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Review

Creating a low enteric methane emission ruminant: what is the evidence of success to the present and prospects for developing economies?

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Abstract. Enteric methane emissions from livestock constitute a greater part of anthropogenic greenhouse gases (GHGs) in Africa, than in more industrialised economies, providing a strong incentive for the development of low methane phenotype ruminants. Although dietary and husbandry options already exist for lowering methane production, means of changing 'methane status' of animals enduringly has a strong appeal. This paper is a critical review the empirical success to date of attempts to alter this status. Introduction of reductive acetogens, defaunation, anti-methanogen vaccines, early life programming and genetic selection at both the rumen and animal level are considered in turn. It is concluded that to date, there is little *in vivo* evidence to support the practical success of any of these strategies, save selective breeding, and this at a high cost with unknown efficacy. Finally, it is suggested that for developing economies management and nutritional strategies to reduce emissions will have the greatest and most immediate impact, at the lowest cost.

Additional keywords: anti-methanogen, defaunation, early life programming, reductive acetogenesis, rumen biome.

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Introduction

Enteric methane emissions from ruminants are of wide concern to government, to environmentalists, aid organisations, and arguably to the wider or general community, because of the interrelationships between food production, human health and enteric methane's adverse effect on climate. It has been argued that it would be best if domestic ruminants were eliminated as a human food source (Sabaté and Soret 2014); however, others suggest that such an action would not be an efficient use of available resources and question if the future human population could meet its food needs if such a course were adopted (Rojas-Downing *et al*. 2017). This is crucially so in sub-Saharan Africa where the consumption of animal-based proteins is low, but essential to the basic nutritional requirements of some of the world's most economically vulnerable people. Unfortunately, large livestock numbers with low productivity and relatively low levels of industrial development mean that the ruminant contribution to anthropometric greenhouse gas (GHG) emissions on the African continent is the largest component of national GHG inventories in many countries. This provides a compelling case for developing and implementing practical and effective strategies to reduce GHG emissions in African livestock.

Enteric emissions can be reduced or mitigated in several ways. Several dietary manipulations such as the use of lipids (Machmüller *et al*. 2000) or chemical feed additives (e.g. bromochloromethane; (Denman *et al*. 2007), have been demonstrated to reduce enteric methane production in ruminants. Improving animal productivity and thereby decreasing emissions per unit of animal product produced is well recognised as an effective way to reduce the carbon footprint of livestock. Dietary and management strategies to ameliorate the impact of ruminants on GHG levels have been the subject of considerable scientific enquiry and has been well summarised in several authoritative reviews (Beauchemin *et al*. 2008; Hegarty *et al*. 2010).

Regardless of known and effective strategies for reducing enteric emissions, the impetus to create, or develop ruminants that emit lower levels of methane permanently or semi-permanently, without the need for ongoing (human) intervention is strong. Theoretically, as many have claimed (Iqbal *et al*. 2008; Mitsumori and Sun 2008; Kumar *et al*. 2014) this should be achievable, and realising this goal would be a game-changer, particularly in developing economies where ruminant livestock production systems are a significant contributor to emissions The process of enteric methane production is a complex one, involving multiple microbial consortia and their interactions with the host itself (Leng 2018). Thus, there are several potential modalities for interfering or modulating the process of methanogenesis at either at the level of the rumen biome or the mammalian host level. Permanently altering the rumen environment by introducing organisms that compete with or are inimical to rumen *Archea*, inducing a host immune response to methanogens or genetic selection of a low-methane phenotype animal are all paths by which, at least in theory, enteric methane production may be permanently reduced. However, results

in vivo frequently fail to match those predicted by modelling, molecular or *in vitro* studies. This review specifically examines the animal experimental evidence to date for producing the 'low methane' ruminant, whether by manipulating the rumen population or the host, or both.

