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Indications for a lower methane yield from digested fibre in ruminants digesting fibre more efficiently

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Abbreviations: ADFom, ash-free acid detergent fibre; aNDFom, ash-free neutral detergent fibre treated with α-amylase; CH_4, methane; CI, confidence interval; DM, dry matter; DMI, dry matter intake; OM, organic matter; VFA, volatile fatty acid.

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A B S T R A C T

It is assumed that the absolute amount of methane (CH_4) produced on a given diet increases proportionately (i.e., in a linear manner) with the amount of digested fibre. Therefore, the CH_4 yield per unit of digested fibre is considered constant for a given diet. This conceptually matches findings of lower digestibility in low-CH_4 emitting animals, and of lower CH_4 yield at higher intake levels due to shorter digesta passage and hence reduced digestibility. Irrespective of these observations, this

general assumption was challenged by findings in one study where CH_4 yield per unit of digested fibre had unexpectedly declined in individuals digesting the fibre provided by the same diet more efficiently. To investigate this finding in more detail, we collated a dataset from 16 studies with cattle and sheep with a total of 61 forage-based diet groups consisting of at least five animals each (472 animals in total). We assessed whether there was a linear relationship between the daily CH_4 emission and the amount of digested fibre, both within the same and across the different diet groups. Across diets, CH_4 emissions did not increase linearly with the amount of digested neutral or acid detergent fibre in either species. Within diet groups, the majority of cases also showed evidence for less-than-linear increase of CH_4 emissions with increasing amount of digested neutral or acid detergent fibre, even though the 95% confidence intervals could not rule out a linear relationship in many cases. Reasons why this phenomenon was not described earlier may include that the great individual variation associated with an accumulation of errors in the variables concerned often prevented statistical significance in individual studies. Although the findings across diets concerning the variation in CH_4 yield per unit of digested fibre do not exclude some diet-specific effects, the within-diet assessment clearly points towards individual animal effects in microbial fibre digestion in a way that CH_4 production is proportionately lower when fibre is digested more efficiently. Mechanistically, animals with a more efficient fibre digestion might produce volatile fatty acids at a higher rate and have a locally lower ruminal pH, favouring microbiota of propionate-producing pathways. The presence of animal-individual differences in CH_4 yield per unit of digested fibre with varying efficiency of fibre fermentation should be confirmed in a specific experiment where also the reasons for such a phenomenon are further investigated.

1. Introduction

For quite some time, microbial digestion of the plant cell wall is commonly understood as a sequence of steps carried out by different groups of microbes (Van Soest, 1994). Organic polymers like cellulose are first hydrolysed into soluble sugars. These are subject to primary fermentation into

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'intermediate products' such as pyruvate, lactate, succinate, formate, and then via secondary fermentation to the volatile fatty acids (VFA). These steps also release hydrogen and carbon dioxide. In order to maintain the sequence of reactions required for fibre digestion, the hydrogen must be removed from the system, for instance via methanogenesis. This intuitively suggests that there is a proportional relationship between the amount of digested plant cell wall, the amount of hydrogen and released and hence the amount of methane (CH_4) produced. Therefore, CH_4 yield per unit of digested fibre should represent a constant across diets dominated by roughages (e.g., Dittmann et al., 2014).

Within diets, this concept can be reconciled with several findings made repeatedly in studies on CH_4 production in ruminants. For example, the reduction in CH_4 yield per unit of dry matter (DM) ingested, observed when animals increase their intake level on the same diet, is typically linked to the digesta passage-accelerating effect of higher intakes and the concomitant depression of digestibility (Hammond et al., 2014; Goopy et al., 2020). Ruminants classified as low CH_4 emitters have repeatedly been found to have shorter digesta passage and lower digestibility (Goopy et al., 2014; Stepanchenko et al., 2023). A proportional relationship between CH_4 production and fibre digestion aligns with these findings.

Across diets, differences in the CH_4 yield per unit of digested fibre would be expected, because of the differences in the profiles of digestible fibre fractions, and because the fermentation of non-fibre diet components will make different contributions to overall CH_4 production beyond that linked to fibre degradation. Diet specificity is, for example, part of the concept of a 'partitioning factor' that describes the contribution to gas and microbial mass production per unit of degraded substrate for different diets (Blümmel et al. 1997a, b). As these variations do not occur within diet, constancy of the CH_4 yield per unit of digested fibre is the default expectation for a given diet. Mathematically, this means that we expect daily CH_4 production to increase linearly with the amount of digested fibre for a given diet (Fig. 1A left) and form a horizontal line when the amount of digested fibre is related to the corresponding CH_4 yield (Fig. 1A right).

Having this concept in mind, an evaluation of a dataset from one extensive study (Grandl et al., 2018) unexpectedly revealed that the CH_4 yield per unit of digested fibre significantly increased with decreasing fibre digestibility across individual cattle fed the same diet. Additionally, a meta-analysis of data available for a large variety of domestic and non-domestic mammal species fed roughage-based diets indicated the same pattern, namely an increase in CH_4 yield per unit of digested fibre with decreasing fibre digestibility or amount of digested fibre (Clauss et al., 2020). Also, several studies in which forage-to-concentrate ratios (Hindrichsen et al., 2005, 2006; Klevenhusen et al., 2011a, b) or forage types (Hess et al., 2004; Staerfl et al. 2012b) were varied showed an increase in CH_4 yield per unit of digested fibre when fibre digestibility decreased. Overall, these findings suggest that CH_4 production indeed may not be constant with digestion of fibre from the same diet. If further corroborated, these observations might have far-reaching consequences. For example, they raise the question of whether more efficient digesters might use different key members of rumen microbiome or different fermentation pathways for fibre digestion or both, resulting in a proportionately lower CH_4 formation.

However, a literature-based systematic evaluation of the phenomenon of individual animal differences is difficult, as such individual animal data are rarely reported. Therefore, to confirm or disprove the observation described by Grandl et al. (2018), we collated individual animal data from ten and six of our own experiments with cattle and sheep, respectively. These animals had been fed a total of 61 distinct diets (Table 1), and individual intake, fibre digestibility and CH_4 emissions had been quantified. The null hypothesis was that absolute CH_4 production increases linearly with the amount of digested fibre for a given diet, and that the CH_4 yield per digested fibre is therefore independent from the amount of digested fibre (Fig. 1A). If, however, the phenomenon found by Grandl et al. (2018) is true, an exponent of lower than 1 (less-than-linear) would occur (Fig. 1B left), with a corresponding decrease of CH_4 yield per digested fibre at increasing amount of digested fibre (Fig. 1B right). As the composition of neutral detergent fibre (aNDFom) can vary distinctively, and hence also its digestibility between diets and animals, we also included acid detergent fibre (ADFom),

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which is generally more uniform in composition. The hypothesis of a more efficient fibre digestibility with proportionately less CH_4 production would therefore gain support if the same pattern could be detected for ADFom. Because most diets included in our dataset had a low number of observations, a reliable statistical demonstration of the kind of scaling (linear, lower, or higher) was not expected in most cases. However, we postulated that a bias in the total of exponents would be an indication of the overall pattern. In other words, for a linear scaling, we expected a widely random distribution of numerical exponents around 1, whereas for a less-than-linear scaling, the majority of numerical exponents should be <1.

