Sveriges lantbruksuniversitet
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Macroalgae as feed supplement for reduction of methane emission in livestock

- Overview of current knowledge and potential Nordic species

Makroalger i foderstat för reduktion av metangasutsläpp hos nötkreatur

- Översikt av nuvarande kunskap och potentiella Nordiska arter

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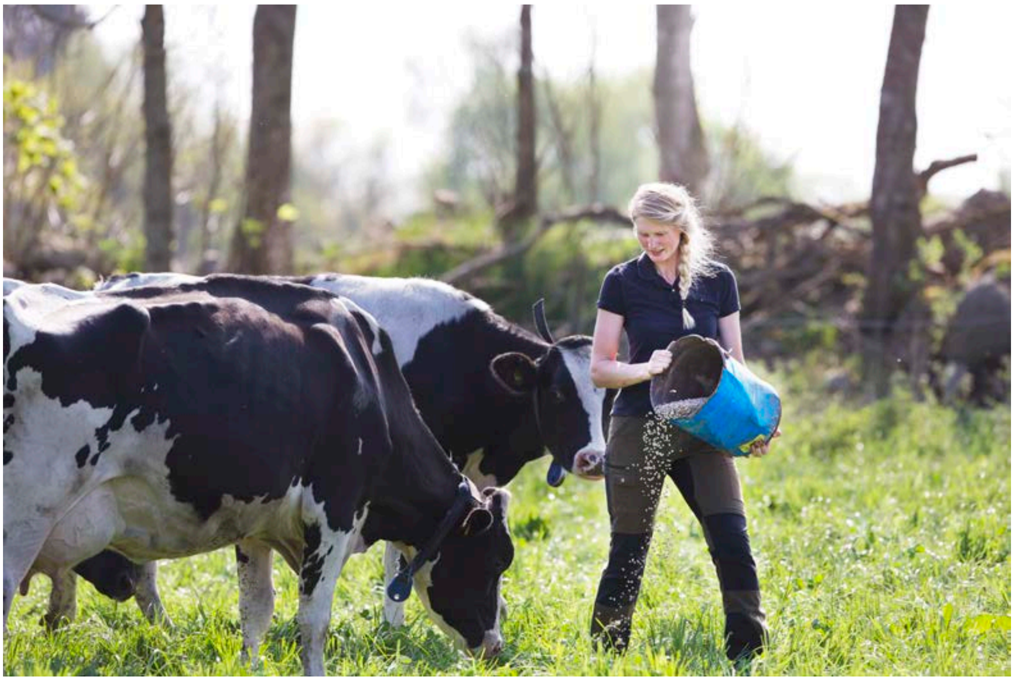

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Abstract

Climate change is a fact and production systems are in need of modernization and sustainable development. Methane is a problematic and potent greenhouse gas and is emitted as a natural byproduct from livestock metabolism.

Asparagopsis taxiformis is an exotic alga that has been found to reduce methane production in livestock rumen by 99% when ingested with everyday feed, at as low inclusion rates as 2% of total organic matter. The biochemical mechanism behind the methane emission reduction is an inhibition of methanogens in the final enzymatic step of methanogenesis in the rumen. This inhibition is conducted by algae secondary metabolites, especially bromoform.

Algae species found in Swedish watercourses are plenty, however only few species contain the desired secondary metabolites of interest. Red algae seem to be the most potent producers of antimethanogenic secondary metabolites. These algae can potentially be produced in open or closed systems and thus be used as supplements in livestock feed for methane reduction. However, the possibility for sustainable largescale algae production and effects on animal health has to be investigated properly before algae can be used commercially. A possible future product has to be accepted by the farmers and be easily integrated with the basal feed.

The market in Sweden has few actors that work with algae. Because of this there is need of further research and development of this sector. Nevertheless, this can mean the rise of a potential new niche on the countryside.

Sammanfattning

Just nu är klimatförändringen ett faktum och detta medför att produktionssystem är i behov av modernisering och hållbar utveckling. Metan är en problematisk och potent växthusgas och emitteras som en naturlig biprodukt i nötkreaturs metabolism.

Asparagopsis taxiformis är en exotisk alg som i vetenskapliga försök har kunnat reducera metangasproduktion med 99% vid så låg inblandning som 2% av totala organiska materialet av fodret. Den biokemiska mekanismen bakom denna metangasreduktion är inhibering av metanogener i det sista enzymatiska steget av metanogenes i vommen. Denna inhibering sker med hjälp av algens sekundära metaboliter, speciellt bromoform.

Algarter i de svenska vattendragen är många men få arter innehåller de sekundära metaboliterna av intresse. Röda alger verkar vara de alger som är mest potenta producenter av sekundära metaboliter som reducerar metangasbildning. Dessa alger kan produceras i öppna eller stängda system och användas som supplement i nötkreaturs foder för reduktion av metangasbildning. Riskerna med storskalig produktion av alger samt inverkan på djurets hälsa måste undersökas grundligt innan alger kan användas kommersiellt. En möjlig framtida produkt måste accepteras av bönderna på marknaden och vara lätt att integrera i foderstat.

Marknaden i Sverige innehåller få aktörer som arbetar med alger. På grund av detta finns det behov av fortsatta studier och utveckling inom sektorn. Icke desto mindre kan detta betyda startskottet för en potentiell ny nisch på landsbygden.

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Dictionary

Bromochloromethane = BCM

Bromoform = BF

Carbon dioxide = CO_2

Coenzyme M (CoM)

Degradability of organic matter = OMdeg

Dibromochloromethane = DBCM

Dry matter = DM

Halogenated methane analogues = HMAs

Hydrogen = H_2

Methane = CH_4

Methyl-coenzyme M = methyl-CoM

Organic matter = OM

Volatile fatty acids = VFA

1 Introduction

Algae are organisms that can range in size, from microscopic microalgae to large seaweeds (macroalgae) (Algae Biomass Organization, 2018). The most common algae are red, brown and green algae and these algae species differ considerable and contain different amounts of protein, lipids, photosynthetic residues and secondary metabolites. Despite of their differences, they play a big role in many ecosystems and thus they are found almost everywhere on the planet. In aquatic food chains, they build the foundation for sustainable fish populations and on land we can benefit from them as well, since they produce about 70% of all the air we breathe.

In some cultures, such as in Asia and Hawaii, algae has been consumed as animal feed and food and can even be seen as a delicacy (Borowitzka, 1998). Algae have great nutritional value and globally, in times of bad economy or insufficient harvests, algae has been used to feed livestock. In present day there is more knowledge regarding algae and thus they have come to be useful in many ways. They are used to produce medicine, cosmetics, biofuel, fertilizers and purifying wastewaters (Oilgae, 2018). Algae can be used in so many versatile ways, but how can we benefit from them even more? And how can algae be applied in the agricultural sector?

The agricultural sector contributes to global warming, both with usage of machines and animal production. According to Eurostat (2018), in 2016, the collection of cows' milk by dairies in EU was a total of 153.2 million tons. Livestock in the dairy industry produce methane, which is a problematic greenhouse gas, as a natural byproduct when fermenting ingested feed. To be able to feed increased human population the demand for dairy and animal derived products will increase. However, the amount of cattle has not increased in the last couple of years, but so has pigs and poultry which have a lower carbon footprint than beef cattle (Statista, 2018). With an increased demand for animal protein, it will be evident that more greenhouse gases be emitted and thus to some extent contribute to the global warming (NASA, 2018). Recent discoveries has shown that an exotic algae species, *Asparagopsis taxiformis*, can be used as feed supplement to reduce methane production in the rumen (Tompkins & Kinley 2015; Kinley et al., 2016; Maia et al., 2016; Vucko et al., 2016). In this thesis, the mechanism behind this reduction will be explored. If methane emissions can be reduced, the dairy industry can benefit from algae and in the same time become more environmentally friendly. Regardless area of study or interest, it is evident that all sectors has to find innovative means of action to develop in a sustainable way. Algae might be the innovative solution that the dairy industry needs to be able to continue as an environmentally sustainable actor in Sweden, and an interesting question that will be investigated in this thesis is if we have any useful macro algae species of in our own watercourses.

