

### **REVIEW ARTICLE**

## Genetic aspects of enteric methane emission in livestock ruminants

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### Abstract

This review summarizes the importance of enteric methane (CH<sub>4</sub>) emission in ruminants and summarizes the current state of knowledge relevant to genetic aspects on enteric methane production, highlighting future research needs and directions. Global average temperature has increased by about 0.7°C in the last century. The Intergovernmental Panel on Climate Change (IPCC) reported that anthropogenic greenhouse gases (GHG), including carbon dioxide (CO<sub>2</sub>), CH<sub>4</sub>, nitrous oxide (N<sub>2</sub>O) and halocarbons, have been responsible for most of the observed temperature increase since the middle of the twentieth century. Agriculture, particularly livestock, is increasingly being recognized as both a contributor to the process and a potential victim of it. Policy interventions and technical solutions are required to address both the impact of livestock production on climate change and the effects of climate change on livestock production. Food and Agriculture Organization (FAO), declared that in the next 50 years, the world's farmers will be called upon to produce more food than has been produced in the past 10,000 years, and to do so in environmentally sustainable ways. Therefore, the GHG reduction should be treated as a public good. The United States congress is prospecting to define a price on GHG emissions. Limiting the concentration of CO<sub>2</sub> and other GHG in Earth's atmosphere requires a technological and economic revolution. A costeffective way could be the genetic improvement of livestock, which produces permanent and cumulative changes in performance. Animal variation in enteric CH<sub>4</sub> emission has been reported in the literature, providing potential for improvement through genetic selection.

### Introduction

Enrichment of the atmosphere with methane (CH<sub>4</sub>), as one of the most important greenhouse gases (GHG), is strongly linked to global warming. Global average temperature has increased by about  $0.7^{\circ}$ C in the last century, as reported by the Intergovernmental Panel on Climate Change (IPCC, 2007). The IPCC concludes that anthropogenic GHGs, including carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and halocarbons have been responsible for most of the observed temperature increase since the middle of the twentieth century.

The aim of this review is to summarize the current knowledge of genetic aspects of enteric CH<sub>4</sub> production, highlighting future research needs and directions.

# Methane balances in atmosphere

Methane is removed from the atmosphere (*i.e.*, converted to less harmful products) by a range of chemical and biological processes, which occur in different regions of the atmosphere. These include tropospheric oxidation, stratospheric oxidation and uptake by soil. The Environmental Change Institute (2012) of Oxford estimated that oxidation of CH<sub>4</sub> in the troposphere is the largest CH<sub>4</sub> sink, removing 506 Mt of this gas per year from the global CH<sub>4</sub> burden. Therefore, changes to the chemistry and composition of the troposphere will dominate the future environmental impact of CH<sub>4</sub> emissions.

Stratospheric oxidation of CH<sub>4</sub> consumes 40 Mt per year. The third process for removal of CH<sub>4</sub> from the atmosphere occurs at the ground-atmosphere interface. Approximately 30 Mt per year of CH<sub>4</sub> are removed annually from the atmosphere by soil uptake. Therefore, the total sinks of CH<sub>4</sub> are estimated on 576 Mt per year while the overall emissions are estimated on 598 Mt of CH<sub>4</sub> per year.

Soil is an important source of sink of CH<sub>4</sub>, and it contains populations of methanotrophic bacteria that can oxidise CH<sub>4</sub>, by a process known as high affinity oxidation. These bacteria consume CH<sub>4</sub> that is in low concentrations, close to that of the atmosphere (<12 ppm). The bacteria favour upland soils, in particular forest soils. Surprisingly, the bacteria responsible for high affinity oxidation processes remain largely unidentified. It is known, howCorresponding author: Prof. Marcello Mele, Department of Agricultural, Food and Agri-Environmental Science, University of Pisa, Italy. E-mail: mmele@agr.unipi.it

Key words: Animal genetic variation, Enteric methane emission, Ruminants, Livestock systems, Greenhouse gases.

Acknowledgments: the authors would like to thank the Animal Science and Production Association (ASPA, Italy), for supporting this review and the referees for the suggestions provided.

