

Can geophagy mitigate enteric methane emissions from cattle?

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ABSTRACT

Mitigation of ruminant methane (CH₄) production remains a formidable challenge for both improving feed conversion efficiency and decreasing emissions of this potent greenhouse gas but no viable solutions are yet available. We have taken a novel approach to addressing this challenge, based on our understanding of soil microbial ecology and clay mineralogy, and the practice of geophagy, that is, the deliberate consumption of soil materials including clay minerals by animals. In a series of preliminary *in vitro* studies, we have found that some clays can significantly, albeit inconsistently, reduce CH₄ production. A hydrothermally-derived kaolinite gave the most consistent results when the initial pH of the cow rumen content, used as an inoculum, was in the range of 6.0 to 6.2. In one *in vitro* incubation, this kaolinite (7.5 and 15 mg clay g⁻¹ minced alfalfa), a condensed tannin (7.5 mg g⁻¹ minced alfalfa), and a 1:1 kaolinite/condensed tannin mixture all caused a marked reduction in CH₄ production. Kaolinite has a lower surface area than the other clay minerals tested, so this finding suggests a common biological process that is related more to the pH-variable charge characteristics than the surface area of the clay sample. While the underlying mechanism is yet to be clarified, the use of suitable clays could potentially offer an animal- and environment-friendly approach to limiting enteric CH₄ emissions, especially when the rumen pH is depressed from feeding silage or grain.

Keywords: mineral consumption, ruminants, methane, clays

INTRODUCTION

Ruminant animals emit appreciable amounts of methane (CH₄) into the atmosphere, representing a significant loss of their digestible energy intake as well as a major source of this potent greenhouse gas. To date, no viable technologies are available for reducing these emissions [1,2]. The mitigation strategies that have been proposed [3] fall into three broad classes: (i) feeds, feeding management, and nutrition; (ii) rumen modifiers; and (iii) improving animal production through genetics. Within the rumen modifier class, several approaches have been used, including direct inhibition of methanogenesis, decreasing the production of, and providing alternative sinks for, protons [4].

Clays and clay minerals are used to modify rumen processes by reducing rumen acidosis and scours, as well as improving animal weight gain. These materials can be introduced into the rumen by dusting on pastures

or as a feed additive. Thus, bentonite (a montmorillonite-rich swelling clay), used as a feed supplement, is known to affect the protozoan population and trace element concentrations in the rumen [5, 6]. Likewise, natural dolomites have the ability to reduce CH₄ emissions *in vitro*, using sheep rumen fluid and fresh feces as inocula, and meadow hay and barley grain as substrates [7].

The deliberate consumption of clay and soil materials by animals and humans, known as geophagy, has been practiced for millennia [8,9]. For example, animals instinctively ingest clay minerals to cure wounds and soothe irritations [10]. Among the potential benefits of geophagy are adsorption of toxins, bacteria and viruses, detoxification of unpalatable and noxious compounds, gastrointestinal cytoprotection, alleviation of gastrointestinal upsets, supplementation of mineral nutrients, and reduction of acidity in the digestive tract [8]. Ruminants might also gain some of these benefits from the inadvertent ingestion of soil during grazing [11]. Because of their small particle size, large surface area, and peculiar surface charge characteristics, clays and clay minerals have a propensity for taking up water, nutrients, organic molecules, and polymers [12, 13,

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14]. Clays can also bind microbial cells [15, 16, 17]. The clay-microbe interaction is complex and conceptually challenging because both microbial cells and clay surfaces usually have negatively charged sites. Nevertheless, bacteria can adhere to clay particles because of the presence of positively charged Fe and Al hydr(oxides) on soil clay surfaces in the pH range commonly found in both soils and rumen (pH 4–7). More often than not, however, microbes bind to clay surfaces through extracellular polysaccharides that their cells produce [16]. As clays can both promote and inhibit the activity of microorganisms in their vicinity, it seems likely they can influence rumen processes, including CH_4 production during enteric fermentation.