2 Background

2.1 Climate change, global warming

”We live in a greenhouse” (NASA, 2018) and life on Earth is in fact depending on the sun and the energy that it provides. What NASA mean with this statement is that some of the radiated energy from the sun is trapped in our atmosphere, due to greenhouse gases (Rummukainen, 2005). The greenhouse effect influence the energy balance of the earth (incoming solar radiation and outgoing thermal radiation) which in turn affects the climate. Anderson et al. (2016) points out that there is a natural greenhouse effect, due to the natural amounts of greenhouse gases and water vapor. Stated by Le Treut et al. (2007), thanks to the greenhouse effect, the average global temperature is 14°C instead of -19°C.

The natural greenhouse effect is also enhanced by human activity, such as burning fossil fuels. According to United States Environmental Protection Agency (EPA, 2017) the main greenhouse gases are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (HFCs, PFCs, NF_3 and SF_6, often referred to as High Global Warming Potential gases). These gases are emitted to the atmosphere by burning of fossil fuels, processing of wood products, agricultural practices and livestock, decay of organic waste and industrial processes. When the amount of greenhouse gases are altered, which according to NASA (2017) we are facing today, the Earth will become warmer on average. The higher temperatures will result in more evaporation and precipitation, warming the ocean and melt glaciers, which contribute to that the sea level rise, and alter the plant dynamics as some species will be favored by higher amounts of CO_2.

The ecosystems as we know them today would look much different if it was not for the greenhouse effect. But in the rate that it is excelling now the Earth is facing changes, which different species cannot adapt to in time (Caiais et al., 2005). Factors such as altered ecosystem dynamics and more evaporation, due to higher temperatures, will cause oxidative stress in plants due to induction of reactive oxygen species (ROS). ROS is a natural part of the plant physiology but increase in response to abiotic stress. Higher levels of greenhouse gases, such as CO_2, increase net photosynthesis and decreasing stomatal opening. But although the increasing levels of CO_2 might favor the plants, the changed pattern of rainfall and higher temperatures will generally result in diminished crop yields, because of ROS (Farnese et al, 2016). What ROS actually do to reduce crop yields is that they cause damage on proteins, alter lipid structure, damage DNA, affect cell structure and deteriorate plant morphology and physiology. This has a negative effect on plant growth and thus crop yield (Frohnmeyer & Staiger, 2003).

In a report from IPCC (2014) it is stated that the CH_4 concentration represents only about 0,5% that of CO_2 in the atmosphere. However, it is considered about 25-30 times as powerful as a greenhouse gas compared to CO_2. In addition to this, EPA (2017) says that methane alone, in the United States, accounted for about 10% of all human activity influenced greenhouse gas emission. Methane is problematic, since it is emitted from natural sources such as wetlands, animals/livestock, decay of organic waste and as well by human activities (EPA, 2017). The natural sources of methane emission cannot be altered in any larger extent but the human activity is flexible. Finding alternative ways to reduce the methane emission, for example in the meat and dairy industry, are important for sustainable agriculture.

2.2 Rumen fermentation and methanogenesis

There are several differences in cattle feed around the world. However, it is often composed of at least some sort of forage, such as silage, legumes and grass.

The rumen enables decomposition and degradation of the forages that is consumed by the cow (Edwards et al., 2004), which is possible due to the microorganisms that are found in the rumen. Once the cow has chewed the feed, it is mixed with saliva and then moved to the rumen, where the fermentation, i.e. microbial degradation of the ingested feed, takes place. The ingested plant material (in shape of protein, carbohydrates and other polymers) are degraded to their respective monomers by primary anaerobic fermenters (Morgavi et al., 2010).

The microbes, which are found in the rumen, are bacteria as well as protozoa, methanogenic archaea and fungi (Edwards et al., 2004). The microbes enables the cow to produce the final nutrients that it needs, but also metabolic residues for their own consumption and wellbeing. The wellbeing of the microbes is essential because the microbes digest about 70-80% of the digestible dry matter in the rumen. Carbohydrates constitutes a large part of the dry matter and the end products, as a result of rumen fermentation, are according to Moran (2005) volatile fatty acids (VFA) (such as acetate, which is important for production of milk fat, propionate and butyrate) and gases (such as CO_2 and CH_4). The gases are most often produced during the carbohydrate fermentation, as VFAs are formed. VFAs are the main source of energy for ruminants and the fermentation efficiency can be measured with production of VFA as indicator.

Fermentation by the glucose that derives from starch or other plant polymers is a part of an anaerobic oxidation, which in the results in reduced co-factors such as NADH (Moss et al., 2000). However the NADH has to be re-oxidized to NAD, otherwise the fermentation of sugars cannot be completed. This results in a regeneration of NAD^+ by electron transport-linked phosphorylation (which takes place in the microbial cells) and results in generation of ATP. The potential of the electron carriers controls production of H_2, which according to Moss et al. (2000) is one of the quantitative biggest product of the fermentation. But H_2 is not accumulated in the rumen, because of interspecies hydrogen transfer. Iannotti et al. (1973) refer to interspecies hydrogen transfer as the process when microbial fermenting species and H_2-utilising methanogens collaborate with each other in the rumen. Thus, methane production is a result of the microbial fermentation, though not a directly produced metabolite.

2.2.1 Microbial ecosystem in the rumen

The microbial ecosystem in the rumen is complex. Edwards et al. (2004) estimates that there are between 300-400 phylotypes of bacteria in the rumen alone. Additional to this there are the protozoa, fungi, bacteriophages and the methanogen archaea.

The domain archaea contains the methanogens, which according to Morgavi et al. (2010), are necessary for the methanogenesis in livestock rumen. Jansen and Kirs (2008) made surveys and their pooled data shows that the *Methanobrevibacter* clade dominated the rumen archaea. Methanogens are according to Garrity et al. (2007) classified into 28 genera and 113 species, but they predict that more species will occur in nature. However, the numbers of methanogens actually cultured from the rumen are no more than seven (Jensen and Kirs, 2008) and are identified as *Methanobacterium bryantii, Methanobacterium formicicum, Methanobrevibacter millerae, Methanobrevibacter ruminantium, Methanobrevibacter olleyae, Methanomicrobium mobile*, and *Methanoculleus olentangyi.*

Jansen and Kirs (2008) also states that *Methanobrevibacter* spp are hydrogen-utilizing methanogens, which means that it is important that they coexist with hydrogen-producing organisms and sufficient H_2 derives from the fermentation process as described above.

2.2.2 Ruminant livestock methanogenesis

When the fermentation process in the rumen in completed there are access amounts of H_2 and CO_2 (Iannotti et al., 1973). As Iannotti et al. (1973) mentioned, the microbial fermenting species in the rumen collaborate with the H_2-utilising methanogens. Since H_2 and CO_2 constantly is produced in the rumen, the methanogens are needed in the process of reducing the amounts of H_2 (particularly) and CO_2. Thus, the production of methane is essential due to the need of H_2 reduction. If the levels of H_2 exceed a certain level, this might inhibit enzymatic processes that are involved in microbial controlled electron transfer reactions. One example is inhibition of the enzyme NADH dehydrogenase, thus if H_2 levels are too high this will result in NADH accumulation and ultimately reduce the rumen fermentation process.