Received for publication: 11 October 2012. Revision received: 4 June 2013. Accepted for publication: 8 June 2013.

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ever, that exposure of soils to high ammonium concentrations leads to a loss of methanotrophic bacteria and a subsequent reduction in the rate of  $CH_4$  oxidation. The use of artificial fertilisers containing ammonia is therefore detrimental to the removal of  $CH_4$  (Bodelier, 2011).

# Greenhouse gas emissions of livestock

Livestock contributes to climate change by emitting GHG, either directly (e.g., from enteric fermentation) or indirectly (e.g., from feedproduction activities, deforestation, manure, etc.). Greenhouse gas emissions can arise from all the main steps of the livestock production cycle, but the amount emitted varies with animal species (Table 1). Ruminants contribute to GHG emissions with the production of 250 to 500 L of CH<sub>4</sub> per day per animal accounting for approximately 8% to 10% of global warming in the next 50-100 years. Emissions from feed-crop production and pastures are linked to the production and application of chemical fertilizer and pesticides, to soil organic-matter losses and to transport. When forest is cleared for pasture and feed crops, large amounts of carbon stored in vege-



tation and soil are released into the atmosphere. In contrast, when good management practices are implemented on degraded land, pasture and cropland can turn into net carbon sinks, sequestering carbon from the atmosphere.

At the farm level,  $CH_4$  is emitted from enteric fermentation and manure. In ruminant species (*i.e.*, cattle, buffalo, goat and sheep), microbial fermentation in the rumen converts dietary structural charbohydrates into products that can be digested and utilized by the animals. Therefore,  $CH_4$  is physiologically emitted as a by-product of the digestive process. Methane is also generated when manure is stored in anaerobic and warm conditions. Finally, the slaughtering, processing and transportation of animal products cause emissions mostly related to use of fossil fuel and infrastructure development.

In ruminants, enteric CH<sub>4</sub> is produced mainly in the rumen (87%) and, to a smaller extent (13%), in the large intestine (Murray *et al.*, 1976). Enteric CH<sub>4</sub> is produced from methanogenic archaea in anaerobic conditions, using CO<sub>2</sub> and H<sub>2</sub> to form CH<sub>4</sub>, and thus reducing the metabolic H<sub>2</sub> produced during microbial metabolism (McAllister and Newbold, 2008). Opportunities for nutritional and microbial manipulation to reduce enteric CH<sub>4</sub> emissions from livestock have been extensively studied and reviewed by several authors (Beauchemin *et al.*, 2008; McAllister and Newbold, 2008; Martin *et al.*, 2010).

# Animal genetic variability on methane emission

Interestingly, variation in enteric CH<sub>4</sub> emission has been reported between animals, among breeds and across time (Herd *et al.*, 2002; Hegarty *et al.*, 2007; Cassandro *et al.*, 2010; Cassandro, 2013), providing potential for improvement through genetic selection.

A potential mitigation measure would be the

use of individual variation within breed selecting for animals with lower CH<sub>4</sub> yield (g of CH4/kg of dry matter intake; Cavanagh et al., 2008; Vlaming et al., 2008). Recent forums have begun to address the potential effect of animal genetics on emission intensity at the individual animal and whole-farm levels (Chagunda et al., 2009; Wall et al., 2010). Genetic improvement of livestock is a particularly cost-effective way, producing permanent and cumulative changes in performance. However, scarce information is available on opportunities to mitigate enteric CH<sub>4</sub> via animal genetics. In particular, more knowledge is needed on the impact of current breeding programmes on CH<sub>4</sub> emissions on phenotypes that are more useful to achieve accurate estimation of breeding values for CH<sub>4</sub> reduction.

# Breeding for reducing enteric methane emissions at individual level

The genetic improvement is a tool that can be used to reduce emissions and at present there are 3 ways to obtain it: intensification of animal production; improving of system efficiency and the direct reduction of GHG emissions by breeding for reduced predicting animals that are high or low GHG emitters.

# Reduction of enteric methane by intensification of animal production

The first one is based on the intensification of animal production: breeding for improved efficiency of the animal leads to reduction of the total number of heads required to meet a given production level. An estimated drop of 8% of emissions might be obtained by reducing the declining animal number (Jardine *et al.*, 2012).

