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Review article

## Methane production by ruminants: its contribution to global warming

Angela R. MOSS<sup>a\*</sup>, Jean-Pierre JOUANY<sup>b</sup>, John NEWBOLD<sup>c</sup>

<sup>a</sup> ADAS Nutritional Sciences Research Unit, Alcester Road, Stratford Upon Avon,  
Warwickshire CV37 9RQ, UK

<sup>b</sup> INRA, Centre de Recherches de Clermont-Ferrand-Theix,  
63122 Saint-Genès-Champanelle, France

<sup>c</sup> Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK

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**Abstract** — The aim of this paper is to review the role of methane in the global warming scenario and to examine the contribution to atmospheric methane made by enteric fermentation, mainly by ruminants. Agricultural emissions of methane in the EU-15 have recently been estimated at 10.2 million tonnes per year and represent the greatest source. Of these, approximately two-thirds come from enteric fermentation and one-third from livestock manure. Fermentation of feeds in the rumen is the largest source of methane from enteric fermentation and this paper considers in detail the reasons for, and the consequences of, the fact that the molar percentage of the different volatile fatty acids produced during fermentation influences the production of methane in the rumen. Acetate and butyrate promote methane production while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen. The many alternative approaches to reducing methane are considered, both in terms of reduction per animal and reduction per unit of animal product. It was concluded that the most promising areas for future research for reducing methanogenesis are the development of new products/delivery systems for anti-methanogenic compounds or alternative electron acceptors in the rumen and reduction in protozoal numbers in the rumen. It is also stressed that the reason ruminants are so important to mankind is that much of the world's biomass is rich in fibre. They can convert this into high quality protein sources (i.e. meat and milk) for human consumption and this will need to be balanced against the concomitant production of methane.

**methane / ruminants / global warning / reduction strategies**

**Résumé** — **Production de méthane par les ruminants : sa contribution au réchauffement de la planète.** Cet article examine le rôle du méthane dans le processus de réchauffement de la planète et évalue la contribution au méthane atmosphérique des gaz d'origine digestive issus principalement des ruminants. Les émissions annuelles de méthane d'origine agricole dans l'Europe des quinze ont été estimées récemment à 10,2 millions de tonnes et représentent la principale source des entrées

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\* Correspondence and reprints  
e-mail: angela.moss@adas.co.uk

atmosphériques de méthane. Parmi celles-ci, approximativement les deux tiers proviennent des fermentations entériques et un tiers des lisiers. Le méthane ruminal représente environ 90 % de l'ensemble des fermentations digestives. Le présent article analyse en détail l'impact des orientations fermentaires sur la production de méthane dans le rumen. L'acétate et le butyrate favorisent la production de méthane tandis que la formation de propionate constitue une voie alternative d'utilisation de l'hydrogène dans le rumen. Les différentes possibilités offertes actuellement pour diminuer les émissions de méthane sont analysées, à la fois en terme de réduction par animal et par unité de produit animal. Les voies d'approche les plus prometteuses pour réduire la production ruminale de CH<sub>4</sub> consisteraient à rechercher de nouveaux produits doués d'activité antiméthanogénique ou à favoriser la formation d'accepteurs d'électrons autres que CO<sub>2</sub> ou le formate, ou à agir dans le sens d'une réduction de la population de protozoaires. Enfin, cette réflexion globale sur la contribution des ruminants à l'effet de serre doit tenir compte du fait que ces animaux jouent un rôle essentiel dans l'équilibre de notre écosystème en transformant l'importante biomasse végétale mondiale en protéines animales (viande et lait principalement) qui constituent la base de l'alimentation humaine. Cet aspect doit contrebalancer les aspects négatifs liés à la production de méthane et à ses conséquences.

### **méthane / ruminants / réchauffement de la planète / stratégies de réduction**

## **1. THE GREENHOUSE EFFECT AND METHANE CONTRIBUTION**

### **1.1. Evolution of the Earth's atmosphere during the last century**

There has been much interest in the composition of the Earth's atmosphere over the last few decades as a result of the observed increase in atmospheric temperatures. The observed increase in concentration of many gases in the troposphere has been related to the increase in global temperatures. The past

and current concentrations of the main greenhouse gases, rates of increase and atmospheric lifetimes are summarised in Table I.

### **1.2. Description of the greenhouse effect**

The greenhouse effect is thought to be due to the absorption of solar infrared (IR) radiation by gases and the earth's surface, which, as a result, are heated and then re-emit IR radiation at low frequency with a

**Table I.** The tropospheric concentrations, residence times and atmospheric trend of various greenhouse gases. Source: IPCC [54, 55].

	CO <sub>2</sub>	CH <sub>4</sub>	CFC-11 <sup>1</sup>	CFC-12 <sup>2</sup>	N <sub>2</sub> O
Atmospheric concentration	(ppmv)	(ppmv)	(pptv)	(pptv)	(ppbv)
Pre-industrial	280	0.8	0	0	288
Present (1990)	355	1.72	280	484	310
Current rate of change (% per year)	0.5	0.9	4	4	0.25
Atmospheric lifetime (years)	50–200	10	65	130	150
Relative radiative effectiveness					
Per molecule	1	21	12 400	15 800	206
Per unit mass	1	58	3 970	5 750	206

<sup>1</sup> chlorofluorocarbon 11; <sup>2</sup> chlorofluorocarbon 12. ppmv: parts per million volume; ppbv: parts per billion volume; pptv: parts per trillion volume.

high absorptive power. In fact greenhouse gases in the atmosphere are essential for maintaining life on earth, as without them the planet would be permanently frozen because all of the incoming heat from the sun would be radiated back into space by the earth's surface (see Moss [98] for a review). The threshold concentration of these gases at which their greenhouse effect would be minimised is not known, but it is accepted that their concentrations in the atmosphere should not be allowed to continue to rise. As a result of this acceptance international organisations like the IPCC (Intergovernmental Panel on Climate Change) have asked the governments of developed nations to evaluate the amount of gases produced in their country and to develop research to limit emissions further.

Warming of the earth's surface is achieved by solar energy being radiated, mainly in the visible part of the spectrum (wavelength 0.4 to 0.7  $\mu\text{m}$ ) and passing through the atmosphere of the earth without being absorbed. Some of the solar energy is reflected back into space by clouds and about 7% is radiated in the ultra-violet region of the spectrum (below 0.4  $\mu\text{m}$ ) which is absorbed by the ozone layer in the atmosphere. The solar energy reaching the earth's surface warms the earth and is radiated back from the surface in the infra-red region of the spectrum (4–100  $\mu\text{m}$ ). Approximately 70% of this radiation is in the wavelength band between 7 and 13  $\mu\text{m}$ , which can pass back through the atmosphere into space. The remaining radiation is absorbed, essentially by water vapour and carbon dioxide, thus there is warming of the lower layer of the atmosphere (troposphere), which in turn radiates heat, keeping the earth warmer than it would otherwise be [46].