In the process of methane production, the methanogens use three major substrates (figure 1). These are CO_2, acetate and different compounds that contain a methyl group (Liu and Whitman, 2008). However, the most common pathway is the hydrogenotrophic using of CO_2 as carbon source and H_2 as electron donor, see figure 1 for pathway of methanogenesis. Depending on which substrate is used by the methanogens, they are subdivided into two major groups, the slow-growing methanogens (generation time ≈ 130 hours, producing CH_4 from acetate) and the fast-growing methanogens (generation time ≈ 4-12 hours, producing CH_4 from reduction of CO_2 with H_2). Despite which pathway is used by the methanogens to produce methane, all three pathways converge on a reduction of methyl-coenzyme M (methyl-CoM), with coenzyme B, to form methane. In all cases there is an electrochemical gradient generated and due to this electrochemical gradient ATP can be synthesized. The chemical reaction to produce methane, performed by the methanogens, can be described by the following simplified and balanced chemical reaction:

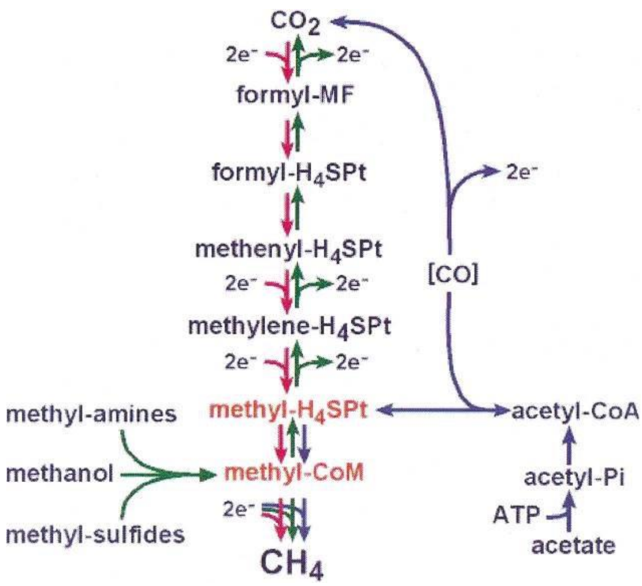

Figure 1: Three pathways of methanogenesis. Hydrogenotrophic pathway (red), methylotrophic pathway (green) and acetoclastic pathway (blue). By James E. Galagan et al., Cold Spring Harbor Laboratory Press (2002). *Genome Research*. 2002. p. 533. (CC BY-NC 4.0)

$$4H_2 + CO_2 = CH_4 + 2H_2O$$

2.3 Substrates used for reduced methane emission

Investigations and research have been conducted regarding different substrates for methane emission reduction in livestock. Van Gastelen et at. (2018) conducted research regarding linseed oil and Patra and Saxena (2010) investigated different plant secondary metabolites (such as tannins, essential oils, organosulfur compounds and saponins) to inhibit methanogenesis in the rumen. In addition there has also been research done regarding usage of ionophore compounds, some forage species such as legumes (containing condensed tannins), chemical compounds (halogenated CH_4 analogs, amichloral, chloroform, chloral hydrate, bromochloromethane and 2-bromoethanesulfonic acid, iodopropane and some nitro compounds such as nitroethane, 2-nitroethanol and 2-nitro-1-propanol). Defaunation (removal of protozoa from the rumen) and altering of microbial ecosystem composition has also been studied. Some of these treatments, especially with chemical compounds such as chloroform and chloral hydrate, have resulted in liver damages, altered amounts of red and white blood cells, toxicity for the animal or microbial ecosystem and in some cases even death of the animal (Patra, 2011). Usage of synthetic chemical compounds such as bromoform (BF) and bromochloromethane (BCM) has however been tested on animals with positive outcome and does not seem to damage the animal. Despite this, research has been conducted regarding the microbial ecosystem and when treated with BF and BCM the microbial community in the rumen was altered.

2.4 Usage of algae in livestock feed

Besides the desired property of methane reduction, algae has been used for other reasons, as mentioned in the introduction. Algae possess good nutritional properties and can contribute to both ingestion of macro- and micronutrients (Yaakob et al, 2014). As the human population is increasing, the demand for protein and dairy is increased on the market and because of this, there is a desire for high quality feed supplement such as algae.

Algae are a reliable source of essential amino acids for the livestock. Becker (2004) even states that the protein contained and produced by algae are better in quality than protein that derives from plant material. Some algae species (such as *Arthrospira*) can consist of about 60-70% protein in dry matter. However, algae can provide low amounts of cysteine and methionine and does not provide sufficient amounts of protein sulfur.

Besides protein, Becker (2004) also states that algae consists of high amounts of carbohydrates. This is important, as mentioned before, for the gastrointestinal health and wellbeing of both animal and microbial ecosystem of the rumen. The algae can provide livestock with large amounts of dietary fiber. As primary producers, algae also often possess important bioactive compounds and fatty acids (Madeira et al., 2017).

2.5 *Asparagopsis taxiformis*, the frontrunner for methane reduction

The genus *Asparagopsis* has caused great confusion and the only species within the genus to be recognized are *A. taxiformis* and *A. aramata* (Bonin and Hawkes, 1987).

AlgaeBase and Guiry (2018) gives an overall picture of the *A.taxiformis* taxonomy (empire: Eukaryota, kingdom: Plantae, subkingdom: Biliphyta, phylum: Rhodophyta, subphylum: Eurhodophytina, class: Florideophytina, subclass: Rhodymeniophycidae, order: Bonnemaisoniales, family: Bonnemaisoniaceae, genus: *Asparagopsis*).

A. taxiformis is distributed in tropical to warm temperate regions and can be found throughout the warm parts of the Atlantic and the Indo-Pacific (Silva et al. 1996). *A. taxiformis* has a haplodiplophasic lifecycle where every developmental phase is morphologically distinct. It is also characterized by alternating erect gametophytes (Zanolla, 2015) and spring/summer filamentous tetrasporophytes. They live as free floating or entangled.

Figure 2: *Asparagopsis taxiformis. Asparagopsis taxiformis* Réunion by Jean-Pascal Quod. 2013. (CC-BY-SA-3.0)

3 Aim and research questions

3.1 Aim

The purpose of this literature study is to compile published research regarding usage of algae in livestock feed, in purpose of methane reduction. Additional purpose of this study is also to initiate a process to identify possible Swedish algae species for inclusion in livestock feed to reduce methane emissions.

3.2 Research questions

The thesis focus on the following questions

- Which is the biological mechanism responsible for the algae induced methane emission reduction in livestock?
- Which algae species can potentially be used to achieve methane emission reduction in livestock?
- How should the algae be used and processed to achieve the desired effect?

3.3 Limitations

This bachelors thesis focus on methane emission reduction achieved by usage of algae in livestock feed. Thus, other substances or activities that can affect the methanogenesis are not in scope of this study. In addition, effects besides reduction of methane due to inclusion of algae in livestock feed is not in focus in this thesis. Restrictions regarding ruminants will also be done, no other ruminants than cows will be under consideration.

4 Methodology

The thesis is based on a literature study. Information from articles regarding the chosen topic was searched for on various search engines, such as PubMed, Google Scholar, PRIMO, DiVA portal, ArtDatabanken and National Center for Biotechnology of Information (NCBI).

Words that were used in browsing for articles and literature were rumen fermentation, methane, methane emission, rumen methane, *Asparagopsis taxiformis*, algae, algae feed, livestock, livestock methane emission, climate change, agriculture methane emission, rumen fermentation, methanogenesis, rumen microbiology and methane mitigation. ArtdataBanken has been used to search for specific species.

5 Result

5.1 Usage of *Asparagopsis taxiformis*, compilation of published research

Because of the high amount of secondary metabolites, *A. taxiformis* has been investigated in many studies. Investigations has been done regarding quantification and determination of secondary metabolites, dosage efficiency and effect on host animal.

The majority of research done regarding usage of *A. taxiformis* as feed supplement is done in Australia, since this is where *A. taxiformis* is a native species (FloraBase, 2006). The macro algae was recently discovered as an antimethanogenic organism and thus the majority of research conducted is published in the recent years. In 2014 Greff et al. published a study regarding the chemical compounds in *A. taxiformis,* which could help further studies such as when Machado et al. (2015; 2017), Kinley et al. (2015) and Maia et al. (2016) investigated the effect these chemical compounds had on both methane production and animal wellbeing when *A. taxiformis* was studied.

