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The Microbiome of Ruminants and its Relation to Methane Emissions

Abstract

Methane emissions is at the forefront of any discussion on climate change and politicians across the globe are calling for ways to mitigate greenhouse gases (GHGs) from agricultural production. Cows and other ruminants produce methane through a process known as methanogenesis by archaea in the *Methanobacterium* species. Numerous studies show a strong correlation between *Methanobateria* and high levels of methane emission. Altering the microbiota of a cow's rumen (stomach) by changing a cow's diet has shown promise in reducing methane emissions. However, these mechanisms are not yet fully understood. Promoting the cultivation of methanotrophs (bacteria that process methane as part of their metabolism) in a cow's rumen and its natural environment may be an effective and practical way to counter methane emissions.

Introduction

Methane is a GHG that is 28 times more potent than carbon dioxide in terms of increasing global warming.[1] While most methane is produced by fossil fuels, the dairy and meat industry is estimated at 4% of the total methane emission nationwide.[2] This number may seem insignificant in the grand scheme of total GHG production but it is still a formidable problem that the industry and global economy must eventually mitigate or eliminate. Researchers at the University of California at Davis have conducted trials that show that pastes of red algae, when mixed with a cow's normal feed, significantly reduce methane emissions.[3] The mechanisms by

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[1] Ilma Tapio, Timothy J. Snelling, Francesco Strozzi, and R. John Wallace, "The ruminal microbiome associated with methane emissions from ruminant livestock," in *Journal of Animal Science and Biotechnology,* (2017), 8:7.
[2] Sam Bloch, "Sorry, Alexandria Ocasio-Cortez, but "farting cows" aren't the problem," in *New Food Economy,* Mar 7, 2019, accessed at https://newfoodeconomy.org/alexandria-ocasio-cortez-green-new-deal-livestock-cow-greenhouse-gas-emissions-climate-change/ on March 16, 2019.
[3]Judith Lewis Mernit, "How eating seaweed can help cows to belch less methane," in *Yale Environmental 360,* July 2, 2018. Accessed at https://e360.yale.edu/features/how-eating-seaweed-can-help-cows-to-belch-less-methane on March 7, 2019.

which red algae reduces methane emissions and whether this is a sustainable practice is not fully understood. Minerals in the red algae may allow for the growth of methanotrophs inside the cow's rumen and these bacteria may feed off the methane emitted before it is belched into the air. If so, other food supplements that are rich in minerals, specifically copper (Cu), would also have a positive effect on decreasing methane emissions. Furthermore, fertilization and grazing practices alter the microbiome of the natural landscape and may also affect high methane emissions from dairy and meat farms. A multi-faceted and holistic approach involving science and policy is the most effective means to reduce methane emissions from ruminants.