### **1.3. Consequences of the greenhouse effect on our environment**

The consequences of the increases in concentration of the gases that generate the

greenhouse effect is that average global temperatures will rise, along with many consequences on human life. The degree to which these changes are projected to occur is dependent upon a reliable greenhouse gas policy model and a range of scenarios for the levels of greenhouse gas emissions. By the year 2030 the world is likely to be 1–2 °C warmer than today, although given the full range of uncertainties, the range could be from 0.5 °C to 2.5 °C. The concomitant rise in global mean sea level is 17 to 26 cm, with a full range of 5 to 44 cm, due mainly to thermal expansion of the oceans and increased melting of ices in the Arctic and Antarctic areas.

### **1.4. Consequences on humans and animals**

The projected climatic changes in the next century due to the greenhouse effect are likely to have an effect on water supplies and the increase in temperature will induce a new distribution of deserts and wet areas in the world and will alter the range or numbers of pests that affect plants or diseases that threaten animals or human health. Also of interest are the effects on unmanaged ecosystems, mainly forests.

### **1.5. Contribution of methane to the greenhouse effect**

While carbon dioxide receives the most attention as a factor in global warming, there are other gases to consider, including methane, nitrous oxide ( $\text{N}_2\text{O}$ ) and chlorofluorocarbons (CFCs).

The presence of methane in the atmosphere has been known since the 1940's when Migeotte [90] observed strong absorption bands in the infra-red region of the solar spectrum which were attributed to the presence of atmospheric methane. Numerous measurements since have demonstrated the existence of an average temporal increase

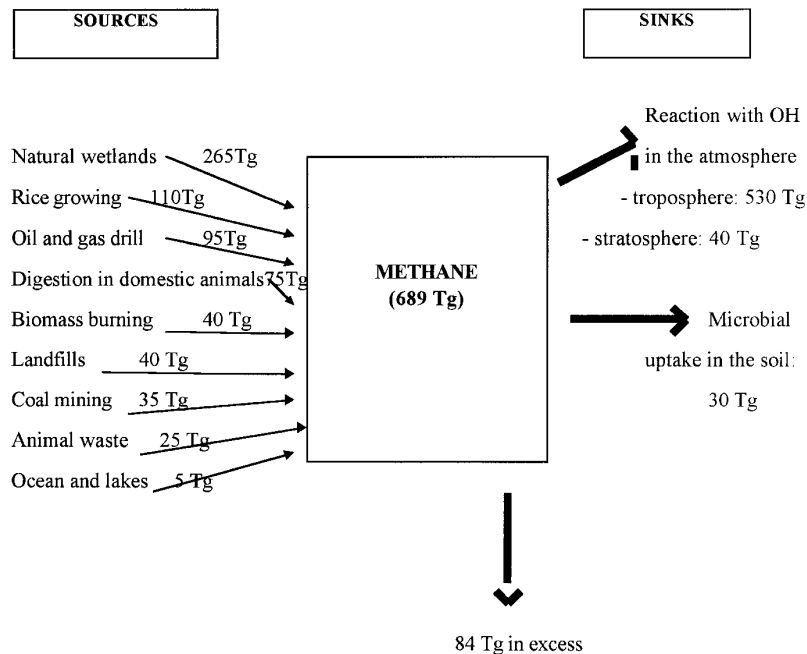
of atmospheric methane during the period 1980–1990 of about 18 ppbv (parts per billion per volume) per year [119]. The current rate of increase in atmospheric methane concentration has subsequently slowed to about 10 ppbv per year [129], but the reason for this is uncertain [152]. The current global average atmospheric concentration of methane is 1720 ppbv, more than double its pre-industrial value of 700 ppbv [8]. The concentration of methane in the Northern hemisphere is about 100 ppbv more than in the Southern hemisphere, indicating either greater source or lower sink strength in the Northern hemisphere [152].

The rising concentration of methane is correlated with increasing populations and currently about 70% of methane production arises from anthropogenic sources and the remainder from natural sources. Agriculture is considered to be responsible for about two-thirds of the anthropogenic sources [36]. Biological generation in anaerobic envi-

ronments (natural and man-made wetlands, enteric fermentation and anaerobic waste processing) is the major source of methane, although losses associated with coal and natural gas industries are also significant. The primary sink for methane is reaction with hydroxyl radicals in the troposphere [23, 24, 42], but small soil [97, 130, 153] and stratospheric [23, 24] sinks have also been identified. The major sources and sinks of methane are shown in Figure 1.

Agriculture contributes about 21–25, 60 and 65–80% of the total anthropogenic emissions of carbon dioxide, methane and  $N_2O$  respectively [36, 56, 152]. Agriculture is also thought to be responsible for over 95% of the ammonia, 50% of the carbon monoxide and 35% of the nitrogen oxides released into the atmosphere as a result of human activities [56].

The release of an estimated 205 to 245 million tonnes of methane per year from agricultural sources is shown in Table II.



**Figure 1.** Sources and sinks for methane on the earth and atmosphere.

**Table II.** Methane emission rates from agricultural sources. Source: Watson et al. [152].

Agricultural sources	Methane emission rates (million tonnes per year)
Enteric fermentation	80
Paddy rice production	60–100
Biomass burning	40
Animal wastes	25
<b>Total</b>	<b>205–245</b>

The soil sink strength for methane appears to have been reduced by changes in land use, chronic deposition of nitrogen from the atmosphere and alterations in nitrogen dynamics of agricultural soils [62, 97, 126, 130]. Ojima et al. [110] estimated that the consumption of atmospheric methane by soils of temperate forest and grassland eco-systems has been reduced by 30%. Without the temperate soil sink for methane, the atmospheric concentration of methane would be increasing at about 1.5 times the current rate.

Since atmospheric methane is currently increasing at a rate of about 30 to 40 million tonnes per year, stabilising global methane concentrations at current levels would require reductions in methane emissions or increased sinks for methane of approximately the same amount. This reduction represents approximately 10% of current anthropogenic emissions. The major agricultural sources of methane are flooded rice paddies, enteric fermentation and animal wastes. Decreasing methane emissions from these sources by 10 to 15% would stabilise atmospheric methane at its present level and is a realistic objective [35].

In 1990, agricultural emissions of methane in the EU-15 were estimated at 10.2 million tonnes per year and were the greatest source (45%) of methane emissions in the EU. Of these, approximately two-thirds came from enteric fermentation and one-third from livestock manure.

