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Principles of enteric methane reduction in ruminants

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Microbes in the rumen of ruminants ferment cellulose and other plant polysaccharides to acetate, propionate, butyrate, CO_2 and methane (CH_4) with H_2 being an important intermediate (Figure 1). H_2 is formed in exergonic reactions during metabolism of the polysaccharides. However, the H_2 concentration does not build up because its concentration is kept low by methanogenic archaea that convert H_2 and CO_2 or H_2 and methanol to methane more rapidly than H_2 is formed, with hydrolysis of the polysaccharides generally being the rate limiting step. The methane gas is released by ruminants into the atmosphere, mostly by a process called eructation, which is similar to burping. A dairy cow burps up to 700 L of methane per day. Between 3% and 14% of the energy of the digestible part of the ruminant feed is thus lost into the atmosphere (Johnson and Johnson 1995). This is of concern not only because the lost energy is not available to the ruminant but also because methane is a potent greenhouse gas, whose concentration is increasing in the atmosphere contributing significantly to climate change. All these are reasons why reduction of enteric methane formation in ruminants is an active area of research.

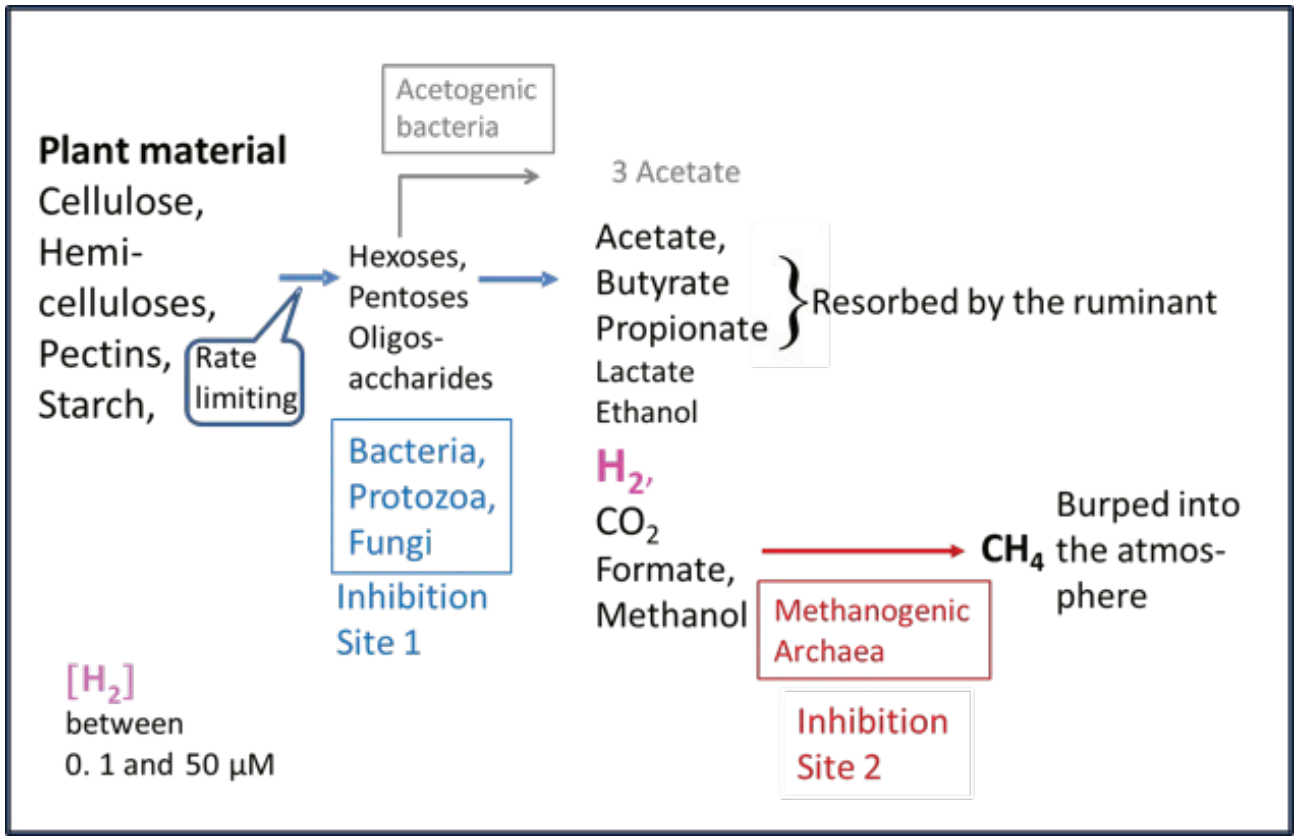

Figure 1. Scheme of polysaccharide fermentation in the rumen highlighting the role of H_2 as intermediate and the two sites of methanogenesis inhibition.

