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# Reducing Enteric Methane Losses from Ruminant Livestock – Its Measurement, Prediction and the Influence of Diet

M. J. Bell and R. J. Eckard

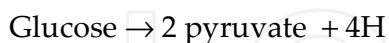
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## 1. Introduction

Ruminant livestock systems contribute significantly to global anthropogenic methane emissions, with about 50% or more of the GHG emissions produced coming from enteric fermentation [1]. The loss of dietary energy in the form of methane has been extensively researched and reviewed [2, 3, 4]. Microorganisms called methanogens produce methane (methanogenesis) in the digestive tract as a by-product of anaerobic fermentation. Briefly, the process of methanogenesis [see 5, 6 for a more detailed summary] consists of:

1. Glucose equivalents from plant polymers or starch (cellulose, hemicellulose, pectin, starch, sucrose, fructans and pentosans) are hydrolysed by extracellular microbial enzymes to form pyruvate in the presence of protozoa and fungi in the digestive tract:

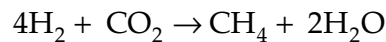


2. The fermentation of pyruvate involves oxidation reactions under anaerobic conditions producing reduced co-factors such as NADH. Reduced co-factors such as NADH are then re-oxidised to NAD to complete the synthesis of volatile fatty acids (VFAs) with the main products being acetate, butyrate and propionate (anions of acetic, butyric and propionic VFAs):



3. The VFAs are then available to be absorbed through the digestive mucosa into the animal's blood stream. The production of acetate and butyrate production provides a

net source of hydrogen or alternatively propionate can utilise any available hydrogen. Methanogens eliminate the available hydrogen by using carbon dioxide (CO<sub>2</sub>) to produce methane:



In ruminants, some 87 to 93% of methane production occurs in the foregut, with the highest rate of production being after eating [7]. In sheep, almost 90% of the methane produced in the hindgut has been found to be absorbed and expired through the lungs, with the remainder being excreted through the rectum [8]. Rectum enteric methane losses have been estimated at 7% [9] and 8% [10] of methane output in dairy cows compared to the 1% found in sheep [8].

Reductions in enteric methane production from ruminants can result from a reduction in rumen fermentation rate (suppression in microbial activity) or a shift in VFA production [11]. An inverse relationship exists between the production of methane in the rumen and the presence of propionate. If the ratio of acetate to propionate was greater than 0.5, then hydrogen would become available to form methane [12]. If the hydrogen produced is not correctly used by methanogens, such as when large amounts of fermentable carbohydrate are fed, ethanol or lactate can form, which inhibits microbial growth, forage digestion, and any further production of VFAs [13]. In practice, ethanol or lactate may form, but any excess hydrogen is simply eructated.

The methods for sampling, measuring and predicting enteric methane production (using studies on dairy cattle as an example), and the influence of dietary components on methane production are reviewed.

## 2. Methods used to sample and measure methane production

Estimates of methane output from livestock can be costly and difficult to make, especially from larger ruminants. Standard methods for measuring the methane concentration in air are by infrared spectroscopy, gas chromatography, mass spectroscopy or a tuneable laser diode. In a controlled and enclosed environment (i.e. chamber) the gas concentration can be calculated directly from the difference between ingoing and outgoing air, but in less contained environments a tracer gas is required as a marker, which is often the inert sulphur hexafluoride (SF<sub>6</sub>) gas.

Of the methods summarised [from the reviews of 7, 12] in Table 1 that can be used to sample air for its methane concentration, the open-circuit indirect respiration calorimeter (chamber) is acknowledged as currently providing the most reliable and repeatable method of obtaining an estimate of individual whole animal enteric methane emissions (including eructated and flatulence emissions) over a continuous sampling period [7]. If this method becomes less costly to implement, direct selection of animals on methane output could become possible. In some cases, there are suggestions that this technique may affect the

behaviour of the animal causing depression of appetite [14, 15], which may be avoided by making the walls of the enclosed environment transparent. A more mobile chamber that has been used is a polythene tunnel. Due to the polythene tunnel being mobile it is adaptable to different feeding systems such as grazing animals [14, 16]. However, difficulties in controlling the tunnel's temperature and humidity have been found, resulting in a lower estimate of methane production compared to chamber measurements [14, 16].