From a range of production and fitness traits, breeding studies found feed efficiency to have a large impact on reducing the GHG emissions from dairy systems (Jones *et al.*, 2008; Bell *et al.*, 2011). The genetic correlation between feed efficiency and enteric CH<sub>4</sub> emissions is positive and ranges from 0.18 to 0.84 (de Haas *et al.*, 2011). Feed efficiency can be assessed by feed intake required per unit product (gross efficiency) or by net or metabolic efficiency commonly calculated as residual feed intake (RFI; Jones *et al.*, 2008). Residual feed intake is a measure of the predicted intake of the animal on the basis of published feeding standards (based on milk yield, live weight and weight changes) in comparison to the observed feed intake (Archer *et al.*, 1999).

Residual feed intake is thus independent from production levels and this led some authors to suggest that it may represent inherent variation in basic metabolic processes (Kelly et al., 2011). Studies looking at selecting beef cattle for reduced RFI found that growth performance was not compromised and the lower expected feed intake resulted in less CH4 production (Okine et al., 2003; Hegarty et al., 2007; Nkrumah et al., 2006). Similar results were found also in meat sheep (Muro-Reves et al., 2011). However, some authors reported that cattle with low RFI may have the potential to contribute to reduced CH4 emissions under grazing systems only when provided with a high nutritional quality pasture source (Jones et al., 2011). Heritability estimates for feed efficiency ranged from 0.16 to 0.46 (Crews, 2005; Jones et al., 2008; Cassandro et al., 2010; de Haas et al., 2011). Low genetic correlations between RFI and other production traits imply that little or no genetic improvement has been previously obtained for RFI in beef cattle as a result of selection for production traits (Jones et al., 2008). According to Alford et al. (2006), the inclusion of RFI as a breeding objective for beef cattle will lead to an annual reduction of CH4 emissions by 3.1%. In dairy cows, an accurate estimation of RFI is difficult because changes in body tissue composition need to be fully accounted for, otherwise RFI is mathematically equivalent to energy balance (Veerkamp, 2002).

#### Table 1. Impact of livestock species in different production systems on greenhouse gas emissions.

	Ruminant species		Monogastric species	
	Extensive grazing	Intensive systems	Traditional systems	Industrial systems
CO <sub>2</sub> emissions from land-use change for grazing and feed-crop production		-	ns	
CO <sub>2</sub> emissions from energy and input use	ns		ns	
Carbon sequestration in rangelands	++	ns	ns	ns
Methane emissions from digestion			ns	

