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# Supplementation of ensiled *Saccharina latissima* to calves as a mineral replacement

## Master Thesis in Animal Science – 45 ECTS

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# Preface and acknowledgements

This thesis terminates my master's degree in Animal Science at the University of Copenhagen, Faculty of Health and Medical Sciences. The project proceeded from March to November 2020 with the experimental work carried out in March and April 2020. It was in collaboration with, and funded by, Lerøy Ocean harvest.

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## Abstract

The Danish dairy production is increasing with a growing demand for sustainable feed solutions. One such solution is utilizing the sea instead of producing feeds on land. The production of seaweed in integrated multi-trophic aquaculture can decrease the environmental impact from the fish production and the seaweed can thereafter be used as an environmentally friendly feedstuff. A challenge of feeding seaweed could be its greater mineral content compared to terrestrial plants. Therefore, the objective of this thesis was to investigate if ensiled *Saccharina (S.) latissima* can be used as a feed supplement to replace or decrease mineral supplements in calves' diet without resulting in toxic or unfavourable concentrations of iodine, arsenic or cadmium.

A feeding trial with 12 calves, divided into a control group and a seaweed group, was conducted. The control group was fed with a feed ration containing 57% grass silage and 43% wheat with a mineral mix, while the seaweed group was fed the same ration where 5% of the grass silage was substituted with 5% *S. latissima* on a dry matter basis. The trial lasted 15 days, with a three-day sampling period at the end with measurement of; feed- and water intake, blood sampling, total collection of faeces and grab sampling of urine. Feed samples, pooled faeces samples and pooled urine samples were analysed for mineral content to estimate the mineral retention within the groups. The blood samples were analysed for iodine content.

All minerals were fed and retained in sufficient amounts according to the National Research Council recommendations and requirements. However, there was an increased retention of sulphur, potassium, sodium, iodine and arsenic in the seaweed group compared to the control group. The increased retention of these minerals (except iodine) did not seem to cause problems, as the intake was below maximum tolerable and permitted levels. An increased iodine concentration was found in the plasma of the seaweed group compared to the control group. This reflected the toxic level of iodine in the seaweed feed, which also resulted in symptoms (coughing and nasal discharge) related to iodine toxicity in all calves from this group. There was a tendency for a lower growth in the seaweed group as well, which could be caused by iodine toxicity.

In conclusion, it is not possible to supplement with a 5% dry matter inclusion of *S. latissima* in calf feed, as it will most likely result in iodine toxicity. However, if the iodine can be removed from the seaweed or the absorption can be inhibited, *S. latissima* could be a possible feed supplement to replace mineral supplementation in calf feed.

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# 1. Introduction

In Denmark, the production of milk has increased during the last decade (Statistikbanken 2019). The production of milk demands a high production of feed, which results in negative environmental consequences in the form of emission of greenhouse gases, decrease in biodiversity, degradation of land, water depletion and pollution (Steinfeld et al. 2006). Recently, there has been an increasing awareness regarding sustainability. This was expressed in a market analysis by the Danish Agriculture and Food council from 2017 (Preus et al. 2017). They found that some of the most important issues for the consumers, other than transparency and healthy food, were ecology, chemical-free and ethically sound products, which could be summarised to sustainable products (Preus et al. 2017). Hence, the agricultural industry must become more environmentally sustainable to maintain the consumers' goodwill.

The use of seaweed as an additive or a feedstuff has been suggested for a more sustainable production of feed compared to present practices. Seaweed removes excess nutrients from the water, in which it is produced, (Marino et al. 2015) and therefore decreases eutrophication. This process can be utilized by growing seaweed near fish productions. Furthermore, seaweed reduces the need to cultivate feed for livestock on land. The seaweed seems to be digestible and there are indications it can decrease the emission of methane from the rumen. A study showed *in vitro* lab fermentation of dried *Saccharina (S.) latissima* with either maize silage or sugar beet pulp decreased the methane production per organic matter (OM). The decrease in methane production from the *in vitro* fermentation was 19.8% and 10.8% compared to incubation of the maize silage and sugar beet pulp, respectively. There was only a slight decrease in dry matter (DM) degradation between 2-3% (Satessa et al. 2018). Another study reported lab fermentation of *S. latissima* with meadow hay or corn silage did not decrease the *in vitro* fermentation however, this study did not find a significant decrease in methane production (Maia et al. 2016). The two studies indicate that inclusion of *S. latissima* does not decrease the digestibility of the feed ration and might decrease the methane production.

As in the study of Satessa et al. (2018), the ash fraction is often omitted when looking at the digestibility of seaweed as most studies look at the digestible OM and not on the digestible DM. Since the ash faction does not contribute with digestible energy and the fraction is usually higher in *S. latissima* than in terrestrial plants (Rupérez 2002), omitting it results in a seemingly higher digestibility of the seaweed. In addition, most earlier studies have focused on digestibility of the OM and not on the utilization of the mineral fraction. *Saccharina latissima* contains on average 20-40% ash depending on the season of harvest according to research by

Manns et al. (2017) and Schiener et al. (2015). Ignoring or removing the ash fraction will increase the price of the seaweed substantially by loss of product and the cost of removal. Therefore, the aim of this thesis is to investigate whether the minerals in the ash of *S. latissima* are metabolised by calves, and thereby could increase the value of the seaweed for the producers and the farmers.

### 1.1 Objective

The objective of this Master thesis is to examine if farmed ensiled *Saccharina latissima* can be used as a feed supplement to replace, or decrease, the mineral supplements in the diet to calves without resulting in toxic or unfavourable concentrations of iodine, arsenic or cadmium.

### 1.2 Hypotheses

- Calves will reduce their daily feed intake when offered up to 5% *S. latissima* of the total ration dry matter.
- Farmed ensiled *S. latissima* cannot be used as a dietary supplement to replace mineral supplementation in calf diets
- Farmed ensiled *S. latissima* as a 5% dietary supplement on dry matter basis results in unfavourable and/or toxic levels of iodine, arsenic and cadmium in calves

### 1.3 Delimitation

This Master's thesis will only focus on the use of *S. latissima* as a feed supplement to calves and will not focus on the use of other seaweeds. In relation to the minerals, the focus will be on the mineral retention through the absorption and excretion. Furthermore, the focus will only be on certain essential minerals; Ca, P, Mg, Na, K, S, I, Fe, Cu, Zn, Mn and the potentially toxic minerals; As and Cd.

## 2. Background

The purpose of this literature review is to establish background knowledge about, the mineral content of *S. latissima* and which environmental conditions that could affect it. Furthermore, it aims to provide the needed information about the function, absorption, nutrient requirements and toxic levels of minerals in relation to calf feeding. Finally, it will also contain a small review of earlier studies investigating the potential of using *S. latissima* as a feed supplement with focus on mineral utilization. This information will be used in the evaluation and discussion of the results of the feeding trial.

## 2.1 Mineral content in *Saccharina latissima*

The mineral content in *S. latissima* varies and depends on environmental factors. To ensure an optimal product when using the seaweed as a mineral supplement for cattle, it is important to investigate the individual mineral concentrations and the environmental factors affecting these.

### 2.1.1 Essential minerals

In Table 1 macro-mineral concentrations from different sources of *S. latissima* are listed including the origin of the seaweed and the time of harvest. When evaluating the literature in Table 1 it seems the most abundant macro-minerals are; K > Na > S > Ca > Mg > P, which is also the case in the studies analysing for all macro-minerals (Sharma et al. 2018; Ometto et al. 2018) expect for Ca in Manns et al. (2014). In Table 2 the most abundant and relevant (for this study) trace elements in *S. latissima* are listed. When looking at the concentrations across the literature the order from most to least abundant trace elements are Fe > Zn > Mn > Cu.

*Table 1. Overview of macro-mineral content in S. latissima. Unless otherwise listed the origin is rope culture and the concentrations are given in g/kg DM.*

| Author | Origin | Harvested | Ca | P | Mg | Na | K | S |
|---|---|---|---|---|---|---|---|---|
| **Cabrita et al. (2016)** | IMTA[a] in tanks, Portugal | 2013 | 9.59 | 2.26 | 5.31 | - | - | - |
| **Manns et al. (2014)** | Baltic sea, Denmark | April (year unknown) | 1.29 | 4.439 | 7.969 | 12.26 | 25.53 | 12.11 |
| **Marinho et al. (2015)** | Horsens fjord, Denmark | May 2013-May 2014 | - | 2-17 | - | - | - | - |
| **Neto et al. (2018)** | Brittany, France | April 2015 | 9.19 | - | 6.11 | 30.48 | 38.69 | - |
| **Nielsen et al. (2016)** | 10 stone reefs in inner Danish waters | Aug 2012 | - | 1.0-5.6 | - | - | - | - |
| **Ometto et al. (2018)** | Wild from Trondheim, Norway | Aug 2014 | 16 | 2.4 | 8.2 | 48 | 64 | 11 |
| | | Oct 2014 | 16 | 3 | 7.9 | 42 | 86 | 15 |
| | | Feb 2015 | 17 | 5.5 | 8.3 | 47 | 120 | 14 |
| | | May 2015 | 13 | 0.71 | 9.5 | 46 | 25 | 34 |
| **Sharma et al. (2018)[b]** | Frøya, Norway (Depth 3 m) | May 2015 | 7.9 | 2.8 | 7.2 | 48.1 | 80.5 | 8.8 |
| | Frøya, Norway (Depth 8 m) | May 2015 | 8.6 | 2.8 | 7.1 | 44.9 | 82.1 | 8.4 |
| | Wild from Frøya, Norway | July 2014 | 17.8 | 1.9 | 6 | 33.3 | 58.9 | 9.7 |
| **Stévant et al. (2018)** | Brittany, France | May2015 / June 2015 | - | - | - | 36 / 38.5 | 65 / 81.7 | - |

a) Integrated multi-trophic aquaculture b) Did not remove epiphytes

*Table 2. Overview of trace element content in S. latissima. Unless otherwise listed the origin is rope culture and the concentrations are given in mg/kg DM.*

| Author | Origin | Harvested | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|
| **Cabrita et al. (2016)** | IMTA[a] in tanks, Portugal | 2013 (month unknown) | 30 | 3.91 | 1.17 | 41.6 |
| **Manns et al. (2014)** | Baltic sea, Denmark | April (year unknown) | 133.9 | 10.4 | 2.3 | 44.4 |
| **Neto et al. (2018)** | Brittany, France | April 2015 | 1854 | 5.6 | 38.6 | 38.6 |
| **Nielsen et al. (2016)** | 10 stone reefs in inner Danish waters | Aug 2012 | 21-434.3 | 3-34.1 | 0.29-11.75 | 7.5-68.9 |
| **Ometto et al. (2018)** | Wild from Trondheim, Norway | Aug 2014 | 230 | 4.4 | 3 | 38 |
| | | Oct 2014 | <150 | 4.8 | 2.4 | 32 |
| | | Feb 2015 | 170 | 7.2 | 2.9 | 54 |
| | | May 2015 | <150 | 13 | 6.5 | 66 |
| **Schiener et al. (2015)** | Wild from Scotland | May 2011 | 1159 | 35 | 4 | 29 |
| **Sharma et al. (2018)[b]** | Frøya, Norway (Depth 3 m) | May 2015 | 53.7 | 5.6 | 1.3 | 44.7 |
| | Frøya, Norway (Depth 8 m) | May 2015 | 55.8 | 5.6 | 1.2 | 54.7 |
| | Wild from Frøya, Norway | July 2014 | 111.1 | 4.2 | 4 | 33.7 |

a) Integrated multi-trophic aquaculture b) Did not remove epiphytes

When comparing the literature in Table 1 and 2, it becomes evident, that the concentrations of the macro-minerals and trace elements in *S. latissima* fluctuates. The different concentrations could be due to spatial differences as three studies investigated and found an effect of the spatial area on the mineral concentrations investigated (Nielsen et al. 2016; Roleda et al. 2018; Roleda et al. 2019). The fluctuations in the concentrations could also be due to seasonal changes, which was the case in three other studies (Marinho et al. 2015; Schiener et al. 2015; Ometto et al. 2018). The literature does not agree on a clear pattern of the seasonal differences, but it seems that the mineral content (mostly Na and K) is highest in the winter and early spring (Scheiner et al. 2015; Ometto et al. 2018). Some of the differences in the reported mineral content between the studies could also be attributed to methodological differences. Sharma et al. (2018), for example, did not remove epiphytic growth, which could affect the analysed P content (Marinho et al. 2015) and it might affect other minerals as well.

