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Asparagopsis taxiformis a literature review of antimethanogenic properties

The case for Asparagopsis taxiformis as a feed additive

Globally, methane emissions from livestock enteric fermentation makes up about 8-10% of total greenhouse gas emissions (CGIAR, 2020). Methane has a global warming potential 28 times higher than CO2 over a hundred year period. Reducing methane emissions is essential to controlling global warming and ensuring a sustainable ecosystem for humans and nonhumans alike. In 2016, the California Senate passed Senate bill 1383 on Short-lived climate pollutants, calling for a 40% decrease in methane emissions from livestock by 2030. Organizations like PETA are also lobbying for the implementation of a meat tax to cover the health and environmental costs of meat production, similar to alcohol and cigarette taxes. In order for small farmers to avoid additional taxes and reduce their carbon footprint, Symbrosia is working to scale the production of Asparagopsis taxiformis, a species of red alga that has been shown through various studies to drastically reduce methane production.

It has been shown that macroalgae contain secondary metabolites that reduce methane production in the rumen by manipulating the microbial populations of the rumen (Machado 2015). Asparagopsis taxiformis accumulates compounds such as bromoform and dibromochloromethane, antimethanogenic compounds that inhibit enzymes crucial to methane production (Roque 2019). In testing the seaweed's ability to decrease methane production, scientists also tracked Volatile Fatty Acid (VFA) concentrations. VFAs are the primary source of energy for livestock and are created during digestion (Kinley 2016). A decrease in VFA concentrations could signify potential issues of livestock productivity including weight gain and milk production. However, it has been found that Asparagopsis taxiformis has negligible effects on VFA production and livestock productivity, making it an ideal candidate for a methane-reducing feed material. The following literature review offers a comprehensive summary of some of the research that has been done with A taxiformis to date and includes both in vitro (in a laboratory) and in vivo (animal testing) experiments that spans several years and countries including Australia, Sweden, and the United States.

Senate Bill No. 1383 signed into California law in 2016 requires a 40% reduction in methane emissions compared to 2013 levels by 2030.

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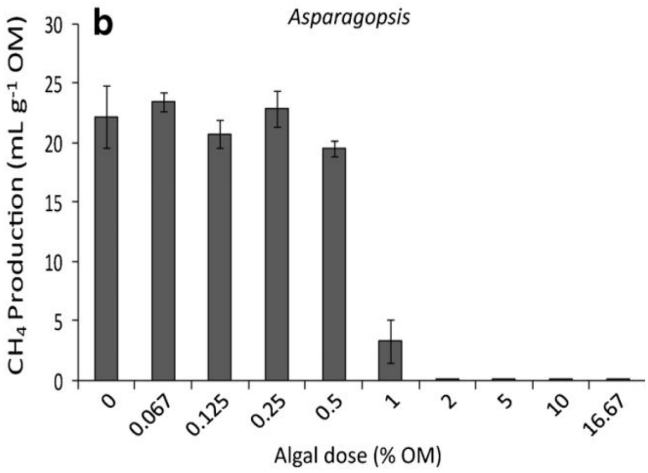
Dose-response effects of Asparagopsis taxiformis and Oedogonium sp. on in vitro fermentation and methane production

Machado and colleagues tested the in vitro effects of *A. taxiformis* and *Oedogonium* on cattle methane production in a dose-dependent manner. Both seaweeds show potential as a methane-reducing feed additive. *A. taxiformis* was tested at total feed percentages ranging from 0-10% and 16.7%. *Oedogonium* was tested at 0, 10, 16.7, 25, 50, 75, and 100% feed. Rumen fluid was collected from Brahman steers and incubated with hay feed samples and seaweed at each concentration for 72 hours. At ≥75% inclusion, *Oedgonium* reduced methane production by at least 50%, and at 100% inclusion, methane production decreased by 61.6%. Comparatively, at 1% inclusion *A. taxiformis* reduced methane production by 84.7% and at doses ≥2%, methane production was reduced by over 99%, practically undetectable.