## 1.6. Recommendations in European post-Kyoto policy

Facing the serious visible signs of global warming, the United Nations (UN) created the Framework Convention on Climate Change (UNFCCC) with the missions of preparing the Conferences of the Parties (COP) for international decisions on gaseous emissions and collecting information on climatic changes through the Global Impact of Environmental Change (GIEC). Delegates from nearly all the countries in the world work in COP according to the UN rules, with the exception that only countries which ratified the Rio Convention are allowed to vote. The other countries are only allowed to propose amendments to the texts submitted to COP for approval. The Subsidiary Body for Implementation (SBI) was created to elaborate recommendations for the COP and to control the enforcement of decisions. It collaborates with the Subsidiary Body for Scientific and Technological Advances (SBSTA) which is in charge of co-ordinating scientific studies with the information given by international organisations and the needs of the COP.

The COP1, COP2, COP3, COP4 met respectively in Berlin (1995), Geneva (1996), Kyoto (1997) and Buenos-Aires (1998) to decide on strategies of reduction of radiatively active trace gases. After a 14 day-meeting, 174 countries took the following decisions registered in an agreement called the “Kyoto Protocol” produced during COP3, which now have to be applied:

- a decrease of greenhouse gas emissions by an average of 5.2% below 1990 level during the period 2008–2012 in industrialised countries;
- the level of allowed emissions during this period varies according to the countries: +8% for Australia; –8% for EU; +10% for Ireland; –6% for Japan; +5% for Norway and –7% for USA;
- the agreement is applied only to 6 greenhouse gases: carbon dioxide, methane, nitrous oxide, two fluorocarbons and sulphur hexafluoride.

Since the objective is an effective reduction at a global level, the protocol introduced a very complex system allowing industrial countries to exchange or postpone in time, part of their reduction. According to their economics, countries are allowed to sell or buy some emission rights provided that the joint obligations on total reductions are respected.

Such decisions raise several questions:

- 1) guidelines for national greenhouse gas inventories must be proposed;
- 2) unanimous decisions must be taken at the European level to reach a total decrease of 8% during the 1st decade of the third millennium;
- 3) appropriate controls must be put into place to supervise the effective reduction in each country;
- 4) Penalties must be applied to infringers.

Because about 60% of the methane arises from agricultural activities in Europe, much of the effort in the near future will concern this economic sector. Anaerobic digestion in the forestomach of ruminants is a major source of methane emissions. The contribution of other livestock such as horses, rabbits, pigs or poultry is much less significant. As indicated below, mitigation scenarios based on a scientific knowledge of methanogenesis must be proposed to the European Commission and European Parliament to provide guidance on how to fulfil the mandate outlined in the Kyoto protocol.

## 2. METHANE PRODUCTION AND HYDROGEN SINKS IN THE RUMEN

### 2.1. Fermentative reactions in the rumen and caecum involving H<sub>2</sub> production and H<sub>2</sub> sinks

#### 2.1.1. Fermentation in the rumen

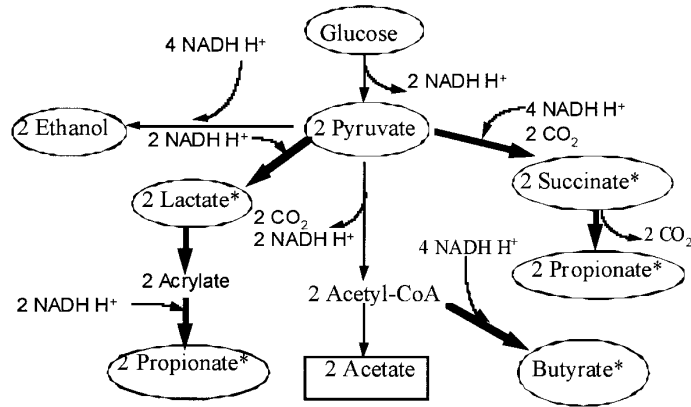
Ingested feed macromolecules are degraded in the digestive tract into small molecules that are then transferred into the

blood flow through the digestive mucosa. Such hydrolysis is performed by enzymes of both endogenous and microbial origin. Although the anatomy and physiology of the digestive tract varies widely in the animal kingdom, enzymatic digestion is generally located at the beginning of the digestive tract while microbial digestion takes place at the end. Ruminants and some other animals considered as pseudo-ruminants like *camelidae*, other animals like the bird Hoatzin have in addition large anaerobic fermentative chambers located at the beginning of the tract. Such anatomical characteristics with a small intestine flanked by two microbial compartments at both ends are much more efficient for the digestion of carbohydrates and for the degradation of plant cell walls. Furthermore, microbial protein synthesised in the forestomachs is then available for digestion in the small intestine where they supply more than 50% of the amino acids entering the blood stream.

Fermentation of glucose equivalents released from plant polymers or starch, is an oxidative process under anaerobic conditions occurring in the Embden-Meyerhof-Parnas pathway and giving reduced cofactors like NADH (see Fig. 2). These reduced cofactors have to be re-oxidised to NAD to complete the fermentation of sugars. NAD<sup>+</sup> is regenerated by electron transfer to acceptors other than oxygen (CO<sub>2</sub>, sulphate, nitrate, fumarate). Electron transport-linked phosphorylation inside microbial bodies is a way of generating ATP from the flow of generated electrons through membranes, if the required co-factors are present [37]. Production of H<sub>2</sub> is a thermodynamically unfavourable process that is controlled by the potential of the electron carrier [158]. Even traces of H<sub>2</sub> inhibit the hydrogenase activity, but more H<sub>2</sub> is tolerated if bacteria have ferridoxin-linked pyruvate oxidoreductases [92].

Although H<sub>2</sub> is one of the major end products of fermentation by protozoa, fungi and pure monocultures of some bacteria, it does not accumulate in the rumen because

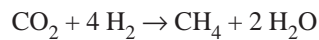




**Figure 2.** Metabolism of  $\text{NADH H}^+$  and the electron sink products\* in anaerobiosis.

it is immediately used by other bacteria which are present in the mixed microbial ecosystem. The collaboration between fermenting species and  $\text{H}_2$ -utilising bacteria (e.g. methanogens) is called "interspecies hydrogen transfer" [51]. Some physical associations between fermentative species and  $\text{H}_2$ -users may facilitate interspecies transfer in the rumen. Attachment of methanogens to the external pellicle of protozoa has been reported by Krumholz et al. [66] and Stumm et al. [133].

