

METHAGONE

BUSINESS PLAN

## TABLE OF CONTENTS

EXECUTIVE SUMMARY.....	3
COMPANY OVERVIEW.....	4
PROBLEM & SOLUTION .....	5
THE PROBLEM.....	5
OUR SOLUTION .....	5
TARGET MARKET.....	6
MARKET SIZE & SEGMENTS.....	6
COMPETITION.....	7
CURRENT ALTERNATIVES TARGET BUYERS ARE USING.....	7
OUR COMPETITIVE ADVANTAGES .....	7
PRODUCT OR SERVICE OFFERINGS.....	8
PRODUCT OR SERVICE .....	8
MARKETING .....	8
.....	9
MARKETING PLAN .....	9
TIMELINE & METRICS.....	10

TIMELINE	
.....	
10 MILESTONES	10
.....	
KEY PERFORMANCE METRICS	10
.....	
FINANCIAL FORECASTS	11
.....	
ASSUMPTIONS	11
.....	
FINANCING	12
.....	
SOURCES OF FUNDING	12
.....	
USE OF FUNDING	12
.....	
...12	

## EXECUTIVE SUMMARY

MethaGone is a Limburg-based company that aims to decrease greenhouse gas emissions by creating a pill that can be given to cows to decrease their methane output. The unique product is a capsule that contains *E.coli* bacteria which have been modified using genes found in the red seaweed (*Asparagopsis taxiformis*) that are known to produce bromoform (Thapa et al. 2020). Bromoform decreases methane production in the cow's rumen as it inhibits archaea from synthesizing the gas (Machando et al. 2017). Instead of losing energy in the form of methane, this energy can be used to increase the animal's weight, therefore also its meat and milk production (Martinez-Fernandez et al. 2017).

The company is situated in the Netherlands which has a cow population of 1.59 million. Recent strife between farmers and governmental regulations that have been researched by our company has led to the need for an innovative solution that would decrease global warming concerns but increase food production for the growing global population.

MethaGone is still currently in the research and development phase, having created suitable genetic vectors that can be inserted into the bacteria and have begun ruminal testing. Further lab and field tests would be conducted within the next 5 to 7 years, thereafter quality assurance and further improvement testing will continue indefinitely. The aim for when the product has been completed is to target bovine farmers in the Netherlands after field tests have been conducted. The production of Methagone can then be scaled for worldwide distribution.

Methagone runs on a simplified management structure, with a general management team and specific sector managers. This collaborative structure allows for an innovative approach to research and business.

# COMPANY OVERVIEW

## VISION

To reduce the climate impact of the livestock industry beneath planetary boundaries without replacing it.

## Mission

Creating mass-producible and sustainable alternatives to red seaweed, while compensating for the green premium by acting as a weight gain agent and expanding our operations by developing more long effect microbial feed additives.

## OBJECTIVES

- To have high volume production and sales of our microbial feed additive.
- To ensure the intended effects are working without any adverse effects.
- To improve and diversify our product range.
- To develop our capability of developing long term effect feed additives.

# PROBLEM & SOLUTION

## THE PROBLEM

Global warming is a pressing issue that is being talked about all over the world because of the impacts that a growing human population is having on the environment. The human population has grown from approximately 2.6 Billion in 1950 to around 7.8 Billion nowadays (United Nations, 2021). A secondary effect of this growing population is an increasing demand for food. A common source for food-related products such as dairy and meat are cows, as well as sheep for their wool and meat. These animals all belong to the suborder Ruminantia, meaning that they have a four-chambered stomach, the first one of which is the rumen (Britannica, 2019). Within the rumen, microorganisms can digest cellulose taken up as grass, hay, straw, etc., into compounds that can be taken up by the animal as an energy source. The main electron acceptors and donors in this rumen fermentation process are hydrogen (H<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) (Matthews et al. 2018). In a reduction pathway, these compounds are used by archaea in the rumen to produce methane (CH<sub>4</sub>) (Kinley et al. 2020). CH<sub>4</sub> is a compound that can be seen as a loss of energy when it is formed and eradicated, representing a loss of up to 12% of gross energy intake.