The effect of *Asparagopsis taxiformis* on methane production at various inclusion levels (in percent of organic matter OM) after 72 hours of in vitro incubation.

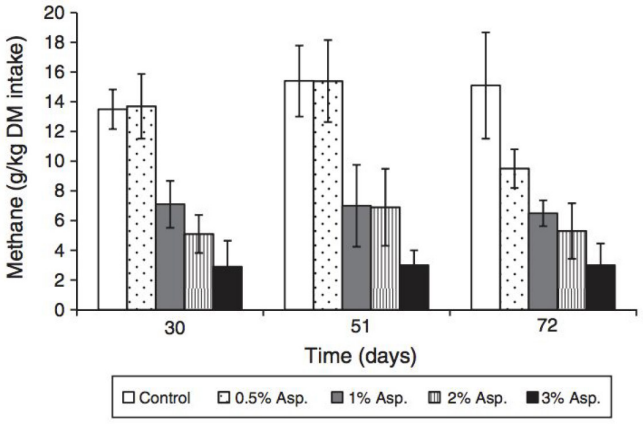
Organic matter degradation (OMdeg) and volatile fatty acid (VFA) production are standard indicators of fermentation efficiency. It was found that both *A. taxiformis* and *Oedogonium* reduced VFA production. Only at values ≥10% inclusion does *A. taxiformis* significantly inhibit OMdeg, suggesting that at lower inclusion levels (<5%) *Asparagopsis* can significantly reduce methane production while minimally affected rumen fermentation processes.

Machado et. al, 2015

Asparagopsis taxiformis decreases enteric methane production from sheep

Li and her colleagues fed Merino wethers sheep *Asparagopsis taxiformis* at five inclusion levels for 72 days. The seaweed inclusion levels were 0%, 0.5%, 1%, 2% and 3% of the OM. The *A. taxiformis* was mixed with crushed lupin seeds and sprinkled into regular feed. Every 21 days sheep were isolated in collection chambers to measure gas emissions and take blood and rumen samples. At the end of the 72 days, 3 sheep from each inclusion level were euthanized, fat and muscle samples were taken for analysis.

The results found that while *A. taxiformis* did not affect the sheeps' weight, there were significant reductions in methane production compared to sheep on the control diet. Because the sheep were able to sort of the *A. taxiformis,* the researchers could not confirm that methane reduction is dose-dependent, although the results strongly suggest that. They did find that at inclusion levels ≤ 1%, the sheep willingly ate all the *A. taxiformis*. Sheep at 2 and 3% inclusion levels saw methane production reductions up to 80%. Tests on the fat and muscle tissues found that there were no detectable levels of bromoform or dibromochloromethane in the samples, suggesting that this methane reduction method would be safe for meat-production facilities.



Mean methane emissions measured on three days for sheep eating *A. taxiformis* at inclusion levels of 0% (control), 0.5%, 1.0%, 2.0% and 3.0%.

Li et. al, 2016

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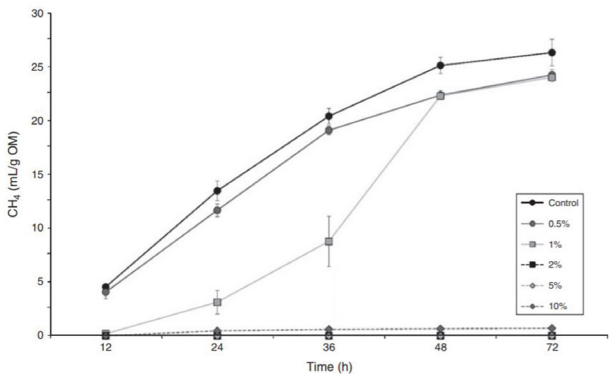
Australia

The red macroalgae Asparagopsis taxiformis is a potent natural antimethanogenic that reduces methane production during in vitro fermentation with rumen fluid