2. Material and methods

2.1. Database development

Data were obtained from the 16 experiments performed at ETH Zurich and described in Table 1. A forage-only diet (grass, grass hay, grass silage, or maize silage) had been applied in seven of these experiments (two of which also investigated a mixed diet), 11 experiments had used diets with a forage proportion in dry matter of ≥ 0.5 of total, and one experiment used a forage proportion of 0.4 of total (Table 1). Feed intake had not been deliberately varied within or across the studies, being simply appropriate for the respective dietary requirements of the animals. The database was constructed from experiments where feeds and faeces had been analysed for contents of dry matter (DM), organic matter (OM), dietary neutral detergent fibre (assayed with α-amylase and without residual ash, aNDFom; AOAC International (1995) index no. 2002.04), and acid detergent fibre (without residual ash, ADFom; AOAC International (1995) index no. 973.1). Individual feed intake had been determined by weighing (manually or automatically), digestibility by total faecal collection, and CH_4 emissions in open circuit respiration chambers. In addition to aNDFom and ADFom digestibility, digestibility, and amount of digested OM and non-aNDFom OM were calculated where possible. Considering these boundary conditions, 472 individual animal data from ten experiments with cattle (most with dairy cows, one with heifers and one with beef cattle) and six experiments with

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sheep were available for the statistical evaluation (Table 1). Data comprised of 295 individual cattle observations and 177 with sheep. In most experiments, more than one kind of diet had been used, resulting in a total of 31 different diet groups for cattle and 30 for sheep. Only diets fed to at least five animals were accepted. The ADFom data was only available for 24 and 14 diet groups and that on the amount of digested OM for 25 and 24 diet groups with cattle and sheep, respectively (cf. Table 1).

The descriptive statistics for all individual diet groups for body mass, the intake of DM, OM, aNDFom and ADFom, the apparent digestibility for, and the amount of, digested OM, aNDFom and ADFom as well as the daily CH_4 emission are provided in Tables S1 (cattle) and S2 (sheep) in the supplementary material.

2.2. Data analysis

The null hypothesis of a linear increase of absolute daily amount of CH_4 with the amount of digested fibre implies a linear scaling of CH_4 ~ digested fibre$^{1.00}$ (Fig. 1A left). This means that the exponent is 1.00, and would therefore not necessarily have to be specified. When applying linear models to untransformed data, an exponent of 1.00 is the default assumption. When expressed as a ratio or 'yield' (CH_4 per unit of digested fibre), this translates into an absence of scaling of the yield with the amount of digested fibre, i.e., CH_4 per digested fibre ~ digested fibre$^{0.00}$ (Fig. 1A right).

When expecting the absolute daily amount of CH_4 to increase less pronounced with increasing amount of digested fibre, the expected relationship is CH_4 ~ digested fibre^z with $0 < z < 1$ (Fig. 1B left). Hence, the yield is expected to show a negative scaling with CH_4 per unit of digested fibre ~ digested fibre^{z-1} (Fig. 1B right).

We statistically assessed only the relationships displayed on the left side of Fig. 1, to avoid the potential of a spurious negative relationship between a ratio (y/x) with its denominator (x); the correlation of a ratio (y/x) with its denominator (x) will produce a negative relationship if the data for both y and x are completely random (Atchley et al., 1976; Atchley and Anderson, 1978). However,

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because it is visually much easier to detect differences in the pattern displayed on the right side of Fig. 1 than on its left side, we also display the results plotting CH_4 per unit of digested fibre against digested fibre. Additionally, we show the results by plotting CH_4 per unit of digested fibre against digested fibre digestibility.

Because digestive physiology differs systematically between cattle and sheep (e.g., Pfau et al. 2023), statistical analyses were performed individually for each species, and individually for the larger datasets containing information about aNDFom, and the smaller datasets with additional information on ADFom, OM and non-aNDFom OM. Following a long-established analytical practice (Glazier, 2021), log-transformed data were used for all analyses, where $y = a\ x^b$ is transformed into $\log(y) = \log(a) + b \log(x)$, using linear regression to estimate b, including its 95% confidence interval (CI). This was done individually for all diet groups and for the entire respective datasets (cattle or sheep; aNDFom, ADFom, OM and non-aNDFom OM, respectively). For the latter, diet was included as a random factor in linear mixed effects models, using R package lmerTest (Kuznetsova et al., 2017). The resulting exponents determined in the individual diet groups were displayed with their 95% CI. Additionally, to assess the effect of intake level (measured as DM intake (DMI)), we assessed a potential effect of DMI on the apparent digestibility of aNDFom in the same way (for all diet groups separately, and for the entire cattle and sheep datasets).

3. Results

In cattle (Fig. 2 top), for 25 out of 31 diet groups (81%), the estimated scaling exponent for digested aNDFom was less-than-linear. In 16 (52%) of these cases, the 95% CI of these exponents did not include 1.0. In case of digested ADFom, the estimated scaling exponent was less-than-linear for 21 out of 24 diet groups (88%), and in 14 (58%) of these cases, the 95% CI of these exponents did not include 1.0. For the overall datasets, the average exponents were 0.81 (95% CI: 0.74, 0.88) for aNDFom and 0.37 (95% CI: 0.26, 0.48) for ADFom, respectively (grey symbols in Fig. 2).

In sheep (Fig 2 bottom), for 28 out of 29 diet groups (97%), the estimated scaling exponent for digested aNDFom was less-than-linear. In, 17 (59%) of these cases, the 95% CI of these exponents excluded 1.0. In case of digested ADFom, the estimated scaling exponent was less-than-linear for 12 out of 14 diet groups (86%), and in 4 (29%) of these cases, the 95% CI of these exponents did not include 1.0. For the overall datasets, the average exponents were 0.26 (95% CI: 0.15, 0.38) for digested aNDFom and 0.22 (95% CI: 0.12, 0.33) for digested ADFom, respectively (grey symbols in Fig. 2).

The relationships of CH_4 production and amount with digested fibre, as well as CH_4 yield per unit of digested fibre determined in the individual diet groups is illustrated in Fig. 3 for cattle and Fig 4 for sheep. These illustrations, especially those for CH_4 yield per unit of digested fibre, also indicate that there could be non-linearity in both animal species and both fibre fractions.

The scaling of CH_4 emissions with digested OM and digested non-fibre-OM generally followed a similar pattern (Fig. 2, Supplementary Fig. S1 and S2). All calculated exponents with their 95% CI are listed in Tables S3 (cattle) and S4 (sheep), together with the proportion of forage in the respective diet. Less-than-linear scaling occurred not only in forage-only diets, but also in diets of lower forage proportion (Table S3-S4).

Across all diets, DMI did not have an effect on the apparent aNDFom digestibility in cattle (exponent: 0.03, 95% CI: -0.03, 0.08) or sheep (exponent: 0.09, 95% CI: -0.06, 0.24) as the 95% CI of the exponent included zero (no effect) in both cases (Fig. S3, Table S5). In the individual diet groups in cattle, eight had a negative exponent (which was significant in only two cases) and 23 a positive exponent (which was also significant in only two cases) for this relationship; in sheep, 8 had a negative exponent (which was never significant) and 22 a positive exponent (which was significant in only two cases) for the relationship of DMI with apparent aNDFom digestibility (Table S5). Thus, intake did not appear to have a systematic effect on digestibility in this dataset.