- , scarce effect; +, positive effect; ns, not significant





Residual feed intake is an overall index of digestive and metabolic efficiency and it is considered a useful trait for studying the physiological mechanisms underlying variation in productive efficiency (Richardson, 2003). Digestibility, protein turnover, tissue metabolism and stress, physical activity and heat increment for fermentation account for more than 70% of the variation of RFI (Richardson and Herd, 2004). Basal metabolic rate is associated with cellular processes, as protein turnover and mitochondrial function, and accounts for the largest fraction of total energy expenditure and for the large majority of interanimal variations. A recent research (Kelly et al., 2011) has examined these processes in productive cattle, providing evidence of association between mitochondrial biogenesis and energy efficiency and suggesting that the expression of some genes and their transcriptional regulators may provide potential indicators for genetic variation in feed efficiency. Several neuropeptides are involved in the complex metabolic regulation of feed intake, such as orexigenic neuropeptide Y or the anorexigenic transcripts pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript, which regulate the expression of some key genes and of leptin, GH and ghrelin receptors (Niemann et al., 2011). Moreover, some recent insight about the association of specific SNPs of leptin and ghrelin genes with voluntary feed intake are a first step to apply the genomic selection also for latter trait (Lagonigro et al., 2003; Banos et al., 2008). Genomic selection is especially promising when phenotypes are available only on a representative sample of the population, because measure of traits is difficult or expensive. Genomic breeding values are calculated as the sum of the effects of dense genetic markers that are approximately equally spaced across the entire genome, thereby potentially capturing most of the genetic variation in a trait. Here the prediction equation is formed in a reference population with genotype and phenotype data. The equation can be used to predict breeding values in animals that are genotyped but without phenotypic data. The availability of SNP chips at affordable prices has made implementation of this technology commercially feasible. For example, many countries now publish genomic breeding values for bulls on a range of traits of economic importance. Recently, three different genomic association studies revealed 150, 161 and 111 SNPs, associated with a significant allele substitution effect on RFI in beef cattle (Barendse et al., 2007; Sherman et al., 2010; Bolormaa et al., 2011). In Holstein cattle, 8 SNPs with large effects on RFI were located on chromosome 14. These SNPs may be associated with the gene NCOA2, which has a role in controlling energy metabolism (Pryce et al., 2012). Collaborative efforts among research organizations in the Netherlands, the UK and Australia have already demonstrated that the accuracy of genomic predictions of dry matter intake can be increased by combining datasets (de Haas et al., 2011). Since RFI seems to be a trait reflecting inherent inter-animal variation for some biologically relevant processes, which are related to the metabolic efficiency, it is likely that a single biomarker would not be an accurate index of this phenotype, especially if an absolute measure is required (Herd and Arthur, 2009). A relationship between metabolic profiles in biological fluid (blood, urine, milk) and productive efficiency of the animals has been extensively reported. Among metabolic profiles, free fatty acid concentration, 3hydroxybutyrate and urea in blood, 3-hydroxybutyrate and urea in milk (Kelly et al., 2010; 2011), total purine derivatives excretion (Stefanon et al., 2001) or total purine derivatives to creatinine ratios (Susmel et al., 1995) in blood and urine are often suggested as potential candidate biomarkers of feed and metabolic efficiency. According to Spicer et al. (1990) and Herd and Arthur (2009), the significant variations of blood metabolites are not only related to feed intake, diet composition, and physical activity, but also to the genotype of the animals.

Dairy animals experience a large variety of stressors that can modify normal behaviour and growth, leading to losses in performance (Amadori et al., 2009). Normal physiological events such as calving, milk yield, weaning and group rearrangement can cause metabolic and environmental conditions which lead to stress and a consequent loss of animal welfare (Stefanon et al., 2005; Gygax et al., 2006; Lykkesfeldt and Svendsen, 2007) and a consequent decrease of safety and quality of products. Under these stressful conditions, the hypothalamic-pituitary-adrenal axis, the autonomic nervous system and the immune system are called into action to reestablish homeostasis (Fukasawa and Tsukuda, 2010; del Rosario González-de-la-Vara et al., 2011). Stress modifies the secretion of various hormones, which differentially affect the immune system and blood constituents with consequences that depend on the type of stimulus, the species, the sex and the individual considered (Amadori et al., 2009). Among the stress responsive hormones, glucocorticoids play an important role in shaping immunity by influencing immune cell trafficking to sites of

inflammation and altering downstream, adaptive immune responses by causing a shift from cellular (Th1/inflammatory) to humoral (Th2/anti-inflammatory) type immune responses (Elenkov and Chrousos, 1999). Moreover, activated macrophages, endothelial cells, lymphocytes and other immunity related cells induce the synthesis of pro-inflammatory cytokines (Steptoe et al., 2001), which stimulate the liver to synthesize positive acute phase proteins (APP+), such as haptoglobin and ceruloplasmin (Bionaz et al., 2007). Another important consequence of animal response to stress can be the increase of oxidative metabolism of glucose and lipids. Cortisol diverts body resources to the blood stream to face with the increasing demand of energy and protein substrates required to respond to the stressors, reducing the overall efficiency of the animal (Blecha, 2000). All these phenomena can lead to an increase of free radicals, which give rise to cellular damages and alteration of homeostasis (Sgorlon et al., 2007). However, the biological response to stress is variable and depends on the physiological conditions (not cognitive stress) and on how the animal perceives the environmental conditions (cognitive stress), but a common feature of these situations is an increase cortisol secretion. Even though many methods of welfare evaluation are reported in the literature, cortisol is one of the gold standards among the biomarkers to depict the level of animal response to stress. Association studies between cortisol secretion of the genetic variation of hypothalamic-pituitary-adrenal axis can assist in the selection of dairy cows with an higher adaptation to environmental and physiological stresses, decreasing the negative effects of immune suppression and catabolic activity and enhancing the quality of life of the animals in the farm.