CH<sub>4</sub> is a greenhouse gas with higher potency than CO<sub>2</sub>. This means that, although

there is less of it in the atmosphere, its global warming potential is 86 times stronger per unit of mass than CO<sub>2</sub> over a period of 20 years and 28 times stronger on a time span of 100 years (Jackson et al. 2020). Since global warming is an ever-increasing problem worldwide, CH<sub>4</sub> emissions from ruminants, especially cows, are getting more and more attention. Therefore the emission of methane from ruminants has been experimentally reduced in various ways. One example is the addition of canola oil to cow feed, which can reduce methane emissions by up to 20%. Another method is using 3-nitrooxypropanol as a food additive, which has even been proven to reduce methane emissions by up to 80% (Zhang et al. 2021). An alternative approach of reducing the methane output by ruminants is the usage of the red seaweed *Asparagopsis taxiformis*, which, depending on the feed amount, could reduce methane output by up to 98% (Kinley et al. 2020). This ability of *A. taxiformis* is due to its ability to generate halogenated compounds, amongst them bromoform (Machando et al. 2017), which is the major halogenated product of these red algae (Machando et al. 2016). Bromoform has the effect of inhibiting a cobamine-dependent methyltransferase, which is required for the synthesis of methyl-coenzyme-M, the key enzyme in the final part of methanogenesis (Machando et al. 2016), ultimately reducing methane production if present in the cow rumen (Kinley et al. 2020). These methyl-coenzyme-M enzymes are present exclusively in methanogens (Zhu et al. 2021), whereof the archaeal genus *Methanobrevibacter* is the most abundant and most studied (Danielsson et al. 2017). Of the many ways of reducing methane emission, *A. taxiformis* shows the strongest dose-dependent CH<sub>4</sub> mitigating effects, while having the least impact on rumen fermentation (Chagas et al. 2019). Unfortunately, the life cycle of *A. taxiformis* consists of three life stages. Aquafarms have yet to grow the algae through all of these, meaning that the cycle could not be closed. This hinders the farming of the species on a global scale dramatically (Zhu et al. 2021). In 2020, Thapa et al. identified the genes from different groups of *A. taxiformis* which are involved in the production of bromoform, as well as the genes involved in bromoform production in the algae *Chondrus crispus*, amongst others.

## OUR SOLUTION

MethaGone has the aim to use synthetic biology in order to create a solution. Bacterial host cells will be manipulated to take up the gene sequences involved in making bromoform-producing enzymes. These bacterial cells will be encapsulated and then given to ruminants in order to colonize their rumen, producing bromoform and therefore reducing methane emissions. This would result in a resource- and time-favorable scaling of the production of methane-inhibiting bromoform within the cow.

Inhibiting methanogenesis can lead to an accumulation of H<sub>2</sub> (Duin et al. 2016). The excess H<sub>2</sub> may be belched out by the ruminant (E. Ungerfeld, pers. comm.), or even redirected into propionate biosynthesis, the main glucose precursor for ruminants (Li et al. 2016; Kinley et al. 2020; E. Ungerfeld, pers. comm.; Kinley et al. 2016; Roque et al. 2021). Indeed, alternative H<sub>2</sub> sinks, such as the volatile fatty acids acetate, butyrate, and aforementioned propionate are in constant competition with methanogens for H<sub>2</sub>, yet have a higher threshold of H<sub>2</sub> concentration than methanogenesis, and are therefore outcompeted (Greening et al. 2019). This means that excess energy can be

rechanneled into fatty acid production, and therefore leads to higher weight gain for the animal.