4. Discussion

The results of the present study challenge the concept that the amount of CH_4 produced is completely proportional to the amount of digested fibre. Rather, the results propose that animals that digest fibre better do so with a proportionately lower CH_4 production. We could demonstrate this phenomenon in cattle and sheep, and individuals of most diet groups followed this relationship. Across diet groups, the effect was also non-linear.

In this context, the question is important whether the observed effects are biologically meaningful. Indeed, at a first glance, this finding appears to be counterintuitive. However, similar relationships have been found in a different data compilation joining domestic and nondomestic mammalian species (Clauss et al., 2020). This suggests that the effect may be repeatable. It is difficult to compare the results with other studies because many *in vivo* studies that relate CH_4 production to DM intake, OM intake, or the amount of digested DM or OM, provide no information about fibre digestibility on an individual animal basis, and *in vitro* characterizations of forages usually use DM or OM disappearance as a basis for CH_4 yield, but not fibre disappearance.

In the following, we first discuss aspects of the findings across different diets, which include the influence of diet composition. Subsequently, we discuss aspects of the findings within diets, where factors of influence were without diet composition effects and which relate to differences between animals, including differences in intake, digestive anatomy, physiology, and microbiome.

4.1. Non-linearity of methane yield per unit of digested fibre between different diets

Fibre can only be degraded via microbial fermentation to compounds which are digestible, whereas other components of the diet can be degraded by both, the microbiome or the ruminant's own digestive enzymes. Nevertheless, the majority of components will be degraded by the microbiome of the rumen; here, it is well-known that non-fibrous components are less methanogenic than fibrous components (e.g. Wang et al., 2018).

The dataset of the present study (cf. the scaling exponents for digested OM and non-aNDFom-OM in Fig. 2, and the corresponding patterns in Supplementary Fig. S1 and S2) confirmed that the amount of CH_4 released per amount of digested OM and non-fibre OM decreases with increasing OM and non-fibre OM digestibility and thus is not constant. Similarly, Pacheco et al. (2014) found a decreasing CH_4 yield per unit of digested OM with increasing OM digestibility across a variety of forages fed to sheep, even within different batches of these forage species, and termed this 'seemingly paradoxical'. Still this effect may mainly reflect differences in the proportions of fibre and other nutrients between and within diets.

By contrast, to our knowledge it has not yet been suggested that feed with a higher fibre digestibility should release relatively less CH_4 per unit digested fibre during that fibre's fermentation. This appears particularly remarkable in our data collection because it does not only refer to aNDFom, but also to ADFom. Differences between diets might have been more expected with aNDFom, because aNDFom contains a greater variability of different types of cell wall constituents, which are potentially targeted by a larger variety of microbes and may vary in digestibility. This is, for example, suggested by the two distinct clusters of diet groups in sheep (Fig. 4) in which, at the same amount of digested fibre, different levels of CH_4 were emitted. However, the similarity in the pattern for those experiments where ADFom was available points towards a fundamental principle considering fibre digestion.

Summarizing the explanation of the across-diets finding, non-linear scaling of CH_4 emissions with digested OM and digested non-fibre-OM is typically related to mechanisms based on varying nutrient composition as outlined above. Yet, the parallel non-linear scaling of CH_4 emissions with digested aNDFom and digested ADFom across the same diets cannot be explained by these mechanisms. Therefore, we suggest that also effects other than differences in nutrient composition should by explored for the background of the effects of digestibility on CH_4.

Several alternative pathways for the use of hydrogen other than for methanogenesis are recognized, including the formation of propionate, homoacetogenesis, nitrate and sulphate reduction,

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biohydrogenation of unsaturated fatty acids, and the synthesis of microbial biomass (Wang et al., 2023). The alternative explanation that there are different fibre degrading pathways with putative differences in hydrogen production appears far less likely. Although feeds of different fibre digestibility might differ in any of the mentioned factors, a shift towards more propionate-releasing fermentation along with higher fibre digestibility appears particularly plausible. In parallel to a shift towards propionate fermentation with the lower pH triggered by concentrate feed added to forage (Lana et al., 1998; Russel, 1998; Wang et al., 2023), fibre of a higher digestibility will most likely be digested at a faster rate. Thus, more VFA are released per time, hence decreasing the pH and thus creating slightly more favourable conditions for lactate and propionate producing bacteria.

4.2. Non-linearity of methane yield per unit of digested fibre within diets, i.e., between individuals

Within diets, the same major mechanisms may be responsible for a systematic shift from methanogenesis to other hydrogens sinks – in particular, propionate and microbial biomass production. But here, the effect cannot be triggered by the diet itself; rather, it must be caused by differences among individual animals.

An important factor shown repeatedly to reduce the yield of CH_4 per unit of DMI is an increasing intake level (Hammond et al., 2014; Goopy et al., 2020). This is likely the result of the often found decrease in fibre digestibility and an increase in digesta passage at higher intakes, with an increasing contribution of the hindgut to fibre digestion that, however, mostly does not compensate for the general digestibility reduction (Staples et al., 1984; Firkins et al., 1986; Le Liboux and Peyraud, 1998). In the studies included in the present evaluation, DMI was not manipulated intentionally but corresponded generally to the requirements of the animals when fed on the respective diets, and, as expected, DMI did not have a relevant effect on fibre digestion (Fig. S3, Table S5). We cannot completely exclude that the data reflect a systematic effect of a kind that animals that digested a higher amount of fibre within a diet group did this with a higher contribution of the hindgut (where CH_4 production per fibre digestion is proportionately lower than in the rumen; Immig, 1996).

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However, given the absence of previous findings on such a systematic relevance of the hindgut, we consider this unlikely.

In this context, characteristics of individual ruminants classified as low CH_4 producers might be helpful for clarification of the effect of host genetics and their microbiome, even though we do not claim a parallelism between this classification and the effect observed here. Findings include lower rumen capacities and shorter digesta retention compared to high CH_4 producers (Pinares-Patiño et al., 2003; Goopy et al., 2014; Bond et al., 2019) – factors rather associated with a lower, not a higher fibre digestibility, and therefore unlikely to be related to the phenomenon described here. Similarly, low CH_4 producing cows are often characterized by lower OM and fibre digestibility than high producing cows, again stressing that our findings should not be equated with a generally low CH_4 emission. Documented differences in the microbiome between high and low CH_4 producers include microbial diversity in general (less diversity = less CH_4) (Ben Shabat et al., 2016; Saborío-Montero et al., 2022), the abundance of protozoa (less protozoa = less CH_4) (Guyader et al., 2014; Saborío-Montero et al., 2022) or of specific bacteria like *Quinella*, *Prevotella, Sharpea* or *Succinovibrionaceae* (Kittelmann et al., 2014; Wallace et al., 2015; Kamke et al., 2016; Danielsson et al., 2017; Aguilar-Marin et al., 2020; Kumar et al., 2022; Stepanchenko et al., 2023) (all ultimately associated with increased propionate production), and abundance of methanogenic archaea (lower = less CH_4) (Arndt et al., 2015; Wallace et al., 2015; Aguilar-Marin et al., 2020). A similar abundance of methanogenic archaea at a reduced transcription of methanogenesis pathway genes would have the same effect (Kittelmann et al., 2014; Shi et al., 2014; Greening et al., 2019). In terms of fermentation products, low CH_4 producers have been reported to have higher ruminal proportions of propionate (Kittelmann et al., 2014; Ben Shabat et al., 2016; Danielsson et al., 2017; Lyons et al., 2018; Stepanchenko et al., 2023) or of lactate (Kamke et al., 2016). To our knowledge, none of these factors have been linked directly to a higher fibre digestion capacity. Yet, they could be the consequence or expression of a faster-fermenting (and hence locally pH-reducing) microbiome.