The two phenotypes are part of the same adaptative response of the animal to the environment. Correlation between stress response and cortisol secretion and metabolic efficiency of cattle, measured as RFI has been recently investigated (Montanholi et al., 2010). In beef steers, to evidence for genetic associations for RFI with plasma cortisol and blood cell contents have been published, indicating that animals with high-RFI (low efficiency) are more susceptible to stress (Richardson and Herd, 2004). It is likely that only a system biology approach will help to identify the factors driving the productivity of the dairy cows that can be associated to genotypes and integrated in genomic selection.





# Reduction of enteric methane by improving of system efficiency

Similarly to the first way, the second way is based on the improving of system efficiency, but taking into consideration functional traits that can reduce wastage from the system and therefore GHG emissions. Garnsworthy (2004) reported a reduction of 10-11% in CH<sub>4</sub> emissions if dairy fertility is improved. Fertility has a major effect on the replacement rate of the herd because scarce reproductive performances are associated to a higher number of young livestock to be reared. Moreover, although first calving at 24 months of age is a target, many herds calve heifers at an older age. All these aspects have a direct effect on the total herd emissions of CH<sub>4</sub>. At the genetic level, the improvement of milk yield over the past 20 years has been associated to a decrease of fertility levels (Royal et al., 2002). Increased milk yield is beneficial to reductions of CH<sub>4</sub> emissions per unit of product, but it is important that effects of reduced fertility do not outweigh them. Therefore, over the long term, fertility traits included in a selection index should be considered a positive way to reduce environmental impact as much as to preserve fertility.

# Reduction of enteric methane by direct selection of best animals greenhouse gases emitters

The third way is based on the direct reduction of GHG emissions using selection for reduced predicting CH<sub>4</sub> emission by individual level defining animals that are high or low GHG emitters (knowing the different diets and different systems conditions). Many factors influence ruminal CH<sub>4</sub> emissions, including feed intake and composition and alterations in the ruminal microflora (Chilliard et al., 2009). Different strategies have been used to suppress methanogenesis, including chemicals, antimicrobials, vaccination, organic acids and microbial feed additives, each with limited success (Hook et al., 2009). Recently, host genetics has demonstrated to play a role in determining the microflora composition in the gut of model organisms (Benson et al., 2010) and in the rumen of dairy cows (Garnsworthy et al., 2012).

These possibilities open the option of using an integrated approach to reduce carbon footprint in dairy farms, for which in addition to dietary and manure management strategies it is possible to include also animal selection for lower environmental impact, to reach a permanent decrease of GHG emissions from ruminants.

To pursue this strategy a substantial number of animals have to be measured individually for CH<sub>4</sub> production, a task still too demanding in terms of cost and efforts to be applied routinely.

# Direct and indirect measures of methane emission

The gold standard to measure CH<sub>4</sub> and GHG emissions is the respiratory chamber, but this technique is not easily applicable, because is time consuming and costly, especially if a large number of measurements are needed, as in the case of breeding schemes. The prediction of CH<sub>4</sub> emission can be obtained with several systems, that can be classify as feed intake records, breath analysis and cow characteristics and milk composition records (Table 2).

Methane emissions can be accurately measured by placing animals in sealed chambers with appropriate measures of gas flow and composition (Blaxter and Clapperton, 1965;