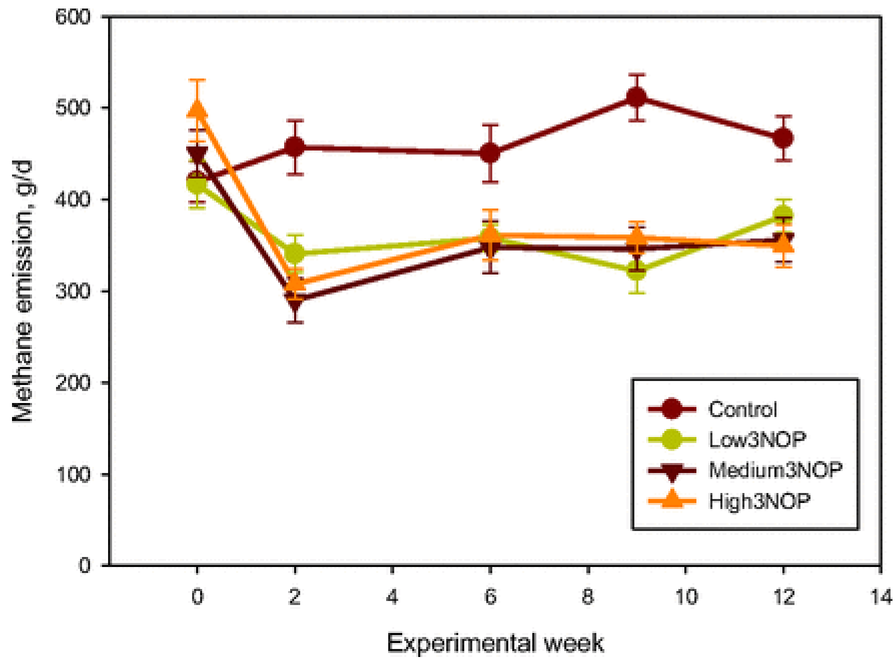
Methanogenesis in Ruminants

Methanogenesis is the process by which H_2 (Hydrogen) and CO_2 (Carbon Dioxide) are converted to CH_4 (Methane) with the help of various enzymes that are either digested or internal to a bovine's gut. The protozoa, bacteria, and fungi that are within the rumen provide the hydrogen and carbon dioxide necessary for the archaea to produce the methane as part of their metabolism. The most prevalent species of archaea found in a cow's rumen is *Methanobrevibacter*.[4]

Several European companies have tested a methane inhibitor, 3-nitrooxypropanol (3NOP), which acts upon methyl coenzyme-M (CoM) reductase. This prevents the last step in methanogenesis without specifically targeting the *Methanobrevibacter*. 3NOP essentially acts as a competitive inhibitor which means it can be overcome if enough CoM and / or other substrates were added. Results have shown that 3NOP can reduce methane emissions in cows by up to

[4] Ilma Tapio, Timothy J. Snelling, Francesco Strozzi, and R. John Wallace, pg 2.

30% and this product is currently being tested and used throughout the European Union and other parts of the world.[5]



(Figure showing Methane Emissions and levels of 3NOP)[6]

Though this inhibitor shows promise in clinical trials of dairy cows, it is not the panacea for reducing methane emissions throughout the agricultural industry.

Another method for targeting methanogenesis is the use of ionophores. Ionophores, like monensin and lasalocid, transport ions across lipid membranes in both Gram-positive and Gram-negative bacteria and cause these cells to cease to divide or to undergo apoptosis (cell death). Bacterial species such as *Clostridia* and *Ruminococcus* produce hydrogen ions which aid in the formation of methanogenesis. If these species are reduced in the rumen microbiome then there will be less hydrogen for methanogenesis to occur. Ionophores do not completely eliminate the

[5] Alexander N. Hristov, Joonpyo Oh, Fabio Giallongo, Tyler W. Frederick, Michael T. Harper, Holley L. Weeks, Antonio F. Branco, Peter J. Moate, Matthew H. Deighton, S. Richard O. Williams, Maik Kindermann, and Stephane Duval in "An inhibitor persistently decreased enteric methane emission in dairy cows with no negative effect on milk production," in *Proceedings of the National Academy of Sciences,* August 25, 2012, 112 (34): 10663-10668. Accessed at https://www.pnas.org/content/112/34/10663 on March 11, 2019.
[6] Ibid.

production of methane but they can reduce methane emissions by up to 10 or 30% based upon the concentration of supplement.[7]

Regardless of the methods used to try and prohibit methanogenesis is the underlying fact that a cow's ruminary microbiome has a propensity to return to its original state if these mitigation measures are not administered in perpetuity. A common practice in the beef industry is to restrict the amount of feed during the winter months based on cost, location, etc. This has a temporary effect on the different populations of microbes within the rumen. A 2015 Irish study showed that *Methanobrevibacter* flourished during feed restriction times but post-55 days of feed restriction, the rumen microbiome had reverted to normal population and density of all species.[8] The fact that methane producing archaea flourish in times of feed restriction may suggest that there is a tremendous evolutionary or adaptive benefit that has not yet been explored. Therefore, the elimination of methanogenesis in the rumen may have undesirable side effects that have not yet manifested in studies.

Methanotrophs

Methanotrophs are gram-negative bacteria that rely on methane for their source of energy and they are considered an integral part of the carbon cycle on earth. They are ubiquitous throughout the environment and, like their name implies, thrive in and around environments that