The lack of microbial data in the present dataset leaves the question open about the characteristic microbiome of less and more efficient fibre fermenters. A closer look at the bacteria involved in fibre digestion pathways may help to look for promising candidates in future studies of the phenomenon. In Fig. 5, the variation in abundance of several microbial species with variation in the dietary forage proportion is summarized. These microbes also seem to be important for feed efficiency and, thus, fibre digestive efficiency. Among the fibre fermenters, *Fibrobacter succinogens* is of particular importance, because it is a specialised cellulose digester that does not produce hydrogen and henceforth does not contribute to CH_4 emission (Gokarn et al., 1997; Stewart et al., 1997; Joblin et al., 2002; Chaucheyras-Durand et al., 2010). The abundance of *F. succinogens* is associated with both high forage diet and high feed efficiency (Fernando et al., 2010; Henderson et al., 2015; Elolimy et al., 2018; McGovern et al., 2018). Besides *F. succinogens*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are the best known cellulose degraders (Miller and Wolin 1973; Latham and Wolin 1977; Rooke et al., 2014; Zheng et al., 2014). They may construct extracellular enzyme scaffolds known as cellulosomes to digest cellulose. The abundance *R. albus* and *R. flavefaciens* is also related to feed efficiency, but both, positive and negative association have been observed (Carberry et al., 2012; McGovern et al., 2018). The species that are important in the breakdown of hemicellulose, xylan and pectin are *Butyrivibrio spp.*, and *Lachnospira multiparus*; however, the association with feed efficiency still lacks concrete evidence (van Gylswyk et al., 1996; Fernando et al., 2010; Mayorga et al., 2016; Emerson and Weimer, 2017; McGovern et al., 2018; Bowen et al., 2020; Salfer et al., 2021).

The methanogen composition may actually play a smaller role than bacterial diversity when it concerns feed efficiency (Henderson et al., 2015). Rather, it is the activity of the methanogens that is most closely associated with actual CH_4 emission (Söllinger et al., 2018), which is governed by the substrate availability from bacterial or protozoal fermentation. However, a lower abundance of *Methanobrevibacter ruminantium* (Ben Shabat et al., 2016; Delgado et al., 2019) and *Methanobrevibater* AbM4 (Arndt et al., 2015) has been found in more feed efficient cows.

It is generally accepted that the level of CH_4 emissions represents a heritable trait (Difford et al., 2018; de Haas et al., 2021; Mahala et al., 2022), also because the genetic properties of the animals affect microbiome composition and metabolism (Saborío-Montero et al., 2022) and VFA proportions are heritable as well (Jonker et al., 2019). Heritability estimates for fibre digestibility have not been made to our knowledge. Selecting animals with a lower residual feed intake was hypothesized to be a strategy for selecting low CH_4-emitters. But even in these considerations, the contribution of fibre digestibility to the overall feed efficiency was rarely mentioned or investigated, except by Potts et al. (2017) for dairy cows on a low-starch diet. In addition, Arndt et al. (2015) found a lower CH_4 yield per unit of digested NDF in highly feed efficient dairy cows, but da Silva et al. (2020) did not corroborate this in heifers.

Other potential individual factors that might concomitantly affect fibre digestion and CH_4 production include chewing intensity. This property has been shown to differ between individual cattle (Dado and Allen, 1994) and to be related to digesta turnover characteristics (Zhang et al., 2023a), and can also be expected to be related to the rate of particle size reduction. Smaller particles typically have a faster fermentation rate (Bjorndal et al., 1990; Lowman et al., 2002), which might cause local reductions in ruminal pH. Another hypothesis links a higher rumen turnover not only to selecting for generally more microbial growth (Zhang et al., 2023b) but also to selecting bacteria characterised by fast heterofermentative growth that produce less hydrogen (Kittelmann et al., 2014).

4.3. Study limitations

While the dataset of the present study is comprehensive, the described statistical effects are not unambiguous; this most likely due to the low number of animals per diet group. Although the null hypothesis expectation would have been that roughly equal numbers of diets show a relationship below and above linearity (which was clearly not the case when counting the estimates for b in Fig. 2), the 95% CI excluded linearity only in a lower number of cases. Therefore, while the results show a clear trend, additional data from other studies, or specific studies targeting the observed relationship

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with high numbers of individuals, would be welcome. The low number of individuals per diet group precluded a more comprehensive evaluation of within-diet effects by using multiple regression parameters. Another study limitation is that the data originated all from a single research group with three generations of respiration chambers and results obtained over 17 years. While this ensured a certain degree of method consistency of the data collection over several years, it would be clearly desirable if other research groups with individual-based data would apply similar tests to their data to ensure a repeatability of the observed effects. The datasets used in the present evaluation did not include information about the rumen microbiome and other ruminal characteristics. Therefore, the discussion about possible reasons had to remain speculative. Confirmation of the presence of the phenomenon indicated from the present evaluation, namely that individual animal differences in fibre digestibility are associated in a non-linear relationship with CH_4 emissions, could be for instance obtained by an experiment involving a sufficient number of animals receiving the same amount and type of feed. Confounding factors such as intake level and fibre content of the diet could be excluded with such an experiment, leaving the varying fibre digestibility of individuals as the main factor of influence. Samples for microbial abundance and transcriptome as well as genetic characterisation of the hosts should be taken and analysed once this confirmation is obtained.

5. Conclusion and outlook

Previous studies have focussed solely on differences between high and low CH_4 producing individuals, whereas our study suggests that a concomitant characterisation of the microbiome of high or low fibre-fermenting individuals might be important to understand the conditions favouring the low-CH_4 microbiome. This may be especially relevant because, as the phenomenon found contradicts the finding that selecting for low-CH_4 animals obviously results in a lower capacity for fibre digestion (Løvendahl et al., 2018). Of course, our data do not allow claiming that a low CH_4 yield per unit of digested fibre is necessarily linked to an overall low absolute CH_4 emission or a higher feed conversion efficiency. However, the results should encourage the exploration of the details of

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different fibre digestion strategies, including the importance of the individual's rumen microbiome and metabolome, beyond those commonly associated with rumen volume and digesta retention time.