Method of measurement	Description
<b>Whole animal emissions measured</b>	
Chamber	Open-circuit indirect respiration calorimeter. Air blown in and extracted out of a chamber. Air concentrations between the incoming and outgoing air are continuously monitored using gas analysers. Chamber conditions are controlled and monitored usually for 48 hours.
Polythene tunnel	Air blown in and extracted out of tunnel. Air concentrations between the incoming and outgoing air are continuously monitored.
Room tracer gas	Tracer gas is released into a ventilated room until a steady concentration is reached, after which air samples can be collected. Background air samples are required.
Mass balance micrometeorological	Background air samples and a high precision gas analyser are required. Sampling downwind (and up) of the source.
<b>Eructated emissions measured</b>	
Head box, hood or mask	Respired gas volume can be sampled at regular intervals.
ERUCT (Emissions from ruminants using a calibrated tracer)	Typically using the inert sulphur hexafluoride (SF <sub>6</sub> ) tracer gas. Assumes that the emitted tracer gas from a permeation tube in the rumen simulates the diffusion of any methane emitted. Respired air collected via a capillary tube near the animal's nostrils into a vessel.

**Table 1.** A general summary of a few methods used to collect air samples to measure whole animal enteric methane emissions or solely eructated emissions

In comparison to methods that use a controlled and enclosed environment, methods that use a tracer gas such as SF<sub>6</sub> as a marker tend to be less costly and more applicable to use on a greater number of animals. The room tracer [17] and mass balance micrometeorological methods, where a known amount of gas i.e. a tracer gas or the gas of interest are released from fixed points [18, 19, 20], both require careful monitoring of the sampling environment and diffusion of the gas of interest (in this case methane) needs to be tested prior to

commencing sampling. The temperature, air pressure, humidity and air speed should also be monitored for their consistency in a non-enclosed sampling environment. Controlling the sampling environment would make replicating these techniques consistently on commercial farms difficult. Also, in some countries the use of SF<sub>6</sub> is not permitted and there may be a withdrawal period on products from animals exposed to the gas [7]. The ERUCT (emissions from ruminants using a calibrated tracer) technique [9, 21] or a head box, hood or mask [22, 23] estimate eructated methane emissions from individual animals. This ignores enteric methane from the rectum, which could be 1 to 8% of total enteric methane production of an animal as previously discussed. The ERUCT technique was devised to allow measurement of methane emissions from free ranging and feedlot animals. The ERUCT technique has been found to be suitable for estimating respired methane emissions from high forage fed animals and not with animals on diets that result in greater post-ruminal digestion [21, 24]. Even though the ERUCT technique is more open to errors in estimates compared to using a chamber, these errors could be reduced by removal of outlying estimates and replicating sampling over several days [10]. More invasive methods of estimating methane production from rumen fluid involve injecting radioactively labelled methane (isotope dilution technique) [8, 25] or ethane [26] into the rumen.

### 3. Methane output measurements

Studies measuring the methane production of livestock have been carried out for over 80 years (Table 2). In the last 20 years the number of studies globally that have measured enteric methane have increased, as have the range of sampling methods used.

In cattle, the use of high energy dense diets has increased the amount of dry matter (DM) that an animal can consume, as a result of improved efficiencies in rumen fermentation and feed digestibility [42]. The level of intake of feed (more specifically organic matter) influences methane production. Dairy cows ranging in live weight from 385 to 747 kg were found to produce between 45 and 199 kg methane/head/yr (14 to 31 g/kg DM intake) of methane and beef cattle of 364 to 627 kg live weight produced between 40 and 92 kg methane/head/yr (13 to 35 g/kg DM intake), with the difference attributed to the amount of DM consumed [43]. Notably in Table 2 the highest DM intake measured was 29 kg/day in two of the studies [33, 41] and the methane production was also the same at 19 g/kg DM intake. Where a high energy dense diet is formulated to meet the nutrient requirements of a high milk yielding animal, it would appear that the methane output per kg DM intake could average about 19 g/kg, but this would be slightly more for high forage diets where potential intake is lower (0.21 g/kg DM or more [44]). As well as the influence of the composition of the diet, reductions in methane losses per kg DM intake appear to be possible by an incremental increase in the level of feed intake, brought about by increasing the proportion of concentrate feed in the diet. It has been suggested that this decrease in the percentage of dietary GE intake lost as methane occurs at an average of 1.6% per unit increase in feed level [12].