Some studies examined the effect of the cultivation depth on the mineral concentration and found no effect. The effect of the depths between seven and sixteen meters did not show any effect on the concentration of P, Fe, As, Cd or Zn (Nielsen et al. 2016). Sharma et al. (2018) analysed the essential mineral and heavy metal content at three- and eight-meter cultivation depth. However, they did not report a statistical analysis on the effect of the depths, probably because no significant difference was found. This is supported by looking at the reported concentrations in Sharma et al. (2018), as the concentrations are similar between the two cultivation depths (See Table 1 and 2).

The water salinity and plant phenology and age seem to affect the content of some minerals. The plant phenology of *S. latissima* can be seen on Figure 1. Water salinity was correlated negatively with Fe and P content and positively with As, Cd, Zn content (Nielsen et al. 2016). A higher concentration of Cu and Mn was detected in smaller fronds (composed of stipe and blade) compared to larger fronds of *S. latissima* in Nielsen et al. (2016). Moreover, Indergaard et al. (1990) found a greater concentration of P in new blades of S. latissima compared to both new stipes and old blades and in new stipes compared to old stipes. This indicates the P content decreases with age and that the greater P content is found in the blades.

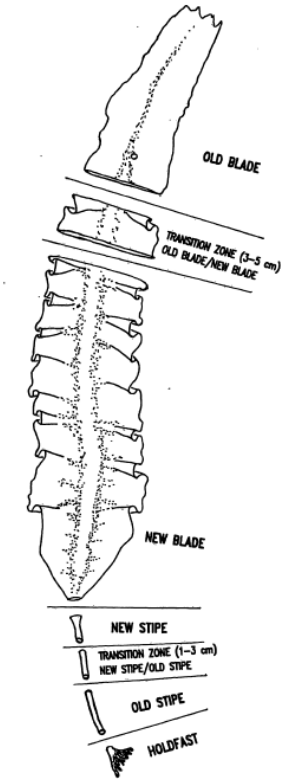


*Figure 1 Plant phenology of Saccharina latissima showing a thallus taken from Indergaard et al. (1990).*

The P content in *S. latissima* seems to be affected by the Phosphate ($PO_4^{3-}$) water concentration. Indergaard et al. (1990) analysed the P content in tank cultured (TC) *S. latissima* with different $PO_4^{3-}$ water concentrations and found a positive correlation between the P content in *S. latissima* and the $PO_4^{3-}$ concentration in the water. The same correlation was found when comparing the P content in *S. latissima* from 10 different stone reefs in inner Danish waters (Nielsen et al 2016). As mentioned above, there was no effect of cultivation depth on the P content in the study by Nielsen et al. (2016). This was despite the $PO_4^{3-}$ concentration was positively correlated with depth.

The biomass source might affect the essential mineral content. A study by Sharma et al. (2018) analysed the mineral content of RC and wild harvested *S. latissima* from the same area. The wild *S. latissima* had a somewhat different concentration of Ca, Na, K, Cl (Table 1), Zn, Cu, Fe (Table 2) and As (Table 4) compared to the RC, which could support the theory that

biomass source has an effect on the mineral content. However, this could be due to other environmental differences between the cultivation sites, which were not analysed or investigated in the study. Another study by Marinho et al. (2015) examined the biomass source's effect on the P content in *S. latissima* by looking at the environmental differences between two sources; integrated multi-trophic aquaculture (IMTA) system and rope culture (RC) placed two km from the blue mussel and trout production in the IMTA. There was no difference in temperature, salinity, and phosphate concentration in the water between the two sites and no effect of the biomass source on the P content in *S. latissima* (Marinho et al. 2015). This might be ascribed to the lack of difference in the P concentrations in the water, as it influences the P content (Nielsen et al. 2016; Indergaard et al. 1990). There were, however, a variation in the P concentration in *S. latissima* over the year with the greatest concentrations in January and the lowest over the spring and summer months (Marinho et al. 2015).

*Table 3. Overview of iodine content in S. latissima. Unless otherwise listed the origin is rope culture and the concentration is g/kg DM.*

| Author | Origin | Harvested | Iodine |
|---|---|---|---|
| **Cabrita et al. (2016)** | IMTA[a] in tanks, Portugal | 2013 | 957.6 |
| **Lüning & Mortensen (2015)** | Horsens fjord, Denmark | May (Year unknown) | 1500 |
| | Faroe Islands | August (Year unknown) | 2700 |
| **Roleda et al. (2018)[b]** | Bodø, Norway | Spring, summer and autumn 2015/16 | 3104 |
| | Trondheim, Norway | | 5171 |
| | France | | 5799 |
| **Schiener et al. (2015)** | Wild from Scotland | May 2011 | 3193 |
| **Sharma et al. (2018)[c]** | Frøya, Norway | May 2015, 3 m | 3600 |
| | | May 2015, 8 m | 3600 |
| | Wild from Frøya, Norway | July 2014 | 3600 |
| **Stévant et al. (2018)** | Brittany, France | May 2015 | 4898 |
| | | June 2015 | 6568 |

a) Integrated multi-trophic aquaculture b) From IMTA, monoculture and wild SL. Iodine content ranged from 1556-7208 mg/kg DM. c) Did not remove epiphytes

### *Iodine*

The iodine (I) content in *S. latissima* is the greatest of the trace elements and ranges from 958-6568 mg/kg DM in the studies shown in Table 3. The environmental effects on the I content will be elaborated in greater detail compared to the other minerals, as the I content of *S. latissima* is greater than of usual feedstuffs and therefore has a greater risk of causing toxicity.

The biomass source and spatial location seems to influence the I content, where fully covered cultivated *S. latissima* in warmer seawater has the greatest concentration (Küpper et al.

2008; Lüning & Mortensen 2015; Roleda et al. 2018). Roleda et al. (2018) examined the effect of season, year, biomass source (wild, monoculture or IMTA) and spatial location on the I content. There was no effect of the season or year, but there were an effect of spatial location and biomass source on the I concentration with the greatest concentrations in the south and the lowest in the north (Roleda et al. 2018). It was speculated that the lower northern temperature might cause this effect, as the I uptake is dependent on an enzymatic system (Küpper et al. 2008), in which the activity seems to be affected by temperature (Almeida et al. 2001). Furthermore, the lower I content in the wild harvested biomass source could be explained by a release of I to detoxify oxidative stress when the wild seaweed is exposed to air during low tides (Küpper et al. 2008). However, the difference in I content could also be explained by differences in the I concentration in the water, as indicated by Lüning & Mortensen (2015). This study found an effect of the biomass source and spatial locations as well, when comparing the I content between RC and TC *S. latissima*. A higher I content was found in RC *S. latissima* from the sea compared to RC near fjords or in TC (Lüning & Mortensen 2015). This could be caused by greater salinity in the seawater compared to fjords and tank water, as the I content seems to increase with salinity in kelp species (Nitschke & Stengel 2014). The I content also decreased with decreasing water turnover, which also indicates the I accumulation is dependent on the I concentration in the water (Lüning & Mortensen 2015). On the other hand, Sharma et al. (2018) did not find a difference in the I content between cultivated and wild *S. latissima* from the same area. This could be explained by similar environments and water turnover between the two sources, but the exact environmental factors for the wild *S. latissima* was not reported, thus it is not possible to compare them.

Two studies investigated the effect of cultivation depth and did not find any differences in I content between cultivation depths of 3 and 8 meters and 2, 5, and 8 meters, respectively (Sharma et al. 2018; Roleda et al. 2018). This could according to Roleda et al. (2018) be due to a similar I concentration within the chosen depth ranges.

The I content seems to be smaller in the youngest parts of the thallus (see Figure 1). The study by Roleda et al. (2018) examined the I content in different parts of the thallus and found the lowest I content per kg DM in older distal parts of the blades and the highest in the holdfasts. This effect was not seasonal, as the same effect was found in 27 independent samples taken over two years. The findings are supported by Nitschke & Stengel (2015), which found the I content per kg DM increased from distal to basal blades and was highest in holdfasts.

### 2.1.2 Potentially toxic heavy metals

In Table 4 studies analysing the heavy metal content of *S. latissima* are listed. From the table it seems content of Arsenic (As) is fairly high compared to the other three heavy metals with Cd being present in the second greatest concentration.

*Table 4. Overview of potentially toxic heavy metal content in S. latissima. Unless otherwise listed the origin is rope culture and the concentrations are given in mg/kg DM.*

| Author | Origin | Harvested | As | Cd | Pb | Hg |
|---|---|---|---|---|---|---|
| **Cabrita et al. (2016)** | IMTA[a] in tanks, Portugal | 2013 | 67.074 | 1.649 | 0.196 | 0.117 |
| **Llorente-Mirandes et al. (2011)** | Galician cost, Spain | Purchased | 52.4 | - | - | - |
| **Manns et al. (2014)** | Baltic sea, Denmark | April (Year unknown) | - | - | 1.5 | - |
| **Nielsen et al. (2016)** | 10 stone reefs in inner Danish waters | Aug 2012 | 29-88.3 | 0.07-2.64 | 0.07-1.66 | - |
| **Ometto et al. (2018)** | Wild from Trondheim, Norway | Aug 2014 | 68 | 0.91 | <0.5 | <0.05 |
| | | Oct 2014 | 120 | 4.6 | <0.48 | <0.05 |
| | | Feb 2015 | 100 | 4.6 | <0.48 | <0.05 |
| | | May 2015 | 28 | 0.76 | <0.49 | <0.05 |
| **Roleda et al. (2019)** | Wild: Bodø, Trondheim, Farmed: Trondheim, France IMTA[a]: Bodø, Trondheim | Spring, summer, autumn 2015+16 | 52.15-99.11 | 0.21-0.99 | 0.03-0.68 | 0.0009-0.1054 |
| **Schiener et al. (2015)** | Wild from Scotland | May 2011 | 73 | - | 1.9 | - |
| **Sharma et al. (2018)[b]** | Frøya, Norway (Depth 3 m) | May 2015 | 55.6 | 0.8 | 0.1 | <0.1 |
| | Frøya, Norway (Depth 8 m) | May 2015 | 54.6 | 0.9 | 0.1 | <0.1 |
| | Wild from Frøya, Norway | July 2014 | 92.5 | 1 | 1.2 | <0.1 |
| **Stévant et al. (2018)[c]** | Brittany, France | May 2015 | 0.16 | 0.22 | - | - |
| | | June 2015 | 0.23 | 0.27 | - | - |

a) Integrated multi-trophic aquaculture b) Did not remove epiphytes. c) Content of inorganic As

The season for harvest might influence the content of the heavy metals but more studies are needed as the literature is conflicting. According to the findings of Ometto et al. (2018) the heavy metal content is lowest in spring. This was attributed to a decreasing As concentration from October to May, as As constitutes most of the heavy metal fraction in *S. latissima*. In contrast to this, Roleda et al. (2019) did not see a significant effect of the season on the As

content, though it was numerically lowest in spring and highest in autumn. The lack of a significant difference could be caused by the high variation in the samples, by a missing observation in the winter or simply because there is no seasonal effect. The observed seasonal effect in Ometto et al. (2018) could also be an indirect effect of the water temperature or the available sunlight, as it usually changes with the season. In addition to this, the age of the seaweed is also dependent on the season and one study showed a higher concentration of As in longer fronds of *S. latissima* compared to smaller fronds (13% increase per meter) (Nielsen et al. 2016). However, this could be due to higher salinity, as the fronds tended to increase with the salinity and increased salinity was correlated with increased As content (Nielsen et al. 2016).