In the rumen, formation of methane is the major way of hydrogen elimination through the following reaction:

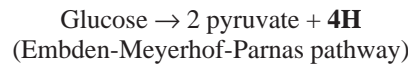


The hydrogen transfer towards methanogens is beneficial to the degradation of cell wall carbohydrates as shown in vitro by Wolin and Miller [159] with bacteria, by Bauchop and Mountfort [4] with fungi, and by Ushida and Jouany [139] with protozoa. These results were confirmed in vivo in gnotoxenic lambs with or without methanogens [40].

Metabolic hydrogen in the form of reduced protons ( $\text{H}$ ) can be also used during the synthesis of volatile fatty acids or incorporated into microbial organic matter. The stoichiometry of the main anaerobic

fermentation pathways can be summarised as follows:

*2H producing reactions:*

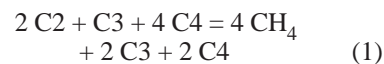


*2H using reactions:*



When  $\text{H}_2$  is not correctly used by methanogens,  $\text{NADH}$  can be re-oxidised by dehydrogenases of the fermenting bacteria to form ethanol or lactate. This situation which occurs in animals fed large amounts of rapidly fermentable carbohydrates, is considered as abnormal and illustrates a real dysfunction of the ruminal ecosystem.

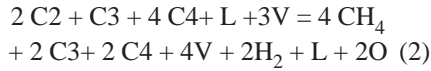
Assuming that the amount of  $2\text{H}$  produced ( $2\text{H}_p$ ) is equal to  $2\text{H}$  used ( $2\text{H}_u$ ) on a molar basis, Demeyer and Van Nevel (1975) proposed the following equation obtained from the previous reactions:



If production of  $\text{H}_2$ , lactate (L), valerate (V), and consumption of  $\text{O}_2$  (O) are considered,



equation (1) can be converted into:

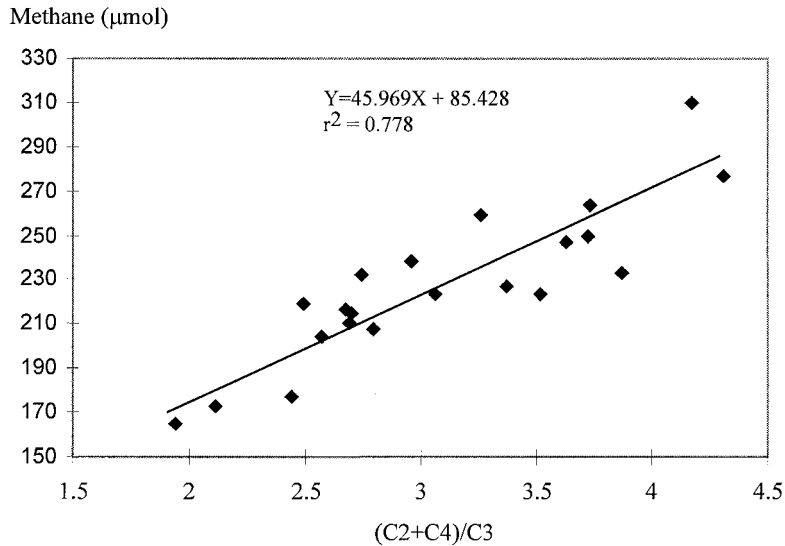


The recovery rate of metabolic hydrogen which is calculated as  $2H_u/2H_p$ , varies between 78 and 96% in the rumen for roughage diets [26]. Considering a mean hydrogen recovery of 90%, then equation (1) allows the calculation of methane production:

$$CH_4 = (1.8 C_2 - 1.1 C_3 + 1.6 C_4) / 4 \\ = 0.45 C_2 - 0.275 C_3 + 0.40 C_4 \quad (3)$$

Clearly, equation (3) indicates that the molar percentage of volatile fatty acids (VFAs) influences the production of methane in the rumen. Acetate and butyrate promote methane production while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen. Such theoretical calculations have been confirmed in vitro where the end products can be easily quantified. Methane production was measured when the molar proportions of individual VFAs, was altered by adding monensin to the diet of animal donors (Fig. 3).

Methane was not correlated to C2 production ( $r^2 = 0.029$ ) but, there was a good negative correlation between methane and C3 ( $r^2 = 0.774$ ). The correlation between methane and C2/C3 ratio ( $r^2 = 0.772$ ) was slightly lower. The ratio  $(C_2 + C_4)/C_3$ , which accounts for acetate and butyrate both of which are involved in  $H_2$  production, and propionate which is involved in  $H_2$  utilisation, improved the relationship slightly ( $r^2 = 0.778$ ). This result is consistent with the idea that propionate production and methanogenesis are competing, and are alternative pathways for regenerating oxidised co-factors in the rumen. However, this result alone gave no information on the regulating mechanisms involved. Van Kessel and Russell [144] observed in vitro, using rumen fluid sampled from animals fed on roughage-based diets, that ruminal methanogens lose the ability to use  $H_2$  at low pH, giving rise to free  $H_2$  in the gas phase when the pH was less than 5.5. Thus on roughage diets a low pH leads to a decrease in methanogenesis independent from propionate formation. On the contrary, starch-fermenting bacteria can compete against methanogens for hydrogen use by



**Figure 3.** Relationship between methane and  $(C_2 + C_4)/C_3$  ratio.

producing large amounts of propionate [123]. However, H<sub>2</sub> accumulated and propionate decreased dramatically while acetate increased when the pH reached non-physiological values below 5.3. This means that the microbial ecosystem involved in propionate formation differs with the dietary conditions. The cellulolytic bacteria *Fibrobacter succinogenes* is the major propionate producers through the succinate pathway in roughage diets, while lactate is the main intermediate in the conversion of starch to propionate. Unlike cellulolytic bacteria and methanogens, lactic bacteria are known to be tolerant to low pH making them able to use H<sub>2</sub> and be competitive with methanogens even in unfavourable pH conditions.

### 2.1.2. Fermentation in the hindgut

In ruminants, large amounts of organic matter can by-pass the rumen and be digested in the hindgut if there is no digestion in the small intestine or if the digestion is incomplete. So ground roughage diets and diets rich in maize starch can supply large quantities of digestible organic matter to the hindgut. It has been estimated that 10 to 30% of digestible organic matter can be digested there. Because the large intestine is the only compartment of fermentation in the digestive tract of simple-stomached species, it plays an essential digestive role, especially in herbivore monogastrics.

The anaerobic bacteria in the hindgut are not very different from those found in the rumen [61]. Other species of protozoa inhabit the large intestine of equines [9], but protozoa are missing from the hindgut of ruminants. Anaerobic fungi are absent in the human large intestine, although they have been found and identified in the large intestine of Equidae [10].