Reference	Dry matter intake (kg/day)	Body weight (kg)	Methane (kg/hd/yr)	Sampling method
[10]	18	496	120	ERUCT / Chamber
[17]	25	-	102	Room tracer (SF <sub>6</sub> )
[18]	-	600	142	Micrometeorological mass balance
[27]	1 - 15	162 - 655	39	Chamber
[28]	9	-	79	Chamber
[29]	-	-	40	Chamber
[30]	8 - 18	-	68 - 122	Chamber
[31]	18	602	137	Micrometeorological mass balance
[32]	-	450 - 700	112	Chamber
[33]	4 - 29	426 - 852	24 - 198	Chamber
[34]	13	402 - 562	96	ERUCT
[35]	13	517	95	Chamber
[36]	14 - 16	595	138	Chamber
[37]	14	-	109	ERUCT
[38]	12	526	84	Chamber / mask / ERUCT / micrometeorological mass balance
[39]	20	572	137	Chamber
[40]	8 - 25	379 - 733	72 - 210	Chamber
[41]	2 - 29	173 - 826	13 - 197	Chamber

\* Most recent reference to data collected is shown and values where available are presented.

**Table 2.** Some of the key experiments globally that have measured methane output from dairy cattle\*

#### 4. Methane output prediction equations

Prediction methods can be either empirical or mechanistic. Several reviews have studied the use and performance of different methane output prediction equations [11, 12, 33, 38, 45, 46, 47, 48, 49].

Mechanistic equations estimate methane output using mathematical descriptions of rumen fermentation. Even though mechanistic equations at present appear to show the greatest degree of adaptability across diet types and intake level [48, 50, 51], they require detailed and complex dietary input values. Published mechanistic equations are not presented in this review but are described in [52] (recommended in [50] and [46]), [53], [54], [55], [56], [57], [58], [59] (recommended in [50]) and [60].

Empirical equations such as those shown in Table 3 offer a more practical solution to predicting methane output using input variables such as digestibility, carbohydrate content, energy and nitrogen intake, milk production and live weight. Table 3 and Figure 1 present

empirical prediction equations for methane output developed using animals that included dairy cattle, with a range of intakes and different diets. Of the empirical prediction equations shown in Table 3, studies have compared the predictions of an equation against methane measurements, with some being recommended such as [29] (recommended in [33]), [61] (recommended in [33], [12], [46] and [47]), [62] (recommended in [63]) and the non-linear equations using DM intake and metabolisable energy (ME) intake by [47] (recommended in [48] and [38]).