Changing the animal: altering the rumen microbiome

The rumen is an ecologically complex environment, but there are documented instances of the successful transfer of novel organisms to improve rumen function (Jones and Megarrity 1986). This section examines the evidence of reducing methane production using modalities that attempt to enduringly alter the rumen environment.

Reductive acetogens

The process of reductive acetogenesis (RA) takes the same reactants used in methanogenesis (CO_2 + H_2) and transforms them into acetate via an alternate biochemical process. This is a process commonly found in anoxic natural systems such as lake sediments, but generally accounts for less than 5% of hydrogen utilisation in these systems (Lovley and Klug 1983) perhaps due to the hydrogen concentration threshold for uptake by methanogens being 10^{-1} to 10^{-2} of that for acetogens (Cord-Ruwisch *et al*. 1988).

Notwithstanding this, establishing the process of reductive acetogenesis in ruminants has become something of a 'holy grail' of enteric methane reduction. If acetogenic bacteria could be established and compete effectively in the rumen, this colonisation would have the dual benefit of reducing (possibly eliminating) the animals' requirement for methanogenesis to dispose of excess hydrogen, while simultaneously providing additional energy substrate for the host animal (resulting in more complete usage of feed). The potential for RA to reduce methane emissions in ruminants was reviewed by Joblin (1999) who concluded that 'it is too early to discard the possibility for reductive acetogens competing with or acting in concert with methanogens'. However, evidence to suggest that acetogenic bacteria will grow in the conditions prevalent in the rumen, using the process of RA, is sparse. RA, along with methanogenesis, is a critical metabolic process in termites, where acetogens apparently co-exist with methanogens (Breznak and Kane 1990). Schmitt-Wagner and Brune (1999) found that both groups are present in termites because they are highly localised, with acetogens existing where there is the highest partial pressure of hydrogen and methanogens predominate more distally where hydrogen concentration is considerably lower. Leadbetter *et al*. (1999) discovered that the separation is facilitated by the attachment of acetogens to spirochetes resident in the termite gut.

RA in the large intestine has been estimated to provide 0.25% of the energy requirements of rats, rabbits and guinea pigs (Yang *et al*. 1970; Prins and Lankhorst 1977). Graeve and Demeyer (1990) found circumstantial evidence for the existence of RA in the hindgut of cattle as well as of pigs, and this was confirmed by later *in vitro* studies (De Graeve *et al*. 1994). Evans *et al*. (2009) latterly identified the existence of RA in the foregut of the tamar wallaby (*Macropus eugen*), whereas in humans, a minority of the population have detectable levels of methanogenic *Archaea* (MA) (Bernalier *et al*. 1996) and it appears that MA have a competitively exclusive relationship with acetogenic bacteria (AB) (Doré *et al*. 1995), a phenomenon also observed in new born lambs (Morvan *et al*. 1994).

RA have been isolated from ruminants including deer (Rieu-Lesme *et al*. 1995) and lambs (Rieu-Lesme *et al*. 1996) as well as cattle (Greening and Leedle 1989). However, attempts to grow acetogens in mixed culture *in vitro* have only been possible using partial pressures of hydrogen far above that encountered in a normally functioning rumen, or chemical suppression of methanogens, or both, to be successful (Nollet *et al*. 1997; le Van *et al*. 1998; Nollet *et al*. 1998; Lopez *et al*. 1999). Early attempts to induce RA *in vivo* have been reviewed by Fievez *et al*. (1999), who concluded that all attempts, even after suppression of MA, were unsuccessful. More recently Fonty *et al*. (2007) reported successfully establishing and maintaining a population of RA species in gnotobiotic lambs; however, methane production was not recorded and hydrogen production and utilisation were estimated indirectly from volatile fatty acids (VFA) stoichiometry. The study concluded that methanogens increased quickly to normal densities when they were introduced to the rumen of acetogen-colonised lambs.