Here in this preliminary investigation we test this new hypothesis *in vitro* by adding a variety of clays and related minerals, together with minced alfalfa, to an artificial rumen fluid (containing a fresh cow rumen inoculum), incubating the mixture under anaerobic conditions, and measuring the total gas, CH_4 , and H_2 produced.

MATERIALS AND METHODS

A series of *in vitro* incubation studies were carried out by adding 2 g of minced alfalfa (dry matter content, 20.2%) to a mixture of McDougall's buffer (artificial saliva) and an inoculum of freshly sampled cow rumen content. One litre of the artificial saliva, comprising NaHCO_3 (9.8 g L^{-1}), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (9.3 g L^{-1}), NaCl (0.47 g L^{-1}), KCl (0.57 g L^{-1}), and MgCl_2 (0.06 g L^{-1}), was freshly made before each incubation. The solution was purged with CO_2 for 45 min before placing in the incubation bottles (50 mL). Anaerobic conditions were maintained by adding a reducing agent (cysteine hydrochloride, 0.315 g; water, 48 mL; 1M NaOH , 2 mL; and Na_2S , 0.315 g), prepared immediately before each incubation, and conducting all manipulations under CO_2 . Each morning (ca 9.00am), a fresh inoculum was collected from the same grass-fed, fistulated Friesian-cross cow, sieved through cheesecloth into a warmed Dewar flask, and transported to the laboratory (ca 5 minutes), where its pH was immediately measured.

One incubation experiment was also conducted when the cow was fed only on hay. All anaerobic incubations were carried out at blood temperature (38 °C) over one digestion cycle (ca 7 h). The pH, measured at the end of each incubation, was always ca. 6.3.

Three treatments (no clay; clay A, 15 mg; clay B, 15 mg), each involving five replicates, were used for each incubation. A total of 16 clays and related minerals (Table 1) were tested. One incubation also included a condensed tannin (15 mg) from *Lotus corniculatus* [18],

which has been shown to reduce CH_4 production in lactating dairy cows [19].

The adopted procedure involved placing the incubation bottles with forage \pm clay added in a water bath (38 °C) for 90 min before adding the rumen inoculum. Each bottle was prepared by adding 12 mL buffer and 0.5 mL reducing agent under a continuous flow of CO_2 . After sealing, the bottles were placed in the water bath for 15 min during which time dissolved oxygen was removed by the reducing agent. Then, 3 mL of rumen inoculum was added to each bottle under CO_2 , the bottle was resealed, and the time noted.

The following order of measurements were made: cow rumen pH (immediately after collection and before incubation commenced), and gas (total gas, CH_4) production during the incubation (after 1, 3, 5 and 7 h). Total gas production was measured by syringe volume, and CH_4 by a Shimadzu GC2010 gas chromatograph [20]. The hydrogen produced in some incubations, as part of total gas, was analyzed by the National Institute of Water and Atmospheric Research using a Reduction Gas Detector (detection limit, 1 ppm).

At the end of the incubation period, the pH of the incubation mixture was measured. Samples (6 mL) were then taken for volatile fatty acid (VFA) analysis by centrifuging the mixture at 13 000 rpm for 5 min, filtering through a 0.45 μm membrane filter, and injecting (25 μL) into a Dionex ion chromatograph.

RESULTS

Gas production

The total gas produced typically increased during the first 3 h of incubation, and then declined slowly up to 7 h as the more readily digested constituents were metabolized (Fig. 1a)

The addition of either allophane or hydrothermally-derived kaolinite (HDK) had no significant effect (relative to the control) on the volume of total gas produced, the major constituent of which was carbon dioxide.

The presence of HDK did, however, have a marked effect on CH_4 emission, decreasing production by 65% over 7 h of incubation relative to the control (Fig. 1b). Of the 16 clays and minerals tested (Table 1), only this particular kaolinite sample showed a consistent capacity for reducing CH_4 emissions. Thus, allophane had no effect (Fig. 1b), while a reduction in CH_4 emission was measured in the presence of zeolite (7, Table 1) but this result could not be reproduced.