Since this research area is in its infancy, few experiments in animals have been conducted. The majority of these studies are performed *in vitro* and commonly stated within the studies is that studies *in vivo* is necessary to further investigate *A. taxiformis* as feed supplement.

Studies with usage of *A. taxiformis* has also been conducted on other animals than livestock, such as goats and sheep (Li et al., 2016).

5.1.1 Methane reduction amount is dose and time dependent

Machado et al. (2015) tested different dosages of *A. taxiformis* together with a basal diet of Rhodes grass for the donor steers and as a substrate for *in vitro* incubations. They found that the production of methane was significantly decreased when the algae was included at 1% of the total organic matter (OM), the decrease was by 84.7%. At doses >2% of OM there was a decrease by >99% compared with the control. These results were achieved after 72 hours incubation. Their conclusion is that the effects of inclusion with *A. taxiformis* is dose dependent and that low doses of *A. taxiformis* is enough to obtain desired result.

In comparison, Kinley et al. (2015) also found that reduction of methane production was dependent on both time and dose. They saw that in 1% inclusion had minimal methane production during the first 24 hours, but was followed by a rapid increase in methane production after about 36 hours. However, after 48 hours the methane production was reduced again. At an algal inclusion of 2%, there was no detectable amounts of methane. Thus, it is not necessary to use more than 2% *A. taxiformis* inclusion in feed supplement.

5.1.2 Effect on fermentation

The amount of produced VFA was previously mentioned as a way to measure the fermentation efficiency. In addition to this, Machado et al. (2015) measured the degradability of organic matter (OMdeg) as another way to measure the fermentation efficiency. Their result showed that inclusion of *A. taxiformis* affects the fermentation efficiency, reflected in altered amount of VFAs and reduced OMdeg after 72 hours incubation. When compared to the control, at doses <5% of OM, *A. taxiformis* had equal or higher OMdeg, however OMdeg was significantly reduced at doses >10% of OM. In comparison, the amount of produced VFA was significantly reduced for doses >0,5% of OM. At doses of 1% or 2% of OM (the most common doses investigated), the total VFA concentration was reduced by 16.6% respectively 25%. This is thought to be linked with a decrease in production of acetate.

The proportions of the different VFAs were also altered. The molar proportions showed an increase of butyrate and propionate while there was a reduction in acetate. At the 2% of OM inclusion acetate to propionate ratio decreased by 63% compared with the control. Similar results have been achieved (Tompkins & Kinley 2015; Kinley et al., 2016; Maia et al., 2016; Vucko et al., 2016), suggesting that *A. taxiformis* affects fermentation efficiency, however only in a greater extent when incubated at >5% of OM. As mentioned in the section above, this is a high amount compared to what is needed for methane reduction.

5.1.3 Response of rumen microbiota to *Asparagopsis taxiformis*

Machado et al. (2017) used quantitative PCR to target genes in archaea and bacteria to be able to measure the relative abundance of the methanogens in the rumen. The result showed that the decrease in abundance of methanogens was positively correlated with the decrease of methane production and that the abundance of the methanogens was time dependent, since it was lower after 72 hours than 48 hours.

When compared to the control, rumen fluid that had been treated with *A. taxiformis* showed a decrease in the ratio between two different bacteria, suggesting that *A. taxiformis* can shift the bacterial community diversity, however when compared to batch fermentation or treatment time the differences at phylum level was minimal. All of the applied treatments that Machado et al. (2017) used with *A. taxiformis* inhibited the growth of methanogens from the *Methanobacteriales*, *Methanomassiliicoccales* and *Methanomicrobiale*.

Machado et al. (2017) also compared treatments with *A. taxiformis* with pure bromoform, which gave similar results, suggesting that the secondary metabolites produced by *A. taxiformis* are responsible for reduction of methanogen abundance in the rumen.

5.2 The biochemistry behind the methane reduction

Several studies has been conducted to find the underlying chemical reaction that is responsible for the methane production and emission reduction (Burreson et al., 1976; Greff et al., 2014; Machado et al., 2016). Greff et al. (2014) found that phytochemical investigations that were performed on *A. taxiformis* gametophyte contain highly brominated cyclopentenones (mahorone and 5-bromomahorone), which are the first derives of 2,3-dibromocyclopentenone which is of natural occurrence. Stated in their report, in a chemical perspective, *A. taxiformis* is particularly interesting since they produce a high diversity of halogenated metabolites.

In 1976 Burreson et al. investigated the content of essential oils from *A. taxiformis* and found that the major content of the essential oil was bromoform (BF, chemical formula $CHBr_3$). However, what they found more interesting in their investigation was that in the same essential oil there was iodide-containing haloform (mostly dibromoiodomethane). In general, halogenated chemical compounds was found in the collected oils from *A. taxiformis*. In this study they also found that *A. taxiformis* contains halomethanes, haloalkanes, haloketones and haloacids. The volatile constituents of *A.taxiformis* is furthermore listed in their report.

Machado et al. (2016) identified the bioactives from *A. taxiformis* whom promoted antimethanogenic activity. Out of the analyzed secondary metabolites, the brominated halomethane (CHX_3) and BF were the most abundant. These are accumulated within vacuoles of specialized gland cells and can be used as defense against herbivores and microbes.

The halogenated volatile organic compounds produced by *A. taxiformis* are diverse and many of these can contribute to the methane reduction. Some halogenated methane analogues (HMAs) other than BF are such as bromochloromethane (BCM, CH_2BrCl), dibromochloromethane (DBCM, $CHBr_2Cl$) dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$). The property of reducing methane in the rumen is, according to

Machado et al. (2016), because of chemicals/secondary metabolites ability to bind with reduced vitamin B_{12} and hence obstruct the cobamide-dependent methyltransferase reaction. This is essential for the composition of methyl-CoM. CoM is a cofactor, which, according to Liu et al. (2011), is found in all methanogens but in no other bacteria or archaea. Because of these abilities, the response to HMAs suggest that they act as direct inhibitors of methanogenesis.

In the study that was conducted by Machado et al. (2016) it was found that *A. taxiformis* produced high concentrations of BF as a secondary metabolite and in dry weight the concentration of BF can vary between 0.17%-1.45% of total dry weight. In comparison to the other secondary metabolites in *A. taxiformis* BF was the most abundant and followed by DBCM, these were also the most active in methane production reduction. However, only BF consisted in high enough quantities in the biomass at 2% algae (which is the most common percentage) organic matter in the feed. In the study it was also found that BF alone, at a concentration of 1µM, reduced methane production by an average 52% compared to the control. But when combined with the other HMAs the level of produced methane was below detection levels. Because of the methane reduction in the rumen, Machado et al. (2016) also found that the production of H_2 significantly increased (as explained in section 2.2.2.)

Wood et al. (1968) described how these HMAs acted as enzymatic inhibitors reducing vitamin B_{12} in the methanogens. In the final enzymatic step of the methyl-transfer reaction, which produces methane from vitamin B_{12}, HMAs acted competitive and inhibited the last step in the reaction. Chalupa (1977) further explained that the mechanism that results in methane reduction involves an irreversible reaction of halogenated methane analogs with reduced vitamin B_{12} and thus this inhibits the previously mentioned cobamide dependent methanogenesis.

Liu et al. (2011) found that structural analogues of coenzyme M (CoM) that are involved in the terminal step of methane biosynthesis could be used to reduce methane biosynthesis. This is due to that CoM is involved in the final step of the methane biosynthesis, in that final step the methyl group carried by CoM is reduced to methane (conducted by methyl-CoM reductase). The inhibition performed by the HMAs is possible since they inhibit the methyl transfer reaction. This is conducted during the final step during methane biosynthesis in methanogens, using H_2 and CO_2. Those methanogenic inhibitors are often the previous mentioned HMAs. Liu et al. (2011) furthermore state that these HMAs can be referred to as ”specific” methanogenic inhibitors, since they are only needed in small concentrations to inhibit all the groups of methanogens.