It appears that the critical requirement for RA to establish as a significant metabolic process in the rumen is a high partial pressure of hydrogen (Weimer 1998). Further, evidence suggests RA cannot occur in the rumen unless MA are permanently suppressed, with unknown consequences for the host animal. Thus, it appears that because MA have such a high affinity for hydrogen, the likelihood of RA being a substantial hydrogen sink, thus reducing production of methane in the rumen is low.

Defaunation

Elimination of protozoa from the rumen (defaunation) has been the subject of considerable interest and investigation over the last 50 years. Although ubiquitous, ciliate protozoa are not essential to proper functioning of the rumen, and it was suggested that their absence may lead to improved production efficiency (BRYANT 1970). Trials undertaken to assess the effect of defaunation on animal productivity have generally shown that growth, and in particular wool growth in sheep, improves, especially where rumen bypass protein is limited (Bird and Leng 1978; Bird *et al*. 1979; Eugène *et al*. 2004*b*) From a meta-analysis of defaunation trials it was concluded that although feed digestibility was lower in defaunated sheep, there were substantial increases in microbial nitrogen outflow with a concomitant decrease in rumen ammonia concentration (Eugène *et al*. 2004*a*) and such changes are consistent with the notion that elimination of protozoa would diminish wasteful lysis and intraruminal recycling of bacteria.

Protozoa do not possess H^+-utilising (i.e. propiogenic) metabolic pathways, and many methanogens form a close physical and probably symbiotic relationship with ciliates (Chagan *et al*. 1999). This has led to the suggestion that defaunation may be an effective strategy to reduce enteric methane production (Hegarty 1999). There is some evidence that defaunation decreases methane production in sheep (Kreuzer *et al*. 1986) and cattle (Whitelaw *et al*. 1984).

Overall the evidence is equivocal, if not contradictory and the effect of defaunation on enteric methane production not clear, with Bird *et al*. (2008) failing to find differences in methane production between faunated and defaunated ewes at 10 and 25 weeks after treatment. In studies using medium chain fatty acids (MCFA) to suppress protozoa in sheep (Machmüller *et al*. 2000, 2003) the authors attributed the decrease in methane to MCFA's inhibitory effect on methanogens themselves, as well as reducing protozoa. A definitive study of lambs born fauna-free to previously chemically defaunated ewes, Hegarty *et al*. (2008) found no difference in enteric methane production between faunated and fauna-free animals. Thus, it appears that although substantial gains in productivity may be realised through the defaunation of ruminants, decreasing energy losses through the diminution of enteric methane production, does not appear to form part of those gains.

Host response: immunisation

Early trials by researchers in Western Australia produced two vaccines designed to induce an immune response to rumen methanogens (Wright *et al*. 2004). Plasma IgA and IgB antibodies titres in sheep showed a significant response post-immunisation. However, extensive testing and repeated applications of the vaccines failed to produce significant reductions in methane production (*P*-value 0.401–0.751), except on one occasion where a 7.7% decrease of CH_4 per unit dry matter intake (DMI) ($P = 0.051$) was observed. A vaccine prepared on a similar principle (i.e. using cell extracts from the target species) with the aim of reducing rumen protozoa (Williams *et al*. 2008), showed little response, as did a vaccine based on five phylotypes of methanogens (Williams *et al*. 2009), suggesting approaches based on a limited number of species of methanogens is unlikely to be effective.

Both Attwood *et al*. (2011) and Wedlock *et al*. (2013) have emphasised the importance of developing an effective methanogen vaccine that targets all methanogens, to prevent non-target species expanding to fill the ecological niches left by those selectively eliminated, while avoiding affecting non-target organisms. This will require the identification of some common features, such as a shared surface protein, unique to Archaea, so as not to interfere with or compromise the function of other bacterial consortia playing crucial roles in ruminal digestion. Recent trials using a recombinant protein as a potential antigen against methanogens elicited strong antibody responses in both cattle (Subharat *et al*. 2015) and sheep (Subharat *et al*. 2016), but neither study quantified rumen methanogens or enteric methane production post-immunisation. Using a different protein, but employing a similar approach, Zhang *et al*. (2015) also observed strong immune responses in saliva and plasma, yet failed to detect any reduction in either rumen methanogens or enteric methane production in inoculated goats. It is concluded that although conceptually appealing, work to date has produced very little actual evidence for the efficacy of methanogen vaccines on the production of enteric methane *in vivo*.