Kinley and his colleagues performed a 72 hour in vitro experiment to determine the effects of *Asparagopsis taxiformis* on methane production and digestibility of feed over time. Rumen fluid from four Brahman steers was used to ferment feed with *A. taxiformis* at five doses (0.5, 1, 2, 5, 10%). It was found that 2% inclusion was near optimal for total gas reduction (around 30% compared to the control) and ≥2% inclusion rates resulted in undetectable methane production. Additionally, in vitro digestibility of the substrate was unaffected at inclusion levels ≤ 5% but significantly affected at 10%, indicating that lower dosage levels of 2% are ideal for reducing methane production without affecting rumen fermentation processes. However, there was a trend of total volatile fatty acid production decrease as inclusion levels increased. Specifically, acetate levels decreased significantly in dosage levels compared to the control, while propionate levels increased; this decrease in acetate:propionate ratio may explain decreased methane production in the dose-dependent manner seen in this study. However, this shift in chemical production did not negatively affect VFA production due to the low inclusion level (<5%) of *A. taxiformis* needed for methane production reduction.

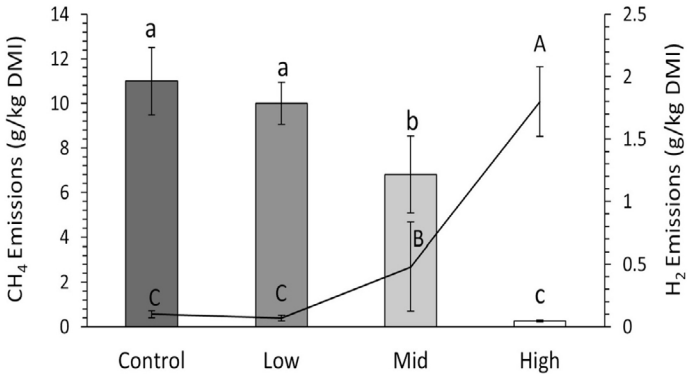


The effect of *A. taxiformis* on mean in vitro methane production over time. Control was Rhodes grass hay.

Kinley et. al, 2016

Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed

Kinley and his team tackled an in vivo experiment to test the effectiveness of *Asparagopsis taxiformis* in reducing methane production in beef cattle. The additive was tested on 20 Brahman-Angus steers at four different inclusion levels (0.0%, 0.05%, 0.10% and 0.20%) for 90 days. Gas, rumen fluid, and stool sample collections occurred five times throughout the experiment. The steers were introduced to their inclusion level of *A. taxiformis* over 30 days and remained at their experimental level for 60 days. Two days after the experiment was completed the cattle were slaughtered and one M. Longissimus dorsi was boned out from every carcass to be judged on flavor, juiciness, tenderness and overall acceptance. Results showed that compared to the control group with no seaweed, the 0.10% and 0.20% inclusion groups had significant methane production decreases of 38% and 98%, respectively. Additionally, the 0.10% and 0.20% inclusion groups had average daily weight gains of 51% and 42%, respectively. Consumer evaluation of the meat found no difference in meat quality from any of the treatment groups and no bromoform residues were found in any meat, fat, kidney, or feces samples collected.



Enteric methane (columns, lowercase letters) and hydrogen (line points, uppercase letters) production from steers consuming *A. taxiformis* at inclusion levels of 0.00 (Control), 0.05 (Low), 0.10 (Mid), and 0.20% (High) of organic matter intake.