There is no clear information on the occurrence of significant methanogenic fermentation in the hindgut of species other than humans and pigs. Only some individuals in the rat population and termite population are able to produce methane [12, 68].

Breath tests were used to screen a large human population. About 0.33 of the individuals have 10<sup>8</sup> to 10<sup>10</sup> methanogens per g digesta. The latter concentration gives enough methane production (30 ml to 3 litres of methane per day) for a breath detection.

As in the rumen, methanogens of colonic fermentation use H<sub>2</sub> to reduce CO<sub>2</sub> to methane [93]. When non-methanogenic fermentation occurs in the hindgut, H<sub>2</sub> is used to reduce CO<sub>2</sub> into acetate [33] according to the following reaction:



Such use of H<sub>2</sub> is of interest for the nutrition of animals since acetate is absorbed into the blood and used as a major source of carbon and energy by ruminants, while methane is lost from the animal. Accordingly, several attempts have been made by scientists to reduce methane production from digestive fermentation and to increase acetogenesis by the microbial community of the rumen. Although the concentrations of acetogenic bacteria in the bovine rumen are similar to those of methanogens [71], Prins and Lankhorst [115] did not observe any formation of acetate from the reduction of <sup>14</sup>CO<sub>2</sub> by rumen contents. Contrary to methanogens, acetogenic bacteria are able to use sources other than hydrogen for their energy supply, which explains why their concentration can be high in the rumen while acetogenesis is negligible.

Demeyer and De Graeve [27] showed that the addition of H<sub>2</sub> to the gas phase of fermenters inoculated with rumen digesta had little impact on VFA production (from - 4% to + 7%), but stimulated methanogenesis significantly (+ 94%). When added to caecal digesta sampled from the same cattle, H<sub>2</sub> significantly stimulated VFA production (+ 10% C2, + 14% C3, + 14% C4) as well as methane production (+ 67%). However, the increase in methane was lower than that noted with rumen digesta. The hydrogen recovery rate was always much lower in the caecum or colon than the rumen

(0.50 to 0.60 vs. 0.85 to 0.90). Such results indicate that methanogenesis is the major pathway for  $H_2$  use in the rumen compared to the use for propionate or butyrate synthesis. When methanogens are present, methanogenesis still remains the major  $H_2$  sink in the caecum, but its contribution is minor when compared to the rumen. Conversely, the contribution of VFAs is larger in the caecum. In experiments carried out in France (Jouany, unpublished data) using incubations with human bowel contents sampled from methane producers or non-methane producers, it was observed that methane emissions from methane producers were low (5% of total C6 fermented estimated as  $C2/2 + C3/3 + C4$ ) and, as a consequence, the other end products of fermentation were not statistically different between the two groups. The hydrogen recovery rate calculated from equations (1) and (2) was low (0.33 in non-methane producers vs. 0.38 in methane producers) which indicates that hydrogen sink reactions other than those considered in the rumen exist in the hindgut, and that these reactions can represent more than 60% of the total hydrogen sink reactions. The recovery values noted above for humans are close to those calculated from work with rabbit digesta [27].

Individual determinations of methane production indicate that large variations occur between animals under the same conditions within a herd [59]. Subtle balances between microbes involved in hydrogen transfer must exist in the digestive ecosystems to explain such variations. Feeding behaviour and animal physiology (rumen motility, flow of digesta, mastication, salivation) are probably determinants of the microbial populations involved in production and use of hydrogen, which explains such an animal effect. This aspect has been confirmed by the higher accuracy in prediction of methane production when mechanistic models are used rather than simple regression equations as shown by Benchaar et al. [5]. The former models [30] integrate some parameters derived from animal

characteristics while the latter are generally associated only with dietary characteristics.

## 2.2. Micro-organisms involved in digestive $H_2$ metabolism

Methane is produced by strict anaerobes belonging to the sub-group of the Archaea domain [155]. Archaea have no peptidoglycan polymer in their cell walls. Also intracellular lipids are different in composition from other bacteria. Triacylglycerol is replaced by ether linkages between glycerol and polyisoprenoid chains. Ribosomal RNA nucleotide sequences of Archaea and other bacteria show an early divergence of the two types of cells during evolution. There is a large phylogenetic diversity of methanogens in natural media. Also, the different genera and species of methanogens have various shapes and physiological characteristics: cocci, rods, spirilla, thermophilic and mesophilic species, motile and non-motile cells.

Rumen methanogens grow only in environments with a redox potential below  $-300$  mV [131]. More than sixty species were isolated from various anaerobic habitats like sanitary landfills, peat bogs, waterlogged soils, salt lakes, thermal environments, and intestinal tracts of animals. Only five of these species belonging to *Methanobrevibacter* and *Methanosarcina* genera, were isolated from rumen digesta. Only two of these species have been found at a population level greater than  $10^6$  ml $^{-1}$ .

Although  $H_2$ , formate, acetate, methanol, mono-, di- and tri-methylamine are all potential substrates for methanogens, only  $H_2/CO_2$  and formate to a lesser degree, are used as methane precursors in the rumen [91]. The reactions involved in methane production in the rumen which have been described by Rouviere and Wolfe [121] are their sole energy-generating mechanism. They show that specific co-factors are needed for the methane to be produced and inhibition of some of them could be a way to

reduce the activity of methanogens. In the intestine, methanogens are able to use other precursors. As an example, *M. smithii* makes methane only by reducing methanol with H<sub>2</sub>, methanol being produced from hydrolysis of pectins and other methylated plant polysaccharides.

Acetogens are the major bacteria involved in H<sub>2</sub> utilisation in the hindgut while their population rarely exceed the concentration of 10<sup>5</sup> ml<sup>-1</sup> found in the rumen of adult ruminants [96]. They appear in the rumen soon after the birth of lambs in herd conditions [95] and their population decreases during the growth of methanogens, confirming the strong competition between the two H<sub>2</sub>-users. When inoculated into gnotobiotic lambs isolated without methanogens, they reached the concentration 10<sup>8</sup>–10<sup>9</sup> ml<sup>-1</sup> which were maintained for the entire experiment. Free hydrogen accumulated and represented 10% of the total gas production in the rumen of gnotobiotic lambs, which indicates that acetogens have a low efficiency in hydrogen use. Inoculation of methanogens in these gnotobiotic animals induced a drop in the concentration of acetogens and a quasi-complete use of H<sub>2</sub> since free hydrogen disappeared. This means that acetogens and methanogens compete for H<sub>2</sub> use, and that methanogens always derive advantage from this competition as confirmed by Demeyer et al. [28], Le Van et al. [73] and Lopez et al. [75].