Besides H_2, also formate is an intermediate but only of minor importance in the interspecies electron transfer. Polymer hydrolysis is the rate limiting step in methane formation with the result that the steady state concentrations of monomeric sugars (10 µM), cellobiose (100 µM) and of H_2 (1 µM) in the rumen are very low (Janssen, 2010; Pinder et al., 2012). The methane yield for cellulose in cows is about 0.28 mol per 100 g, 0.20 mol per 100 g of starch, and 0.21 mol per 100 g of casein (Janssen, 2010). The particulate passage rates through the reticulorumen are in the order of 5% h-1. The fluid passage rates can be twice as high (Lopes et al., 2018; Pino et al., 2018; Wang et al., 2018).

In principle, enteric methane formation from plant material in ruminants can be reduced by interfering at two different sites in the metabolism (Figure 1). (i) Polysaccharide conversion to carboxylic acids, CO_2 and H_2; and (ii) H_2 - oxidation as electron donor for CO_2 and methanol reduction to methane. Inhibition at site 1 decreases the rate of polysaccharide hydrolysis to mono- and oligosaccharides and/or the rate of mono- and oligosaccharides fermentation to carboxylic acids, CO_2 and H_2. Consequently, the rate of methane formation concurrently decreases; the amount of methane formed per kg plant material is predicted to remain essentially constant and the steady state H_2-concentration to decrease relative to the control. Inhibition at site 2 leads primarily to the reduction of the rate of methanogenesis without necessarily affecting the rates of plant material hydrolysis and fermentation. The amount of methane formed per kg plant material is predicted to decrease and the

steady state concentration of H_2 to increase. However, the situation is not always as straightforward as this, e.g., there is indirect evidence that upon inhibition at site 2 the associated increase in the steady state H_2-concentration leads to an inhibition also at site 1 (Ungerfeld 2020) and that an increase in the propionate concentration upon inhibition at site 2 is a signal to the ruminant to decrease its appetite (Allen et al. 2009).

For the reasons outlined above, it is important to measure enteric methane formation in ruminants in mol (or g or liter) of methane formed per day and kg of plant material (dry mass) eaten by the ruminant when methanogenic inhibitors are investigated. Sometimes it is useful to measure enteric methane formation also in mol (or g or liter) of methane formed per day and kg product (e.g. milk or meat). Only those methanogenic inhibitors are of interest that do not concomitantly inhibit polysaccharide fermentation. It also has to be considered that the amount of methane formed per kg plant material digested varies between different ruminants, and is dependent on the feed composition and the percentage of feed fermented by the microbes in the rumen in the given time. The latter in turn is dependent on the growth rates and retention times of the planktonic- and biofilm-associated microorganisms present in the rumen, which can be affected by diet composition, level of feed intake and the animal's genetics. .

Various plant secondary metabolites (tannins, saponins, flavonoids, anthraquinones etc.), seaweed- and garlic extracts (bromoform and allicin), inhibitors of HMG-CoA reductase or of methyl-coenzyme M reductase (the statin lovastin, 3-nitrooxypropanol (3-NOP), chloroform, nitrite and bromoethanesulfonate), lipids, antibiotics (monensin), chemotherapeutics (metronidazole) and antibodies against methanogen strains have been tested for inhibition of enteric methane formation in the rumen. Many of these compounds were found to decrease methane formation in the rumen but in many cases, the site or sites of inhibition remain unclear. One of the reasons for this is that, with a few exceptions, inhibition experiments with pure cultures of methanogens have not been performed. Such inhibition experiments with pure cultures of methanogens have been reported only for 3-NOP, chloroform, C12-C14-fatty acids and bromoethanesulfonate but even in these cases only one of the several methanogens dominating in the rumen have been used as test strains.

There is a third type of inhibitors of methane formation in the rumen, namely nitrate, sulfate, fumarate and poly unsaturated fatty acids. In the presence of these electron acceptors, H_2 is used as electron donor by various rumen bacteria to reduce nitrate to ammonia, sulfate to H_2S, fumarate to succinate and the unsaturated fatty acids to saturated fatty acids in reactions that are far more exergonic than CO_2 reduction with H_2 to methane. As result, the steady state H_2 concentration decreases below the threshold H_2 concentration at which the rate of methanogenesis from H_2 and CO_2 is zero. To the contrary, methanogenesis from methanol and H_2 is exergonic enough to allow methanogenesis in the presence of the more positive electron acceptors.

Few anti-methanogenic compounds are commercially available for purchase by farmers. The reason is that many of the anti-methanogenic compounds are toxic for the ruminant when applied at concentrations high enough to inhibit methanogenesis by at least 30%. Based on this finding the idea has recently emerged to inhibit enteric methane formation in ruminants by a mixture of different anti-methanogenics, each at a non-toxic concentration. Anti-methanogenic diets could contain mixtures of anti-methanogenic compounds, which need to be evaluated considering the principles outlined above.

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