## ORGANIZATIONAL STRUCTURE

The initial organizational structure only consists of the RnD team. In the Gantt diagram (found on the entrepreneurship wiki page) the creation of different departments are marked. The operational structure at the end of the 7th year is given below:

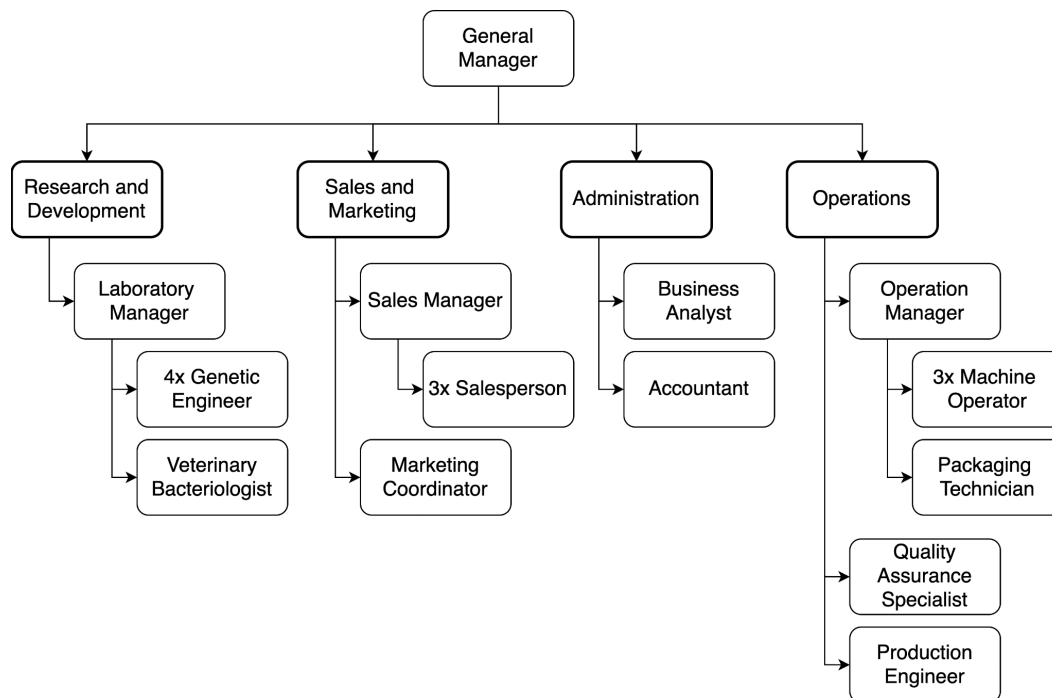


Figure 1 - Organizational Diagram

## TARGET MARKET

### MARKET SIZE & SEGMENTS

Farm sizes:

Small (<100 cows)

Medium(100-200 cows)

Large (>200 cows)

Methagone will target medium to large farms that would be willing to pay for the improvements that the capsules would bring. These farms are also the largest GHG producers in the form of ruminal methane.

Geographic:

Our target market would initially be in Europe because this is where Methagone is situated and is an area of the world that is a lot more environmentally conscious. Contact with the RIVM in the Netherlands has already been made in order to ensure the safety of the capsules when they are prepared for distribution. The European Union owns 8.55% of the world's cow population (Cook 2021). If Methagone was used in all of these cows it would decrease the global methane output by 7.95%. Further branches would be created in South America, India and China, where a large percentage of ruminal animals are based (Cook 2021). Many countries in this area also face problems of poverty and food shortages so an increase in meat production without the need for more land and animals would be beneficial to the farmers residing there.

Product:

The main target audience for this product are cattle farmers, this includes both farmers for meat and milk. The capsules not only have a positive environmental impact but convert otherwise lost energy into a product that can be used by the cows body to produce more meat and a higher fat content milk. The benefit of the solution is that the capsule would be a one time administered product, therefore mitigating recurring costs for the individual farmer and increasing MethaGone's compiled impact.