Successful incubations involving significant reductions in CH_4 production were mostly conducted in spring and early summer, when the cow was feeding on vigorously growing grass with a high to very high

Table 1. Some qualitative properties of the clays and related minerals.

No.	Clay/Mineral	CEC	Particle size	SSA External	SSA Total	Measurable IEP
1	Bentonite-I	H	< 0.5 µm	M	VH	N
2	Bentonite-II	H	< 0.5 µm	M	VH	N
3	Bentonite-III	M	< 0.5 µm	H-VH	VH	N
4	Na-mont-I	H	< 0.5 µm	M	VH	N
5	Na-mont-II	H	< 0.5 µm	M	VH	N
6	Na-mont-III	H	< 0.5 µm	M	VH	N
7	Zeolite-I	Vh	< 100 µm	L	H-VH	N
8	Zeolite-III	VH	< 100 µm	L	H-VH	N
9	Acid (0.2 M HCl)-washed-ze- olite-I	VH	< 100 µm	L	H-VH	N
10	Halloysite-I	L-M	0.5–2 µm	L-M	L-M	N
11	Halloysite-II†	L	0.5–2 µm	L-M	L-M	N
12	Kaolinite HDK*	L	1–2 µm	L	L	Y
13	Allophane-I	L	< 250 µm	VH	VH	Y
14	Allophane-II	L	< 2 µm	VH	VH	Y
15	Layered double hy- droxide	None (has AEC)	~ 2 µm	M?	M?	N
16	Talc	None	< 0.05 µm			

CEC = cation exchange capacity; SSA = specific surface area;

IEP = isoelectric point; AEC = anion exchange capacity; mont = montmorillonite

†surfactant-modified

*tests were conducted on this hydrothermally-derived kaolinite (HDK)

in combination with tannin (12T), and using double the amount of clay (see Fig.4).

Five other kaolinites were also tested, but none were effective (data not shown).

H = high; M = medium; L = low; V = very; N = no; Y = yes

content of soluble sugars and starch. In one experiment, conducted in winter (July) when the cow was on a hay diet, a 1:1 combination of the kaolinite and a zeolite (total 2 g mineral) was used. The gas production was lower than for the grass-fed animal in the spring-summer period, and a modest reduction (ca. 30%, results not shown) in CH₄ emission was recorded.

The kaolinite supplementation was also tested using the standard amount of clay (15 mg), and twice this amount. We also tested a supplement of condensed tannin (15 mg), with and without kaolinite as this substance was known to be effective in reducing CH₄ emissions [19]. Fig. 2a shows that the total gas production, over 5 h of incubation, followed the same pattern as before (Fig. 1a). Interestingly, all the treatments tested (kaolinite, 2× kaolinite, tannin, and a kaolinite-tannin mixture (1:1)) caused a similar but much stronger reduction in CH₄ production (Fig.2b) than shown in the first experiment (Fig. 1b).

Hydrogen analyses were only conducted for those incubations where a reduction in CH₄ production was recorded. Although there was a suggestion of an accompanying increase in hydrogen concentration for a zeolite treatment (7, Table 1), the results for the other clays, including HDK, were inconclusive (results not shown). In addition to the clays and minerals listed in Table 1, we also tested five additional kaolinites (results not shown). Only one kaolinite (HDK) and one zeolite (Fig. 3) caused a reduction in CH₄ production, and only the results using this kaolinite sample could be repeated.

After testing all the clays, the cow rumen pH at the time of sampling appeared to be the common factor influencing the results. Only when the initial rumen pH fell within the range of 6.0–6.2, which was close to the final pH recorded in the buffered suspension at the end of the incubation, did we observe a measurable reduction in CH₄ production in the presence of the kaolinite (Fig.3). In the one successful experiment with a zeolite, the initial rumen pH was 6.38.

