paired each with a hindgut fermenter (the rabbit and the koala, respectively). These datasets might be better comprehensible if no clear separation between general digestion types would be assumed.

Relationships with intake and digesta retention

Abandoning clear categories of CH_4-producing digestion types raises the question whether other rules can be gleaned from the comparative dataset. There are well-established relationships between food intake or digesta retention and CH_4 yield in domestic ruminants (Okine *et al.*, 1989; Lassey *et al.*, 1997; Barnett *et al.*, 2012; Hammond *et al.*, 2014; Barnett *et al.*, 2015) and in individual groups of non-domestic species (Frei *et al.*, 2015; Vendl *et al.*, 2015; Vendl *et al.*, 2016a). In addition, the intra-individual variation in CH_4 emission in domestic ruminants is explained by differences in digesta retention, possibly linked to digestive tract capacity (Pinares-Patiño *et al.*, 2003; Goopy *et al.*, 2014; Cabezas-Garcia *et al.*, 2017). Therefore, species were expected to vary in their CH_4 yield depending on their species-specific food intake levels, digesta retention times and DM gut fill. The first two predictions were met (Supplementary Material Figures S1C and S3B); however, the respective datasets failed to provide an explanation why certain species, such as horses, macropods or rabbits, had lower CH_4 yields at similar intake levels or retention times than others.

Additionally, the combination of an apparent evolution of a high fluid throughput or 'digesta washing' in macropods, and results from various *in vitro* studies led to an additional hypothesis (Vendl *et al.*, 2015): A large difference in the MRT of fluids and particles (measured as a high MRTparticle: MRTsolute ratio and leading to a high outwash rate of microbes from the fermentation chamber) might create conditions favourable of a microbiome tuned towards growth rather than CH_4 production. However, in spite of a similar pattern of fluid and particle retention in pygmy hippos as in macropods (Supplementary Material Figure S4), no similarity in CH_4 emissions were evident between these species. When adding the ratio between particle and fluid retention to a regression of CH_4 yield against particle retention, no significant relationship resulted.

Clauss and Hummel (2017) suggested that adaptations in so-called 'cattle-type' ruminants towards a high fluid throughput might have a similar effect, and that variation in the amount of fluid passing through the rumen, because of differences in saliva production, might contribute to inter-individual variation in CH_4 emissions. The authors specifically referred to evidence from breeding experiments against the susceptibility to frothy bloat in cattle, which is considered linked to low saliva production of individual animals (Gurnsey *et al.*, 1980; Morris *et al.*, 1997). The assumption of Pinares-Patiño *et al.* (2008) that bloat-susceptible cattle, with less fluid flow, have higher CH_4 yields, based on the observation of slightly higher proportions of CH_4 in rumen gases of bloat-susceptible animals (Moate *et al.*, 1997), would match this hypothesis. However, the same authors demonstrated no difference in CH_4 yields between cattle of low and high bloat susceptibility (Pinares-Patiño *et al.*, 2008). Additionally, Grandl *et al.* (2018) did not find an association of inter-individual CH_4 yield differences and the MRTparticle: MRTsolute ratio in cattle. Should the CH_4-saving effect of increased fluid throughput that is so evident in *in vitro* fermentation experiments (Isaacson *et al.*, 1975; Pfau *et al.*, 2019) occur in herbivores *in vivo*, it remains to be demonstrated.

Relationship with fibre digestion

Similar to the moderate scaling of digesta retention with BM detected in the present study, the absence of a body size effect on the digestibility of NDF across herbivore species is in congruence with previous findings (Müller *et al.*, 2013; Steuer *et al.*, 2014). The results indicate that the amount of CH_4 produced per unit of fibre digested is not necessarily constant. The scaling relationship between digestible NDF intake and absolute CH_4 emission included linearity in nonruminants and hindgut fermenters, but this was not the case in ruminants (Supplementary Material Table S8), where digested NDF translated to a less-than-linear scaling

(albeit on a generally higher level) into CH_4. In all data subsets, the relative intake of digestible NDF was negatively related to the CH_4 yield per unit of digested fibre, and in some subsets, a similar negative CH_4 yield scaling was evident with NDF digestibility. While it cannot be excluded that these patterns reflect differences in diet, they also hold for some groups in Supplementary Material Figure S2B fed a consistent diet for the measurements, and thus resemble a finding made in three different cattle feeding groups by Grandl *et al.* (2018). It is tempting to speculate that conditions leading to a higher fibre digestibility are linked to microbiota functions that are characterised by fibre digestion at reduced CH_4 production. While promising, the pattern again cannot explain fundamental differences in CH_4 emission between all species.

Conclusion and outlook

While many of the species investigated by our team have only been assessed once, results on domestic ruminants, South American camelids, horses, macropods and rabbits have been reproduced in more than a single study, suggesting that CH_4-related characteristics represent repeatable, species-specific characteristics. Given the absence of generalisable patterns, all that is left is the suggestion that yet-to-be-defined, species-specific characteristics determine the composition and activity of the microbiota of herbivores, and that across species, these characteristics may not be linked to digesta retention mechanisms of gut anatomy. Such a host specificity has been shown within cattle (Weimer *et al.*, 2010; Weimer *et al.*, 2017) or humans (Goodrich *et al.*, 2014), or across species in a famous cross-over experiment with mice and fish microbiota (Rawls *et al.*, 2006). Differences in the methanogenic potential of the microbiota of cattle have been identified (Zhou *et al.*, 2009; Danielsson *et al.*, 2012; Ben Shabat *et al.*, 2016), and studies indicate the heritability of the microbiome composition, that is, its genetic control (Goodrich *et al.*, 2014; Roehe *et al.*, 2016). These results open the possibility of selecting domestic animals for their microbiome composition. Yet, they also raise again the question of the Introduction why, if microbiome and hence methanogen control by the host is possible, evolutionary adaptations have not led to the exclusion but to a prominent role of methanogens in many herbivore species.

Our study suggests that CH_4 production may be more uniform across many herbivore species, that perceived differences between digestion types are due to a historical focus on certain animal groups, and that ruminants should possibly not be considered peculiar in this respect. Further studies elucidating the peculiar conditions that result in the comparatively low CH_4 emissions in macropods, equids, and, possibly, rabbits are warranted. For a reliable reconstruction of past CH_4 budgets, the use of domestic equids as model animals for 'hindgut fermenters' must be questioned, and additional measurements in other, non-rodent hindgut fermenters would be needed. This could be rhinoceroses, tapirs, (forage-fed and non-fasted) hyraxes, or pigs fed forage-only diets. Finally, the relevance of methanogens for the digestive physiology of primates remains to be explored.

Acknowledgements

The experiments reported here were part of a study financed by the Swiss National Science Foundation 310030_135252/1.
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Declaration of interest

The authors declare no conflict of interest.

Ethics committee

Experiments were performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence no. 142/2011), and with the internal ethics committee of the former Al Wabra Wildlife Preservation.

Software and data repository resources

None of the data were deposited in an official repository, but given as Supplementary Materials S1 (PDF file) and S2 (Excel file).

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119003161

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