Kinley et. al, 2020

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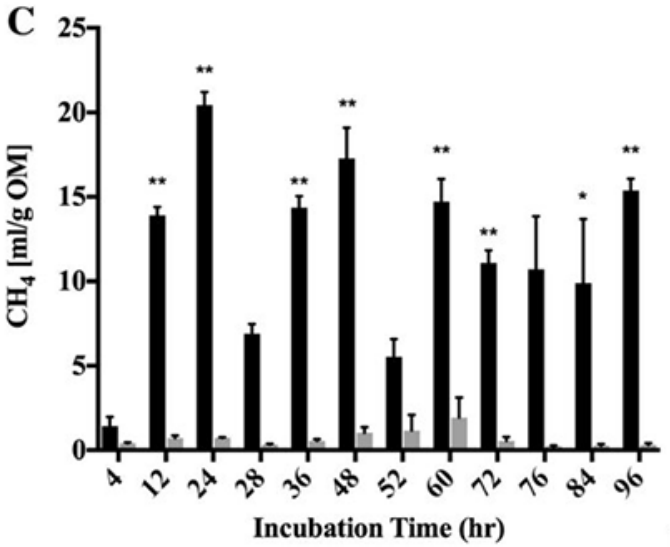
United States

Effect of the macroalgae Asparagopsis taxiformis on methane production and rumen microbiome assemblage

Roque and her team worked at University of California, Davis to test the effectiveness of *Asparagopsis taxiformis* on methane emission reductions in dairy cattle. Using rumen samples from two Californian fistulated dairy cattle, *A. taxiformis* was added to the samples at a 5% inclusion rate (with the remaining 95% being normal feed) and the concoction was fermented for four days in vitro. The results found that total gas production was reduced 51.8% compared to the control and methane production was reduced 95% compared to the control. Volatile fatty acid concentrations were not significantly different in the control and experimental samples. There was an increased propionate:acetate ratio found in the experimental samples. According to this study, the propionate:acetate ratio could affect the milk produced in vivo because a decrease in proionate:acetate ratio is associated with increased milk yield but decreased milk fat. Therefore, dairy cattle that eat a diet with *A. taxiformis* would produce significantly less methane but they could also produce more milk with less fat.

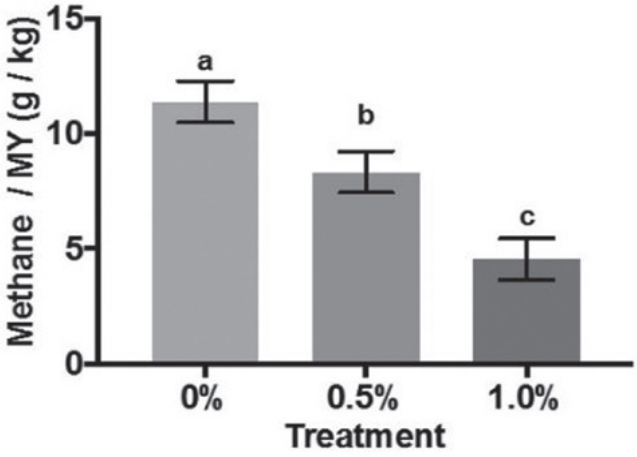


Methane production in vitro over the course of 96 hours. Measurement were performed in triplicates. "**" indicates significant difference (p value ≤0.05), "*" indicates trend toward significance (0.05 > p value ≤0.1).

Roque et. al, 2019

Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent

Roque followed up her in vitro research with an in vivo experiment to test the effects of *Asparagopsis armata* on methane production and milk yield. 12 lactating Holstein cows were assigned to three treatments: control, 0.5% (low), and 1% (high) inclusion levels for 21 days. While methane production was significantly reduced (26.4% at low inclusion and 67.2% at high inclusion), feed intake was also reduced with increased inclusion of the seaweed. Feed intake was reduced by 10.8% and 38% in cows fed the low and high inclusion diets, respectively. Bromoform concentrations in milk were greater in cows that ate *A. armata* at both inclusion levels compared to the control. However, the differences were not considered significant. Cows in the high inclusion level gained less weight than the control and low inclusion groups and produced 11.6% less milk. Milk protein also decreased with more seaweed (significant only for the high inclusion level) but the milk fat wasn't affected. These findings show that while *A. armata* has antimethanogenic properties, it is not as effective as *A. taxiformis*, which reduced methane production by 98% at 0.2% inclusion (Kinley 2020).