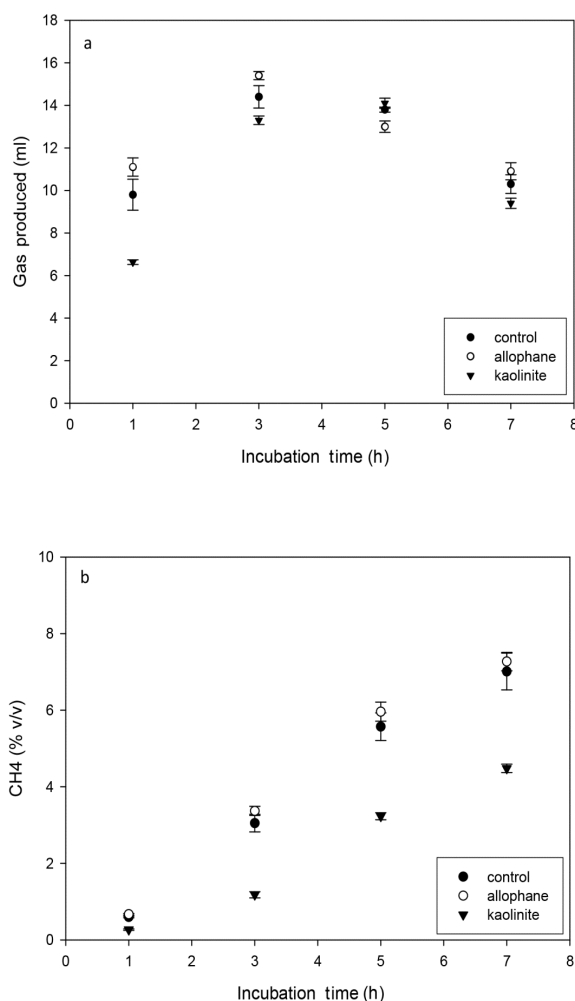


Figure 1. A comparison of the *in vitro* effect of allophane and kaolinite additives on (a) total gas production and, (b) CH₄ production. The initial rumen pH was 6.03. Uncertainties shown are one standard deviation

Volatile fatty acid (VFA) analyses were also made in instances where clay supplementation led to a reduction in CH₄ production. The acetate/propionate ratios (not shown) ranged from 2.0 to 2.6, and did not vary greatly during a given experiment or between experiments. This finding indicated that fermentation was proceeding normally during incubation.

DISCUSSION

Continuing efforts are being made to identify and develop strategies for reducing CH₄ production in the rumen of cattle and sheep [1, 2, 3]. Here we took a novel approach to meeting this challenge based on the finding that bacterial sorption to clay particles in soils increases with clay content, and varies with bacterial species [16]. The activity of soil bacteria and other components of the microbial biomass can also be strongly influenced by clay surfaces [21, 22]. Like soil, the rumen is a complex ecosystem where bacteria, fungi, and protozoa function

in a dynamic relationship with the animal in breaking down plant material anaerobically. While ingestion of soil particles is common to all grazing animals, the introduction of specific clays might be expected to influence some rumen processes, including methanogenesis. An example is the *in vitro* use of natural dolomites to reduce CH₄ production, in part by significantly reducing the rumen protozoan population [7].

Our working hypothesis was that specific clays could directly inhibit methanogenesis by limiting methanogen activity, and hence reduce CH₄ production. Research to test this hypothesis has been partially successful in that one particular clay mineral was capable of limiting CH₄ production in some *in vitro* anaerobic incubations. The sample in question was a hydrothermally-derived kaolinite from New Zealand whose presence in a rumen inoculum could markedly reduce CH₄ production. Under the same conditions, allophane and two halloysites were ineffective. This result suggests that surface area is not an important factor as allophane, in particular, has a much larger surface area than kaolinite [16]. The 'effective' kaolinite was naturally formed by percolating acidic geothermal waters. As such, the clay surface could contain an amorphous, protonated silica phase [23, 24], contributing to the reduction of CH₄ emissions. The isoelectric point (IEP) of 3.9, derived from zeta potential and electrophoretic mobility measurements, is consistent with this possibility. Further, the exchange sites of the mineral would predominantly be occupied by Al³⁺ and polyhydroxy-Al cations, facilitating the sorption of negatively charged methanogens through electrostatic interactions, 'water bridging', and 'ligand exchange' [16, 25].