#### Table 2. Methods to predict methane emission (PME) using different variables.

Method	r	Reference
PME from breath analysis		
Respiratory chamber	0.96	Place <i>et al.</i> , 2011
Head hoods	0.96	Place <i>et al.</i> , 2011
SF6 tracer technique	0.83	Muñoz et al., 2012
Green feeder	0.89	de Haas <i>et al.</i> , 2011
Laser methane detector	0.80	Chagunda and Yan, 2011
FTIR- Fourier Transform Infrared Spectroscopy	0.89	Garnsworthy et al., 2012
PME from milk records		
$CH_4$ (g/kg DM) = 24.6 (± 1.28) + 8.74 (± 3.581)×C17:0 anteiso - 1.97 (± 0.432)	0.85	Dijkstra <i>et al.</i> , 2011
×trans-10 + 11 C18:1-9.09 (± 1.44)×cis-11C18:1 + 5.07 (± 1.937) ×cis-13C18:1		-
PME from feed intake records		
$CH_4 (MJ/d) = 3.23 (\pm 1.12) + 0.809 (\pm 0.0862) \times DM$ Intake (kg/d)	0.65	Ellis <i>et al.</i> , 2010
$CH_4$ (Mcal/d) = 0.814 + 0.122* Nitrogen Free Extracts (kg/d) + 0.415*	0.72	Moe and Tyrrell, 1979
Hemicellulose (kg/d) + 0.633 * Cellulose (kg/d)	-	(cited from Demeyer and Fievez, 2000)
		Van Es, 1978, IPCC, 2000, 2006
$CH_4$ (g/d) = feed intake (kg of DM/d) × 18.4 (MJ/kg of DM)/0.05565 (MJ/g)		Bannink <i>et al.</i> , 2011
$\times 0.06 \times \{1 + [2.38 - \text{level of intake (multiples of maintenance level)}] \times 0.04\}^{\circ}$		
$CH_4$ (g/d) = [grass or grass silage (kg of DM/d) × 21.0 (g/kg of DM) + concentrates	-	
(kg of DM/d) $\times$ 21.0 (g/kg of DM) + corn silage (kg of DM/d) $\times$ 16.8 (g/kg of DM)]		
$\times \{1 + [2.38 - \text{level of intake (multiples of maintenance level}) \times 0.04]\}^{\#}$		

r, correlation with respiratory chambers; °18.4 MJ/kg: energy released by each unit of feed DM (Van Es, 1978), 0.05565 MJ/g: energy generated by methane (IPCC, 2006), 0.06 × gross energy intake (GE, MJ/d): methane production level in MJ/d (IPCC, 2000), 2.38 × maintenance feed intake level: energy requirements scaled to an average cow at feed intake level, 0.04: correction factor of 0.04 per unit feed intake level; <sup>1</sup>g/kg of DM: CH<sub>4</sub> production for 1 kg DM of grass, grass silage or concentrate, 21 g/kg of DM: CH<sub>4</sub> production for 1 kg DM corn silage.





Moe and Tyrrell, 1979); however, diets eaten by cows in chambers may differ from that selected by grazing animals (Clark, 2002). The majority of ruminants graze under extensive conditions, are free ranging, and select a variety of forage types. Their CH<sub>4</sub> production must be determined to calculate inventory. The sulfur hexafluoride  $(SF_6)$  tracer technique is often used to measure CH4 emissions from grazing ruminants (Johnson et al., 1994; Woodward et al., 2006; Lassey, 2007; Pinares-Patiño and Clark, 2008), and although data appear to be defensible and repeatable, additional validation would provide a degree of certainty to CH<sub>4</sub> inventory. Studies with beef cattle and sheep indicate that CH4 estimated with the SF<sub>6</sub> tracer technique is 93 to 95% of that measured using whole-animal chambers (Johnson et al., 1994; Ulyatt et al., 1999; McGinn et al., 2006) and 105% of that measured using hood chambers (Boadi et al., 2002). The lower estimates using the SF<sub>6</sub> tracer technique are partly explained by the CH<sub>4</sub> released via the rectum (Murray et al., 1976). Measuring CH<sub>4</sub> production directly from animals under practical conditions is currently difficult and hampers direct selection on reduced enteric CH<sub>4</sub> in practice.