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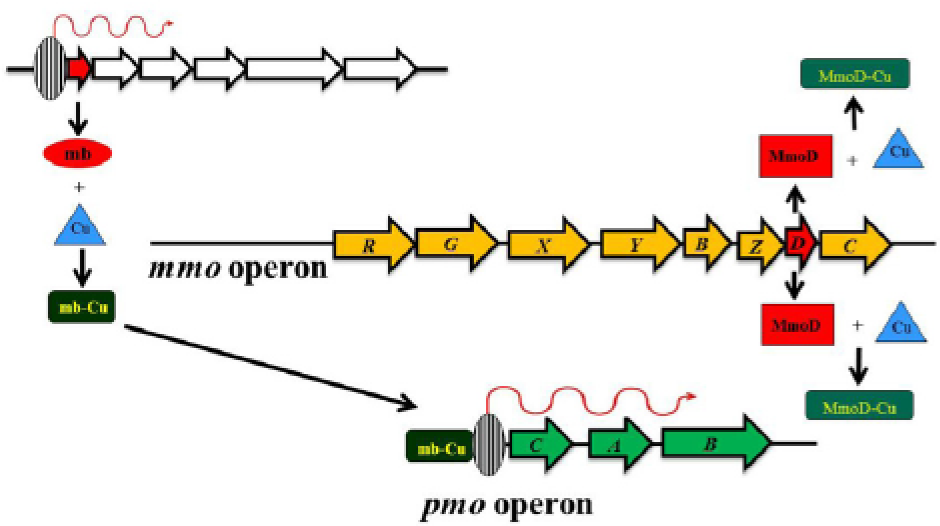
[7] Amlan Patra, Tansol Park, Minseok Kim and Zhongtang Yu, "Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances" in *Journal of Animal Science and Microbiology,* (2017), 8:13. Accessed online at https://jasbsci.biomedcentral.com/articles/10.1186/s40104-017-0145-9, on March 14, 2019.

[8] Matthew Sean McCabe, Paul Cormican, Kate Keogh, Aaaron O'Connor, Edin O'Hara, Rafael Alejandro Palladino, David Anthony Kenny, Sinead Mary Waters, in "Illumina MiSeq Phylogenetic Amplicon Sequencing Shows a Large reduction of an Uncharacterised Succinivibrionaceae and an Increase of the Methanobrevibacter gottschalkii Clade in Feed Restricted Cattle" in *Public Library of Science*, July 30, 2015, pg 3-21.

are high in methane. They are ubiquitous organisms in almost every environment on earth and are often found in especially high concentrations within forests, soils, and grasslands.[9]

Methanotrophs utilize an enzyme known as methane monooxygenase (MMO) in order to process methane into CO2 or methanol. There are two known types of MMOs: sMMO and pMMOs, the latter of which has a higher affinity for methane and is expressed by the pmo operon. These enzymes are regulated by a protein called a methanobactin (mb) that binds to copper (Cu). As seen in the illustration below, when copper and the methanobactin interact then that results in higher expressions of the pmo operon and subsequently, the enzyme, pMMO.



(Figure showing affinity of Cu and Mb and subsequent gene expression)[10]

Therefore, the more copper, the greater expression of pMMO and oxidation of methane. Indeed, some methanotrophs are known to have a very high requirement for copper.[11]

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[9] Richard Hanson and Thomas Hanson, "Methanotrophic Bacteria," in *Microbiological Reviews,* Vol 60, No. 2, June 1996, 439-471.

[10] Jeremy D. Semrau, Sheeja Jagadevan, Alan A. Dispirito, Ashraf Khalifa, Julie Sanlan, Brandt H. Bergman, Brittani C. Freemeier, Bipin S. Baral, Nathan L. Bandow, Alexey Vorobev, Daniel H. Haft, Stephane Vuilleumier and J. Colin Murrell, "Methanobactin and MmoD work in concert to act as the 'copper-switch' in methanotrophs," in *Environmental Microbiology* (2013), Vol 15(11), 3077-3078.

[11] Ibid, 3078.

Methanotrophs are the only known prokaryotes that uptake copper for gene expression. Until recently, methanobactin proteins on methanotrophs were unknown to scientists.[12] The role that these organisms have in the carbon cycle is well established but the mechanisms by which they act and proliferate, especially as part of the microbiota of a ruminant's stomach and its natural environment, warrants further study.

The Role of Red Algae (Asparagopsis) in Methane Reduction

Researchers at the University of California at Davis claim that grinding up red algae (*Aspargopsis taxiformis*) and feeding it in a paste form to cows along with their normal feed can reduce their methane emissions by up to 58%. The claim is that *Asparagopsis taxiformis* has enzymes that specifically inhibit methane production.[13] Researchers at UC Davis have yet to present their findings formally in a paper and are yet unable to share their research with respect to methane emissions and its relationship to the microbiome.[14] The same researchers do caution that more evidence and scientific research is needed to ascertain whether this process is a viable and sustainable solution. Ironically, certain press elements inflated these claims and stated that seaweed (of any type) can eliminate up to 99% of methane emissions.[15] This media incorrectly referenced an in vitro test vice in vivo measurements.