Incubation experiments with a positively charged layered double hydroxide, however, showed negligible reduction in CH₄ production, suggesting that another mechanism may be operating. Investigations on the sorption of *Pseudomonas putida* to clay minerals by Huang and co-workers [26, 17, 27] showed that non-electrostatic forces (e.g., H-bonding, ligand exchange) played an important role in the bacterium-clay interaction. They also found greater and stronger sorption to kaolinite than montmorillonite although the (external) surface area of kaolinite was five times smaller. These results are not inconsistent with the involvement of extracellular polysaccharides in the sorption process.

A comparison of the results for kaolinite in Fig. 1 with those shown in Fig. 2, indicated that a reduction in CH₄ production (~ 65%) occurred regardless of variations in the treatment. Thus, doubling the amount of clay, or introducing the condensed tannin, gave the same result as the standard kaolinite treatment, provided the initial rumen pH fell within the range of 6.0–6.2. This finding suggests the process is biologically controlled.

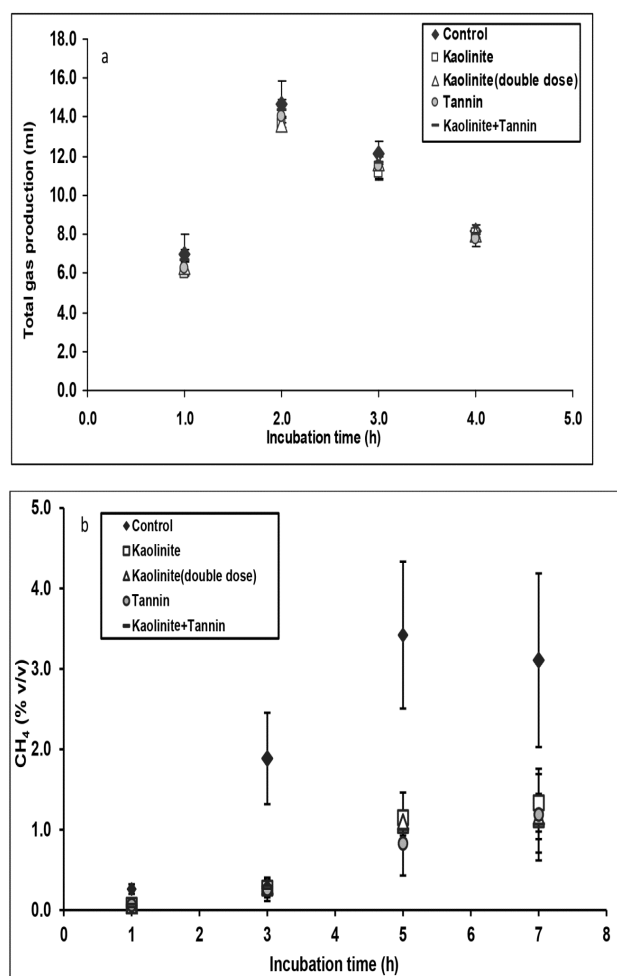


Figure 2. Effect on total gas (2a) and CH₄ production (2b) of kaolinite (15 and 30 mg) with and without condensed tannin, and tannin alone. The initial rumen pH was 6.09. Uncertainties shown are one standard deviation

Repeating the tannin experiment in the presence or absence of the kaolinite several weeks later, when the initial rumen pH was 6.61, failed to reduce CH₄ production. This observation would indicate that the effectiveness of condensed tannin in reducing rumen CH₄ emissions from lactating dairy cows [19] was not primarily due to its structural chemistry. Rather, the condensed tannin effect is related to its capacity for binding plant proteins in the rumen, reducing protein degradation to ammonia, and increasing absorption of essential amino acids from the small intestine [28, 19].