Host response: early life programming (ELP)

The influence of diet during early life on the bacterial community (Eadie *et al*. 1959) and physical structure of the rumen (Greenwood *et al*. 1997) is well recognised. This interrelationship has led to speculation that dietary or other interventions during early life might be able to influence the life-time microbial community of ruminants (Morvan *et al*. 1994) and thus affect life-time methane production. Abecia *et al*. (2013, 2014) explored the effect of ELP on enteric methane production in lambs using bromochloromethane to suppress methanogens in new-born lambs and their dams, but reported that the reduction in methane production lasted only as long as treatment persisted, although noting a longer term change in the archaeal community. In contrast, De Barbieri *et al*. (2015*a*) observed persistent changes in the rumen microbial community of lambs inoculated with rumen content from different sources; however, this did not translate into differences in enteric methane production (De Barbieri *et al*. 2015*b*). This approach appears to have some promise, but it is clear our understanding of effects and modalities of ELP are at a preliminary stage (Yáñez-Ruiz *et al*. 2015) and there are no practical applications to reduce enteric methane at present.

Conclusion

There are several potential modalities available to reduce enteric methane emissions by altering the rumen population through extraneous means. Although initially promising, after extensive testing it seems clear that the introduction of reductive acetogens and elimination of ciliates will not produce the desired effect. Although attempts are still ongoing, there has been a similar lack of success in producing an effective methanogen vaccine and understanding of ELP is still at a preliminary stage. Thus it is concluded that at present there are no demonstrated technologies that will reduce enteric methane by altering the rumen biome in an enduring manner.

Changing the animal: the low-methane phenotype

Substantial differences in methane production have been observed between animals consuming the same quantity of a given diet (Blaxter and Clapperton 1965; Lassey *et al*. 1997; Ulyatt *et al*. 1999; Hegarty *et al*. 2007). There is evidence to indicate that digestibility of feed is inversely related to methane production per unit intake (e.g. (Gordon *et al*. 1995; Yan *et al*. 2000), although intake and digestibility are frequently conflated in the literature. In any case, these factors fail to explain why animals under identical conditions should have different methane yields (MY; gCH_4/MJ of digestible energy intake). Observed differences between animals in MY under equivalent conditions may be due to differences in the animals' digestive physiology, in the rumen microbial community, or a combination of both. Partially shifting the site of digestion, or alterations in the bacterial and ciliate populations each have the capacity to change the amount of methane generated per unit of energy ingested through changing the amount and profile of VFA, the partitioning of energy between cell growth and maintenance, and ultimately the amount of hydrogen generated. The extent to which each of these factors are important in determining between-animal differences in MY, and the degree to which they are labile to manipulation will determine the theoretical potential of developing a 'low methane phenotype' (LMP) animal.

Contribution of digestive physiology

Residence time of digesta, along with composition of the microbial population, may each be influenced by mean (rumen) retention time (MRT). Although level of food intake is negatively correlated with MRT (Grovum and Williams 1973; Evans 1981), voluntary feed intake is positively related to rate of eating (Forbes *et al*. 1972), which is itself highly repeatable among diets and over time for individual animals, but is highly variable between individual animals (Frisch and Vercoe 1977). So it can be deduced that individual variation in rate of eating may be expressed in differing MRT and it has also been demonstrated that whole tract digesta time varies more between animals than for individuals across diets (Bines and Davey 1970). Thus, variables directly under the control of the animal will affect important facets of digestive physiology in the ruminant, but the case for digestive physiology having a direct impact on MY goes well beyond intake and rate of eating.