The concentration of As is also affected by the spatial location, but does not seem to be influenced by the biomass source or year of harvest (Roleda et al. 2019). This is supported by a significant effect of the spatial area on the content of all four heavy metals (Nielsen et al. 2016; Roleda et al. 2019). The differences could be due to availability of heavy metals in the water, as the concentrations of heavy metals are expected to be different between locations. It could also explain the rather high As and Pb content found in May by Schiener et al. (2015), since the seaweed was grown in Scotland as opposed to Denmark, Norway and France, where the majority of the studies in Table 4 were conducted. The study of Roleda et al. (2019) also examined the effect of biomass source (IMTA, MC or wild) and year of harvest (2015 and 2016) on the concentration of all four heavy metals but found no effect of either, except on the Pb content between the two years.

The As fraction in *S. latissima* is mostly constituted of organic As (oAs) in the form of arsenosugars as opposed to inorganic As (iAs). The study of Llorente-Mirandes et al. (2011) showed that arsenosugars constituted 98.5% of the 52.4 mg/kg DM As fraction in the *S. latissima* and no iAs was detected. Whereas another study found a concentration of 0.16 and 0.23 mg iAs/kg DM in May and June, respectively (Stévant et al. 2018). The small difference in iAs concentrations could be due to methodological differences, as they used different extraction methods.

## 2.2 Mineral requirements for calves

This section will give the needed knowledge of absorption, function, interactions, deficiency, toxicity and requirements of essential minerals for calves. The essential minerals are important for multiple physiological, structural, and regulatory functions in the animal (McDonald et al. 2010). In this thesis, the focus is on 11 of the essential minerals (macro-minerals and trace elements), which were all classified as essential by 1950 (McDonald et al. 2010). Furthermore,

two potentially toxic minerals, As and Cd, will be addressed, as they are commonly found in *S. latissima* (see Table 4).

### 2.2.1 Mineral function

Structural minerals are minerals, which constitutes a structural element in the body, such as the bones and tissue (McDonald et al. 2010). Calcium, P and Mg are the most abundant mineral constituents of bones and about 99%, 80% and 60-70% of the three minerals in the body are found in the bones, respectively (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Furthermore, the trace element Mn is vital for the formation of the bones (Suttle 2010). Other than serving as a framework for muscle attachment and protection of the organs, the bones serve as a storage of Ca and P, which can be mobilized and utilized in situations of deficiency or when the requirements increase drastically, for example in start lactation (Suttle 2010). Magnesium is also bound in proteins in the soft tissue, where it serves as a catalyst in binding enzymes and substrates for example in phosphorylation's in adenosine triphosphate (ATP) formation (NRC 2001, 2005; Suttle 2010). Another mineral vital for structural elements in the body is S, which is essential for the formation of some amino acids and proteins present in muscle tissue (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). In addition, S is present in the epidermal derivatives and connective tissue (McDonald et al. 2010; Suttle 2010). Copper is also found in linking in connective tissue and Fe is incorporated in proteins vital for the formation of the connective tissue (NRC 2005; Suttle 2010).

Some minerals such as K, Na, Cl, S, Ca and Mg have a physiological function in for example maintaining the acid-base balance, electrochemical potentials, osmotic pressure and in cell signalling. Potassium is an important intracellular ion in tissues, where it acts in the acid-base balance, osmotic regulation, alters enzyme activity, respiration and nerve and muscle activation and function (NRC 200, 20051; McDonald et al. 2010; Suttle 2010). Furthermore, minerals as Na and Cl are the major anion and cation in the extracellular fluid and play an important role in the acid-base balance, osmotic regulation, electrochemical potentials and water metabolism function (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). The S, which is not incorporated in proteins, plays a role in the acid-base balance (NRC 2005). The remaining 1% of the Ca in the body functions in blood clotting and in signalling such as nerve conduction and muscle contraction (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Magnesium is also involved in nerve impulses, muscle contraction and membrane integrity (NRC 2001; Suttle 2010).

Other minerals have regulatory functions in metabolism or in replication and transcription of genes. These are often incorporated in proteins and enzymes and act as cofactors in different reactions. Examples hereof could be Mg's function as a catalyst or functioning in the rumen microorganisms' digestion of nutrients (NRC 2005; McDonald et al. 2010; Suttle 2010). The remaining 20% of the P, that is not found in the bones, functions in some metabolic processes such as energy utilization (ATP) and protein synthesis and is incorporated in for example DNA and phospholipids (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Sulphur is not only a constitute of some proteins but also functions as a binding site for substrates and prosthetic groups in for example enzymatic reactions (Suttle 2010). Zink is involved in a high number of biochemical processes and many enzymes and proteins are dependent on Zn. These enzymes and proteins are involved in transcription of genes, different metabolic pathways such as protein metabolism, synthesis of DNA and absorption of fat and vitamins (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Manganese is involved in activation and a constitute of enzymes for metabolic pathways (McDonald et al. 2010; Suttle 2010). One of these is carboxylase, which is activated by an Mg dependent enzyme and plays a role in lipid and carbohydrate and lipid metabolism (Scrutton et al. 1966; McDonald et al. 2010; Berg et al. 2015a). Copper is essential for the formation or activity of some cofactors, enzymes, and proteins such as haemoglobin. Iron is incorporated in different proteins, where haemoglobin and myoglobin, responsible for transporting oxygen in the blood and muscle tissue, respectively, are the most abundant (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Iron is also incorporated in proteins essential in the electron-transfer chain (NRC 2001; McDonald et al. 2010; Suttle 2010) and in the Krebs cycle, where it removes potentially toxic products (NRC 2005; Suttle 2010; Berg et al. 2015b).

The role of Fe in haemoglobin is called a unique function which is when a mineral is an important constituent of a specific molecule (McDonald et al. 2010). This is also the case for S incorporated in insulin, oxytocin, and some vitamins and P in the formation of phospholipids and ATP (McDonald et al. 2010; Suttle 2010). Furthermore, it is the case for I in thyroid hormones, which is I's only known function in the body (NRC 2005; McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016a). The active form of the hormone is Triiodothyronine (T3), and it regulates the transcription of genes and thereby influences the protein synthesis in the animal. This means the hormone is vital for growth, muscle function, immunity, circulation and fetal development (Hillman & Curtis 1980; NRC 2005; McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016a). Triiodothyronine also increases leptin production from the adipose tissue, which regulates appetite and affects reproduction (Suttle 2010).

### 2.2.2 Mineral retention

Understanding the absorption pathway of the individual minerals and the excretion of them are important when investigating their retention as not all the minerals in the feed will be absorbed or retained in the animal. Furthermore, an understanding of the interactions between the minerals is equally important, as some minerals can affect the absorption of others. Therefore, the absorption, excretion and mineral interactions need to be considered when evaluating a feedstuff as a mineral supplement.

The uptake of some minerals is hormonally regulated. This is for instance the case with Ca, which is passively and actively absorbed between epithelial cells and through Ca channels in the intestines, respectively. The number of Ca channels and the resorption in the kidneys are regulated by the active form of vitamin $D_3$ (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016b). The activation is stimulated by parathyroid hormone, which is secreted from the parathyroid gland in response to small reductions in the ionic Ca concentrations in the extracellular fluid. When there is excess Ca in the intestines, the absorption is downregulated by calcitonin to passive absorption, which also works as negative feedback on vitamin $D_3$ (McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016b). Calcium absorption is evidently regulated and therefore the absorbed Ca is a good measure for the retention, as only a small amount of Ca is excreted through the urine (Suttle 2010; Sjaastad et al. 2016b). The homeostasis of Ca and P are linked since they are the major minerals in the bones (NRC 2001; McDonald et al. 2010; Suttle 2010). However, the absorption of P is less regulated and happens linearly with the content in the feed. It takes place in the small intestine and mainly by diffusion (NRC 2001, 2005; Suttle 2010). Active absorption can occur when the P content in the feed is low. This is also regulated by vitamin $D_3$, which is increased when the concentration of P is low in the blood (NRC 2001, 2005). The saliva is the primary form of excretion of P, though it is easily absorbed in the intestines, which means the urinal excretion might constitute the largest excretion of P (NRC 2005; McDonald et al. 2010; Suttle 2010). Magnesium is also a constituent of bones but does not seem to be hormonally regulated in its absorption and is instead regulated through excretion in the urine, which starts when the concentration in the kidneys exceed a certain threshold (Suttle 2010). Magnesium is both actively and passively absorbed through the rumen, where the passive absorption is driven by a negative potential over the membrane (Martens and Schweigel 2000, NRC 2005). When the concentration of Mg increases, two active carrier mediated transporters start to dominate the absorption (Martens and Schweigel 2000). Evidence shows that a high pH in the rumen can decrease the solubility of Mg and therefore decrease the absorption (Dalley et al. 1997).

Some minerals are readily absorbed, which means there is little regulation of the uptake and the retention is therefore highly dependable on the excretion. This is often the case with monovalent ions (Sjaastad et al. 2016c) such as Na, K, Cl, I and S. The absorption of K is almost absolute, as K is very soluble and there is no regulation of the absorption. It is linear and takes place both in the rumen and the small intestine (Khorasani et al. 1997) by diffusion (NRC 2005; Sjaastad et al. 2016c). The regulation of K in the body is mainly performed by the kidneys and excess K is excreted in the urine. Furthermore, K is excreted in the sweat and saliva (NRC 2001; McDonald et al. 2010; Suttle 2010). Sulphur is also absorbed from both the rumen and intestine. In the rumen about 50% is absorbed as sulphate ($SO_4^{2-}$) in sheep (Kandylis and Bray 1987) and constitutes the potential for toxicity (Suttle 2010). Sulphur incorporated in proteins from both the feed and microbial protein is absorbed in the intestine (NRC 2001, 2005; Suttle 2010). Excess S is excreted in the urine as $SO_4^{2-}$ and the excretion can be rapid. Some excretion also occurs through the saliva and as undegraded protein in the faeces, which depends on the blood concentration of S and the microbial protein synthesis, respectively (Suttle 2010). The absorption of Na happens by active transport in the entire digestive tract (NRC 2001). Sodium is used to transport other elements or nutrients into or out of cells by using the osmotic gradient produced by actively transporting Na out of the cell. Therefore, Na is usually co-transported with glucose, amino acids, I, Cl or other nutrients (NRC 2001, 2005; Suttle 2010; Sjaastad et al. 2016c). As Na absorption is not regulated in the gut, the loss in the urine is regulated and can be almost non-existent. Sodium is also excreted in the saliva, sweat and in milk (NRC 2001; Suttle 2010). Iodine is also readily absorbed throughout the gastrointestinal tract, but mostly from the rumen depending on the feed source. The excess I is excreted in both milk and urine, but also into the abomasum, though most will be reabsorbed (Miller et al. 1975; NRC 2005). The excretion of the daily intake of I at normal feeding levels (defined here as <10 μg I/kg BW) is divided as follows; 30% in faeces, 40% in urine and 8% in milk (Miller et al. 1975).