The same result has been achieved by many scientists and it can therefore be concluded that the biochemistry behind the methane reduction depends effect on the final enzymatic step of the methane biosynthesis.

5.3 Scandinavian algae species for methane reduction

Species found to affect methanogenesis in a study conducted by Maia et al. (2016) were *Ulva* sp. (green macroalgae), *Laminaria ochroleuca* (brown macroalgae), *Saccharina latissima* (brown macroalgae), *Gracilaria vermiculophylla* (red macroalgae) and *Gigartina* spp. (red macroalgae). The algae were added to rumen fluid and studied *in vitro*, without any other

Table 1: The effect of different seaweed (algae) regarding gas production and composition, pH, VFA, and fermentation efficiency form *in vitro* 24 hours incubation. Maia et al., Springer Nature (2016). (CC-BY-4.0)

	Seaweed							
	Control	*Ulva* sp.	*Laminaria ochroleuca*	*Saccharina latissima*	*Gigartina* sp.	*Gracilaria vermiculophylla*	SEM	*P*
Gas, mL	23.7[a]	20.3[c]	21.2[c]	21.1[c]	16.0[b]	19.6[c]	7.94	<0.001
Gas, mL g^{-1} DM	100.5[a]	86.2[c]	89.5[c]	89.9[c]	67.5[b]	82.5[c]	33.65	<0.001
Methane, mL	0.413[a]	0.308[b]	0.472[a]	0.425[a]	0.266[b]	0.255[b]	0.0780	<0.001
Methane, mL g^{-1} DM	1.754[a]	1.301[b]	1.984[a]	1.813[a]	1.117[b]	1.072[b]	0.3322	<0.001
pH	5.94	6.03	6.02	5.99	6.02	6.02	0.076	0.306
NH_3-N, mg g^{-1} DM	3.43[a]	4.47[b]	3.52[a,d]	3.86[a,d]	6.07[c]	4.18[b,d]	0.681	<0.001
Total VFA, mmol g^{-1} DM	3.38[ab]	3.20[a]	3.03[a]	3.60[b]	3.21[a]	3.20[a]	0.220	0.033
Acetic acid, %	61.3[a]	63.4[b]	62.8[ab]	64.3[b]	62.8[ab]	63.4[b]	2.61	0.023
Propionic acid, %	24.0	22.8	22.6	22.4	23.2	22.7	4.67	0.086
Iso-butyric acid, %	0.832	0.997	1.033	0.882	0.994	0.983	0.2834	0.231
Butyric acid, %	10.4[b]	9.6[ac]	10.0[bc]	9.3[a]	9.6[a]	9.5[a]	1.11	<0.001
Iso-valeric acid, %	1.25[ab]	1.24[ab]	1.18[a]	1.12[a]	1.39[c]	1.31[bc]	0.056	<0.001
Valeric acid, %	1.60	1.45	1.57	1.44	1.48	1.49	0.415	0.599
Caproic acid, %	0.551	0.484	0.650	0.469	0.492	0.486	0.2377	0.176
Acetic:propionic acid ratio	2.82[a]	3.09[b]	3.03[b]	3.14[b]	3.00[b]	3.10[b]	0.684	0.002
H_2 generated, mmol L^{-1}	62.4	58.9	56.0	65.4	59.3	59.4	5.84	0.118
H_2 consumed, mmol L^{-1}	21.4	22.2	19.6	19.2	23.5	20.2	1.01	0.161
Recovery, %	36.9	34.8	34.7	33.6	35.0	35.1	4.79	0.233
Fermentation efficiency, %	75.5	74.8	74.7	74.5	74.9	74.8	2.01	0.280

supplement and the effects were observed after 24 hours incubation. The table is an unmodified replica from the study conducted by Maia et al. (2016) and the parameters pH, NH_3-N ml g^{-1} DM, H_2 generated/consumed mmolL^{-1} and Recovery % are not of any further relevancy in the following sections.

The capability to decrease methane production differed between the algae species, a significant reduction in methanogenesis, expressed as ml g^{-1} dry matter (DM), was observed with *Ulva* spp, *Gigartina* spp and *Gr. vermiculophylla*. The most noticeable effects were found with the red algae *Gr. vermiculophylla* and *Gigartina* spp (table 1). This suggests that red algae are the most efficient to reduce methanogenesis in the rumen and this can be connected to the secondary metabolites produced by the algae. Blunt et al. (2007) states that red algae have more than 1500 secondary metabolites of all classes, especially halogenated compounds with bromine or chlorine. In comparison, brown algae has more than 1100 reported secondary metabolites Green algae possess the lowest amount of secondary metabolites of all algae, with only about 300 found compounds.

Carpenter and Liss (2000) conducted a study regarding production and emission of bromoform, which was identified as an inhibitor during methanogenesis, in different algae species. In this study, they found that brown algae from *Laminaria* spp appeared to be the most efficient producers of bromoform within fairly cold waters, such as North Atlantic and Pacific regions. Furthermore they also found that some species within the order Fucaceae, such as *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum* produced high amounts of bromoform. Machado et al. (2014) also found one brown algae of interest, which showed similar methane reduction as *A. taxiformis*, which is called *Dictyota* spp. *Dictyota* spp, reduced methane production by over 92% compared to the used control.

A. taxiformis is a red alga and if other red algae that can be found in Swedish watercourse are to be compared to this alga there is one species that has similar properties. The alga is called *Bonnemaisonia hamifera*, previously known as *Asparagopsis hamifera* (Hariot) Okamura 1921 (currently not an accepted name).

All mentioned algae above can be found in table 2.

5.3.1 *Ulva* spp.

Ulva spp. reduced methane production to 55% of the control when incubated with meadow hay (Maia et al., 2016). As seen in table 1, P<0.001 show that usage of *Ulva* spp. as a direct supplement reduces methane production significantly. Regarding *Ulva* spp., the following species can be found around the Swedish coast: *Ulva lactuca* (figure 3), *Ulva intestinalis*

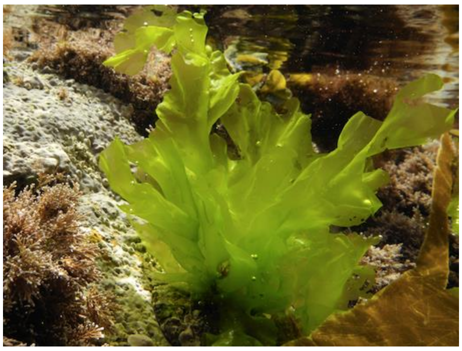

Figure 3: *Ulva lactuca* Used with permission by M.D. Guiry (2000-2018)

Figure 4: *Ulva intestinalis* Used with permission by M.D. Guiry (2000-2018)

(figure 4), *Ulva compressa*, *Ulva procera* and *Ulva prolifera* (Tolstoy et al., 2003). According to Artdatabanken (2018) they can be found mostly around both the east and west coast, from about Stockholm/Gothenburg and down to Skåne. However, *U. intestinalis* can be found around almost every coastal area in Sweden. The algae within *Ulva* spp in Sweden are categorized as viable but some colonies are temporary established.

5.3.2 *Dictyota* spp.

Within *Dictyota* spp, *Dictyota dichotoma* (figure 5) exists in Sweden and has been encountered in Bohuslän. It is categorised as near threatened since it can only be found in a few places around the Bohuslän coast. There are fluctuations in occurrence between different years.

Figure 5: *Dictyota dichotoma*. Used with permission by M.D. Guiry (2000-2018)

5.3.3 *Laminaria* spp.

When used as a direct supplement, without incubation of other substrates methane production was reduced significantly (P<0,001), see table 1.

Outside of the Norwegian coast and around the Shetland Islands there has been isolations of *Laminaria ochroleuca*, according to Smirthwaite at The Marine Life Information Network (2007). Otherwise *Laminaria ochroleuca* can be found on the coast of southwest England (including Lundy, the Isles of Scilly, south Devon and Cornwall).