Our study suggests that an increased digestion of fibre from a given diet may be associated with a less than proportionate increase in CH_4 production. This finding could be a first step with far-reaching consequences for the mechanistic understanding of fibre digestion in ruminant. Our results indicate that it might be beneficial to include fibre digestibility measurements in studies focussing on CH_4 emissions, although being possibly more labour intensive than respiration measurements only. We hope that our findings incite other research groups that they use available, or create new, datasets on an individual animal basis that include fibre digestibility and CH_4 to assess whether the patterns we found can be confirmed. If it turns out that the phenomenon is indeed heritable, breeders might be particularly interested in the determination of the individual's fibre digestibility and its rumen microbiome. Both variables are not easy to quantify and may require suitable proxies, but their implementation in breeding schemes would result in animals with a higher efficiency of fibre utilisation at concomitantly limited extra CH_4 emissions. One such proxy could consist of *in vitro* assessments focussing on rumen fluid from individual animals (e.g., breeding bulls) fed the same diet.

cRediT authorship contribution statement

M. Terranova: Data compilation, Validation, Writing–- original draft. **A. Schwarm**: Data provision, Writing–- review & editing. **Y. Li**: Visualisation, Writing–- review & editing. **M. Kreuzer:** Data provision, Writing–- original draft. **M. Clauss:** Formal analysis, Methodology, Writing–- original draft.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

Data availability

The data are not deposited in an official repository but will be made available by the corresponding author upon reasonable request.

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Appendix. Supplementary data

The following is Supplementary data to this article: *download homepage to be added*

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Figure captions

Fig. 1. Depiction of potential relationships between the absolute amount of digested fibre and the absolute daily CH_4 production, or – for the same hypothetical data – the relationship between the corresponding CH_4 yield (per unit of digested fibre) and the amount of digested fibre. (A) hypothetical dataset assuming a proportionate increase in CH_4 with digested fibre, i.e., a linear relationship with a scaling exponent of 1.00 (which implies a constant CH_4 yield); (B) different hypothetical dataset assuming a disproportionate increase (less-than-linear with a scaling exponent of 0.75) in CH_4 with digested fibre (which implies a decreasing CH_4 yield at increasing amount of digested fibre).

Fig. 2. Exponents *b* (incl. their 95%CI; the exponent has not unit) for the relationships between CH_4 (g/day) and amounts of digested neutral detergent fibre $(aNDFom)^b$, acid detergent fibre $(ADFom)^b$, non-aNDFom organic matter $(OM)^b$ and OM^b in individual diets in cattle (top) and sheep (bottom). Note that an exponent of 1 (i.e., a linear relationship) is the null hypothesis. Diets sorted by increasing *b* for the digested aNDFom dataset; in the other displays, the same sequence of diets is maintained. The grey symbols at the top of the graphs represent the scaling exponent for the complete dataset determined while having diet as a random factor.
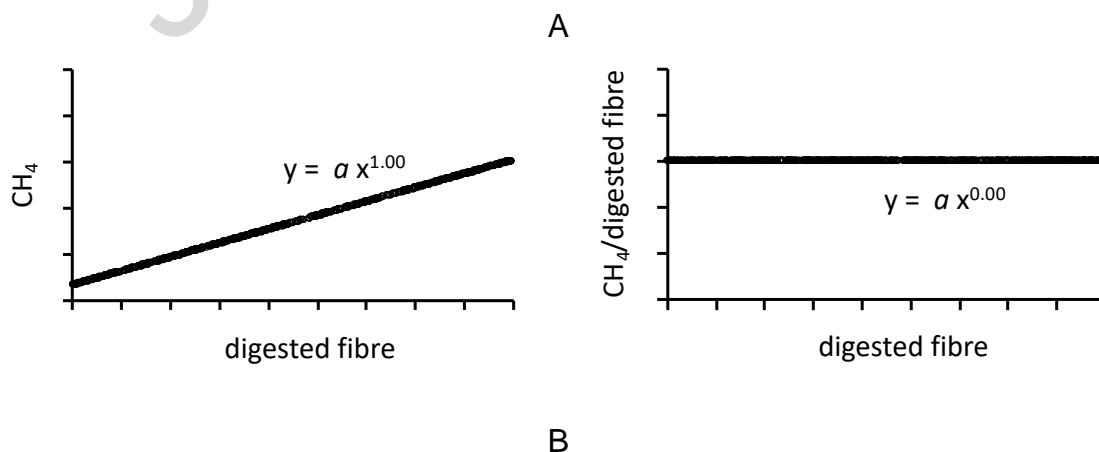
Fig. 3. Visualisation of (**A**) the relationship between absolute daily CH_4 production and the amount of digested fibre (neutral detergent fibre aNDFom on the left, and acid detergent fibre ADFom on the right), (**B**) of the relationship between CH_4 yield per unit of digested aNDFom or ADFom and the amount of digested aNDFom (left) and ADFom (right), and (**C**) of the relationship between CH_4 yield per unit of digested aNDFom or ADFom and the apparent digestibility (aD) of these fibre fractions in cattle. Dots are measured values, curves are the results regressions within diets.

Fig. 4. Visualisation of (**A**) the relationship between absolute daily CH_4 production and the amount of digested fibre (neutral detergent fibre aNDFom on the left, and acid detergent fibre ADFom on the

right), (**B**) of the relationship between CH4 yield per unit of digested aNDFom or ADFom and the amount of digested aNDFom (left) and ADFom (right), and (**C**) of the relationship between CH4 yield per unit of digested aNDFom or ADFom and the apparent digestibility (aD) of these fibre fractions in sheep. Dots are measured values, curves are the results of regression analyses within diets.