### 2.3. Effect of feeding characteristics on methane production

#### 2.3.1. Digestible OM or energy

Methane emissions are closely related to the amount of rumen fermented OM or the amount of digestible OM since more than 50% of digestion occurs in the rumen. When the digestibility of energy increases by 10%, energy losses as methane increase by 0.47 points in a roughage diet and by 0.74 points in a mixed diet [6]. However,

Johnson and Johnson [59] showed from 118 experiments that digestibility of dietary energy explained only 5% of the variation in proportion of gross energy lost as methane. Digestible energy does not take into account the nature of fermented OM (FOM) and a possible shift of digestion from the rumen to intestine.

#### 2.3.2. Residence time in the rumen and level of intake

A reduction in methane production is expected when the residence time of feed in the rumen is reduced since ruminal digestion decreases and methanogenic bacteria are less able to compete in such conditions. Furthermore, a rapid passage rate favours propionate production and the relevant H use. According to Kennedy and Milligan [63] and Okine et al. [111], a 30% decline in methane production is observed when the ruminal passage rate of liquid and solid phase increased by 54 to 68%. Mean retention time was shown to explain 28% of the variation in methane emissions [111].

An increase in feeding level induces lower methane losses as a percentage of daily energy intake [6, 7, 100]. Johnson and Johnson [59] noted that methane losses expressed as the proportion of gross energy intake declined by 1.6 percentage units for each multiple of intake. The major effect of feeding level is explained by its consequences on passage of feed particles out of the rumen [113].

#### 2.3.3. Source of C and pattern of fermentation

Because proportions of the individual VFAs is influenced by the composition of OM of the diet, mainly by the nature and rate of fermentation of carbohydrates, these dietary characteristics will have large effects on methane production. Diets rich in starch which favour propionate production will decrease the methane/FOM ratio in the rumen. As discussed before, the effect of

such diets on ruminal pH can also explain the observed effect on methane emission. Conversely, a roughage-based diet will increase the ratio. As an example, the level of methane losses was 6–7 or 2–3% of energy intake when forages were fed at maintenance or when high grain concentrates were fed *ad libitum* respectively [59]. Some other feed characteristics can affect methane production. It increases when mature dried forages are fed [134] or when they are coarsely chopped rather than finely ground or pelleted [50, 99], and decreases when forages are preserved in ensiled form [99]. Because they stimulate the rumen degradation of plant cell walls, alkali-treatments of poor-quality forages have been shown to increase the amount of methane emissions [100].

### 3. MITIGATION SCENARIOS FOR METHANE EMISSIONS FROM RUMINANTS

#### 3.1. Methane inhibition

##### 3.1.1. Direct inhibition

Direct inhibition of methanogenesis by halogenated methane analogues and related compounds has been widely demonstrated *in vitro* [146] and some have been tested *in vivo*. Chloroform reduced methanogenesis *in vitro* and *in vivo* [3, 19], but is obviously not suitable for use in practice. Chloral hydrate, which is converted to chloroform in the rumen [114, 117], inhibited methane production *in vivo* [86] but lead to liver damage and death in sheep after prolonged feeding [70]. Amichloral (a hemiacetal of chloral and starch) appeared to be safer and increased liveweight gain in sheep [137], but unfortunately its antimethanogenic activity declined with prolonged feeding [21, 57]. Similarly the effects of trichloroacetamide and trichloroethyl adipate on ruminal methanogenesis were apparently transient [19, 20, 138]. The anti-methanogenic activity of bromochloromethane was also

reported to be transient [125], however May and colleagues [88, 89] suggested that a combination of bromochloromethane and  $\alpha$ -cyclodextrin was more stable and capable of suppressing methane emissions in sheep and cattle over a prolonged period.

2-bromoethanesulfonic acid (BES), a bromine analogue of coenzyme F involved in methyl group transfer during methanogenesis, is a potent methane inhibitor [80, 156]. BES is a specific inhibitor of methanogens and does not appear to inhibit the growth of other bacteria [124, 128]. However, unfortunately when tested *in vivo* the inhibition in methanogenesis was transient suggesting that adaptation of the methanogenic population occurred [146].

Recently, 9,10-anthraquinone has been shown to inhibit methanogenesis by mixed rumen micro-organisms *in vitro* [43, 67] and to depress methane production in lambs over a 19 day period [67]. Garcia-Lopez et al. [43] speculated that 9,10-anthraquinone inhibited the reduction of methyl co-enzyme M to methane by uncoupling electron transfer in methanogenic bacteria.

##### 3.1.2. Ionophores

Inhibition of methane production is normally accompanied by an increase in propionate production, and a negative relationship between methanogenesis and propionate production has been clearly established in work on interspecies hydrogen transfer [157]. Ionophoric antibiotics such as monensin have been shown to depress methane production by mixed rumen microbes *in vitro* [145]. This decrease in methanogenesis is not due to a direct effect of the ionophores on methanogenic bacteria but rather results from a shift in bacterial population from gram positive to gram negative organisms with a concurrent shift in the fermentation from acetate to propionate [18, 104]. Van Nevel and Demeyer [146] found that *in vivo* monensin depressed methane production by 25% when averaged over 6 studies, however unfortunately some



long term in vivo trials have shown that the inhibition of methanogenesis by monensin did not persist [58, 122]. This appears to be in conflict with the observation that altered patterns of volatile fatty acid production persist in monensin treated animals during long term trials [118, 120]. The effect of salinomycin on methane production however seemed to be more persistent [149].

### 3.1.3. Propionate enhancers

Awareness over antibiotic residues in animal products and the threat of bacterial antibiotic resistance in the wider environment has led to an increasing interest in alternatives to antibiotics as growth promoters. Martin has suggested that dicarboxylic organic acids such as malate may alter rumen fermentation in a manner similar to ionophores [79]. Lopez et al. [75] observed that when fumarate, a precursor of propionate, was added to rumen simulating fermentors, propionate production increased with a stoichiometric decrease in methane production. Ouda et al. [112] found that acrylate, an alternative precursor of propionate, also depressed methane production in rumen simulating fermentors, but to a lesser extent than an equimolar addition of fumarate. Asanuma et al. [1] also found fumarate depressed methane production in vitro and suggested that fumarate could be an economical feed additive in Japan. Malate, which is converted to propionate via fumarate, also stimulated propionate production and inhibited methanogenesis in vitro [83]. However Carro et al. [14] found that malate actually increased methane production in a rumen simulating fermentor, although this was largely explained by stimulation in fibre digestion and methane produced per unit of dry matter fermented actually fell. Malate failed to stimulate rumen propionate concentrations in the rumen of cattle and did not affect estimated methane production [85, 94] although malate did stimulate average daily gain in steers [85].