Figure 6: *Laminaria digitata*. Used with permission by M.D. Guiry (2000-2018)

In Sweden, some species within *Laminaria* spp. are *Laminaria digitata* (figure 6), *Laminaria hyperborea*, *Laminaria fascia* (more known as *Petalonia fascia*), *Laminaria plantaginea* (more known as *Punctaria plantaginea*) and *Laminaria saccharina* (more known as *Saccharina latissima*) (Artdatabanken, 2018). Since *Laminaria* spp. were found to produce high amounts of bromoform these species might be of interest. Within Sweden the mentioned *Laminaria* spp. above can be found mostly around the west coast, from about Gothenburg and down towards the north of Skåne coast. Some colonies are temporary established, some are permanently established and within *Laminaria* spp. the algae are considered viable in Sweden.

5.3.4 *Saccharina latissima*

Figure 7: *Saccharina latissima.* Used with permission by M.D. Guiry (2000-2018)

When used as a direct supplement *Saccharina latissima* (figure 7) reduced methane production significantly (table 1) (Maia et al., 2016).

Artdatabanken (2018) describes *S. latissima* as resident, reproducing and categorized as viable. It can be found mainly along the west coast of Sweden, from about Skagerrak in the north and down to the very south of Skåne in the Öresund region. It can be found in both marine and brackish habitats.

5.3.5 *Gracilaria vermiculophylla*

Gr. vermiculophylla reduced methane production significantly when supplemented to livestock. When incubated with corn silage it decreased methane production to 63% less of the control (Maia et al., 2016).

Gracilaria vermiculophylla is not a domestic species in Sweden. It comes from east/southeast Asian origin, but was found outside of Gothenburg in 2003 (Axelius & Karlsson, 2004). Artdatabanken (2018) does not possess enough information regarding *Gr. vermiculophylla* and thus it is categorized as not applicable. However, it can only be found around Gothenburg.

5.3.6 *Gigartina* spp.

Gigartina spp. reduced methane production to 44% of the control when incubated with meadow hay (Maia et al., 2016). P<0,001 shows that *Gigartina* spp. significantly reduced

Figure 8: *Gigartina clavellosa (Lomentaria clavellosa).* Used with permission by M.D. Guiry (2000-2018)

Figure 9: *Gigartina subfusca* (*Rhodomela confervoides).* Used with permission by M.D. Guiry (2000-2018)

methane production when supplemented to livestock.

Within *Gigartina* spp., these following species can be encountered: *Gigartina clavellosa* (more known as *Lomentaria clavellosa*) (figure 8), *Gigartina lubrica* (more known as *Gloiosiphonia capillaris*), *Gigartina plicata* (more known as *Ahnfeltia plicata*), *Gigartina purpurascens* (more known as *Cystoclonium purpureum*) and *Gigartina subfusca* (more known as *Rhodomela confervoides*) (figure 9). The species can be found around the west coast, *Gigartina plicata* can however be found in small colonies around Gotland, Skåne and Västerbotten and *Gigartina subfusca* can be found around all coastal areas of Sweden but not further than Västerbotten. All algae within *Gigartina* spp. are categorized as viable.

5.3.7 *Bonnemaisonia hamifera*

Since this macroalgae once shared a common genus with *A. taxiformis* there are evidently some similarities between the two algae. *B. hamifera* (figure 10) can according to Artdatabanken (2018) be found around the area of Gothenburg. However, the species is categorized as not applicable and not enough information is gathered regarding the species.

Figure 10: *Bonnemaisonia hamifera.* Used with permission by M.D. Guiry (2000-2018)

Table 2. All suggested algae species that can be found in Scandinavia, with focus on Sweden. Potential species for methane reduction.

Class	Species	Location	Category
Phaeophyceae (B)*	*A. nodosum*	West coast, Skåne, Västergötland, Dalsland.	Viable
Phaeophyceae (B)	*D. dichotoma*	Bohuslän	Near threatened
Phaeophyceae (B)	*F. serratus*	Götaland	Viable
Phaeophyceae (B)	*F. vesiculosus*	All coastal areas	Viable
Phaeophyceae (B)	*L. digitata*	West coast	Viable
Phaeophyceae (B)	*L. fascia*	Skåne & west coast	Viable
Phaeophyceae (B)	*L. hyperborea*	West coast, Västergötland, Dalsland	Viable
Phaeophyceae (B)	*L. ochroleuca*	Norway/UK	**
Phaeophyceae (B)	*L. plantaginea*	West coast	Viable

Phaeophyceae (B)	*S. latissima*	West coast, Skåne, Västergötland, Dalsland.	Viable
Ulvophyceae (G)	*U. compressa*	Götaland	Viable
Ulvophyceae (G)	*U. intestinalis*	All coastal areas	Viable
Ulvophyceae (G)	*U. lactuca*	Götaland	Viable
Ulvophyceae (G)	*U. procera*	West coast, Skåne, east Svealand/Norrland	Viable
Ulvophyceae (G)	*U. prolifera*	Gothenburg, Stockholm	Viable
Florideophyceae (R)	*B. hamifera*	Gothenburg	Not applicable
Florideophyceae (R)	*Gi. clavellosa*	West coast	Viable
Florideophyceae (R)	*Gi. lubrica*	Gothenburg	Viable
Florideophyceae (R)	*Gi. plicata*	West coast, Skåne, Gotland	Viable
Florideophyceae (R)	*Gi. purpurascens*	West coast	Viable
Florideophyceae (R)	*Gi. subfusca*	All coastal areas	Viable
Florideophyceae (R)	*Gr. vermiculophylla*	Gothenburg	Not applicable

* B = brown, G = green, R = red. ** = Not found in Sweden.

6 Discussion

6.1 The importance of native algae species

The most effective algae species to inhibit methanogenesis is without doubt *A. taxiformis* based on current knowledge, but this is an exotic species and cannot live in the Swedish watercourses. *A. taxiformis* could be farmed in Australia and shipped to Swedish farmers. However, this transport would probably contribute to more greenhouse gas emissions than what would be reduced with contribution of the algae. One solution, if *A. taxiformis* is to be used in Sweden, is to grow it in pools or other controlled environments, think of it as a greenhouse but only for algae. On the other hand, the amount of resources such as space, nutrition and electricity to be able to create these exotic environments would probably not be environmentally sustainable. Since it is not environmentally sustainable to work with this exotic alga, it is important to give the native species their needed attention.

It is important to enlighten the differences between species occurring in the Swedish watercourses, regarding their origin. The species can be very viable in the Swedish climate but in the same time an alien species. A personal reflection regarding the relationship between native and alien species is that the native species could be affected in two different ways in their natural ecosystem. One way is that they are ousted since some alien species are invasive. The second is that due to competition with these alien species, their defense mechanisms has to be strengthened and this might favor secondary metabolite production, thus becoming better as feed supplement. It is hard to predict how the laws of nature will act in the algal natural habitats.

In table 2 the suggested species are listed and categorized regarding their viability. Most of the algae are viable and thus they can be collected from their natural habitat. Nevertheless, it is important to keep in mind that when these algae are collected from their natural habitat, the ecosystem in which they exist will be disturbed. As a personal suggestion, it would be of greatest interest to collect algae species that are washed up on shores or possess a threat for other species, such as invasive algae species. The species that can only be found in temporary colonies or are threatened should not be collected.

Cultivation of species could be made from natural habitats, such as ponds or parts of the ocean or in controlled environments. Algae production systems are currently under development and according to Algae Biomass Organization (2018) no industrial algae production is the other alike. The suggested systems for production of algae are open pond systems, closed systems, fermentation, hybrid systems, integrated systems and excretion processes. The vast majority of these systems are used to produce algae or extract products from them in purpose of biofuel production but they can just as easily be used for other purposes. There are nevertheless risks with open production systems or if the algae are to be produced in sealed parts of other watercourses. The risks are connected with bromoform, the desired secondary metabolite. In the marine ecosystems, these algae produce and release high quantities of bromoform, which has been shown to affect chemical reactions in the troposphere and stratosphere (Ziska et al., 2013). One particular chemical reaction involves ozone, which is destructed when there is a flux of bromoform between air and marine environments. Since algae produce high quantities of bromoform, among many other halogenated organic compounds, it is important to monitor the amount of released bromoform to the troposphere and stratosphere. If cultivation of algae in open systems produce high amounts of bromoform that can flux between the water and air in the open systems it would not be environmentally sustainable to grow them in open systems, due to an increased destruction of ozone.