Fig. 5. Key members of rumen microbiome associated with fibre digestion or feed efficiency or both. O, microbial species; Δ or ∇, increased or decreased abundance observed in efficient animals or forage diets. References for the eight identities: (1) Gokarn et al. (1997), Stewart et al. (1997), Joblin et al. (2002), Fernando et al. (2010), Henderson et al. (2015), Elolimy et al. (2018), and McGovern et al. (2018); (2) Marounek and Duskova (1999), Fernando et al. (2010), Emerson and Weimer (2017), and Salfer et al. (2021). (3) Latham and Wolin (1977), Rooke et al. (2014), Zheng et al. (2014), and McGovern et al. (2018); (4) Miller and Wolin (1973), Carberry et al. (2012), Rooke et al. (2014), Zheng et al. (2014), and McGovern et al. (2018); (5) van Gylswyl et al. (1996), Mayorga et al. (2016), and McGovern et al. (2018); (6) Dušková and Marounek (2001), and Bowen et al. (2020); (7) Prabhu et al. (2012), Ben Shabat et al. (2016), and Yoshikawa et al. (2018); (8) Arndt et al. (2015), Ben Shabat et al. (2016), and Delgado et al. (2019).
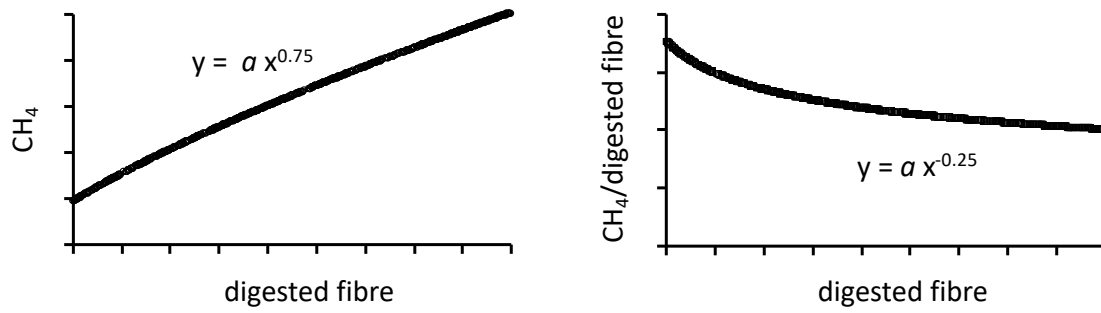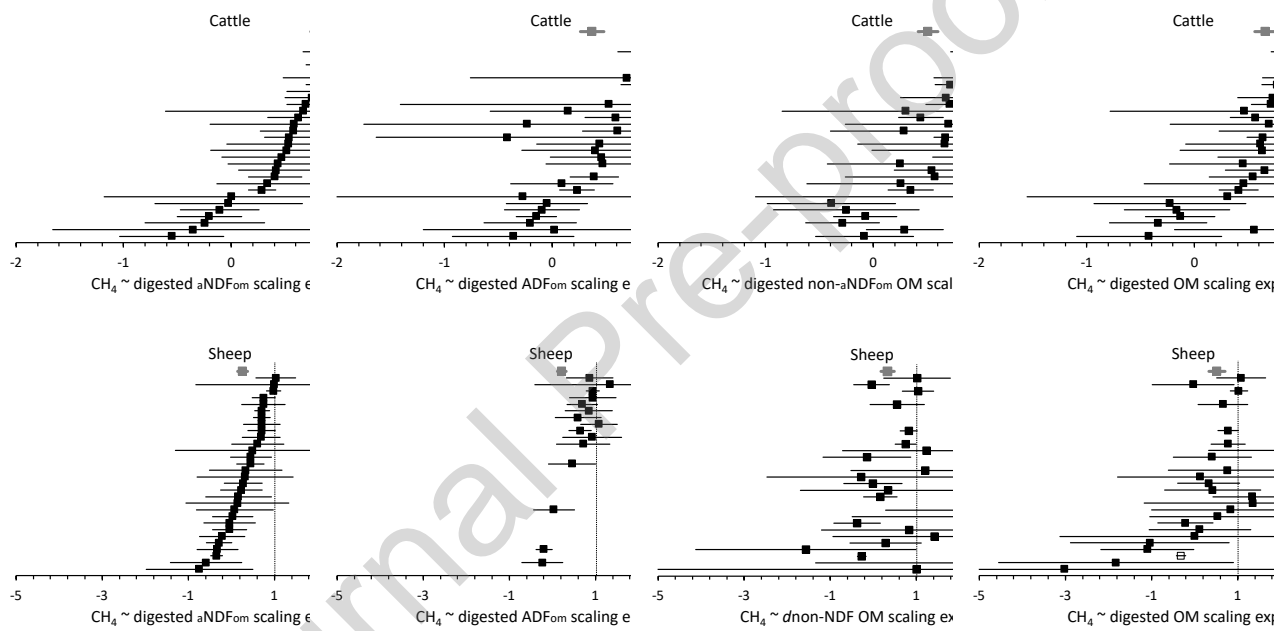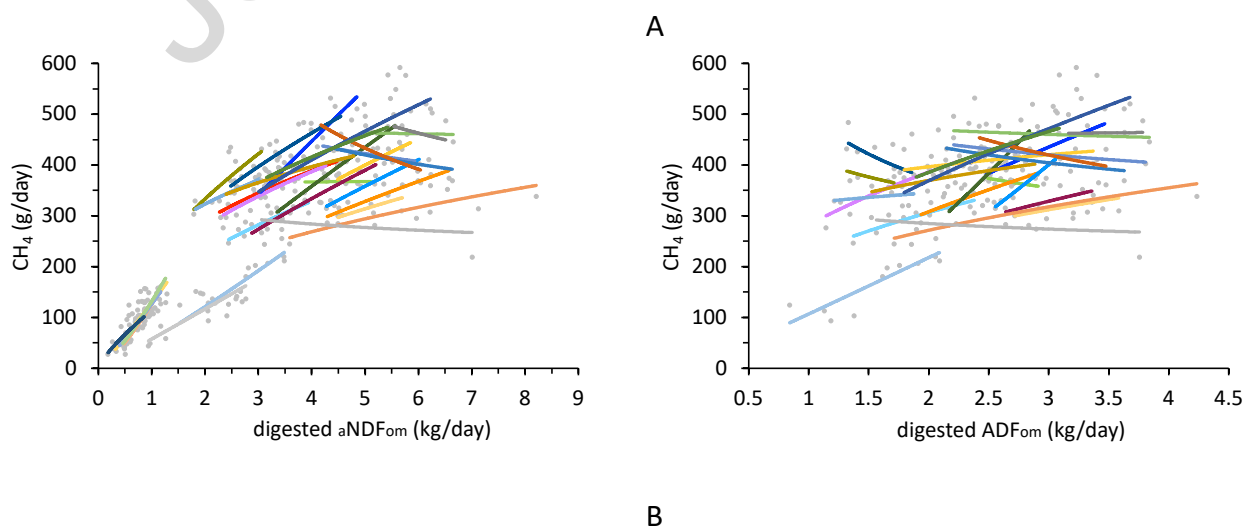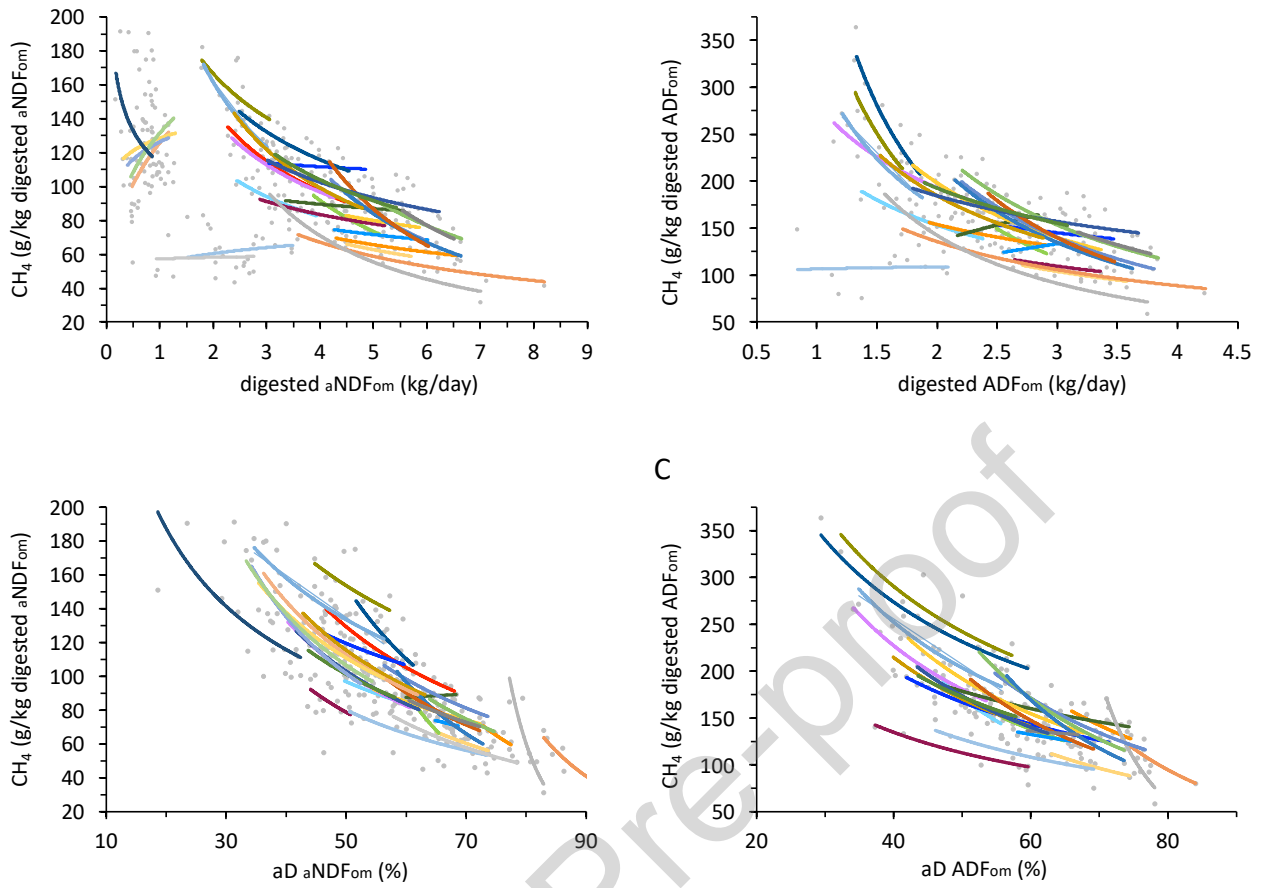
A