The results, shown in Fig. 2, may alternatively be explained in terms of the presence of microbially-derived inhibitory substances in the rumen. It is known that some small peptides ('bacteriocins'), produced by rumen bacteria, can inhibit Gram-positive bacteria, and decrease both CH₄ and protein production. For example, a bacteriocin produced in the rumen by *Streptococcus bovis* (bovicin HC5) can substantially reduce CH₄ production *in vitro*, with no evidence that the methanogens

can adapt and become resistant to it [29]. Furthermore, the activity of this bacteriocin is greatly enhanced at acid pH, being 10-fold more active at pH 5.5 than at pH 7 [30]. These results are consistent with our observation that the initial rumen pH needed to be in the range of 6.0–6.2 for the kaolinite to produce a measurable reduction in CH₄ production (Fig. 3). Indeed, even for the combined kaolinite-zeolite hay experiment, which gave a reduction in CH₄ production of ca. 30%, the initial rumen pH was 6.03.

While the zeolite may have contributed to this reduction (see Fig. 3), at this pH the presence of the effective kaolinite (HDK) appears to be mainly responsible. That this is the only clay tested capable of repeatedly reducing CH₄ production, suggests that the mineral enhances the effect of a bacteriocin by providing a template to which the peptide is only loosely attached. By contrast, clays that can bind peptides more strongly, such as allophane and montmorillonite, would inactivate a bovicin HC5-like bacteriocin, and hence have the opposite effect. Furthermore, as *Streptococcus bovis* is only weakly anionic at pH ~ 6, it can get close to the slightly negatively charged kaolinite surface, allowing strong cell-clay interactions to be established by means of extracellular polysaccharide bundles [15]. As kaolinites can act as specific templates for the polymerization of adsorbed amino acids [31], they can behave like biological agents such as enzymes, nucleic acids and membranes [32]. This close cell-to-clay association, and the propensity of kaolinite for adsorbing peptides, are conducive to the retention and stabilization of CH₄-reducing bacteriocins in the rumen.

The question arises whether the presence of kaolinite would enhance or prolong this inhibitory effect. Maximum uptake of proteins by clay minerals is known to occur at a pH close to the IEP of the protein [13]. Some uptake, however, can still occur at pH > IEP through non-electrostatic interactions, such as hydrogen bonding, van der Waals interactions, hydrophobic forces, and entropy effects [33]. Thus, the answer to this question is a tentative 'yes'.

What evidence is there, however, for the existence of such an inhibitory proteinaceous substance in the rumen of cattle, and in grass-fed New Zealand cattle in particular? The extent and diversity of bacteriocins, produced by *Streptococcus bovis* and similar species, have yet to be determined, but another bacteriocin-like inhibitor (bovicin 255) has been found in rumens [34], and isolated from cattle and sheep in New Zealand [35].

The rumen is a highly diverse and dynamic ecosystem in which populations of bacteria, fungi, and protozoa can change markedly, often over short periods. As a result, the concentration of inhibitory substances, like bacteriocins, would also be expected to fluctuate