6.1.1 Algae production in Sweden

Algae production already exists in Sweden. The company Simris Alg produce algae, but they are specialized in microalgae (2018). They have found algae to be excellent producers of essential substances that are important for both animals and humans and produce some microalgae in closed and very controlled systems to maintain high quality algae. They work intense with processing of algae to make nutritional supplements such as Omega-3-oil and are experts in refining the algae.

SeaFarm (2018) is a research project with marine biologists, chemists, engineers, and economists from four different Swedish universities. The researchers at SeaFarm work with macroalgae since they find them to be renewable and durable, which is needed for sustainable production now and in the future. In this project, they collaborate and grow macroalgae to be used in several different purposes. They state that the Swedish coast is suitable for growing algae, since we have a long coastline and a big archipelago, which means many protected areas alongside the coast. They also state that when growing them alongside the coast as they do, no additional energy has to be used in purpose of algae growth. The algae also help to reduce the effects of eutrophication.

SeaFarm has five areas of main study, where they work separately with every step of the algae production. They investigate everything regarding the algae, from establishment to analysis of the process chain. In the different steps no aspect is left out, which is necessary since algae are living organisms and thus possess a risk for ecosystems. They also investigate the chemical composition of the algae, which in case of algae production for methane reduction would be necessary, since the algae would be ingested and thus has to be free from toxins. Since SeaFarm conduct so many projects in different purposes, they might be suitable as managers for algae production for further studies, perhaps to investigate the methane reduction potential of the suggested species in table 2.

If any of the suggested macroalgae in table 2 are found suitable in purpose of methane reduction, SeaFarm could potentially develop a production system and analyze how more large-scale production would affect the environment. In this scenario, Simris Alg could also be an important actor assisting in processing the algae into a final product and reaching out to the market.

Another company that produce and have knowledge regarding algae is KosterAlg (they produce algae in both sea based cultures and in closed tanks). They grow one species, which happens to be mentioned in table 2 (*S. latissima*) and plan on growing either of or both *U. intestinalis* and *U. lactuca*. Thus, they possess great knowledge regarding these species and could assist in investigating these algae more in purpose of large-scale production.

It seems like there is a big market in Sweden for algae production and development. The few companies or establishments that exist today are pioneers in this area and ”sea farmers” might be a sector worth looking into more, since algae production today is very diverse.

6.2 The potential of a new feed product in Sweden

In Sweden today, there are no products that contain algae in purpose of reducing methane production and emission. Izabella Rosengren (2017) interviewed Rebecca Danielsson at SLU who said that it would probably take time before the Swedish farmers embrace a potential method to reduce methane emissions. The thoughts are divided between researchers, the common consumer and the farmer. Danielsson highlights that on the farm, the farmer thinks that the best for the climate is effective production and to reduce the amount of days that the animals are not productive. The farmer often also puts the animal health high up in the priorities and the health related uncertainties regarding usage of algae as supplement in the feed would not be easy to overcome with the farmer. However, veganism and dairy resistance movements have started a discussion regarding production of meat and dairy due to animal rights and their impacts on the environment. To be able to keep up in this debate and maintain a modern livestock business the farmers has to be able to adapt. When this adaptation has been obtained and the farmer can accept an algae based product in purpose of methane reduction, the final product must be easily used and function as optimal as possible.

6.2.1 Can farmers produce algae on their own?

Taking under consideration the potential risks of growing algae in open systems, it would be hard for the farmer to be able to grow them on their farm. It seems like it is hard to work with algae and to be able to eliminate potential environmental risks time has to be put into it. The common farmer would probably not be able to spend time away from the animals or fields in the way that would be necessary to maintain a good and safe algae production. However, it would without doubt open up for a new kind of specialized farmer that can grow algae. Knowledge regarding algae production is scarce and if it could be applied to the agricultural sector, it would not only open up new business opportunities in Sweden but also develop the countryside.

A dream scenario would be that the farmer could maintain a cycle within the farm and in the end benefit from the algae as feed supplement. If a dream farm was to be created, the algae could be applied to polluted watercourses or eutrophied areas within the farm. The algae would feed on the nutrition and neutralize potential toxic substances, leaving the farmer with clean water and a greater amount of algae. The farmer could then process the algae, feed it to the livestock and reduce methane production. This scenario would be great. But if the disadvantages and risks were to be taken under consideration, it would seem not fitting for the farmer to apply this cycle in the farm.

Risks with this cycle could be regarding ousting other species in the watercourses that the algae would be applied to, releasing of bromoform (which mentioned before would deplete ozone), the algae species could potentially spread uncontrollably to other areas or not grow in the area where they were applied and thus become an economical constraint. The livestock could also be affected negatively form the toxins that were absorbed/consumed by the algae. If the toxins that were ingested by the algae would be passed on to the livestock it could make the animal sick and possess a threat to consumers of dairy or meat products. If a circle like this is to be applied, personal recommendations is that it is only done under extremely controlled circumstances and that proper research is done before it can be a potential common practice.

6.2.2 Algae processing

In the studies that have been processed in this thesis, the used algal material has been freeze dried and applied to the rumen fermentation process. However the studies has not questioned how the effects of the algae, *A. taxiformis* in particular, is affected by the treatment and if the methane reduction potential is affected by any other alternative post-harvest processing methods. Vucko et al. (2016) found the importance to analyze the antimethanogenic capacity and concentration of the secondary metabolites found in *A. taxiformis.* In the study they processed *A. taxiformis* using a factorial design based on rinsing, freezing and drying (freeze-dried, kiln-dried and dehydrated) and investigated the methane reduction potential. They found that freezing and then freeze-dried treatments reduced methane most effectively and that unrinsed material, regardless other treatment, contained the highest amount of BF. However, all treatments that contained more than 1 mg g^{-1} BF in the dry weight inhibited methane production by 100%, demonstrating that the threshold for complete inhibition for methane *in vitro* is 1 mg g^{-1} BF in dry weight.

The mentioned macroalgae in table 2 are species that can be found in our Swedish watercourses. The species are suggestions based on research regarding species that contain the desired secondary metabolite bromoform and occurrence in the Swedish watercourses. How these species are to be treated, if based on the research conducted by Vucko et al. (2016), seems to be most effective as not rinsed and freeze-dried. However it is evident to keep in mind that the suggested species differ from *A. taxiformis* and that when freezing them, the cellular content might not react in the same way. It is of big economic and environmental interest that the steps in processing and maintaining the concentration of secondary metabolites is optimized for large-scale production for each alga species and thus they should all be evaluated individually.

Potential risks with the mentioned processing methods can be connected to the cellular structures. Some secondary metabolites does not respond well to cold and some to heat. Freezing might cause ice formation within the algae cells, causing cell damage and potential leakage of secondary metabolites. Nevertheless, it is possible that the species in Sweden are more adapted to cold and that the potential risk of damage to the secondary metabolites is reduced due to natural adaptation. To use unrinsed algae could also be a potential risk. If the algae are collected from natural habitats and not grown in controlled environments there can be harmful objects, toxic components or too high amounts of salt from the ocean. If the algae are not to be rinsed, it would be suggested that they are grown in controlled environments to ensure that they do not harm the consuming livestock, although it is not the most effective post-harvest processing method.