B

Fig. 1.

Fig. 2.

33

Fig. 3

Fig. 4

Cellulosomes

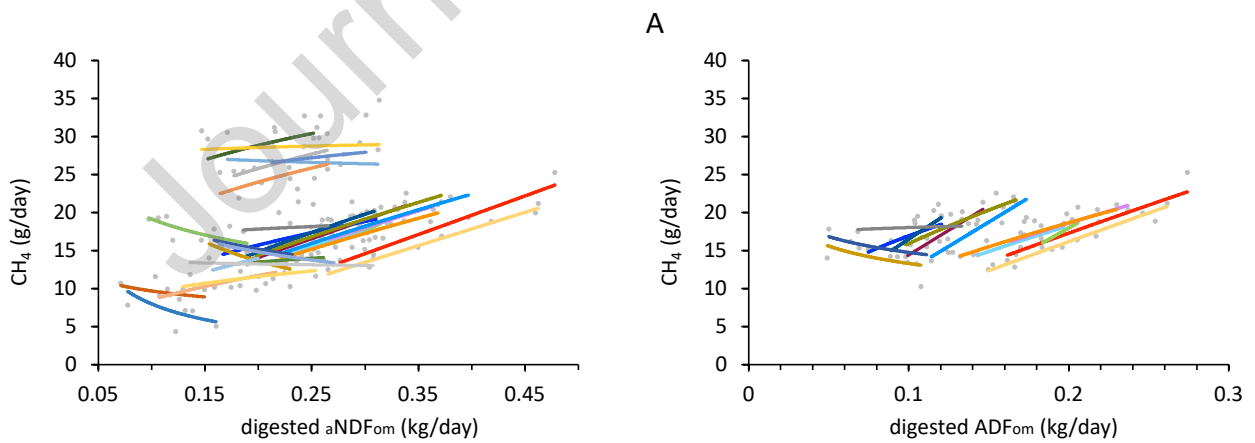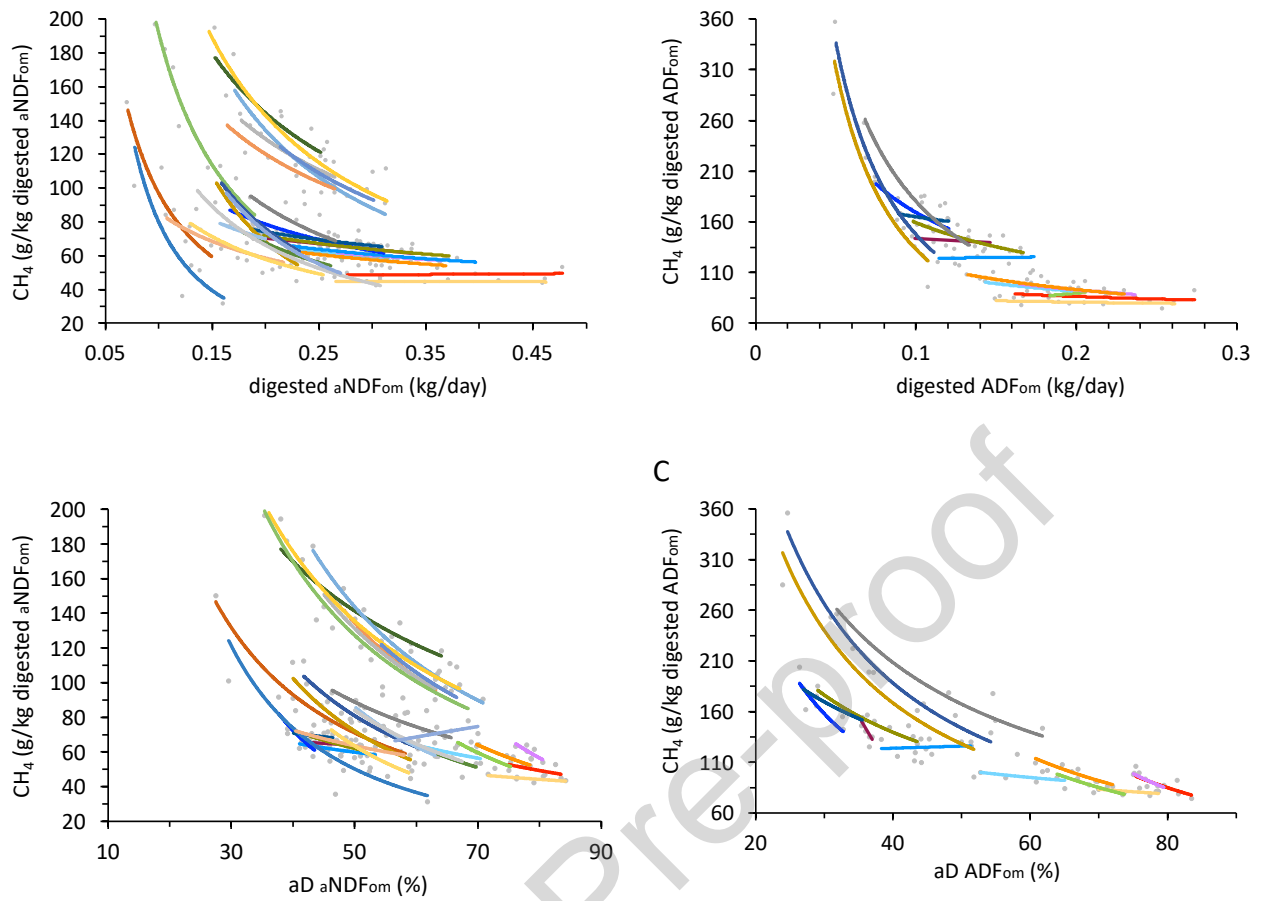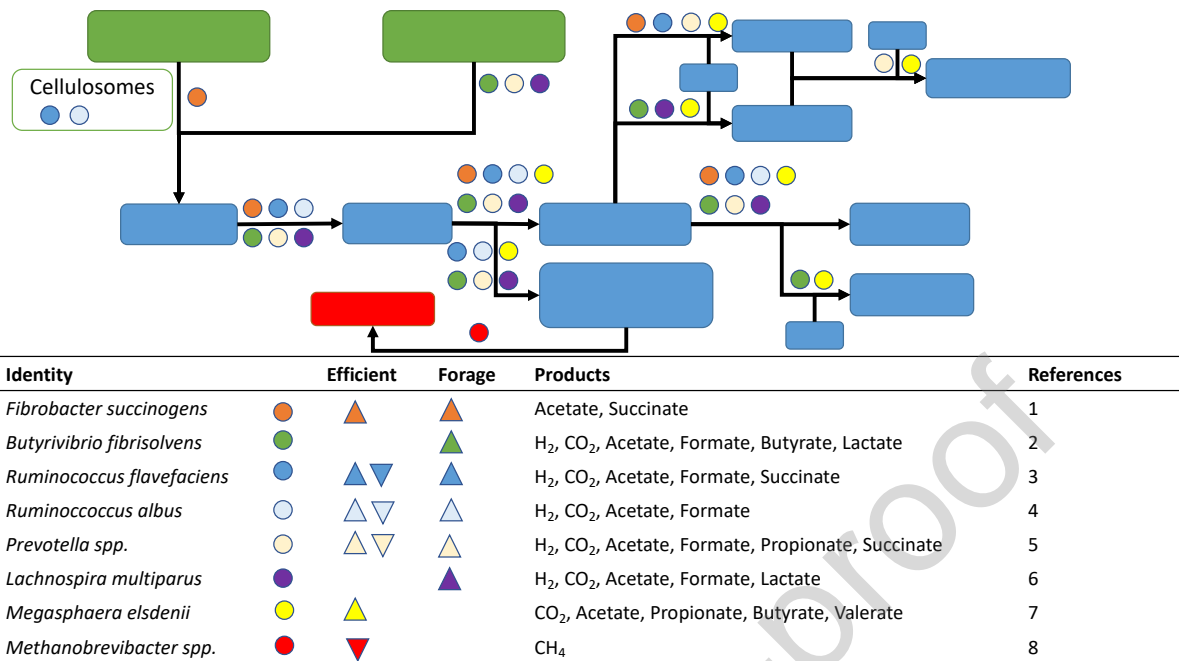| Identity		Efficient	Forage	Products	References
Fibrobacter succinogens	●	▲	▲	Acetate, Succinate	1
Butyrivibrio fibrisolvens	●		▲	H_2, CO_2, Acetate, Formate, Butyrate, Lactate	2
Ruminococcus flavefaciens	●	▲▽	▲	H_2, CO_2, Acetate, Formate, Succinate	3
Ruminoccoccus albus	○	△▽	△	H_2, CO_2, Acetate, Formate	4
Prevotella spp.	○	△▽	△	H_2, CO_2, Acetate, Formate, Propionate, Succinate	5
Lachnospira multiparus	●		▲	H_2, CO_2, Acetate, Formate, Lactate	6
Megasphaera elsdenii	●	△		CO_2, Acetate, Propionate, Butyrate, Valerate	7
Methanobrevibacter spp.	●	▼		CH_4	8