Feeding cows seaweed is not necessarily a novel practice. Evidence suggests that farmers in the ancient world would engage in similar practices and farmers in Canada have

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[12] Nicolas Vita, Semeli Platsaki, Arnaud Baslé, Stephen J. Allen, Neil G. Paterson, Andrew T. Crombie, J. Colin Murrell, Kevin J. Waldron, and Christopher Dennison in "A four-helix bundle stores copper for methane oxidation" in *Nature*, Vol. 3, September, 2015, 525.

[13] "Seaweed additive for methane reduction in cows," in *UC Davis Microbiome Special Research Program,* July 2, 2018. Accessed at https://microbiome.ucdavis.edu/news/seaweed-additives-methane-reduction-cows on February 7, 2019.

[14] Email from Dr. Ermias Kebreab to Dr. Richard Calderone and Jay Zwirblis, Feb 24, 2019.

[15] Josh Gabbatiss, "Feeding cows seaweed cuts 99% of their methane emissions from their burps," in *The Independent,* May 25, 2018. Accessed online at https://www.independent.co.uk/environment/cows-seaweed-methane-burps-cut-greenhouse-gas-emissions-climate-change-research-a8368911.html on Feb 25, 2019.

noticed that dairy cows that are fed local seaweed may produce more milk.[16] While these observations hold promise, they are not based fully on scientific trials and are largely anecdotal. Some cows may graze on seaweed directly while other breeds may prefer to eat it in a paste cut with a more palatable substance such as molasses or salt.[17]

Harvesting seaweed, especially the type of red algae that was so promising in observations at UC Davis, would be difficult to upscale production. *Asparagopsis* species of seaweed can be very invasive to certain marine ecosystems and harvesting them in laboratories has proven more difficult than standard algae.[18] But in certain parts of the world where *Asparagopsis* is not invasive and / or other potent forms of seaweed harvesting are not damaging to marine ecosystems, their farmed cultivation may hold promise as an efficacious and sustainable business model.

<u>Alternatives to Seaweed</u>

A hypothesis of why certain types of seaweed may not necessarily be their ability to inhibit methane but rather the high amounts of copper which they contain. As mentioned before, this mineral helps upregulate certain operons and phenotypes of methanotrophs. It stands to reason that introducing other enriched foods that are high in copper could also have similar methane reducing effects and not alter the microbiome of the rumen and digestive tract.

Most humans get a majority of their copper intake from seafood in the form of fish or shellfish and algae and seawood tend to also have high amounts of copper. Leafy greens such as kale, or swiss chard as well as nuts and legumes tend to contain slightly less amounts of mg of copper per 1 cup serving but they may still be palatable alternatives to seaweed for cows and

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[16] Lewis Mernitt.

[17] Phone Interview with Mr. Steven Goocey, Organic Farmer in Danville, PA, on February 10, 2019.

[18] Patrick Cage, "Seaweed Diet for Burping Cows?", at *Greenhouse Gas Institute,* July 18, 2018. Accessed at https://ghginstitute.org/2018/07/18/seaweed-diet-for-burping-cows/ on March 15, 2019.

other ruminants.[19] Researchers in Mexico have been adding cosmos flowers to the feed of cattle and this has shown a positive decrease in methane emissions by up to 25%.[20] Cosmos flowers have naturally occurring tannins that are known to kill bacteria but they are also extremely rich in copper relative to other nutrients (0.2g of Cu per 100g of flower).[21]

If foods rich in copper are truly a mechanism which promotes the growth of methanotrophs in a cow's rumen, then mixing foods such as kale, or leafy greens in with feed may have as much of an impact on methane reduction as *Asparagopsis.* This may be extremely beneficial in places where the cultivation and delivery of algae on a massive scale is not practical. Unlike *Asparagopsis*, foods high in copper may reduce methane in the rumen without interfering with methanogenesis itself. This could potentially be beneficial without disrupting the microbiota species of the rumen and may prohibit any potentially unknown deleterious effects that eliminating species such *Methanobrevibacter* might have long-term on a cow's health.