MethaGone would also approach organic producers who are concerned about their animals and the environment. These companies may actually be more willing to use our project as it is not only beneficial to them and the cow but also helps reduce their environmental impact allowing for better advertising.

## COMPETITION

### CURRENT ALTERNATIVES TARGET BUYERS ARE USING

We have identified 4 major competitors that we have identified

1) Bovaer - this is a food additive created by DSM that when fed to cows reduces their methane emissions by 30% by means of suppressing the enzyme that produces methane in archaea. This product needs to consistently be given to cows in order to continue to function (DSM 2021).

2) Mootral\_- this natural feed is made of garlic and citric acid and is said to reduce enteric methane emission by 38% by inhibiting the activity of the archaea (Mootral 2021).



3) Blue Ocean Barns and Symbrosia are both companies that produce *Asparagopsis taxiformis* as a feed additive that helps decrease emissions but over 90%. These companies are part of the research efforts that have inspired our own produce. A big disadvantage of cultivating the seaweed is that it is very difficult to scale hence its idea of using the affective genes from this organism was created (Blue Ocean Barns, 2021; Symbrosia 2021).

These are the players that we have currently identified in the market, however because of the novelty of the research more such products may arise in the near future. However they will also have to undergo tremendous amounts of testing and so may not enter the market in the foreseeable future.

4) UltraCruz® Livestock Weight Gain for Cattle, Goats, Sheep and Pigs is a supplement that supports the weight gain of livestock. It is priced at €39 for a month's supply. As with other products it has to be fed consistently for it to function, so for a single cow that will be slaughtered at 18 months old will require a €702 of this product.

## OUR COMPETITIVE ADVANTAGES

Our largest competitive advantage for farmers is that the capsule is administered only once in a cow's lifetime instead of most feed additives that need to be added every day. This saves farmers money and allows for more cows to benefit.

Beginning our research now into using recently discovered genes puts us one step ahead of potential competitors. The genes that we use have recently been discovered (Thapa et al. 2020) and hence our product testing and release will be before any others that use this pathway.

## PRODUCT OFFERINGS

MethaGone offers microbial feed additives for cows that reduce methane emissions by up to 98% and increases the average weight gain by up to 53% (Kinley et al. 2020). On top of that MethaGone collects data from farmers to further enhance its products so that farmers can increase their efficiency even further.

## MARKET ANALYSIS

### PEST Analysis

## Political

The Dutch Biosafety Authority (RIVM) has strict laws that need to be considered about GMO development and testing that need to be considered. Within the project, we have had contact with the organization in order to put together safety measures that would be up to standard.

Research and development is a competitive, ever-growing field so patents would have to be procured in order to protect the intellectual property of our idea. This is a costly and time-consuming process and patents tend to only be recognized in a specific area, say national, continental, or globally, which is very financially dependent.

## Economic

The economic issues that a company in the synthetic biology sector is the cost of skilled labour and researchers needed for further innovation have to have had a high level of education. However with the rapid growth in this economic sector it is becoming a profitable field of business. The CAGR of synthetic biology is estimated at 21.9%, this along with the CAGR of 6.0% of the animal feed is indicative of other growth in a business that focuses on a product in these fields (Allied Market Research 2021). Furthermore, once the bacteria cultures are set up they are relatively inexpensive to maintain as the only variable expenses that would occur are the bacterial food source, cyclodextrin capsules and packaging of the products. Another selling point of the product is that the cows produce more meat, providing a financial incentive to farmers to purchase our produce over others. With the continued growth of the farming industry and the increased need for food without negative environmental side effects, MethaGone is set to solve multiple problems at the same time.

## Social

One cannot talk about the cattle industry without the backlash that comes from organizations that promote vegetarianism or veganism being brought into the conversation. These organizations tend to stand against food additives in favor of a meatless diet. However this is not a viable option for everyone, so Methagone aims to improve an existing industry.