The evidence for MRT and associated rumen parameters being a significant determinant of MY is quite compelling. Okine *et al*. (1989) found cattle with weights in their rumens produced 29% less methane than control animals fed the same amount of the same diet and that methane production was inversely correlated (r = –0.53) with outflow of rumen particulate matter. Similarly, Smuts *et al*. (1995) demonstrated that sheep selected for high wool growth had higher rumen outflows and consequently, higher microbial outflow from the rumen than low-wool growth sheep. Pinares-Patiño *et al*. (2003) observed that rumen outflow rate accounted for ~57% of the difference in MY in sheep fed a restricted diet (1.3 times maintenance), whereas Barnett *et al*. (2012) clearly demonstrated that manipulating gut motility and reducing transit time would decrease MY in sheep at a given intake. The critical role of rumen digestion parameters in determining enteric methane production has been confirmed in studies using previously identified low MY (LMY) and high MY (HMY) sheep., where LMY was strongly associated with not only decreased MRT in both solid and liquid phases, but also smaller rumen volume and differences in rumen contents (Goopy *et al*. 2014; Bond *et al*. 2017). Daily Methane Production and more recently, MY have been shown to have a low but significant heritability (h^2 = 0.13) with distinct sire differences and so there is scope for genetic selection (Robinson *et al*. 2010; Pinares-Patiño *et al*. 2011, 2013). Because MY is a complex trait that is technically challenging to measure, discovering the mechanism(s) by which animal genotype affects MY may help in the identification of proxies which are indicative of MY. This is consistent with the findings reported by Barnett *et al*. (2012), who demonstrated that a reduction in whole-tract MRT (induced by injections of triiodothyronine every second day) also reduced MY, identifying the possibility that blood triiodothyronine concentration may be a factor by which animal genotype affects MRT and so a possible indicator of proxies for MY. To this end, Clauss and Hummel (2017) suggested that selective breeding of ruminants for increased liquid digesta flow rates is likely to be an efficacious strategy to reduce MY, although how this might be undertaken in practical terms, is not addressed. Although it is yet to be investigated empirically, a final consideration is the possible impact of selection for LMY on animal productivity. If decreased rumen volume and MRT are the physiological drivers for LMY, it may be posited that to select for LMY will diminish an animal's ability to assimilate nutrients from low-quality roughages frequently encountered under rangeland conditions, with potentially deleterious effects.

Contribution of microbial genomics

Meng *et al*. (1999) demonstrated *in vitro* that increasing dilution rates were associated with improved microbial efficiency and increased VFA concentrations, while lowering ammonia concentrations in rumen fluid. By quantifying rRNA, Weimer (1998) determined that there were differences up to 8-fold in relative abundance of the three main cellulolytic species between a small number of cattle consuming the same *ad libitum* diets. Chen and Weimer (2001) have demonstrated *in vitro* that varying dilution rates in continuous cultures has substantial effects on the relative abundance of key cellulolytic and amyolytic bacteria. Studies using PCR–DDGE have identified clear differences in microbial communities of steers selected for divergent feed efficiency (Guan *et al*. 2008), indicating clear interrelationships between rumen microbial population structure and host physiology. Thus, evidence for a nexus between LMP animals and differentiated microbial communities, is mounting.

Kittelmann *et al*. (2014) found two distinctly different rumen bacterial communities in LMY sheep, and suggested that the predominant metabolic pathways used by the main species in the communities would result in the production of lower levels of H^+. Separately, Shi *et al*. (2014) discovered that gene pathways involved in methanogenesis were differentially expressed in high and low MY sheep, even though total numbers of methanogens did not differ. In contrast, Wallace *et al*. (2015), using a metagenomics approach to explore rumen microbial community difference in LMY and HMY cattle, reported a much greater abundance of methanogens in HMY cattle, and observed similar differences in methanogenic gene expression between groups.