In the literature several linear and non-linear models have been developed to predict individual CH4 emission, starting from diet composition and feed intake records (Ellis et al., 2009; 2010). Also, the feasibility of using some milk components as predictor of methane emission has been evaluated. Milk odd and branched chain fatty acids can predict CH<sub>4</sub> production in dairy cattle as a consequence of their high potential to predict rumen volatile fatty acids. There is, in fact, a molar stoichiometrical relations between rumen volatile fatty acids and CH<sub>4</sub> proportions. Analysing data from 3 experiments of dairy cattle with a total of 10 dietary treatments and 50 observations, Dijkstra et al. (2011) demonstrated that milk odd and branched chain fatty acids profiles could predict CH<sub>4</sub> production in dairy cows. In this case CH4 was measured using open circuit indirect respiration calorimetry chambers. The C14:0 iso, C15:0 iso and C17:0 anteiso are positively related to CH<sub>4</sub> production, whereas C15:0 and C17:0 are negatively related to CH<sub>4</sub> production (Fievez et al., 2012). The use of mid-infrared technologies seems to be a promising approach to decrease the cost for CH4 measuring. In the last five years, several studies demonstrated the feasibility of mid-infrared spectroscopy to predict fatty acid composition of milk (Soyeurt et al., 2006; De Marchi et al., 2011) that, in turn, may be used to predict CH<sub>4</sub> emission. Recently, the prediction of  $CH_4$  emissions directly from mid-infrared spectra of milk has been proposed. In fact, the milk spectrum reflects the milk composition, which is considered linked to  $CH_4$  because of the relationship of both phenomena to ruminal fermentation (Dehareng *et al.*, 2012).

A huge community of ciliate protozoa, anaerobic fungi, bacteria and Archaea live in the rumen. Archaea make up only a small part of the microbial biomass but are the largest CH<sub>4</sub> producing populations in the rumen. The molecular analysis of archaeal population indicated that methyl coenzyme-M reductase gene is common to all methanogen species and will be used as biomarker for the quantification of methanogenic population. Archaeal membrane lipids can also be used as biomarkers to quantify the Archea rumen population, since they are different and can be distinguished from bacterial membrane lipids (Gill *et al.*, 2011).

Therefore, further research should focus more on the following aspects which have some evidence of correlation to CH<sub>4</sub> emission in dairy cattle: i) the quantity of methyl coenzyme-M reductase genes in cow faeces; ii) the quantity of archaeol in cow faeces. Archaeol is a cell wall component specific of ruminal Archaea, the major CH<sub>4</sub> producers in the rumen; iii) milk fatty acid profile; and iv) midinfrared spectra of milk, which can be used to predict individual CH<sub>4</sub> emission.

## Breeding for reducing enteric methane emissions at feeding system level

Domestic animals are the world's largest users of land resources, with grazing land and cropland dedicated to the production of feed. The livestock sector uses around to one-third of total cropland, equivalent to 3.4 billion hectares for grazing and 0.5 billion hectares for feed crops (Steinfeld *et al.*, 2006). Management practices and use of pastureland vary widely, as does the productivity of livestock per hectare. In arid and semi-arid rangelands, where most of the world's grasslands are found, intensification of pastures is frequently technically unfeasible or unprofitable.

On the other hand, in intensive systems due to the profitability of Holstein cows, Holstein genes are present in a large proportion of dairy cows, particularly by North American. Larger North American Holstein-Friesian cows have been found to show better response in milk yield with higher proportion of concentrates in their diet than smaller genotypes such as the New Zealand Holstein-Friesian, which have been selected for higher milk yield performance from pasture (Dillon et al., 2006). Cows which were ~88% North American Holstein and selected on increased milk fat and protein production were found to grow faster and had increased kg milk per kg of DMI during their productive life when on a high energy dense diet, compared to cows unselected on the same diet (Bell et al., 2010). Selected animals have high genetic potential for mobilising body energy reserves for production, which has been found to have deleterious effects on health and fertility (Pryce et al., 1999; Dillon et al., 2006), particularly later in life (Wall et al., 2010). Selected cows produced lower CO<sub>2</sub>-eq. emissions per kg energy corrected milk compared to non-selected cows both on low and high content of forage in the diet (Figure 1).

Systems emissions can be reduced by enhancing herd health and fertility and by reducing the number of replacement animals retained on the farm to reduce wastage (Garnsworthy, 2004; O'Mara, 2004; Tamminga et al., 2007). Cows of predominantly North American Holstein genes may be better suited to high energy dense feeding systems, rather than a diet containing a high proportion of forage. In contrast, the performance of animals of New Zealand origin had higher yields of milk solids and better fertility compared to cows of North American origin when compared on a range of grazing systems (MacDonald et al., 2007). Therefore, selecting animals for an environment is important. In a study in the US (Capper et al., 2009), good health and welfare in modern high input systems (cows of 90% Holstein genes) was reported, with better production efficiency and CO<sub>2</sub>-eq. emissions per unit product compared to the past. This may be explained by optimal nutrition being provided to these animals, which may not hold true for the same cows on a lower quality forage diet.