Reference	Units	Equation
[27]	g/day	$= 18 + 22.5 \times \text{DMI}$
[28]	MJ/day	$= -2.07 + 2.63 \times \text{DMI} - 0.105 \times \text{DMI}^2$
[29]	MJ/day	$= [1.3 + 0.112 \times \text{D} + \text{FL} \times (2.37 - 0.05 \times \text{D})/100] \times \text{GEI}$
[32]	g/day	$= 10.0 + 4.9 \times \text{MY} + 1.5 \times \text{LWGT}^{0.75}$
[37]	g/day	$= 17.1 \times \text{DMI} + 97.4$
	g/day	$= 84 + 47 \times \text{C} + 32 \times \text{S} + 62 \times \text{DS}$
	g/day	$= 91 + 50 \times \text{C} + 40 \times \text{HC} + 24 \times \text{S} + 67 \times \text{DS}$
	g/day	$= 123 + 84 \times \text{C} - 30 \times \text{HC} + 58 \times \text{S} + 73 \times \text{DS} - 95 \times \text{L}$
[38]	MJ/day	$= 8.56 + 0.14 \times \text{FP}$
	MJ/day	$= 3.23 + 0.81 \times \text{DMI}$
[41]	MJ/day	$= 74.43 - (74.43 + 0) \times e^{-0.0163 \times \text{DMI}}$
	MJ/day	$= 74.43 - (74.43 + 0) \times e^{[\text{cx}]}; \text{cx} = -0.0187 + 0.0059 / [1 + \exp(\text{S}/\text{TADF} - 3.1003)]/0.6127 \times \text{DMI}$
	MJ/day	$= (7.16 - 0.101 \times \text{DMI})/100 \times \text{GEI}$
	MJ/day	$= 2.6861 + 0.0779 \times \text{DEI}$
[47]	MJ/day	$= 5.93 + 0.92 \times \text{DMI}$
	MJ/day	$= 8.25 + 0.07 \times \text{MEI}$
	MJ/day	$= 7.30 + 13.13 \times \text{N} + 2.04 \text{TADF} + 0.33 \times \text{S}$
	MJ/day	$= 1.06 + 10.27 \times \text{FP} + 0.87 \times \text{DMI}$
	MJ/day	$= 56.27 - (56.27 + 0) \times e^{-0.028 \times \text{DMI}}$
	MJ/day	$= 45.89 - (45.89 + 0) \times e^{-0.003 \times \text{MEI}}$
	MJ/day	$= 45.98 - (45.98 + 0) \times e^{[\text{cx}]}; \text{cx} = -0.0011 \times (\text{S}/\text{TADF}) + 0.0045 \times \text{MEI}$
[61]	MJ/day	$= 3.38 + 0.51 \times \text{NFC} + 1.74 \times \text{HC} + 2.652 \times \text{C}$
[62]	MJ/day	$= \text{DEI} \times [0.094 + 0.028 \times (\text{FADF}/\text{TADF})] - 2.453 \times (\text{FL}-1)$
	MJ/day	$= \text{DEI} \times [0.096 + 0.035 \times (\text{FDMI}/\text{DMI})] - 2.298 \times (\text{FL}-1)$
[64]	g/day	$= 4.012 \times \text{TC} + 17.68$
[65]	% GEI	$= 2.898 - 0.0631 \times \text{MY} + 0.297 \times \text{MF} - 1.587 \times \text{MP} + 0.0891 \times \text{CP} + 0.1010 \times [(\text{FADF}/\text{DMI}) \times 100] + 0.102 \times \text{DMI} - 0.131 \times \text{F} + 0.116 \times \text{DMD} - 0.0737 \times \text{CPD}$
	% GEI	$= 2.927 - 0.0405 \times \text{MY} + 0.335 \times \text{MF} - 1.225 \times \text{MP} + 0.248 \times \text{CP} - 0.448 \times [(\text{ADF}/\text{DMI}) \times 100] + 0.502 \times [(\text{FADF}/\text{DMI}) \times 100] + 0.0352 \times \text{ADFD}$
	% GEI	$= 227.099 - 2.783 \times [(\text{ADFD}/\text{DMI}) \times 100] - 6.0176 \times \text{ADFD} + 3.607 \times \text{CPD} + 1.751 \times \text{NDS} - 1.423 \times \text{CD} + 1.203 \times \text{HD}$



Reference	Units	Equation
[66]	g/day	$= 41 + 30 \times DS + 6 \times S + 51 \times DCW$
[67]	MJ/day	$= 1.36 + 1.21 \times DMI - 0.825 \times CDMI + 12.8 \times NDF$
[68]	L/day	$= 38.92 + 26.44 \times DMI$
[69]	L/day	$= 47.82 \times DMI - 0.762 \times DMI^2 - 41$
[70]	L/day	$= 38.2 + 4.89 \times FP \times DMI - 0.719 \times DMI^2 - 20$
	L/day	$= 0.666 \times LWGT + 2.868 \times MY + 75$
	L/day	$= 39.2 \times DMI - 0.588 \times DMI^2 + 0.370 \times LWGT - 1.698 \times MY - 134$