Another social issue that such products face is the negative social opinion on GMOs. In order to research the best way to quell these doubts, creating, distributing and analyzing an international survey in collaboration with teams internationally can inquire and address these concerns. In this same strain of thought, there may be concerns from farmers when it comes to using such new technology because cattle are their livelihood. However with continued testing and farmer input, we hope to make this product accessible to all.

## Technological

Due to the nature of our research being into a very innovative field, it is possible that there may be other companies that emerge at relatively the same time as we launch

that we were previously unaware of. This is because of the long testing time needed to bring a GMO product to the market.

The capsules produced will also contain bacteria and in order to avoid them being damaged or denatured there, we would probably need to have a specific way in which the capsules are transported and stored before they are used.

## **SWOT Analysis**

<b>Strengths</b>	<b>Weaknesses</b>	<b>Opportunities</b>	<b>Threats</b>
<ul style="list-style-type: none"> <li>• Increasing weight gain</li> <li>• Scalability</li> <li>• One-time administration</li> <li>• Reduced emissions</li> <li>• High adaptability</li> </ul>	<ul style="list-style-type: none"> <li>• Radical design</li> <li>• Long RnD</li> <li>• Animal health</li> </ul>	<ul style="list-style-type: none"> <li>• Large market</li> <li>• Licencing</li> <li>• Tax reduction</li> <li>• University interest</li> </ul>	<ul style="list-style-type: none"> <li>• Competition</li> <li>• Patenting issues</li> <li>• Reservations</li> </ul>

### **Strengths - Increasing Weight Gain**

- Animals which are fed our product have increased weight gain, as energy is not lost as methane, and is instead kept within the animal. This allows for conversion of such energy that can be beneficial for the animal in the form of volatile fatty acids.

### **Strengths - Scalability**

- The scalability is very high, as bacteria are an almost inexhaustible source.

### **Strengths - One-time administration**

- The one time administration of our product makes it an attractive alternative to continuous additives, such as canola oil.

### **Strengths - Reduced emissions**

- Farmers that decide to use our product have reduced GHG emissions, and can use this as green advertisement.

### **Strengths - High adaptability**

- As there are multiple gene sources of bromoform producing vanadium-dependent haloperoxidases, there are many opportunities to create different kinds of feed additives. Additionally, varying promoters can reduce methane in different percentages, allowing control of the amount of methane reduced, and therefore of animal weight gain.

### **Weaknesses - Radical design**

- Our product is a novel approach, which may cause suspicion and fear, as a GMO is added to the intestines of a live animal which serves as a food product for humans.

#### **Weaknesses - Long RnD**

- The timeframe for research and development is large, as much testing and modelling is required. Furthermore, legislation and public opinion must be addressed, which adds an aspect that is very sensitive to public opinion.

#### **Weaknesses - Animal health**

- The animal's health and safety needs to be certain. Research on this field is novel, and therefore resources need to be diverted for veterinarian studies that analyze the animal's health undergoing MethaGone treatments.

#### **Opportunities - Large market**

- Beef is produced in many countries worldwide, and therefore a large target market is available. Climate awareness is increasing, and interest in being part of the solution as well. Therefore we predict that in the future our product is considered a necessity for a sustainable and profitable farm.

#### **Opportunities - Licencing**

- If worldwide legislation would allow MethaGone feed additives to be administered, demand may exceed supply. Therefore, the use of patents could further bring in profit, as product licences can be sold to enhance supply.

#### **Opportunities - Tax reduction**

- If GHG taxes are enforced, a marketing point is gained, as reduced methane emissions from cows can lead to direct tax reductions, making our product an attractive purchase.

#### **Opportunities - University interest**

- Funding interest from universities and other academic institutions may increase the pace of research and development.

#### **Threats - Competition**

- A threat is that other companies release similar products with patents, creating early competition in an unexplored field, which is highly dynamic and can change tides quickly.

#### **Threats - Patenting issues**

- Early exposure of details of our product, or inadequate explanations can lead to a patent application refusal.