A secondary benefit of adding copper enriched foods in a cow's diet is the effect it would have on the microbiome of the surrounding terroir. Organic fertilizer in the form of manure can promote the growth of methanotrophs in the soil whereas soil that is inundated with synthetic fertilizers may not.[22] Higher amounts of unutilized copper that is digested and expelled in the form of manure may enable methanotrophs to grow more abundantly and efficiently in soils where nutrients have been depleted from years of overuse. Certainly, a combination of practices

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[19] Helen West. "8 Foods that are high in copper," in *Healthline,* October 25, 2018. Accessed at https://www.healthline.com/nutrition/foods-high-in-copper on March 17, 2019.

[20] Ned Rozell, "Scientists find methane reducing cow diet," at *University of Alaska, Fairbanks* January 17, 2017. Accessed at https://news.uaf.edu/scientists-try-to-mitigate-methane-from-cows/ on March 17, 2019.

[21] Shi-Hui Cheng, Mohd Yusof Barakatun-Nisak, Joseph Anthony and Amin Ismail, "Potential medicinal benefits of *Cosmos caudatus* (Ulam Raja): A scoping review," in *Journal of Research in Medical Scienes*, Oct 20(10), 2015, 1000-1006.

[22] P.J. Strong and W. Clarke, *Agro-Environmental Stability,* (Springer International Publishing, 2017), 24.

in terms of feed and land management use may be necessary in order to achieve a lasting solution to anthropogenic methane emissions.

Proposed Experiment

To test the hypothesis that copper has a positive influence on the composition of rumen microbiota and a reduction in methane levels emitted by cows may require both in vitro and in vivo experiments. As mentioned before, trials in cultured tissues or fluids may yield significantly different results and. The experiment will be a two-phase process and each phase will have a control group and a test group.

The ruminal contents of a statistically significant number of cows could be measured for bacterial species using real-time polymerase chain reaction (PCR). Assuming most of the microbial contents in the ruminal fluid remained intact, varying quantities of copper could be added to that fluid. After some time, real-time PCR could be used again to quantify the population density and species of both methane producing and methane consuming bacteria. If the population of methanotrophs is greater than before it would indicate that copper may have a positive influence on methanotrophic activity in the rumen. Another assay could also be used to measure the methane production of the sample before and after the addition of copper supplements. Regardless of results, it is important to remember that tissue samples and microbial communities may react very differently when exposed to the same nutrients and / or substances when they are cultured as opposed to a live subject. It is also important to note that collecting a statistically significant number of rumen samples may be difficult and constrained to current practices. The experiment would also have to control for various breeds of cows, their age, diet, feed patterns, etc.

The second phase of the experiment would be to feed cows various foods that are high in copper and measure their methane exhalation in relation to a control group. This is a similar setup that researchers have used many times before; the cows grow accustomed to eating through feed bags and their methane emission is captured by a special type of breathalyzer. The timeline of the experiment would also have to account for variables such as timeline, location, soil contents, etc. Quantifying the microbial species within a living cow's rumen is possible but is a labor-intensive task. Cows must first be restrained and then a large tube inserted down their esophagus and into their rumen. Measuring the contents of the rumen before and after the induction of foods rich in copper may be costly and more difficult in terms of accuracy and precision.

If the hypothesis that copper enzymes do not significantly advance the proliferation of methanotrophs in a cow's microbiota nor have a positive influence on reducing methane then by process of elimination, the experiment is still a success. Directly targeting bacterial species such as *Methanobrevibacter* or suppressing the paths of methanogenesis may be the best way to reduce methane. There may also be other factors within the rumen that suppress the development of methanotrophs regardless of which operons are expressed. The pH of the rumen and how it reacts to copper supplements is worth observation and may also have an effect on the growth of various microorganisms.

Conclusion

The problem of GHG and methane emissions will likely continue to be at the forefront of any conversation about the sustainability of the dairy and meat industries. Calls for action seem to continue to revolve around appropriate land use or consumers cutting back on beef. Those courses of action may be part of a larger holistic solution. Examining the elements of the microbiome within a cow's rumen and its environment is relatively new in terms of scientific research. However, a lot of misinformation is propagated in the public: 99% in vitro does not equate to 99% in vivo, nor are cow's flatulence the primary cause of methane emissions. There is promise in recent studies but none examine the development of methanotrophs within a cow's rumen. As with any experiment, there may be long term negative consequences for the industry or the health of the cow based on yet unknown variables.

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