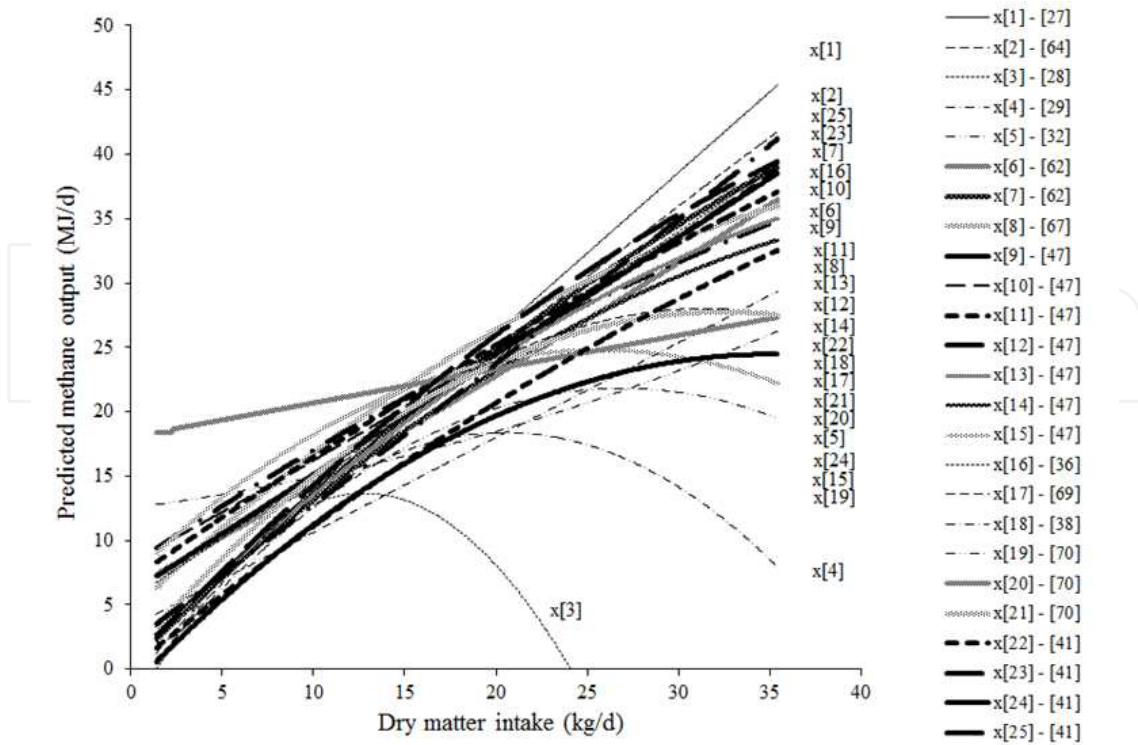
DMI = dry matter intake (kg/day); CDMI = concentrate DMI (kg/day); FDMI = forage DMI (kg/day); TC = total NDF, sugar and starch (100 g/day); D = digestibility of gross energy at maintenance (%); NFC = non-fibre carbohydrate (kg/day); HC = hemicellulose (kg/day); C = cellulose (kg/day); MY = milk yield (kg/day); MF = milk fat composition (%); MP = milk protein composition (%); CP = crude protein (% DMI); F = fat (% DMI); DMD = DM digestibility (%); CPD = CP digestibility (%); ADFD = acid detergent fibre digestibility (%); NDS = neutral detergent solubles digestibility (%); CD = cellulose digestibility (%); HD = hemicellulose digestibility (%); DS = sugars (kg/day); DCW = digested cell walls (kg/day); L = lignin (kg/day); LWGT = live weight (kg); DEI = digestible energy intake (MJ/day); MEI = metabolisable energy intake (MJ/day); GEI = gross energy intake (MJ/day); FADF = forage ADF (kg/day); TADF = total ADF (kg/day); FL = multiples of MEI over maintenance; NDF = neutral detergent fibre (kg/kg DM); FP = forage proportion (kg/kg DM); N = nitrogen (kg/day); S = starch (kg/day).