#### **Threats - Reservations**

- Ethical reservations and government legislation against GMOs are a large hindrance, as this product needs to convince legislators, scientists as well as the public.

### **Strategies to make use of Opportunities through our Strengths**

MethaGone provides a huge weight gain and efficiency advantage. Every animal that is not fed with our product causes profit loss compared to their competitors. Therefore, an aggressive marketing strategy to maximize usage and demand is proposed.

Since the large and fast scalability allows for reduced pricing, we have a market competitive product which stands out, as it is a one-time feed additive.

### **Strategies to prevent Threats through our Strengths**

The reservations that are present in the population and regulation mean that other entrepreneurs may shy away from such an undertaking. Therefore we are ahead of the innovation curve, and we plan to maintain this through continuous research and adaptation of our product. This also includes human practices to make our product more approachable for the general public. Our team has experience in both science communication, human practices as well as integrated human practices, which allows us to understand a correct marketing and publicity strategy for MethaGone.

### **Strategies to make use of Opportunities to minimize Weaknesses**

The fact that such a large market exists reduces the weakness of such a radical idea, as still a considerable percentage of consumers would choose to use our product for their cows, which in absolute numbers still is a large market.

The worldwide pressure to reduce GHG emissions may cause more research to be conducted around animal health issues surrounding methanogenesis inhibition. This will only advance the application of our product on a larger scale, as more proof of animal health and safety will increase trust in our product.

### **Strategies to minimize the potential dangers lying in sectors where Weaknesses meet Threats**

Using a wide range of patents and varying products, we can outcompete new companies with similar products, although we do have a long research and development phase.

The radical design of our product may clash with public reservations against GMOs. Herein lies one of our strengths, which is human practices and integrated human practices. We have conducted lessons in science communication where feedback is an essential part. Hereby we plan to introduce our idea using an integrative approach with the general population.

## **TIMELINE & METRICS**

### MILESTONES

ACTIVITY	DESCRIPTION	COMPLETION DATE
----------	-------------	-----------------

<b>Patenting</b>	A legal firm will be hired to do a novelty search and file the patents.	Y3.
<b>Start of Test</b>	Working with a research farm to administer our supplement in varying doses and observe the effects and (if any) side effects. This will be done to build on the laboratory tests.	Q3 of Y2.
<b>Pilot Production</b>	To test different production methods to optimize full production. This stage also includes the ramp up. This stage is supplemented by tests in partner farms.	Y5.
<b>Full Scale Production</b>	In this stage we will start producing for >10 farms. Marketing and sales team will be operating at full capacity.	Y7.

## KEY PERFORMANCE METRICS

<b>ACTIVITY</b>	<b>DESCRIPTION</b>	<b>KEY METRIC</b>
<b>Sales</b>	MethaGone's only revenue driver is sales. Therefore increasing sales volume is a KPI.	Sales volume.
<b>Marketing</b>	The limiting factor of how much we can expand is how many farmers are willing to pay for this product. Therefore marketing is the key aspect of our sales growth.	Sales growth
<b>Quality Assurance</b>	Happy cows lead to happy farmers. We will be surveying the farmers and their cows performances to further improve the product.	Weight gain and CH4 data from farmers along with qualitative reports.
<b>Administration</b>	MethaGone requires many highly talented employees. To attract and retain them, surveying joyfulness effectively is crucial.	Employee joyfulness.

## FINANCIAL FORECASTS

### KEY ASSUMPTIONS

**Development time assumptions:** The assumptions are based on the risk assessment. The assets required and monthly expenses for each item of the start to production Gantt diagram, and the monthly expenses of the operations until the end of first year of production are listed in appendix 1.