### 3.1.4. Stimulation of acetogens

An alternative strategy to reduce ruminal methanogenesis would be to re-channel substrates for methane production into alternative products. As noted above acetogenic bacteria, in the hindgut of mammals and termites, produce acetic acid by the reduction of carbon dioxide with hydrogen and reductive acetogenesis acts as an important hydrogen sink in hindgut fermentation [27, 68]. Reductive acetogenesis occurs in the intestine of non-ruminants, sometimes along with methanogenesis and sometimes replacing methanogenesis [11, 34]. Bacteria carrying out reductive acetogenesis have been isolated from the rumen [44, 45, 95], but they are few in number, and attempts to increase acetogenesis have not been successful, largely because under rumen conditions the reductive acetogens have been unable to compete with the methanogenic archaea [28, 53, 107, 108]. Lopez et al. [74] found that acetogens depress methane production when added to rumen fluid in vitro and suggested that even if a stable population of acetogens could not be established in the rumen it might be possible to achieve the same metabolic activity using the acetogens as a daily feed additive.

### 3.1.5. Methane oxidisers

Global methane accumulation is the difference between methane production and methane oxidation. Methane oxidising bacteria have been isolated from a wide range of environments [47], including the rumen [132]. Studies with  $^{13}\text{CH}_4$  tracers suggest that oxidation of methane to  $\text{CO}_2$  is of little quantitative importance in the rumen [142] but may be more important in the gut of pigs [49]. Valdes et al. [143] isolated a methane oxidising bacterium from the gut of young pigs which decreased methane accumulation when added to rumen fluid in vitro, however the validity of this approach in vivo has yet to be tested.

### 3.1.6. Defaunation

Methanogenic bacteria have been observed on the exterior surface of rumen ciliate protozoa [148] and as endosymbionts within the ciliates [39]. Newbold et al. [105] estimated that methanogens associated with ciliate protozoa were responsible for between 9 and 25% of the methanogenesis in rumen fluid and the removal of protozoa from the rumen (defaunation) has been associated with decreases in methane production [141]. However these effects are apparently diet modulated with greater responses on high concentrate as opposed to high forage diets [140]. A variety of techniques to remove protozoa from the rumen have been tested experimentally, but none is used routinely, because of toxicity problems, either to the rest of the rumen microbial population or to the host animal [154]. Recently, there has been an increased interest in plant secondary metabolites for use as possible defaunating agents. In particular, saponin-containing plants show promise as a possible means of suppressing or eliminating protozoa in the rumen without inhibiting bacterial activity. Saponins are glycosides which apparently interact with the cholesterol present in eukaryotic membranes but not in prokaryotic cells [17]. A decrease in protozoal numbers was reported in the rumen of sheep infused with pure saponin [76] or fed saponin-containing plants [29, 102, 106, 109, 135, 136]. However, even if a practical on-farm method to remove protozoa from the rumen can be found, the effects of defaunation on methane emissions can not be considered in isolation. Rumen ciliate protozoa play an active role in ruminal fibre breakdown [22] and defaunation has been shown to adversely impact fibre digestion in the rumen [60]. However, protozoa also have a negative impact on animal productivity in that the engulfment and digestion of bacteria by protozoa [150] significantly lowers the flow of microbial protein leaving the rumen [60]. Thus the use of defaunation to decrease methane production from

ruminants would have to be balanced against the effects on fibre and protein metabolism in the rumen.

The inclusion of fat in ruminant diets depresses protozoal numbers [25, 52] and the use of lipids as a defaunating agent has been suggested [104]. Fat inclusion in the diet causes a marked decrease in methane production by rumen fluid, with the effect being at least partly governed by the fat source used [32, 78]. However, the effects of fat on methane production are not limited to those mediated via the rumen protozoa and lipids have been shown to inhibit methanogenesis even in the absence of rumen protozoa [13, 31], possibly due to the toxicity of long chain fatty acids to methanogenic bacteria [48, 116]. However, as with defaunation the effect of fat supplementation can not be viewed in isolation. Fat inclusion in the diet (particularly at levels above 5 g·kg<sup>-1</sup> DM) can significantly inhibit fibre breakdown in the rumen [65, 77], and again the severity of the effect varies with the fat used [78].

### 3.1.7. Probiotics

The most widely used microbial feed additives (live cells and growth medium) are based on *Saccharomyces cerevisiae* (SC) and *Aspergillus oryzae* (AO). Their effect on rumen fermentation and animal productivity are wide ranging and this has been reviewed by several authors [82, 103]. There is very limited information on their effect on methane production and all of this is in vitro. AO has been seen to reduce methane by 50% [41] which was directly related to a reduction in the protozoal population (45%). On the other hand, addition of SC to an in vitro system reduced the methane production by 10% initially, though this was not sustained [101]. In other experiments with AO and SC, an increase in methane production has been reported [81, 84], while Mathieu et al. [87] reported that SC addition did not affect methane in vivo. This suggests that more research is required



before it can be concluded that yeast cultures or AO extracts decrease methane production in vivo.

### 3.1.8. Immunisation

Baker [2] has proposed that it may be possible to immunise ruminants against their own methanogens with associated decreases in methane output. Shu et al. [127] have shown that such an approach can successfully reduce the numbers of *Streptococci* and *Lactobacilli* in the rumen.

## 3.2. Increase in animal productivity

The concept of increasing animal productivity to reduce methane emissions from ruminants is based on the maintenance of overall production output and as a result, increased production of useful product would mean methane production per unit product would decline. A reduction in total emissions of methane would only result if total output levels (e.g. total milk or beef produced) remained constant and livestock numbers were reduced. Possible options for increasing ruminant productivity are discussed in the following sections.

### 3.2.1. Diet type

The type of feed offered to a ruminant can have a major effect on methane production. The forage to concentrate ratio of the ration has an impact on the rumen fermentation and hence the acetate:propionate ratio (declines with F:C ratio). It would therefore be expected that methane production would be less when high concentrate diets are fed [38]. Johnson and Johnson [59] reported a methane energy loss of 6 to 7% of gross energy intake when forages were fed at the maintenance plane of nutrition and this reduced to 2–3% when high grain concentrates (> 90%) were offered at near ad libitum intake levels. Moss et al. [100] found a similar effect when grass silage was supplemented with barley. Van Soest [147] indicated that a high grain diet and/or the

addition of soluble carbohydrates gave a shift in fermentation pattern in the rumen which give rise to a more hostile environment for the methanogenic bacteria in which passage rates are increased, ruminal pH is lowered and certain populations of protozoa, ruminal ciliates and methanogenic bacteria may be eliminated or inhibited. The work of Lana et al. [69] supports this theory confirming that low rumen pH regulates methane production.