**Table 3.** Empirical equations from the literature that predict enteric methane output from dietary inputs and production values for dairy cattle

The success or suitability of an empirical prediction equation for implementation on a data set is dependent on the range of values that the equation was developed on. A comparison of empirical prediction equations from Table 3, which were tested over a range of DM intakes from 1 to 35 kg/d (beyond the range they would have been developed on) for lactating dairy cows fed diets with a high and low proportion of forage content, suggest that the relationship between methane output and intake may be linear up to an average intake of 15 kg DM/d. Above this level of intake, which is more achievable by feeding a higher proportion of concentrates in the diet, the majority of equations showed a decline in methane output per unit intake (due to the increase in the level of intake by feeding a higher proportion of concentrate feed as has been suggested [12]; Fig. 1). This depression in methane lost per kg DM intake at high levels of intake in cattle has also been shown in other studies (reported in [71]). The main difference amongst the performances of methane prediction equations is their ability to give a sensible estimate of methane losses at low (approaching the origin) and high dry matter intakes. Even though some of the variation in the predictive ability of an equation in Figure 1 may be explained by the equation being used on a range of values outside the range it was developed on and the complexity of an equation, there is still considerable variation in methane output for a given level of DM intake [71].

In addition to dynamic and statistical prediction methods, methane output can be estimated based on an animal's predicted energy requirements, which is the technique used in the Intergovernmental Panel on Climate Change (IPCC) methodology [72, 73]. This energy balance approach is suitable as an estimate over a period of time (as used in national inventories based on IPCC methodology) such as a year or lactation [74]. The IPCC methodology is based on production variables that are generally more easily obtained than those used in empirical or even more dynamic enteric methane prediction equations.





**Figure 1.** Average methane output polynomial trend lines for methane output predictions by published equations (in Table 3) x[1] to x[25] across a range of daily dry matter intakes of dairy cows (from [49])

### 5. Effect of diet on methane output

As suggested in Figure 1 and proposed by others [29], increased intake of less digestible feeds such as forage has little effect on methane production per DM intake, whereas an increase in more digestible feeds such as concentrate results in a reduction in methane losses per DM intake. This improvement in the quality of food fed to a ruminant is an effective way to manipulate the diet (particularly in terms of digestible organic matter) to get better animal performance and reduced methane production [40, 45, 70, 75].

Individual feeds can vary considerably in their methanogenic effect based on their chemical composition. An evaluation of chamber measurements of methane from sheep fed different feeds found a range for percentage of GE lost as methane from 3.8% for distillers grains to 12.8% for peas [76]. The authors found that 92% of the variation in methane emission was explained by the equation:

$$\text{Methane output (\% GE)} = -10.5 + 0.192 \times \text{DE} - 0.0567 \times \text{EE} + 0.00651 \times \text{S} + 0.00647 \times \text{CP} + 0.0111 \times \text{NDF}$$

where, DE is digestible energy (% of gross energy, GE), EE is ether extract, S is starch, CP is crude protein and NDF is neutral detergent fibre (all in g/kg DM).

The above equation shows the relative response in methane output to each dietary component, with increases in DE, S, CP increasing methane emissions and increasing EE reducing methane. These parameters and their positive or negative effect on methane are

common inputs to equations in Table 3. However, this would suggest that high starch feeds such as cereal grain would increase methane emissions. But when fed at an increasing level of intake cereal grains have a curvilinear effect on fibre digestion in mixed rations ([71]; expressed as a ratio of starch to acid detergent fibre content in [41, 47]) and result in a depression in methane per unit DM (as in Fig.1 in [47]) and per unit product.