Multiple metals are absorbed by the same transporter in the intestine, but they usually also have a second absorption pathway, which is not necessarily shared with other metals (Suttle 2010). The absorption of haem Fe occurs through a carrier protein and non-haem Fe trough divalent metal transporters (DMT1) (NRC 2005; Suttle 2010). The activity of DMT1 is heavily regulated, which means excretion of Fe after absorption is almost non-existent. When the animal has a high need for Fe or the diet has a low content of Fe, the prevalence and Fe binding properties of DMT1 are upregulated to increase the absorption of Fe (Suttle 2010). Manganese seems to be absorbed in the same manner as Fe, but the excretion is different (Suttle 2010). Excess Mn is mainly excreted through the bile, where it is either lost in the faeces or reabsorbed.

As with Mn, excess Cu is also excreted in the bile, though there is a rather small and constant excretion of Cu in the urine (NRC 2001, 2005; Suttle 2010). This is in accordance with the absorption of Cu, as it is poor in cattle (NRC 2001, 2005), hence, the lack of excretion can ensure retention. The absorption of Cu is also facilitated by DMT1 (Suttle 2010). The absorption of Zn is, in contrast, not facilitated by DMT1 and is mainly absorbed after need through saturable active transport in the small intestine. However, at very high Zn concentration in the feed, the passive absorption may influence the overall absorption, as the active absorption is downregulated to avoid exceeding the animal's needs (NRC 2005; Suttle 2010). The excretion of Zn occurs by endogenous secretion from the pancreas, as the urinary excretion is unaffected by the Zn intake (Suttle et al. 1982; NRC 2005). There is little regulation of the endogenous loss and it is usually rather constant at 100 µg/kg BW (Suttle et al. 1982).

Even though Zn is not absorbed by the same transporter as the other metals, there are interactions between them as the uptake of Zn can be decreased by excess Cu (Suttle 2010) and Fe (NRC 2001, 2005). In the same manner, high content of Zn can decrease the Cu absorption and high content of Cu, Fe or Mn can decrease the absorption of the others (NRC 2001, 2005; Suttle 2010). The most likely mechanism is downregulation of the shared transporter DMT1. Furthermore, deprivation of either Cu, Fe or Mn can increase the risk of toxicity from the others through upregulation of the DMT1 (NRC 2005; Suttle 2010). It seems that excess Ca also can decrease the absorption of Fe (NRC 2005; Suttle 2010) and Zn (NRC 2001) as well as P by forming insoluble complexes with phytases P (NRC 2001, 2005; Suttle 2010). According to Suttle (2010) it is debated whether Ca and P in high concentrations can decrease Mn absorption as well, whereas the NRC (2001) states Ca, P and K decreases the absorption of Mn. However, both, as well as McDonald et al. (2010), state a high concentration of K in the rumen inhibits the passive transfer of Mg by decreasing the negative membrane potential. In the rumen, Cu is released and can form CuS with $S^{2-}$, which cannot be absorbed. Therefore, high amounts of S in the rumen can decrease the absorption of Cu (Bird 1970). In addition to this, S can form cadmium sulphide by binding with Cd, which is insoluble and unabsorbable, therefore deficiency of S can increase the risk of Cd toxicity (Suttle 2010).

### 2.2.3 Mineral requirements and signs of deficiency and toxicity

With the knowledge of the minerals' function and the absorption in the gastrointestinal tract, it is possible to estimate the requirements for maintenance and growth and the absorption coefficients of the minerals. The requirements are then divided by the absorptions coefficients to get the recommendations for the feed content. These calculations are made in Table 5. All

calculations and absorption coefficients are based on the values from the NRC (2001), as these are used internationally. The absorption coefficients and requirement calculations are made on calves weighing approx. 100 kg, growing 700 g/day with a DMI of 3.4 kg/day and a mature weight of 700 kg. If the requirements or coefficients are not mentioned for a calf of this description, the information for the most similar animal/cow is chosen instead.

The feeding level, at which deficiency will occur, is dependent on multiple factors and a specific feeding level is therefore usually not specified. Some of these factors are the interacting effects between the minerals on their absorption, the chemical form of the mineral and the status of the animal (NRC 2005). Hence, the minimal recommended inclusion level of the mineral, before the animal will suffer from depletion or deficiency, is, in this thesis, assumed to be a significantly lower retention than the requirement. Some of the consequences and signs of deficiency are similar between the minerals. For instance, for all minerals, deficiency can lead to reduced feed intake and growth (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). The decrease in growth could partly be due to impairment of the digestion and metabolism as the microbiota in the rumen depends on both P and S for protein synthesis (NRC 2001, 2005; Suttle 2010). To decrease the rate of deficiency of S, an onset of catabolism of protein in less significant tissues (muscles) occurs. This mechanism ensures sufficient S available for the vital functions, but it also reduces the growth of the animal (NRC 2005; Suttle 2010). The decreased growth can likewise be caused by deficiency of P or Mn, as P deficiency can lead to impaired glycolytic metabolism, and deficiency of Mn has been linked to disturbance in lipid and carbohydrate metabolism and decreased fat deposition (Suttle 2010). Phosphorus and Mn also affect the bone growth as does Ca, Mg and Cu. Therefore, deficiency one of them can lead to impaired bone development (NRC 2005; McDonald et al. 2010; Suttle 2010). Prolonged depletion, mostly of Ca and P, results in reduced bone mineralization or demineralization in growing or adult animals, respectively (NRC 2001; Suttle 2010). This can lead to lameness, abnormal gait, enlarged joints, deformation of bones and osteoporosis (McDonald et al. 2010; Suttle 2010). In addition, Ca and Mg affects nerve and muscle stimuli, which means deficiency can lead to loss of nerve and muscle function (NRC 2001, 2005; McDonald et al. 2010: Suttle 2010). In the same manner, deficiency of Mn can result in impaired function of the central nervous system (CNS) and deficiency of K can result in muscular weakness and paralysis, though deficiency of Mn and K is rare (McDonald et al. 2010; Suttle 2010). Copper and I also affect the CNS and deficiency can result in multiple neurological diseases and impaired brain development, respectively (McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016a). Copper deficiency can also result in abnormal development of epidermal derivatives, which is the case

for deficiency of S, I and Zn too (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). Copper and Fe deficiency can also cause anaemia and I deficiency causes thyroid gland enlargement and impaired gonadal function (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016a).

The column named "MTL" in Table 5 refers to the maximum tolerable level of the minerals. This is defined as a dietary level, which will not result in toxicity of the mineral, when fed for a prolonged period of time. These values are based on the NRC (2005) and can vary depending on the same factors as mentioned for deficiency. If the values are exceeded, it can result in toxicity even though there are different pathways in place to prevent this. For example, high regulation of the absorption or excretion after absorption. The absorption of Ca is highly regulated making toxicity uncommon, but calcification of tissues can occur when Ca is ingested in high doses (NRC 2005; Suttle 2010) and it can result in reduced DMI and performance (NRC 2001, 2005). The uptake of Zn is also regulated by need, but if the concentration in the feed is high, the passive absorption can result in toxicity, which can lead to reduced growth, appetite and volatile fatty acid production in the rumen (NRC 2005; McDonald et al. 2010; Suttle 2010). Since the uptake of Cu is also regulated by need, the tolerance of it is high and can in the weaned calf be up to 900 mg Cu/kg DM for several months without symptoms (Felsman et al. 1973). Some of the symptoms are anorexia and liver damage (NRC 2005). Part of the absorption of Fe is regulated in the same manner as Cu, which ensures a high tolerance to high concentrations of dietary Fe. In addition to this, the concentration of Mn and Cu affects the absorption of Fe, therefore, the toxicity of Fe can occur at different levels and can result in anorexia, vascular congestion, irritable gut, oxidative stress, diarrhoea, reduced growth and feed intake (NRC 2001, 2005; Suttle 2010). Peroxidation is also a results of Fe toxicity and the risk is greater when the antioxidant status is low (Vitamin E) or there is increased availability of poly unsaturated fatty acids (McDonald et al. 2010; Suttle 2010). Manganese toxicity is uncommon as the absorption is poor and regulated after need and excess Mn is excreted through the urine (NRC 2001, 2005). The signs of Mn toxicity are decreased appetite and growth, but severe cases can lead to death (McDonald et al. 2010; Suttle 2010). As with Mn, excess P and Mg is excreted in the urine, which decreases the risk of toxicity. However, toxicity can occur and result in urinary calculi (NRC 2001, 2005; Suttle 2010). Furthermore, P toxicity can interfere with Ca metabolism (NRC 2001). Excess I is also excreted in the urine, but toxicity from excessive I intake can occur, and it results in reduced growth and immunity, increased heart and metabolic rate, dermatitis, respiratory problems such as broncho-pneumonia, coughing and nasal and lachrymal discharge (NRC 2001, 2005; Suttle 2010). Sulphur toxicity can also result

in neurological disorders as well as decreased feed intake, rumen function and growth (NRC 2001, 2005; Suttle 2010). Sodium toxicity results in reduced growth, though it normally only occurs if the drinking water contains high concentrations of salt (> 5-7 g/l) depending on the salt (Suttle 2010) or >10 g/l NaCl (NRC 2001). This high tolerance is probably due to the active Na pumps in the intestine, which pumps Na from the cells into the lumen (Suttle 2010).

*Table 5. The mineral requirements for maintenance and 700g growth/day (WG) for a calf with body weight (BW) of 100 kg, a mature weight (MW) of 700 kg and a dry matter intake (DMI) of 3.4 kg/day. The absorption coefficients and feed recommendations for the minerals are also listed. Values and calculations are from NCR (2001) and the maximum tolerable levels (MTL) are from NRC (2005).*

| **Macro minerals** | **Maintenance g/day** | **Growth g/day** | **Absorption %** | **Feed content[t] g/day** | **MTL % of DM** |
|---|---|---|---|---|---|
| **Ca** | 1.54[a] | 10.56[j] | 42.90%[s] | 29.96 | >1.5 |
| **P** | 2.92[b] | 5.82[k] | 78% | 11.73 | >0.7 |
| **Mg** | 0.3[c] | 0.32[l] | 16.00% | 3.98 | >0.6 |
| **K** | 12.64[d] | 1.12[m] | 90.00% | 15.37 | >2 |
| **Na** | 1.5[e] | 0.98[n] | 90.00% | 2.83 | >3 (NaCl) |
| **S** | | | | 0.2% of DM | >0.4 |
| **Trace elements** | **mg/day** | **mg/day** | **%** | **mg/day** | **% of DM** |
| **Cu** | 0.71[f] | 0.81[o] | 4.60% | 34.18 | >0.05 |
| **I** | 0.6[g] | | 85.00% | 0.71 | >0.2 |
| **Fe** | Negligible | 23.8[p] | 50.00% | 51 | >0.05 |
| **Mn** | 0.2[h] | 0.49[q] | 0.75% | 96.67 | >0.004 |
| **Zn** | 4.5[i] | 16.8[r] | 15.00% | 190.00 | >0.005 |
| **As[u]** | | | | | MTL: 0.003 MPL[v]: 0.0002 |
| **Cd** | | | | | MTL: 0.001 MPL[v]: 0.0001 |

a) 0.0154*BW. b) (0.8*DMI)+(0.002*BW). c) 0.003*BW. d) 0.038*BW+2.6*DMI. e) 1.5*BW/100. f) 0.0071*BW. g) 0.6*BW/100. h) 0.002*BW. i) 0.045*BW. j) (9.83*MW^0.22*BW^-0,22)*WG. k) (1.2+(4.635*MW^0.22)*(BW^-0.22))*WG. l) 0.45*WG. m) 1.6*WG. n) 1.4*WG. o) 1.15*WG. p) 34*WG. q) 0.7*WG. r) 24*WG. s) (57% forage*30% absorption)+(43% concentrate*60% absorption). t) (Maintenance + growth)/absorption. u) Based on the NRC (2001). v) Maximum permitted level.