Fig. 5.

Table 1
Description of the respiration chamber experiments with individual animal data included in the present statistical evaluation.

Experiment	Diet groups	Animals total	Obser- vations	Species (purpose)	Variations tested in relation to methane	Forage proportion of total dry matter	References
1	1	28	28	Cattle (dairy)	Low vs high emitting cows	0.95	Denninger et al. (2020)
2a	2	30	30	Cattle (dairy)	Concentrate level and cow age	0.8-1	Grandl et al. (2016a; 2016b)
2b	1	12	12	Cattle (heifers)	Heifers (vs. dairy)	1	Grandl et al. (2016a; 2016b)
3	3	6	18	Cattle (dairy)	Low vs. high-sugar grass	1	Staerfl et al. (2012a; 2013)
4	3	17	17	Cattle (dairy)	Hay- vs. maize- and barley-based diets	0.45 and 1	Klevenhusen et al. (2010; 2011b)
5	2	11	11	Cattle (dairy)	Fresh clover vs. fresh grass	0.82-0.86	van Dorland et al. (2006; 2007; 2008)
6	4	22	22	Cattle (dairy)	Ensiled clover vs ensiled grass	0.72-0.82	van Dorland et al. (2006; 2007; 2008)
7	2	10	10	Cattle (dairy)	grass silage + hay vs. grass silage + maize silage	1	Hindrichsen et al. (2006)
8	6	12	36	Cattle (dairy)	Carbohydrate type in concentrate	0.5	Hindrichsen et al. (2005)
9	1	18	18[2]	Cattle (dairy)	Lauric and myristic acid vs. stearic acid	0.6	Dohme et al. (2004)

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10[1]	6	36	93[2]	Cattle (beef)	Grass vs. maize silage & various supplements	0.64-0.71	Staerfl et al. (2012b)
Total Sheep	31	202	295				
11	3	6	17[2]	Sheep	Diallyl disulphide and lovastatin	0.5	Klevenhusen et al. (2011a)
12	3	6	17[2]	Sheep	Diallyl disulphide and garlic oil	0.5	Klevenhusen et al. (2011c)
13	6	6	35[2]	Sheep	Tropical tannin-rich legumes	1	Tiemann et al. (2008)
14	6	6	36	Sheep	Clover vs. grass; +/- Acacia tannins	1	Carulla et al. (2005)
15[1]	6	6	36	Sheep	+/-*Sapindus saponaria* fruits	0.67	Hess et al. (2004)
16	6	6	36	Sheep	Forage, myristic acid and calcium	0.4 and 0.67	Machmüller et al. (2003)
Total	30	36	177				
Overall	61	238	472				

[1]No data on the intake of digested organic matter excretion available.
[2]No data on the digestibility of acid detergent fibre (ADFom).

Author statement
M. Terranova: Methodology, Investigation, Data curation, Writing – original draft, Writing – Review & editing; **M. Kreuzer**: Conceptualization, Resources, Methodology, Validation, Supervision, Writing – original draft, Writing – Review & editing; **Y. Li**: Conceptualization, Methodology, Writing – Review & editing; **A. Schwarm**: Conceptualization, Methodology, Writing – Review & editing; **M. Clauss**: Conceptualization, Methodology, Formal analysis, Visualization, Resources, Validation, Data curation, Writing – original draft, Writing - Review & editing.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Highlights

- The amount of methane (CH_4) produced from digested fibre is considered constant.

- We challenged this assumption by evaluating 472 individual data from 61 diets.

- Generally, within diets, yield was lower in animals more efficiently digesting fibre.

- This was found in both cattle and sheep, and for both aNDFom and ADFom.

- Breeding for animals with high fibre digestibility might be useful.