Diet composition can influence rumen fermentation and reduce methane production as a result of more propionate present or less degradation of food consumed in the rumen. Post-ruminal digestion, particularly in the small intestine, is energetically more efficient with lower methane losses than digestion in the rumen, which can be encouraged by more digestible and higher quality food. The amount and type of dietary carbohydrate fermented affects the fermentation rate and rumen retention time of substrate, in addition to the hydrogen supply due to the ratio of acetate to propionate. The passage rate of substrate and rumen fluid dilution rate (influencing the ratio of acetate to propionate) have been found to explain 28% and 25% of variation in an animal's methane production [77]. Cellulose ferments more slowly than hemicellulose, but both these structural carbohydrates ferment more slowly than non-structural and more soluble carbohydrates such as starch and sugars [2]. With regard to forages, increasing the digestibility of forage fed by reducing fibre content can reduce methane production. Feeding maize silage [78] or a legume-based silage [45] rather than grass silage has been found to reduce methane production. Also, silage is generally more digestible than hay [45] and adding molasses or urea to straw made it more digestible [79], which in both cases reduced methane production. Forage methane production can be minimised by lower fibre content and high soluble carbohydrate (influenced by maturity), and C3 grasses rather than C4 [2]. The grinding or pelleting of forage to increase its surface area and digestibility could also help reduce methane production [12, 80].

The additions of feed additives to a ruminant's diet have been and are still being extensively evaluated for their effect on reducing methane emissions. The benefit in animal productivity and reduction in methane production relative to the cost of using different additives is continually being assessed. As previously suggested, the supplementation of diets with additives such as fats can reduce methane production [12, 44, 65, 81, 82, 83, 84] particularly fats with C8 to C16 chain length such as coconut oil [56, 85], however the effect, which is a suppression on fermentation appears to not always last [17, 37]. Suppressing fermentation by supplementing the diet with fat inhibits methanogens and protozoa, and subsequent fibre digestion with a shift towards more propionate present rather than acetate [2]. Likewise, the use of ionophores in feed (particularly monensin and salinomycin) and spices [86] that modify the rumen microflora [87] can reduce methane losses [6, 7, 88, 89] and encourage a shift towards propionogenesis. However eventually the rumen microflora would appear to show some resistance and the suppression ceases [90, 91, 92]. The inconsistent effects of monensin on methane in dairy cattle on forage and grain supplemented diets have also been found [93, 94]. Notably, ionophores are banned within the European Union due to the fears of residues appearing in the milk.

Other feed additives tested include the use of plant compounds such as tannins (inhibiting methanogens) [95] and saponins (inhibiting protozoa), which reduce the digestibility of

dietary fibre [96], and organic acids such as fumarate, malate and acrylate which act as an alternative hydrogen acceptor [97], but results for effects on methane production and animal performance are variable [3]. Probiotics (acetogens and yeast) have been found to reduce methane output, mainly through improving digestion efficiency [88] but not by others [3]. Overall, unless yeast by-products can reliably be used to reduce methane production, the most cost-effective additive for reducing production appears to be the addition of cellulase and hemicellulase enzymes to a ruminant's diet, which not only improved fibre digestion but also productivity [98].

## 6. Conclusions

With the increased importance now attached to enteric methane emissions from ruminants, due its global warming potential, there has been and will continue to be improvements in our understanding of methanogenesis and abatement options. Chamber measurements are costly in comparison to other measurement techniques and prediction methods, and therefore methane predictions using mechanistic models describing rumen fermentation are recognised at present as being more applicable to different feeds and animal species. The methane output from different feeds and animals has been extensively measured, predicted and tested but a robust empirical prediction of enteric methane emissions that can be applied to any ruminant production system is still to be developed. This is partly due to the need for the effect of feeding level to be better defined.

The important variables for predicting enteric methane output are the contents of fermentable carbohydrate, fibre, fat, digestible energy and intake level of a diet. Low enteric methane losses per unit DM appear possible by mechanisms that promote the passage of organic matter to post-rumen digestion and reduce rumen fermentation by high intakes of digestible feed and addition of fats, whilst also reducing emissions per unit product.

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