### 2.2.4 Potentially toxic heavy metals

The absorption of As is debated and can be divided into iAs and oAs. The iAs is presumably absorbed with a coefficient of 0.46, while oAs seems to have a lower absorption coefficient (Bereford et al. 2001). This is however disputed by the NRC (2001) and Suttle (2010), which state oAs and iAs are both well absorbed, but oAs is less toxic. The assumption, that oAs is less toxic is supported by the NRC (2005) and EFSA (2005). Arsenic is easily excreted in the urine (NRC 2005; Suttle 2010), while the retained As is accumulated in the kidneys, liver and muscles (Bereford et al. 2001). Toxicity of As disrupts methylation and oxidative processes and it can alter the metabolism of other minerals (Cu, P and Se), which leads to decreased growth, feed

intake and efficiency (NRC 2005), neurological disorders and problems with balance (Suttle 2010).

The absorption of Cd is very low, about <1% according to NRC (2001) and 16% according to the NRC (2005), and is increased by deficiency of Ca, Fe and Zn, while decreased by excess of the same minerals and Mg (NRC 2005; Suttle 2010). There is very little excretion of Cd after absorption, which means the retention is high and long term. Cd is usually retained in the gut, liver and kidneys, but also in the muscles. Furthermore, there is low excretion into the milk (NRC 2001, 2005; Suttle 2010). Often Cd leads to Zn or Cu deficiency, therefore the signs of Cd toxicity will be those of Cu and Zn deficiency. Also, Cd toxicity can lead to reduced growth, anorexia, anaemia, infertility, kidney damage, cancer, impaired cell reduction-oxidation cycle and Ca signalling probably by decreasing Ca absorption (NRC 2001, 2005; Suttle 2010).

## 2.3 Seaweed as a feed supplement

Previous studies using seaweed as a mineral supplement have not used *S. latissima* as the seaweed source. Furthermore, studies investigating feeding seaweed, as a mineral supplement to calves, is scares. Therefore, the next section will focus on studies feeding different species of seaweed and its effect on the mineral balance and feed intake of cattle.

Inclusion of seaweed in the feed ration to dairy cows does not seem to affect their DM feed intake. This was evident in three studies feeding either brown seaweed residue (800 g/day), brown seaweed waste (3.3% of DM) or fermented brown seaweed waste (0.8% or 1.7% of DM) to dairy cows (Baek et al. 2004; Lee et al. 2005; Hong et al. 2010). In addition, supplementing 0.25% or 0.5% Tasco (processed *Ascophyllum nodosum* into meal-form) to dairy cows did not affect the DM feed intake (Pompeu et al. 2011). Lastly, a study by Singh et al. (2017), that supplemented 20% *Sargassum wightii* in the feed of six dairy cows, showed no difference in DM feed intake between the seaweed supplemented group, the negative control and the positive control (supplemented 2% mineral mix and 1% salt).

Two studies have investigated the effect of feeding seaweed as a mineral supplement on the mineral balance. In continuation of the study supplementing 20% *S. Wightii* mentioned above, the authors also investigated the balance of the minerals Ca, P, Cu, Fe, Zn and Mn. The balance showed a significantly greater Ca retention in the seaweed supplemented group compared to the negative control. Aside from this, no other differences between the negative control and the seaweed supplemented group were found. The positive control group had a significantly greater retention of Zn and Cu compared to the other two groups (Singh et al.

2016). The same results were evident in the plasma samples with the addition that the P concentration was also significantly greater in the positive control compared to the other two groups. Singh et al. (2016) concluded that *S. Wightii* can replace up to 20% of the concentrate mixture without harmful effects, however additional Cu and Zn supplementation is needed. The other study that investigated the mineral balance, supplemented organic dairy cows with a mixture of seaweeds (80% *Ulva rigida*, 17.5% *Sargasum muticum* and 2.5% *Saccorhiza polyschides)* and found a beneficial effect on the mineral status (Rey-Crespo et al. 2014). During the trial, 16 dairy cows were either fed a control feed or supplemented with 100 g/day of the seaweed mixture. The mixture was composed to match the physiological requirements of the fairly I deficient dairy cows used in the study. The seaweed supplementation resulted in an elevated plasma level of I and Se and lower Mo concentration. The greater I and Se plasma concentrations (~0.14 mg/L) were considered beneficial, as the cows at the farm had a low I and Se status (Rey-Crespo et al. 2014).

It seems supplementation with (fermented) brown seaweed waste in the concentration of 0.8%-3.3% can result in elevated I plasma concentration. The studies of Lee et al. (2005) and Hong et al. (2010) did not investigate the mineral balance but did indirectly investigate the concentration of I in the plasma by analysing for the content of T3 and thyroxine (T4). Hong et al. (2010) found an increased concentration of T4 in the seaweed supplemented groups compared to the control group. However, when looking at the T3 and T4 concentrations together, the results were not significant. On the other hand, Lee et al. (2005) found an increased concentration of both T3 and T4 in the seaweed supplemented group. This is indicative of an elevated I retention compared to the control group.

# 3. Material and Methods

The cafeteria trial and feeding trial were both performed on Assendrupgaard in Haslev. Trials at this location are approved and supervised by the research veterinarian Einar Vargas.

## 3.1 Cafeteria trial

A cafeteria trial was performed to test calf preference for four different seaweed inclusion levels in the calf diet. The results of this trial were used to choose the inclusion level in the following feeding trial. Three Danish Red calves with approx. the same weight but ranging from 3.5-5 months of age were used in the trial. They were housed together in deep bedding and were not used in the subsequent feeding trial. The calves were normally fed a dairy cow total mixed ration (TMR), therefore the TMR was used in the trial to avoid multiple changes in the feed. The ensiled seaweed *S. latissima* was mixed into the ration at 0%, 1%, 3% and 5% of DM and

the total DM was 500 g in each treatment. The four treatments were separately mixed into four 10 litre bowls and the four bowls were placed in the two feeding troughs with about one meter between each bowl. The trial took place in the morning for approx. 45 minutes before feeding. At the end, the bowls and orts were weighed to calculate the feed intake of each treatment. This was repeated for three consecutive days, where the placement of the treatments was changed each day to avoid conditional learning and position bias affecting the results.

## 3.2 Feeding trial

### 3.2.1 Feed and timeline

To test the hypotheses a feeding trial was conducted. An overview of the trial with the timeline, diet and sampling can be seen in Table 6. In the trial, 12 Danish Red calves were weaned at the same time and entered an adaption period of nine days. In the adaption period, they were fed a diet without mineral supplement consisting of 57% grass silage and 43% wheat on DM basis. The purpose of this period, other than adaption to the feed, was to remove the effects of the mineral supplement given before the trial. This should ensure the correct response (i.e. the treatments) in the mineral retention was measured during the feeding period. During the last two days of the adaption period, sampling was performed to establish baseline mineral retention. The baseline retention gave an estimate of the mineral status before the seaweed supplementation.

Following the adaptation period, there was a feeding period of 15 days. The calves were either fed a positive control or a diet containing a 5% seaweed inclusion on DM basis. During the last three days of the feeding trial, sampling was performed. The control diet consisted of 57% grass silage and 43% wheat on DM basis and 30 g commercial mineral supplement per calf (in accordance with the recommendations of the mineral supplement used). The seaweed treatment consisted of 52% grass silage, 43% wheat and 5% *S. latissima* on DM basis. The seaweed replaced the grass silage, as they are more comparable with respect to energy content and digestibility than seaweed and wheat. The *S. latissima* was produced and delivered by Lerøy Ocean Harvest (Thormøhlens gate 51 B, 5006 Bergen, Norway). The sticklings were tied to rope and suspended in water in September 2018 in Trælsøy, Norway, and harvested in April 2019, whereafter it was ensiled with silage additives. The seaweed did not contain any epiphytes. The diet of grass silage and wheat was chosen due to practical issues. The calves are normally fed the TMR, but as it contains mineral supplement it was not possible to use it during the adaption period and for the seaweed diet. Therefore, it was decided to use a simpler ration, which was easy and fast to blend evenly each day for the farmer.

*Table 6. Timeline of the feeding trial with diet, weighing, and sampling*

| **Sampling** | **Adaption period** | | | | | | | | | **Feeding period** | | | | | | | | | | | | | | **End** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| **Feed** | Without mineral supplement | | | | | | | | | Control or seaweed diet | | | | | | | | | | | | | | |
| **Weighed** | | | | | | | | x | | x | | | | | | | | | | | | x | | | x |
| **Faeces** | | | | | | | | x | x | | | | | | | | | | | | | x | x | x | |
| **Urine** | | | | | | | | x | x | | | | | | | | | | | | | x | x | x | |
| **Blood** | | | | | | | | x | x | x | | | | | | | | | | | | | x | x | x |

### 3.2.2 Animals and housing

The twelve calves used in the trial were of the breed Danish Red and weaned at 8-13 weeks of age, whereafter the trial started. The calves were weighed on day eight of the adaption period and then randomly divided into the two treatments making sure the average weights and standard deviations (SDs) between the groups were similar (not significantly different). Two of the calves in the control group and three in the seaweed group were full siblings, as their mother was flushed, and the same semen was used on the surrogate heifers. Furthermore, two of the calves in the seaweed group shared father. The calves were housed in deep bedded pens with six in each pen in a natural ventilated barn with temperatures between -5˚and 15℃ during the experimental period. During the two sampling periods the calves were housed in individual boxes bedded with sawdust, located in the same barn and measuring about 1.7 $m^2$ each.

### 3.2.3 Sampling

While the calves were group housed, they were group-fed in large troughs attached to the pens. All sampling occurred while the calves were housed individually. Before and after the sampling periods the calves were weighed and moved between the pen and individual boxes. Each individual box had two 12 litre buckets hanging at the front of the box; one for water and one for feed. The calves had ad libitum access to water and feed, which were measured and given to them in the morning. To decrease selection in the feed, the calves were first provided a known amount of additional feed when the buckets were close to empty.

To calculate the mineral retention, samples of the faeces and urine were taken each sampling day (see Table 6) for later analysis of the mineral content. One sample of each feed was taken for mineral analysis as well. The feed intake was measured, and total collection of the faeces was performed during the sampling periods to calculate the total minerals ingested and absorbed, respectively. Grab samples of the urine were analysed for creatinine to estimate

the total urine excreted according to Chizzotti et al. (2008), which was then used to calculate the individual mineral excretion (See section 3.3. for the calculations).

The faeces were collected into individual five litre buckets as soon as possible after defecation. Caution was taken to avoid sawdust in the samples, though some contamination was unavoidable under the conditions. The urine was caught with small plastic, long handle shovels while the calf was urinating. The samples were taken throughout the day during the three sampling days, until one sample was obtained from each calf, when possible. It was not possible to collect urine samples from all calves during the baseline period, whereas urine samples from all calves except no. 769 were collected every day during the feeding trial. After collection, the urine samples were stored in the barn in individual sealed five litre buckets until the next sampling the following morning. Faeces sampling stopped between 8-8:30 am each day and feed and water orts were measured at this time.