As previously mentioned by Machado et al. (2016) there are many different chemical components in *A. taxiformis* alone that could be responsible for the methane reduction, but it is evident that in this case BF is the most abundant. Blunt et al. (2007) also points out that there are many found secondary metabolites within the different algae species. Because of this, it is important to keep in mind that the different secondary metabolites might affect each other and cooperate in different chemical reactions. Each suggested algae species should be investigated to find if there are secondary metabolites beside BF that affect methanogenesis, and how these affect each other. It is evident that regardless processing and concentration of secondary metabolites, each species differ from the other.

The potential product could, as a suggestion, therefor be processed accordingly: collected, frozen, freeze-dried and packaged in different sized bags, so that it is economically available for both small and large-scale framers.

6.2.3 Practical usage

There are not many countries practicing usage of algae in the feed, and in purpose of methane reduction there is no product on the market. Livestock feed in Sweden today is mostly grown on the farm and some additives are used as complement. Some complements are protein, beans, peas, canola and soy, since the livestock need more protein than what can be found in grass (Edhe, 2018). It has previously been mentioned that algae contain more than the desired secondary metabolites, such as high amounts of fiber and protein. Thus a suggestion would be to investigate both the dietary status of the suggested Swedish algae, table 2, as well as the amount of secondary metabolites.

It is evident that the algae common in Sweden does not possess the radical methane reduction properties as *A. taxiformis*. But if they have high content of protein and sufficient amounts of secondary metabolites, such as BF, to reduce methane emissions they are still desired in a practical usage. Edhe (2018) stated that about 2% of the livestock feed intake is based on soybean protein, which is not produced in high enough amounts in Sweden (Heimer, 2010). In 2008 Sweden imported about 350 000 tons of soybean meal and about 90% was used as animal feed. (mainly used for poultry). The beans are used for many reasons, but according to Heimer (2010) they are particularly used because of their content of the amino acid lysine. Once again, Becker (2007) stated that the algae properties are much desirable, however this time it is referred to the protein and amino acid composition of algae, see section 2.4. They are accordingly a better source of high quality protein than plants and contain all essential amino acids, where lysine is included. To be able to reduce the amount of imported plant protein, some algae in table 2 might be of interest. If they have high enough protein content, they could potentially replace soybeans. This would result in less import, less greenhouse gas emissions connected to import, sufficient protein edition to the livestock and, most important of all, a reduction in methane emission.

The produced amount of algae also has to be taken under consideration. If 2% algae inclusion is to be used, the total amount of algae consumed by one cow/year would be about 150kg. This is a rough estimate and in Sweden only, our livestock would consume more than 50 thousand tons of algae.[1]

What percentage should be used when preparing the feed? *A. taxiformis* can be used in as low dosages as 2% of the OM to achieve the desired effect of complete methane reduction. The amount of BF in the suggested algae, table 2, is probably not as high as in *A.*

[1] Herlin, Anders. 2018. E-mail May 28th. <anders.herlin@slu.se>

taxiformis. Only one of the algae, *D. dichotoma*, gave about the same extreme methanogenesis reduction as *A. taxiformis.* Because of this, the percentile amount of total OM has to be higher to achieve similar effect. When ingested in higher quantities the algae might affect the livestock more, which is seen when *A. taxiformis* is supplemented in 5% or 10% of OM (section 5.1.2). Because of this, the dosage of algae as supplement has to be investigated further with the suggested species in table 2.

Besides the percentage of algae, the basal feed plays a crucial role. When investigating some of the algae species mentioned in table 2, Maia et al. (2016) also found that the basal feed of which the algae were integrated with were of significant importance. Very variable results was observed and increase in methane production was observed compared to the control. Thus, knowledge on interactions between basal diet and specific algal species also have to be developed.

6.3 Algae impact on livestock health

As mentioned in section 5.1.2, the effect on fermentation in the rumen is altered when feeding *A. taxiformis* to livestock. The effect is dose dependent and the fermentation effects, measured in OMdeg, is not lowered at doses <5% of OM. The used amount of OM when supplementing *A. taxiformis* to livestock is 2% of OM, which means that the OMdeg is effective at this dosage. The questionability is regarding the VFA, the proportion between these, since total VFA is lowered and butyrate/propionate ration increased while there was a reduction in acetate. The VFA are important, since they are the biggest source of energy, which means that if total VFA is reduced there might be energy inefficiency. If energy is lost, the animal will suffer from poor productivity. Kinley et al. (2016) investigated how the VFA were affected in the rumen and they reasoned with that the potential reduction in energy efficiency could be compensated. Feed energy is typically lost as methane is produced in the rumen, which can be up to about 12% of the gross energy intake. By using algae, this energy might be conserved in the rumen and be productively used, which would reduce the energy loss due to VFA reduction. Kinley et al. (2016) states that the proportion of retained energy can be quantified with *in vivo* feeding studies when feed intake, methane production and productivity is measured.

Since the suggested macroalgae in table 2 are not as investigated as *A. taxiformis,* it is important to find which dose of total OM will cause loss in VFA and monitor how this will affect livestock productivity. If the loss of VFA and energy derived from them is more than can be obtained from methane reduction, they will not be suitable as feed supplement.

In subsection 2.2 there is also stated that H_2 is one of the quantitative biggest end products of the fermentation, but that it is not accumulated in the rumen because of interspecies hydrogen transfer. Since production of H_2 is controlled by the potential of electron carriers it is important that this chemical reaction is not disturbed, resulting in either H_2 excess or deficit. Despite this fact, the amount of H_2 seems to not exceed far too high levels, which suggests that there are alternative H_2 utilizing pathways besides methanogenesis in the rumen.

The collaboration of fermenting species in the rumen might be affected negatively and it is shown, by Machado et al. (2017), that the microbial ecosystem relationship in the rumen is affected negatively from algae inclusion in the feed. Since there are so many species that coexist in the rumen it is important to keep in mind that these microbial symbionts have adapted to each other and undergone a co-evolving process together with the livestock for millions of years. This entails that they most probably account for some specific function in the rumen, which is essential for livestock life functions and general wellbeing. The secondary metabolites seem to be responsible for the alteration of microorganisms in the rumen, since Machado et al. (2017) obtained about the same result when comparing *A. taxiformis* with BF. The composition of secondary metabolites in the suggested species in table 2 has to be investigated properly to understand the potential impact on the rumen microbial ecosystem. Understanding of microbial activity in the rumen might be the key to overcome obstacles regarding energy inefficiency and ecosystem stability in the rumen.

7 Conclusion

The alga *A. taxiformis* is an exotic species and from an environmental perspective, it is less favorable as feed supplement in purpose of methane reduction in Swedish livestock. Instead, the suggested species in table 2 should be taken under consideration, since they all possess secondary metabolites of interest and can be found in the Swedish watercourses.

The composition of secondary metabolites and concentration of these seem to be the key to reduction of methanogenesis. BF is of especial interest and more research has to be done to find which Swedish algae species has high BF concentration. However, it is evident that the biological mechanism of methane emission reduction in livestock has to be investigated further. Further investigations is necessary since the microbial ecosystem and composition of energetic VFA are affected by livestock algae consumption, which can affect livestock productivity and health. In addition, the basal feed interacts with the efficiency of the methane reduction potential of the algae.

If algae are to be used, research suggests that the algae should be freeze dried and then made into a supplement to the everyday feed. Because of the array of secondary metabolites and yet unknown concentrations within the suggested algae in table 2 the amount of algae supplement in the feed has to be investigated.

It would be of interest to have a cycle within the farm of the livestock keepers, to reduce eutrophication and other environmental pollutions on the farm. But this could possess a potential risk for the animal and consumers of livestock derived products if toxins are stored in the algae or if they spread to other areas. Algae cultivation also seem to require time and knowledge, which the farmer might find troubling since they already have the entire farm to keep in mind. Nevertheless, this opens up for more opportunities on the Swedish market, since algae production is not commonly practiced but promising. The amount of knowledge of algae is big but less accessible. This area of study is very young and the potential of further investigation is both necessary and desirable, since we have to find sustainable means of production systems and consumption.

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