More recently, employing 16S rRNA gene amplicon sequencing from previously identified HMY and LMY sheep, Kamke *et al*. (2016) found increased lactate-producing *Sharpea* spp. in LMY sheep bacterial communities, suggesting that the rumen microbiome in LMY animals support increased lactate production, which in turn, is metabolised to butyrate, resulting in a significantly reduced yield of hydrogen ions. Moreover, the authors of this study concluded that the observed differences in the LMY microbial community are consistent with the hypothesis that a smaller rumen size with a higher turnover rate, (where rapid heterofermentative growth would be an advantage) results in lower H^+ production and lower methane formation, thus explicitly making the link between host digestive anatomy/physiology and the differentiated rumen biome.

Conclusion

There is strong evidence for the existence of a LMP animal in both bovine and ovine populations. However, as studies by Münger and Kreuzer (2008), and more recently, Duthie *et al*. (2017) have shown, such differences are unlikely to conveniently fall along existing breed lines, but individuals with LMP will

need to be identified through rigorous and technically challenging testing of a general population. At present, the 'gold standard' method for identifying individuals with LMY or HMY is measurement under standardised conditions in open-circuit respiratory chambers, which is expensive, laborious and time-consuming. Simpler procedures, using short-term measurements have been developed (Goopy *et al*. 2009, 2011), but are less sensitive and require a greater number of measurements to be useful (Pickering *et al*. 2015). Recent research suggests there is possibly a strong association between particular rumen microbial communities and the physiological/anatomical characteristics of a LMP animal, but this is yet to be conclusively demonstrated. If proven, rumen microbial profiling may provide a long-sought after proxy for identifying LMP animals, but even then testing will most likely need to include the provision of standardised feeding conditions.

Creating the LMP ruminant

It can be said there are two broad approaches to creating the LMP ruminant. The first is to alter the rumen enduringly through exogenous means. This has the attraction of being able to be applied to any and all animals in a population, and would achieve an immediate, one-off decrease in enteric methane emissions, but unfortunately the evidence to date for being able to achieve such a feat is disappointingly lacking. The second approach is that of identifying individuals within the population who possess the desired phenotype, then selecting for those animals. In the case of the LMP, it needs to be considered that: (1) the technical requirements for identifying and testing animals are prodigious; (2) the differences between identified LMY and HMY animals are only in the region of 12–15% (Goopy *et al*. 2014; Kittelmann *et al*. 2014) and there is no evidence that this will be increased through trait selection; and (3) there is little if any, economic benefit to be gained by farmers in selecting for LMP under prevailing economic conditions (Robinson and Oddy 2016). Further, Eckard *et al*. (2010) has warned that even though genetic selection for LMP animals is theoretically possible, the rate of genetic gain for the trait will necessarily be low in any multi-trait breeding program.

Thus, on the basis of current understanding, LMP animals can be identified, albeit with some difficulty. Animals that express the trait produce 6–8% less methane on a given diet (Goopy *et al*. 2014; Kittelmann *et al*. 2014) than the general population. The trait is heritable, but not highly so, and might or might not be more fully expressed over subsequent generations of animals selected for the trait. In any case, such a breeding program would require considerable resources to establish and significant industry participation to be successful over many years – and this in an environment where there is no economic imperative to do so.

In contrast, there are at present, a number of practicable, implementable and financially beneficial management options for ruminant productions systems that will almost immediately reduce enteric methane emissions intensities 2–13% (Alcock and Hegarty 2006). In more industrialised livestock systems where the scope to reduce emissions intensities through improved nutrition, husbandry or health becomes narrower, genetic selection, along with dietary additives, may be the only future options. However in developing, low-intensity production systems where emissions intensities for livestock systems can be reduced by 30% or more (J. Goopy, unpubl. data) by simple dietary interventions that increase productivity, it seems questionable as to whether development of the LMP animal is the first, best, choice.

Conflicts of interest

The author declares no conflicts of interest.

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