At 9 am blood samples were taken from the *Vena jugularis* into vacutainers containing EDTA. EDTA was chosen as anticoagulant based on earlier research trials using EDTA vacutainers for blood sampling, where the blood I concentration was determined (Hillman & Curtis 1980; Franke et al. 2009). Therefore, no interaction between EDTA and I was assumed. The samples were then centrifuged at 4000 rpm for 10 minutes as soon possible after sampling (maximum of 45 min, on average 30 min). The quick handling of the blood samples was undertaken to avoid an increase in variation by interacting effects between duration after sampling and temperature of storage (Lennon & Mixner 1956). Hereafter, 1.5 mL of plasma from each calf was pooled into subsamples of either the seaweed or control group. After pooling, all blood samples and plasma sub-samples were stored at -18 ℃. New feed was thoroughly mixed, and the calves were given fresh water. The feed was weighed and fed to the calves at about 10:30-11 am. The same method of sampling was used each day running 24 hours from morning to morning.

The total amount of individual faeces of the sampling day was weighed. Similar sized subsamples were taken from each bucket and pooled by treatment. Instead of mixing the total amount of faeces, it was decided to take multiple subsamples to avoid contamination from the sawdust, as it would affect the DM content. Care was then taken to avoid sawdust in the subsamples, and they were taken from different layers in the bucket to ensure the most accurate representation of the 24-hour collection. The urine samples were also pooled by treatment. The urine was poured through a strain to avoid contamination. The smallest sample was measured first and thereafter the same amount from each calf was pooled into the subsample, in order to

have the equal amount from each calf. Both faeces and urine samples were stored at -18℃ until further analysis.

### 3.2.4 Methods for dry matter and mineral analysis

The DM content of the faeces, feed and seaweed samples were determined by drying smaller samples at 125℃ overnight. Afterwards, ash determination was performed by combustion at 525℃ overnight. The faeces, feed and seaweed samples were then freeze dried and sent to Eurofins Agro (Ladelundvej 85, 6600 Vejen, Denmark) along with the urine and plasma samples for determination of individual mineral content. The samples were analysed for the different minerals by the methods shown in Table 7. The concentrations in the feed, faeces and seaweed samples were then given in weight per kg DM, while the urine was weight per litre and the plasma samples in ppm.

*Table 7. Methods of analysis used and written by Eurofins Agro.*

| Material | Mineral/Substrate | Princip |
|---|---|---|
| **Feed and seaweed** | Ca, Fe, K, P, Cu, Mg, Mn, Na, Zn | The organic material is destroyed by burning at 550° C for a minimum of 4 hours. Then the sample is dissolved in the mixture of hydrochloric and nitric acid. The minerals are determined using ICP-OES. |
| | S | Opening with HNO3 in microwave oven. Analysis in ICP-OES |
| | As, Cd | Opening with HNO3 in microwave oven. Analysis in ICP-MC |
| **Faeces and urine** | Ca, Fe, K, P, Cu, Mg, Mn, Na, Zn, B, S | Opening with HNO3 in autoclave. Analysis in ICP-OES |
| | As, Cd | Opening with HNO3 in autoclave. Analysis in ICP-MC |
| | Creatinine | With BioChain product Z5030020 Creatinine Assay Kit |
| **Feed, seaweed, faeces, urine, plasma** | I | Opening with base at 100℃. Analysis in ICP-MS |

## 3.3 Calculations and statistical analyses

The feed intake in the cafeteria trial was analysed by a one-way analysis of variance (ANOVA) with the feed intake as the response variable and the inclusion level as the independent variable using the statistical software: R 3.6.3 (R Core Team 2020). The analysis used the following model: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ , $\varepsilon_{ij} \sim N(0, \sigma^2)$, where e $Y_{ij}$was the response variable, $\mu$ was the overall mean of the feed intake and $\alpha_i$ was the inclusion level ($i$ = 0%, 1%, 3%, 5%). Significance of inclusion level was tested and thereafter significant differences between the individual inclusion levels were tested. For all analyses a p-value below 5% was chosen as significant, while p-values 5% ≤ P ≤ 10% were defined as tendencies.

A comparison of the mineral content in the control and seaweed feed was performed. As only one sample of each was analysed due to costs, the uncertainty of the method reported by Eurofins Agro was used to calculate the 95% confidence interval (CI). Thereafter, the results were checked for overlapping. It was assumed the two samples were significantly different, if the mineral concentrations of the feeds were outside each other's 95% CI, while a tendency was reported if only one of the feeds' mineral concentration was outside the other feed's 95% CI.

A statistical comparison of the two groups was carried out by preforming T-tests on the data for the feed intake, energy intake, water intake, calf growth, minerals in faeces and urine, the mineral retention, and the I concentrations in the plasma samples. All T-tests were defined with two tails and Levene tests were carried out on the data to determine whether the data was hetero- or homoscedastic. Furthermore, the baseline results of the mineral concentrations of the faeces, urine, mineral retention and plasma samples were tested using T-tests. The growth results were tested for outliers with the third and first quartile +/- the inner quartile range as the upper and lower boundaries, respectively.

The energy intake was calculated using energy values of grass silage and wheat from Møller et al. (2005) as the actual energy values were not available. The energy in the control feed was calculated with 43% wheat and 57% grass silage, whereas the seaweed feed was calculated with 43% wheat and 52% grass silage. The energy from the seaweed was assumed as zero to calculate the worst possible scenario. The energy level used for grass silage was then 13 MJ/kg DM and the energy level used for the wheat was 16 MJ/kg DM (Møller et al. 2005).

The urine samples were analysed for creatinine content, as it can be used to quantify the total urine volume (Valadares et al. 1999). A study has found a negative linear correlation between daily urinary excretion of creatinine and heifer BW. The concentration varied for growing heifers and decreased from 0.268 to 0.234 mmol/kg BW from 100 kg BW to >500 kg BW (Chizzotti et al. 2008). The heifer calves in the trial weighed about 100 kg, therefore, 0.268 mmol/kg BW was used when calculating the total urine excretion with the following formula: [creatinine in urine] / (0.268 mmol/kg BW * BW).

The mineral retention was calculated by day and by group. The total mineral ingestion was calculated by multiplying the groups' average daily DMI with the individual mineral concentrations in the feed. The amount of minerals excreted in the manure was calculated by multiplying the individual mineral concentrations from the groups' daily pooled faeces samples with their daily average total faeces weights and the daily DM % of the pooled faeces samples. The minerals excreted in the urine were calculated by multiplying the individual mineral concentrations in the pooled urine samples with the group's average daily total urine excreted

(Williams et al. 2019). In all calculations, the mineral concentrations used were the highest possible outcome (just below the detection limit), when the concentration was under the limit of detection. The mineral retention was calculated as: ingested mineral – (excreted mineral in faeces + excreted mineral in urine).

Some samples were excluded or lost during the sampling. The blood sample of calf no. 775 was lost on the first day of the baseline sampling period and therefore not included in the pooled sample for analysis. During the last day of the feeding trial calf 775 had diarrhoea and an unexplained swollen head. She was treated with activated coal (Correct Akta), antibiotics (Engemycin® Vet. 100 mg oxytetracycline/ml), anti-inflammatory painkillers (Metacam® 20mg meloxicam/ml) and fed with hay. Therefore, her urine and faeces samples were not pooled with the other calves' samples. Furthermore, no blood sample was taken from her the last morning.

# 4. Results

## 4.1 Cafeteria Trial

### 4.1.1 Feed intake and preference

The one-sided ANOVA showed a significant difference in feed intake between the different seaweed inclusion levels (P = 0.0276). There was a significantly smaller intake of the 0% seaweed inclusion level compared to the rations including seaweed (1%, 3% and 5%) with the p-values comparing the 0% with the other three shown in the far-right column in Table 8. There was no significant difference in the feed intake between the three rations including seaweed.

*Table 8 Dry matter (DM) intake with standard diviations (SD) and p-vaules when comparing 0% seaweed inclusion with 1%, 3% and 5% inclusion.*

| Inclusion level | DM intake | SD | P-value |
|---|---|---|---|
| **0%** | 161.38 | 145.37 | |
| **1%** | 359.26 | 106.71 | 0.039 |
| **3%** | 387.66 | 70.71 | 0.022 |
| **5%** | 465.32 | 32.04 | 0.005 |

## 4.2 Feeding trial

### 4.2.1 Baseline measurements

There was no difference in the individual mineral concentrations between the control and seaweed group in neither the faeces, urine nor plasma samples (data not shown). This was also reflected in the absence of significantly different mineral retentions between the two groups as shown in Table 9.

*Table 9 The baseline average daily individual mineral retention and standard deviation (SD)*

| Mineral | Control | | Seaweed | | T-test p-value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| **Ca g/kg** | 5.00 | 0.14 | 5.00 | 0.14 | 0.3412 |
| **P g/kg** | 3.01 | 0.16 | 4.18 | 0.75 | 0.1654 |
| **Mg g/kg** | 1.11 | 0.33 | 1.40 | 0.19 | 0.3920 |
| **K g/kg** | 20.68 | 2.15 | 25.93 | 1.76 | 0.1163 |
| **Na g/kg** | 3.08 | 0.05 | 3.38 | 0.37 | 0.3683 |
| **S g/kg** | 1.34 | 0.01 | 1.75 | 0.27 | 0.1611 |
| **Fe mg/kg** | -79.09 | 44.08 | -0.70 | 6.54 | 0.1306 |
| **Mn mg/kg** | 52.40 | 6.17 | 63.43 | 3.89 | 0.1657 |
| **Zn mg/kg** | 6.39 | 11.95 | 10.94 | 0.61 | 0.6445 |
| **Cu mg/kg** | 5.05 | 1.73 | 4.82 | 0.62 | 0.8787 |
| **I mg/kg** | -0.39 | 0.06 | -0.44 | 0.04 | 0.3749 |
| **As mg/kg** | 0.14 | 0.00 | 0.17 | 0.03 | 0.2258 |
| **Cd mg/kg** | 0.17 | 0.00 | 0.19 | 0.02 | 0.3347 |

### 4.2.2 Mineral content of the feed

Table 10 shows the mineral content of the two feeds with the upper and lower 95% confidence interval (CI). The seaweed feed contained a greater concentration of K, Na, I, As and tended to have a greater S concentration compared to the control feed, whereas the control feed had a greater concentration of Ca, Fe, Mn, Zn and Cu.

*Table 10 The mineral content in the control and seaweed diet with the upper and lower 95% confidence interval (CI) calculated from the uncertaincy provided by Eurofins.*

| Feed | Control | | | Seaweed | | |
|---|---|---|---|---|---|---|
| **Mineral** | **Content** | **Upper CI** | **Lower CI** | **Content** | **Upper CI** | **Lower CI** |
| Ca g/kg | **4.19** | 4.6928 | 3.6872 | **3.65** | 4.088 | 3.212 |
| P g/kg | **2.67** | 2.9904 | 2.3496 | **2.5** | 2.8 | 2.2 |
| Mg g/kg | **1.68** | 1.8816 | 1.4784 | **1.75** | 1.96 | 1.54 |
| K g/kg | **14.3** | 16.016 | 12.584 | **18.3** | 20.496 | 16.104 |
| Na g/kg | **1.96** | 2.1952 | 1.7248 | **4.44** | 4.9728 | 3.9072 |
| S g/kg | **1.6** | 1.92 | 1.28 | **2** | 2.4 | 1.6 |
| Fe mg/kg | **157** | 185.26 | 128.74 | **123** | 145.14 | 100.86 |
| Mn mg/kg | **60.9** | 71.862 | 49.938 | **42** | 49.56 | 34.44 |
| Zn mg/kg | **58.3** | 68.794 | 47.806 | **27** | 31.86 | 22.14 |
| Cu mg/kg | **14.2** | 16.756 | 11.644 | **4.08** | 4.8144 | 3.3456 |
| I mg/kg | **1.7** | 2.04 | 1.36 | **300** | 360 | 240 |
| As mg/kg | **<0.1** | 0.12[a] | 0.08[a] | **1.8** | 2.16 | 1.44 |
| Cd mg/kg | **0.075** | 0.09 | 0.06 | **0.075** | 0.09 | 0.06 |

a) Calculated with the highest possible result (0.1 mg/kg).