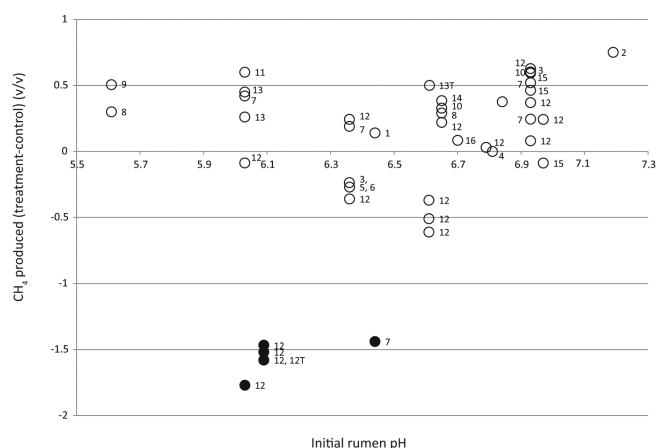


Figure 3. Summary of CH₄ production (clay treatment-control, v/v) for 16 different clays and related minerals listed in Table 1 in relation to initial rumen pH. ●, indicates clays and tannin (T) that are effective in reducing CH₄ production.

with time. Such changes in rumen microbial ecology could also result in some other clays being effective at other times and under different rumen conditions. For example, Zeolite (7) was effective at pH 6.38 (Fig.3). We were unable, however, to test this possibility by conducting incubation experiments involving all clays at one time. What specific role clays may have in such a process remains unclear, apart from providing a reactive and extensive surface to which peptides and proteins can adsorb [22, 13]. At the very least, the intake of clay by ruminants can potentially stabilize proteinaceous inhibitory substances in the rumen, prolonging their biological activity. Although we have no direct evidence for the presence of a bacteriocin in the system, the narrow acid pH range in the rumen required for a clay effect to occur is consistent with a biological mechanism.

Further research would be required to identify the mechanism behind the ability of certain clays to reduce CH₄ emissions *in vitro*. If our hypothesis is correct, we might expect such a geophagic effect to be most pronounced when cows are being fed grain or silage, both of which give rise to acid conditions in the rumen. Addition of a suitable clay mineral to the feed would be an easy, safe, and convenient way of introducing the mineral into the rumen.

CONCLUSIONS

In searching for a way to reduce enteric CH₄ emissions from ruminants, a number of strategies involving rumen modifiers have been proposed and tested. That none have so far been successful is mainly due to a general lack of understanding of the complex

ecosystem processes in the rumen. Here we explore a novel route to tackle this formidable challenge, based on the ancient practice of geophagy, i.e., the eating of soil materials, including clay. Although the results of this preliminary investigation are not definitive, they do indicate that clays can influence metabolic processes in animals, including methanogenesis.

It remains to be seen whether our approach could eventually lead to an effective mitigation strategy for reducing CH₄ emissions from enteric fermentation. What is clear, however, is that a suitable clay mineral could be introduced into the rumen through animal feed without causing adverse effects on either animal health or food safety. In this regard, the hydrothermally- derived kaolinite was found to be very effective in reducing CH₄ emissions *in vitro*, when the initial rumen pH was in the range of 6.0 to 6.2. If a similar effect would apply *in vivo*, the reduction in CH₄ emissions is likely to be less pronounced since the rumen pH usually fluctuates over at least one pH unit during each feeding-digestion event.

As our results are indicative of a biological mechanism, further *in vitro* research should focus on determining the relationship between the surface properties of selected clay minerals, potential inhibitory substances in the rumen, and enteric CH₄ production in ruminants.

FUTURE SCOPE

Our research has suggested that some clay minerals, when fed to cattle, may be effective in reducing CH₄ emissions from the rumen. While we have demonstrated this possibility under certain conditions, further *in vitro* research is needed to (a) test the efficacy of other clay minerals either singly or in combination and, (b) test the biological mechanism we propose, in which the pH-variable charge characteristics of some clay minerals may stabilize and prolong the effectiveness of rumen microbial proteinaceous substances. As to testing other clay minerals, we would suggest using commercially available acid-activated and organophilic samples with well characterized surface properties. As regards proteinaceous substances in the rumen, microbial bacteriocins have the capacity of inhibiting methanogen activity and, therefore, methane production. Future research would require rumen microbial ecologists working closely with clay chemists in devising robust experimental procedures before setting up any expensive trials, involving live animals. A range of different feeds should also be tested, as feed type is known to influence the microbial population and chemical environment of the rumen.

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