The price estimates are made with the following resources:

- Laboratory and Production Assets
  - Alibaba
  - Fisher Scientific
- Laboratory and Office Costs
  - Funda in Business
  - Brightlands Incubator Center
- Salaries and HR
  - salaryexpert.com
  - edume.com
- Cost of Goods Sold
  - post.nl
  - Alibaba

## FINANCIAL FEASIBILITY

We have estimated the running costs of our first year with minimum viable production as €1.453.508,77 (Appendix A - Year 7). This estimation is based on a capacity enough to produce 450 pills per day. As a pill has to be administered only once to have a lifetime effect it can create €702 of value based on the @UltraCruz weight gain supplement explained in the competitor analysis section. Based on this value estimate, and production capacity we estimate to have a €75.816.000 value generation. Therefore even if 2% of our value generation is converted into revenue, we end up with net profit.

The profit margin we estimate shows our product can easily be converted into profits.

## FINANCING

### SOURCES OF FUNDING

**Investors:** We will be needing three series of investments to get the idea to full scale production;

Seed, Series A, Series B.

**Banks:** To compensate for unexpected costs we will keep the option of bank loans.

### USE OF FUNDING

Different levels of funding will be used for the different stages that are in Gantt chart. The amount of costs for the different items in the Gantt chart are provided in the cost structure document. Pre-seed funding will be used to finish the proof of concept of MethaDone. When the proof of concept is achieved, seed investment will be pursued

for starting field tests and expanding the tests gradually. When the tests reach their final stage, series A funding will be started to fund the pilot production, and start of sales.

## Sources

Allied Market Research. (2021). *Animal Feed Additives Market by Size, Share and Industry Forecast 2025*. Alliedmarketresearch.com. Retrieved 19 October 2021, from <https://www.alliedmarketresearch.com/animal-feed-additives-market/amp>.

Blue Ocean Barns. (2021). *Our Solution — Blue Ocean Barns*. Blue Ocean Barns. Retrieved 19 October 2021, from <https://www.blueoceanbarns.com/new-index-1>.

Britannica, T. Editors of Encyclopaedia (2019). Ruminant. Retrieved 16th June, 2021 from: <https://www.britannica.com/animal/ruminant>

Chagas, J.C., Ramin, M., & Krizsan, S.J. (2019). In Vitro Evaluation of Different Dietary Methane Mitigation Strategies. *Animals*, 9(12), 1120. doi:10.3390/ani9121120

Cook, R. (2021). *Ranking Of Countries With The Most Cattle*. Beef2live.com. Retrieved 19 October 2021, from <https://beef2live.com/story-world-cattle-inventory-ranking-countries-0-106905>.

Danielsson, R., Dicksved, J., Sun, L., Gonda, H., Müller, B., Schnürer, A., & Bertilsson, J. (2017). Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. *Frontiers in microbiology*, 8, 226.

DSM. (2021). *Minimizing methane from cattle | DSM*. dsm.com. Retrieved 19 October 2021, from <https://www.dsm.com/corporate/markets/animal-feed/minimizing-methane-from-cattle.html>.

Duin, E.C., Wagner, T., Shima, S., Prakash, D., Cronin, B., Yáñez-Ruiz, D. R., ... & Kindermann, M. (2016). Mode of Action Uncovered for the Specific Reduction of Methane Emissions from Ruminants by the Small Molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences*, 113(22), 6172-6177.

Greening, C., Geier, R., Wang, C., Woods, L.C., Morales, S. E., McDonald, M. J., ... & Mackie, R. I. (2019). Diverse Hydrogen Production and Consumption Pathways Influence Methane Production in Ruminants. *The ISME journal*, 13(10), 2617-2632.



Jackson, R. B., Saunio, M., Bousquet, P., Canadell, J. G., Poulter, B., Stavert, A. R., ... & Tsuruta, A. (2020). Increasing Anthropogenic Methane Emissions arise Equally from Agricultural and Fossil Fuel Sources. *Environmental Research Letters*, 15(7), 071002.