The mineral concentrations of the two feeds are listed with the recommendations from the NRC (2001) and the Danish recommendations in Strudsholm et al. (1999) in Table 11. The concentration of K and Fe in both feeds, I and Na in the seaweed feed and Mn in the control feed are noteworthily greater than both of the daily recommendations, while the concentration of Zn and Cu in the seaweed feed are smaller.

*Table 11 Mineral content of the two feeds compared to the recommendations for calves with a growth of 700 g/day.*

| **Macro minerals** | **Control group intake, g/day** | **Seaweed group intake g/day** | **NRC (2001)[a] g/day** | **Strudsholm et al. (1999)[b] g/day** |
|---|---|---|---|---|
| **Ca** | 14.55 | 12.43 | 28.20<br>Min 8.42[c] | 20 |
| **P** | 9.27 | 8.52 | 11.20 | 9 |
| **Mg** | 5.83 | 5.96 | 3.84 | 4 |
| **K** | 49.66 | 62.34 | 15.29 | 13 |
| **Na** | 6.81 | 15.13 | 2.76 | 3 |
| **S** | 0.16% of DM | 0.2% of DM | 0.2% of DM | 0.16% of DM |
| **Trace elements** | **mg/day** | **mg/day** | **mg/day** | **mg/day** |
| **Fe** | 545.17 | 419.02 | 47.6 | 169.95 |
| **Mn** | 211.47 | 143.08 | 92 | 135.96 |
| **Zn** | 202.44 | 91.98 | 142 | 169.95 |
| **Cu** | 49.31 | 13.90 | 32.93 | 33.99 |
| **I** | 5.90 | 1022.00 | 0.71 | 0.85 |

a) All recommendations are calculated as in Table 5. b) Recommendations for a growth of 700 g/day. c) Minimum recommendation in Nielsen & Volden (2011) from Pehrson et al. (1975).

*Table 12 The two feeds mineral content compared to the maximum tolerable level (MTL). All values are in % of DM.*

| **Mineral** | **Control group** | **Seaweed group** | **MTL (NRC 2005)** | **MTL (Strudsholm et al. 1999)** |
|---|---|---|---|---|
| **Ca** | 0.419 | 0.365 | >1.5 | |
| **P** | 0.267 | 0.25 | >0.7 | |
| **Mg** | 0.168 | 0.175 | >0.6 | |
| **K** | 1.43 | 1.83 | >2 | |
| **Na** | 0.196 | 0.444 | >3 (NaCl) | |
| **S** | 0.16 | 0.2 | >0.4 | |
| **Fe** | 0.0157 | 0.0123 | >0.05 | 0.1 |
| **Mn** | 0.00609 | 0.0042 | >0.2 | 0.1 |
| **Zn** | 0.00583 | 0.0027 | >0.05 | 0.05 |
| **Cu** | 0.00142 | 0.000408 | >0.004 | 0.008 |
| **I** | 0.00017 | 0.03 | >0.005 | 0.005 |
| **As** | <0.0001 | 0.00018 | MTL: 0.003[a]<br>MPL: 0.0002[a] | |
| **Cd** | 0.0000075 | 0.0000075 | MTL: 0.001<br>MPL: 0.0001 | |

a) Based on the NRC (2001)

In Table 12, the MTL of the minerals from the NRC (2005) and the level for toxicity from Strudsholm et al. (1999) are shown next to the mineral content of the control and seaweed feed. According to the NRC (2005) and the Danish recommendations (Strudsholm et al. 1999) the I content of the seaweed feed is the only mineral that exceeds the MTL.

### 4.2.3 Water intake, feed intake, energy intake and growth

Table 13 shows the average daily water, feed and energy intake. The water intake of the control group was significantly greater than in the seaweed group (P = 0.019). The average DMI of the control and seaweed feed through the three sampling days were not significantly different between the groups (P = 0.84). In addition, the energy intake was not significantly different between the seaweed and control group (P = 0.53).

*Table 13 Average daily water, feed, and energy intake with corresponding standard deviations (SD).*

| | Mean | SD | T-test p-value |
|---|---|---|---|
| **Control water intake kg/day** | 3.87 | 1.53 | 0.00186 |
| **Seaweed water intake kg/day** | 2.81 | 0.97 | |
| **Control feed intake kg DM/day** | 3.47 | 3.41 | 0.8436 |
| **Seaweed feed intake kg DM/day** | 0.83 | 1.10 | |
| **Control energy intake MJ/day** | 44.61 | 41.99 | 0.5295 |
| **Seaweed energy intake MJ/day** | 10.71 | 13.50 | |

In Table 14, the start and end weights of the 12 calves during the feeding trial is shown and their growth during the whole period have been calculated. The first T-test including all calves showed a tendency for a greater growth in the control group (P = 0.054). As calf 775 was sick during the last 24 hours, her weight gain during the last three days was calculated. The gain was 4.6 kg, while the average weight gain for the rest of the control group was 1.72 kg (data not shown). Therefore, a T-test excluding calf 775 from the results was performed. It showed a tendency that the control group had a greater weight gain than the seaweed group during the trial (P = 0.096). However, according to the outlier test, none of the weight gains were outliers.

*Table 14 Start weight, end weight and growth of the calves during the feeding trial with groups means and standard deviations (SD).*

| Group | Calf number | Start weight, kg | End weight, kg | Weight gain, kg |
|---|---|---|---|---|
| **Control** | 775 | 88.8 | 102 | 13.2 |
| | 768 | 72.2 | 79 | 6.8 |
| | 773 | 91.6 | 98.6 | 7 |
| | 777 | 92.2 | 102.8 | 10.6 |
| | 766 | 100 | 114.8 | 14.8 |
| | 765 | 106.8 | 118.4 | 11.6 |
| | **Mean** | **91.93** | **102.60** | **10.67** |
| | **SD** | | | **3.25** |
| | **Mean (÷ 775)** | **92.56** | **102.72** | **10.16** |
| | **SD (÷ 775)** | | | **3.36** |
| **Seaweed** | 764 | 117 | 131 | 14 |
| | 771 | 85 | 93.8 | 8.8 |
| | 769 | 84.6 | 84.2 | -0.4 |
| | 767 | 104 | 97.6 | -6.4 |
| | 772 | 92.6 | 97.4 | 4.8 |
| | 770 | 90.2 | 91 | 0.8 |
| | **Mean** | **95.57** | **99.17** | **3.6** |
| | **SD** | | | **7.22** |
| **T.test** | | 0.70702 | 0.72261 | 0.05362 |
| **T.test** | **without 775** | | | 0.09592 |

### 4.2.4 Individual mineral retention

The mineral content of the faeces and urine samples of the two groups are compared in Table 15 and 16. There was a greater content of Ca, Mn, Zn and Cu in the control group's faeces compared to the seaweed group. The seaweed group faeces and urine samples contained a greater content of I and As compared to the control group. In addition, the faeces from the seaweed group had a greater K and Na content compared to the control group.

*Table 15. The average daily mineral content in faeces samples with corresponding standard deviation (SD).*

| Mineral | Control | | Seaweed | | T-test p-value | |
|---|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | | |
| **Ca g/kg** | 9.97 | 1.05 | 6.40 | 0.44 | 0.0056 | * |
| **P g/kg** | 4.83 | 0.32 | 5.33 | 0.57 | 0.2555 | |
| **Mg g/kg** | 3.97 | 0.25 | 4.13 | 0.35 | 0.5406 | |
| **K g/kg** | 6.10 | 1.01 | 8.53 | 0.67 | 0.0255 | * |
| **Na g/kg** | 1.20 | 0.10 | 2.00 | 0.10 | 0.0006 | * |
| **S g/kg** | 2.83 | 0.06 | 3.40 | 0.44 | 0.1510 | |
| **Fe mg/kg** | 463.33 | 30.55 | 446.67 | 85.05 | 0.7654 | |
| **Mn mg/kg** | 250.00 | 26.46 | 133.33 | 20.82 | 0.0039 | * |
| **Zn mg/kg** | 243.33 | 32.15 | 76.67 | 5.77 | 0.0009 | * |
| **Cu mg/kg** | 62.00 | 7.81 | 12.00 | 1.73 | 0.0004 | * |
| **I mg/kg** | 4.13 | 0.81 | 126.67 | 15.28 | 0.0050 | * |
| **As mg/kg** | 0.00018 | 0.00003 | 0.00493 | 0.00067 | 0.0064 | * |
| **Cd mg/kg** | 0.00023 | 0.00003 | 0.00026 | 0.00004 | 0.2980 | |

*Table 16. The average daily mineral content in urine samples with corresponding standard deviation (SD).*

| Mineral | Control | | Seaweed | | T-test p-value | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| **Ca g/kg** | <0.1 | | <0.1 | | | |
| **P g/kg** | <0.05[a] | 0.00 | 0.13 | 0.04 | 0.0257 | * |
| **Mg g/kg** | 0.53 | 0.16 | 0.33 | 0.05 | 0.1002 | |
| **K g/kg** | 12.23 | 4.20 | 12.33 | 0.58 | 0.9694 | |
| **Na g/kg** | 1.34 | 0.79 | 2.83 | 0.57 | 0.0564 | |
| **S g/kg** | 0.52 | 0.05 | 0.57 | 0.02 | 0.1696 | |
| **Fe mg/kg** | <0.001 | | <0.001 | | | |
| **Mn mg/kg** | <0.003 | | <0.003 | | | |
| **Zn mg/kg** | <0.01 | | <0.01 | | | |
| **Cu mg/kg** | <0.005 | | <0.005 | | | |
| **I mg/kg** | 1.63 | 0.12 | 160.00 | 17.32 | 0.0040 | * |
| **As mg/kg** | 0.10 | 0.00 | 0.36 | 0.02 | 0.0000 | * |
| **Cd mg/kg** | <0.01 | | <0.01 | | | |

a) 0.049999 was used in the T-test

The average individual mineral retention of the essential minerals during the three sampling days are shown in Table 17. There was a significantly greater mineral retention of the macrominerals; K, Na and S and the trace element I in the seaweed group compared to the control group ($P < 0.05$). The retention of the rest of the minerals were not significantly different between the two groups ($P > 0.05$).

*Table 17 The average daily individual mineral retention and standard deviation (SD)*

| Mineral | Control | | Seaweed | | T-test p-value | |
|---|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | | |
| **Ca g/day** | 8.40 | 1.36 | 9.10 | 0.42 | 0.4474 | |
| **P g/day** | 6.31 | 0.71 | 5.71 | 0.56 | 0.3127 | |
| **Mg g/day** | 3.16 | 0.35 | 3.71 | 0.46 | 0.1732 | |
| **K g/day** | 40.32 | 6.41 | 53.39 | 2.61 | 0.0279 | * |
| **Na g/day** | 5.48 | 0.31 | 13.10 | 0.67 | 0.0001 | * |
| **S g/day** | 3.60 | 0.46 | 4.85 | 0.47 | 0.0294 | * |
| **Fe mg/day** | 262.35 | 30.88 | 187.00 | 58.71 | 0.1205 | |
| **Mn mg/day** | 58.37 | 21.65 | 73.92 | 15.33 | 0.3676 | |
| **Zn mg/day** | 53.35 | 22.66 | 52.31 | 6.70 | 0.9428 | |
| **Cu mg/day** | 11.33 | 5.25 | 7.67 | 1.42 | 0.3086 | |
| **I mg/day** | 2.62 | 0.64 | 900.43 | 44.99 | 0.0008 | * |

### 4.2.5 Plasma iodine

The I concentration of the plasma samples is shown in Table 18. There was a significantly greater I concentration in the plasma from the seaweed group compared to the control group (P = 0.00076).