### 3.2.2. Forage type and supplementation

Supplementing forages whether of low or high quality, with energy and protein supplements, is well documented to increase microbial growth efficiency and digestibility (see Moss [99] for a review). Milk and meat production will increase as a result. The direct effect on methanogenesis is still variable and unclear, but indirectly, methane production per unit product will decline. The area was recently reviewed [99]. Increasing the level of non-structural carbohydrate in the diet (by 25%) would reduce methane production by as much as 20%, but this may result in other detrimental effects e.g. acidosis, laminitis, fertility problems. Also with the implementation of quotas for milk production in the EU, many producers are optimising milk production from home-grown forages in order to reduce feed costs. Supplementing poor quality forages and chemically upgrading them are good options for increasing productivity and in turn reducing methane emissions per unit product. Reductions of total emissions would only result if livestock numbers are reduced correspondingly.

Feeding of ruminants to optimise rumen and animal efficiency is a developing area and the efficient deployment of this information to all livestock producers would benefit the environment in terms of both methane and nitrogen emissions. This would lead to best practise information and would require good technology transfer. Many farmers within the EU have to pay for

unbiased nutritional advice. If this advice was freely available, there would likely be an increase in productivity and an improvement in the impact of emissions from livestock into the environment. For some of the more productive member states (e.g. Denmark and the Netherlands for milk production) this approach may not be so beneficial.

### 3.2.3. *High genetic merit dairy cows*

Improving the genetic merit of dairy cows has escalated in the last decade with the import of Holstein genetic material from US and Canada for use on the EU native dairy breeds. As a result, average national yields have increased. One of the major improvements is the ability of the cow to partition nutrients into milk preferentially to maintenance and/or growth. This has undoubtedly resulted in increased efficiency. The UK dairy herd has increased its average yield by 8.8% from 1995 to 1997 and the top 10% of herds are averaging 8351 litres per cow. There are additional benefits which include the following:

(i) a cow's lifetime production can be achieved in less lactations, therefore there are less maintenance costs e.g. lifetime production of 30 000 litres achieved as 5 lactations of 6 000 litres or 3 lactations of 10 000 litres;

(ii) a 100 cow herd producing average yield of 6 000 litres = 600 000 l.y<sup>-1</sup> or 60 cow herd producing 10 000 litres, therefore less cows to maintain;

(iii) less replacement heifers to maintain.

Kirchgessner et al. [64] suggested that increasing milk production of dairy cows from 5 000 to 10 000 litres milk annually would only increase methane production by 5% (i.e. from 110 to 135 kg methane per year). Leng [72] indicated that Holstein cattle fed a high quality ration would produce only about 15% as much methane per litre of milk as native Indian cattle on traditional feed.

This could reduce methane emissions by 20 to 30% through reduced numbers. The genetic merit of livestock within the EU is rapidly improving and this will undoubtedly bring with it increased efficiency. The management of these high genetic merit cows will also become more complex and the overall implementation of this may be stalled by animal welfare implications. High genetic merit cows can have increased problems with fertility, lameness, mastitis and metabolic disorders. All these issues will have to be addressed if genetic progress is to be successfully continued.

### 3.2.4. *Ionophores*

The use of ionophores gives rise to improved animal productivity (on average an 8% improvement in feed conversion efficiency [15]) and a possible direct reduction in methane production. This option is in use throughout the EU for beef animals only as its use is not permitted in dairy cows because the product requires a withdrawal period. Its effect is therefore impacting on less than 50% of the methane emissions. As with all measures that reduce methane production by increasing animal productivity, the benefit is only seen if animal numbers are reduced correspondingly.

The use of chemicals/antibiotics to increase animal productivity are increasingly becoming unpopular to the consumers of animal products. It is therefore envisaged that the use of ionophores to reduce methane production is not a sustainable option.

### 3.2.5. *Bovine somatotropin*

Bovine somatotropin (BST) is a genetically engineered metabolic modifier approved for use in some countries to enhance milk production from dairy cows. BST does not affect digestibility, maintenance requirements or the partial efficiency of milk synthesis, nor does it act directly on the mammary gland. BST affects mammary

tissue indirectly by its action on the liver and the kidney to stimulate production of insulin-like growth factors which act on the mammary gland to increase milk synthesis. Nutrients for increased milk yield are provided by increased intake and co-ordination of metabolism to increase supplies to the mammary gland of glucose, amino acids and fatty acids [16]. Given a 15% increase in productivity per animal, there would be a reduction in methane production per unit product. Again, this is not a popular consumer choice for enhancing animal productivity and is actually banned by all EU member states. It is therefore not worthy of pursuance.

### 3.2.6. Probiotics

As noted above the effect of yeast and fungal probiotics on methane production per se are variable. Responses to probiotics have been recorded in dairy cows and growing cattle. From an analysis of published results from more than 1000 cows, Wallace and Newbold [151] calculated that yeast culture stimulated milk yield by 7.8% and from 16 trials using growing cattle, they showed an average increase in liveweight gain of 7.5%. However, many producers are already apparently sceptical about the benefits of probiotics and there is a need to identify the dietary and management situations in which probiotics can give consistent production benefits.

## 4. FUTURE PROSPECTS

Atmospheric methane is currently increasing at a rate of about 30 to 40 Tg ( $10^{12}$  g) per year. Stabilising global methane concentrations at current levels would require reductions in methane emissions or increased sinks for methane of approximately the same amount. This reduction represents about 10% of current anthropogenic sources (of which ruminants contribute about 30%). This is much less than the

percentage reduction necessary to stabilise the other major greenhouse gases. Additionally, because methane has a shorter atmospheric lifetime and greater radiative absorption capacity than carbon dioxide, methane reduction strategies offer an effective means of slowing global warming in the near term.

Methane is an end-product of fermentation of carbohydrates in the rumen. The generation of this can be decreased by promoting a shift in fermentation toward propionate production, but cannot be eliminated completely without adverse effects on ruminant production. Increasing animal productivity seems to be the most effective means of reducing methane release in the short term. It must be borne in mind that this method is only successful if overall production remains constant. The means to achieve this increase in productivity have been discussed, but nearly all involve the increased use of feed containing higher quality/lower fibre sources of carbohydrate. However, the reason that ruminants are so important to mankind is that much of the world's biomass is rich in fibre and can be converted into high quality protein sources (i.e. meat and milk) for human consumption only by ruminants.

The most promising areas for future research for reducing methanogenesis are the development of new products/delivery systems for antimethanogenic compounds or alternative electron acceptors in the rumen and reduction in protozoal numbers in the rumen.

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