Kinley, R. D., de Nys, R., Vucko, M. J., Machado, L., & Tomkins, N. W. (2016). The red Macroalgae *Asparagopsis Taxiformis* is a Potent Natural Anti-methanogenic that Reduces Methane Production during in vitro Fermentation with Rumen Fluid. *Animal Production Science*, 56(3), 282-289.

Kinley, R. D., Martinez-Fernandez, G., Matthews, M. K., de Nys, R., Magnusson, M., & Tomkins, N. W. (2020). Mitigating the Carbon Footprint and Improving Productivity of Ruminant Livestock Agriculture using a Red Seaweed. *Journal of Cleaner Production*, 120836. doi:10.1016/j.jclepro.2020.120836

Li, X., Norman, H. C., Kinley, R. D., Laurence, M., Wilmot, M., Bender, H., ... & Tomkins, N. (2016). *Asparagopsis taxiformis* Decreases Enteric Methane Production from Sheep. *Animal Production Science*, 58(4), 681-688.

Machado, L., Magnusson, M., Paul, N. A., Kinley, R., de Nys, R., & Tomkins, N. (2016). Identification of Bioactives from the Red Seaweed *Asparagopsis taxiformis* that Promote Anti-methanogenic Activity in vitro. *Journal of Applied Phycology*, 28(5), 3117–3126. doi:10.1007/s10811-016-0830-7

Machado, L., Tomkins, N., Magnusson, M., Midgley, D. J., de Nys, R., & Rosewarne, C. P. (2017). In vitro Response of Rumen Microbiota to the Anti-methanogenic Red Macroalgae *Asparagopsis taxiformis*. *Microbial ecology*, 75(3), 811-818.

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., & Cotter, P. D. (2018). The Rumen Microbiome: a Crucial Consideration when Optimising Milk and Meat Production and Nitrogen utilisation efficiency. *Gut Microbes*, 1–18. doi:10.1080/19490976.2018.1505176

Martinez-Fernandez, G., Denman, S. E., Cheung, J., & McSweeney, C. S. (2017). Phloroglucinol Degradation in the Rumen Promotes the Capture of Excess Hydrogen Generated from Methanogenesis Inhibition. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.01871>

Mootral. (2021). *Home - Mootral*. Mootral. Retrieved 19 October 2021, from <https://mootral.com/>.

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (*Asparagopsis taxiformis*) Supplementation Reduces enteric Methane by over 80 Percent in Beef Steers. *Plos one*, 16(3), e0247820.

Symbrosia. (2021). *Seaweed — Symbrosia*. Symbrosia. Retrieved 19 October 2021, from <https://symbrosia.co/seaweed>.

Thapa, H. R., Lin, Z., Yi, D., Smith, J. E., Schmidt, E. W., & Agarwal, V. (2020). Genetic and Biochemical Reconstitution of Bromoform Biosynthesis in *Asparagopsis* Lends insights into Seaweed Reactive Oxygen Species Enzymology. *ACS Chemical Biology*, *15*(6), 1662-1670.

United Nations. (2021). Global Issues: Population. Retrieved 16th June, 2021 from: <https://www.un.org/en/global-issues/population>

Zhang, X. M., Smith, M. L., Gruninger, R. J., Kung Jr, L., Vyas, D., McGinn, S. M., ... & Beauchemin, K. A. (2021). Combined Effects of 3-nitrooxypropanol and Canola Oil Supplementation on Methane Emissions, Rumen Fermentation and Biohydrogenation, and total tract Digestibility in Beef Cattle. *Journal of Animal Science*, *99*(4), skab081.

Zhu, P., Li, D., Yang, Q., Su, P., Wang, H., Heimann, K., & Zhang, W. (2021). Commercial Cultivation, Industrial Application, and Potential Halocarbon Biosynthesis Pathway of *Asparagopsis* sp. *Algal Research*, *56*, 102319. <https://doi.org/10.1016/j.algal.2021.102319>



























# Appendix B- Gantt Diagram