Means and standard deviations of methane intensity in milk yields (MY), for cows supplemented with 0, 0.5, and 1% *A. armata.*

Roque et. al, 2019

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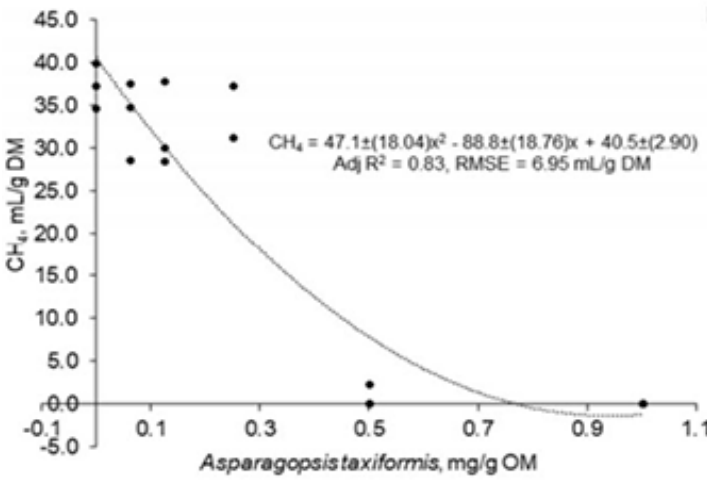
Sweden

In Vitro Evaluation of Different Dietary Methane Mitigation Strategies

Chagas and her team studied 16 different substrates as potential methane mitigation tools in a series of in vitro experiments. Using the rumen from lactating dairy cows, they incubated the rumen samples with feed and each substrate for 48 hours, measuring gas production multiple times throughout. In their first experiment, the three top performers were *Asparagopsis taxiformis*, 2-nitroethanol, and bromoform, reducing in vivo methane production by a predicted 99%, 97% and 95%, respectively. In the second experiment, *A. taxiformis* and 2-nitroethanol were tested to see if they reduce methane in a dose-dependent manner. Asparagopsis taxiformis was tested at inclusion amounts of 0, 0.06, 0.13, 0.25, 0.5, and 1.0 g/kg of diet organic matter and it was found that there was a curvilinear relation between *A. taxiformis* inclusion and methane reduction. Similar to Kinley's 2016 paper, it was found that the molar proportion of acetate decreased while the molar proportion of propionate increased. The research team concluded that out of all substrates tested for methane-reducing diet supplements, *Asparagopsis taxiformis* was most capable of reducing methane in a dose-dependent manner with the least impact on rumen fermentation.



The graph shows predicted in vivo methane production based on analysis of 48 h gas from in vitro incubation of a control diet treated with different levels *A. taxiformis*.

Chagas et. al, 2019

The future of reducing methane emissions in the livestock industry

This research review offers a comprehensive understanding of keystone research on the in vitro and in vivo effects of *Asparagopsis taxiformis* on methane production to date. Covering four years, three countries, and three animal species, the results show that *A. taxiformis* has an incredible potential to reduce methane production by up to 98% with as little as 0.2% inclusion of the livestock's daily feed. No significant increases in bromoform residues were found in the meat or milk products. Additionally it was found that *A. taxiformis* does not significantly affect volatile fatty acid concentrations in ruminal animals, which are traditionally used as markers of productivity and fermentation in the animal. This indicates that the animals' health is not compromised by the use of *A. taxiformis* in livestock feed. Finally, research on the effects of *A. taxiformis* on livestock weight gain is mixed. Li's study on Merino wethers sheep found that the seaweed had no effect on weight while Kinley's study found significant weight gains in cows fed the seaweed material. Roque's study found that *Asparagopsis armata* caused less weight gain at higher inclusion levels. These mixed findings suggest further in vivo research still needs to be done to fully understand the effects of various antimethanogenic substances on livestock health. While such research is currently ongoing, the biggest barrier to market entry for *Asparagopsis taxiformis* remains the difficulty of scaling production, an issue that Symbrosia is actively tackling. As research of products that reduce methane production in ruminant livestock continues, this research review will be updated.

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