However, the intensification of farm systems as a tool to reduce  $CH_4$  emissions has been recently reconsidered, taking into consideration also the link between milk and meat production. Since in many countries dairy cattle population significantly contributes to meat production, the system intensification should lead to a reduction of dairy cattle population and to an increase of beef cattle to maintain the same levels of meat production. In this case the overall  $CH_4$  emission by livestock sector should increase as a consequence of the increasing number of beef cattle (Puillet *et al.*, 2012).

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Figure 1. Carbon dioxide equivalent (CO<sub>2</sub>-eq.) emissions per kg energy corrected milk (ECM) for cows selected for increased milk fat and protein fed a low proportion of forage ( $\bullet$ ) and a high proportion (x) of forage in their diet and cows selected to represent the average for milk fat and protein production fed a low proportion of forage ( $\bullet$ ) and a high proportion ( $\diamond$ ) of forage in their diet (Bell *et al.*, 2011).

### Reduction of enteric methane by technological and management innovations

To bring about reductions in livestock GHG emissions, it has been suggested (Garnett, 2009) that significant technological innovations will be required in the future, in addition to managing our consumption of animal products. Technologies that can bring affordable efficiencies to production are being developed. Using genomic information, such as genomic breeding values for feed related traits described previously, and sexed semen (Weigel, 2004; Cerchiaro *et al.*, 2007) offer the potential for better selective breeding.

Therefore, genetic interactions among diet, animal and rumen microbes should be considered as new target area for animal breeding activity. Moreover, in the next future more research should be done to improve efficiencies in utilising diet (*e.g.*, changing feed, feed additives, genetics, targeting rumen bugs), but also to improve efficiencies in livestock systems (*e.g.*, improving fertility, health, longevity, resource management) and in manure and fertiliser management (*e.g.*, cover tanks, anaerobic digestion).

Reducing the number of unproductive animals on a farm can potentially improve profitability and reduce CH<sub>4</sub>.

Strategies such as extended lactation in dairying, where cows calve every 18 months

rather than annually, reduce herd energy demand by 10.4% (Trapnell and Malcolm, 2006) and thus potentially reduce on-farm CH4 emissions by a similar amount (Smith *et al.*, 2007). With earlier finishing of beef cattle in feed lots, slaughter weights are reached at a younger age, with reduced lifetime emissions per animal and thus proportionately fewer animals producing CH<sub>4</sub> (Smith *et al.*, 2007).

### Conclusions

The livestock sector has enormous potential to contribute to climate change mitigation. Realizing this potential will require new and extensive initiatives at the national and international levels, including: the promotion of research on and development of new mitigation technologies; effective and enhanced means for financing livestock activities; deploying, diffusing and transferring technologies to mitigate GHG emissions; and enhanced capacities to monitor, report and verify emissions from livestock production.

The new rules of livestock sector should account for environment safeguard and climate changes, as limitation of GHG. The application of technologies that improve the efficiency of land use and feed use can mitigate the negative effects of livestock production on biodiversity, ecosystems and global warming. Hence, genetic improvement of livestock population seem to be a cost effective strategy for reducing enteric  $CH_4$  production, and therefore to mitigate GHG emissions.

The current breeding goal includes the impact of production and fitness traits on system profitability but in the future traits that taken into account for mitigation of GHG emissions should be evaluated. New tools that provide accurate measures and estimates of GHG emission from livestock and their systems, supported by breeding tools, such as genomic selection and new goals, should be considered to address environmental impact of livestock species. Indeed, current breeding goals can be developed to consider wider environmental issues because genetic improvement has a role in reducing GHG emissions and is cost-effective. In conclusion, in the near future is neccesary to spend a lot more research in this area to be able to experiment with new tools and define new traits related to GHG emissions.

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