*Table 18 The iodine (I) concentration in the pooled plasma samples*

| Treatment | Day | I mg/kg |
|---|---|---|
| **Control**[a] | 30-03-2020 | 0.23 |
| **Control** | 31-03-2020 | 0.23 |
| **Control** | 01-04-2020 | 0.24 |
| **Seaweed** | 30-03-2020 | 12 |
| **Seaweed** | 31-03-2020 | 13 |
| **Seaweed** | 01-04-2020 | 12 |
| **T-test p-value** | | 0.000757 |

a) Without plasma sample from calf 775.

### 4.2.6 Potentially toxic minerals

Table 19 shows the mineral retention of the potentially toxic minerals; As and Cd in the control and seaweed group. There was no difference in the retention of Cd, while there was a significantly greater retention of As in the seaweed group compared to the control group (P = 0.0003).

*Table 19 The daily average retention and standard deviation (SD) of Arsenic and Cadmium*

| Mineral | Control | | Seaweed | | T-test p-value | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| **As mg/kg** | 0.30 | 0.03 | 6.00 | 0.20 | 0.0003 | * |
| **Cd mg/kg** | 0.26 | 0.02 | 0.25 | 0.01 | 0.7604 | |

# 5. Discussion

## 5.1 Cafeteria trial

The purpose of the cafeteria trial was to evaluate which seaweed inclusion levels the calves would eat and therefore be possible to use in the feeding trial. As there was no difference in the feed intake between the three seaweed inclusion levels, the highest (5%) level was chosen to make sure a measurable effect would occur. Another approach might have been preferable in choosing the inclusion level. Calculating different mineral contents at different inclusion levels could have been used to match the mineral requirements of the calves. Such calculations and comparisons were performed by Cabrita et al. (2016), where it was concluded that a 5.2% inclusion of *S. latissima* would be the MTL for ruminants with the limiting mineral being I. This corresponds well with the inclusion level chosen in this feeding trial, but the concentrations of I in this study and Cabrita et al. (2016) were different. The I content in the *S. latissima* used in Cabrita et al. (2016) was 884 mg/kg DM, while the I content in the *S. latissima* used in this feeding trial was 6100 mg/kg DM. Based on this, the 5% inclusion level might have exceeded the MTL.

From the cafeteria trial, it seemed the seaweed could increase the feed intake of the calves, which in turn could increase their growth. This was not the case in the feeding trial. It might be explained by a bias in the intake during the cafeteria trial, as the seaweed was new and interesting to the calves. This might have led to a higher preference in the short-term without increasing their preference in the long-term or increase their daily feed intake. The long-term preference could have been studied by expanding the cafeteria trial, where the four different seaweed inclusion levels were offered, and total intake was measured daily instead of measuring the feed intake during 45-minute trials. This would have answered the hypothesis about the feed intake and removed the beforementioned bias, while the method used only gave an indication of whether the calves would eat the seaweed or not. As this indication was the main aim of the cafeteria trail, and the cheaper alternative, it was chosen.

## 5.2 Feeding trial

### 5.2.1. Baseline results

The results of the baseline feeding showed no differences in the mineral retentions (see Table 9). These results were as expected since the calves were fed the same baseline feed. However, the retention of Fe and I in the two groups were negative. This should not be possible unless they excreted all the absorbed Fe and I as well as additional Fe and I from the body storages, which is unlikely. The negative retention of Fe in the seaweed group and the I in both groups

are most likely equal to zero, as they were very close. The rather large negative retention of Fe in the control group of -79.09 mg/kg was most likely caused by a mistake in the measurement of samples or the mineral analysis or by contamination of the samples. This uncertainty of the result is also reflected by a large SD value of 44 mg/kg.

### 5.2.2. Feed and energy intake

The results showed no difference in the feed intake (P = 0.84) between the two groups, which is in agreement with earlier trials supplementing seaweed to dairy cows (Baek et al. 2004; Lee et sl. 2005; Hong et al. 2010; Pompeu et al. 2011; Singh et al. 2017). This leads to a rejection of the hypothesis, that the calves would decrease their feed intake, when 5% farmed ensiled *S. latissima* is added to the diet on DM basis.

The energy intake was not significantly different (P = 0.53) between the two groups, when calculated with table values and assuming there was no energy contribution from the seaweed. An analysis of the energy content of the two feeds would have been preferable compared to using the table values, as especially the energy content of grass silage can vary (Møller et al. 2005). Furthermore, the analysis would have included the energy provided by the seaweed, which was not incorporated in the comparison of the energy intakes. This analysis was not economically prioritized in the trial and instead the worst-case scenario was considered by assuming the seaweed did not contribute energy to the feed. If the seaweed contains the same or less energy than the silage, then the energy intake of the two groups would still not be significantly different.

The energy content of the seaweed can be assumed using earlier literature. The gross energy content of wild Canadian *S. latissima* was 13.3 MJ/kg DM in a study by Tibbetts et al. (2016) and according to Makkar et al. (2016), the *in vitro* OM digestibility of *S. latissima* is 81%. Using these values, the available energy in the seaweed would be 10.77 MJ/kg DM, which is similar to the grass silage digestible energy of 13 MJ/kg DM. This energy content of *S. latissima* would therefore not change the results as it is lower than the grass silage energy content. On the other hand, if the seaweed actually contained more energy than the grass silage, then the seaweed group could have had a greater energy intake than the control group. When calculating with increasing seaweed energy content, the two groups energy intakes will only be significantly different, once the energy content is above 85 MJ/kg DM. This is not realistic for seaweed, therefore, it can be assumed, that the groups had a similar energy intake.

### 5.2.3. Growth

There was a tendency (P = 0.054 / without calf 775; P = 0.096) for a greater growth in the control group compared to the seaweed group. This tendency cannot be due to a difference in feed intake, as the feed intake was not significantly different between the two groups. In addition, it cannot be explained by differences in energy intake, as it was also the same between the two groups. The amount of water in the digestive system can also affect the weight of the calves. The control group's water intake was significantly greater (P = 0.019) than the seaweed group's, therefore this could, at least in part, explain the tendency for a greater growth of the control group. The tendency for a greater growth in the control group could, most likely, also be explained by other factors. One factor could be toxic effects of the minerals in the seaweed, as a decrease in growth is a common result of excess minerals (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). This is also the case for excess K, Na, S and I, which retentions were greater in the seaweed group compared to the control group. Excess I intake is the most likely explanation for the tendency for decreased growth, as the content in the feed was 0.03% of DM (300 mg/kg), which is above the MTL of 0.005% of DM reported in both the NRC (2005) and Strudsholm et al. (1999). Earlier studies have found a decrease in growth at lower concentrations; when I was fed at 86 and 174 mg/kg (Fish & Swanson 1982) and 50, 100 and 200 mg/kg (Newton et al. 1974). Deficiency of minerals can also decrease growth (NRC 2001, 2005; Suttle 2010), but the retention of most minerals was greater than the requirements from the NRC (2001) (see Table 20). Furthermore, undetermined potentially harmful compounds in the seaweed, could affect the calves' health or decreased the overall digestibility of the feed, which might be an explanatory factor. However, earlier studies feeding *S. latissima* have not reported significantly lower digestibility (Maia et al. 2016; Singh et al. 2017; Satessa et al. 2018).

It can be discussed whether calf 775 should be included in the growth results. As she was sick, the faeces and urine samples were excluded, and her feed and water intake were not measured on the last sampling day. The sickness did not seem to be caused by the experiment as none of the other calves in her group shared her symptoms and because she was fed the control feed, which did not contain new or uncommon feedstuffs. Moreover, there was no explanation for the swollen head, but the farmer had seen it before, which means it was not a unique event. Based on this knowledge and her sudden weight gain, excluding her from the results might be the correct choice. On the other hand, the weight gain was not an outlier and could be due to a high growth unaffected by the diarrhoea and hay ingestion. If this was the case, it would not be appropriate to exclude her from the results.

There was a high SD (7.2 kg) in the seaweed group. A larger group size in the trial would increase the validity of the results. One calf (767) suffered a weight loss of 6.4 kg during the trial, which seems extreme compared to the other calves. However, the weight loss was not an outlier. Both calf 767 and the high SD could be explained by different tolerances for I retention or individual efficiencies in excreting it. Thereby, the toxicity effects could have started at different time points and affected the calves differently. The most vulnerable calves will have suffered weight loss (such as 767), while others would have impaired growth or were not yet affected by the high I intake. This effect was evident in Newton et al. (1974), where one calf fed 25 mg/kg I showed symptoms of I toxicity and two calves fed 200 mg/kg I had similar weight gain as the control group. It is not possible to evaluate the individual differences in I retention as the samples of faeces and urine were pooled. More research with a greater number of animals and individual sampling are needed before the effect of a 5% inclusion of *S. latissima* on calf growth can be determined.

### 5.2.4. Differences in mineral retention compared to the feed content

Despite multiple significant differences in the mineral concentration between the two feeds, the retentions were only different between the groups in K, Na, S, I and As. These were also the minerals that the seaweed feed had a greater concentration of compared to the control feed. There was no significant difference in the retention of the minerals; Ca, Fe, Mn, Zn and Cu, even though the concentration of them were in greater in the control feed. This effect can be explained by multiple factors. First and foremost, the absorption of all these minerals are regulated in the intestine (Suttle 2010), and therefore, a greater concentration in the feed does not necessarily result in a greater retention. The intestinal regulation was also reflected in the undetectable concentration of these exact minerals in the urine samples of both groups and a significantly greater concentration of them in the control groups faeces except for Fe (see Table 15 and 16). Furthermore, as there was an greater content of the metals in the control feed compared to the recommendations (see Table 11), the interacting effects on the absorption between them can have decreased the absorption of the others for instance by downregulation of DMT1 (NRC 2001, 2005; Suttle 2010).

The minerals, with the greater retention in the seaweed group, are monovalent ions, which are easily absorbed in the intestine and the retention is instead highly dependent on urinal excretion (Sjaastad et al. 2016c). This was somewhat reflected in the results, as there was a significantly greater excretion of I and As and a tendency for a greater Na excretion in the urine of the seaweed group compared to the control group (see Table 16). In addition, the seaweed

group faeces samples showed a greater content of K, Na, I and As (see Table 15), whereas the concentration of S was not significantly different between the two groups in neither the urine nor the faeces samples. This is consistent with the feed content, as the S content was numerically greater, whereas the others were significantly greater in the seaweed feed. The greater excretion in the faeces was probably due to absorption limitations, as there is no regulation of the absorption of these minerals (Suttle 2010; Sjaastad et al. 2016c). Despite the greater excretion in both the urine (for As and I) and faeces, the mechanisms were not able to compensate for the increased content in the seaweed feed. The inability to compensate for the high concentration in the seaweed feed might be because the maximum capacity for urinal excretion was reached, as the concentration of I and As in the seaweed feed exceeded the control feed with 99.43% and >44.44%, respectively. The inability to excrete enough Na and K compared to the control, could be explained by an already increased concentration in the control feed compared to the recommendations from the NRC (2001) as seen in Table 11. Therefore, the calves in both groups might have reached the maximum urinary excretion level, which is supported by the lack of significant difference in the urine excretion.

### 5.2.5. Mineral retention compared to recommendations and requirements

The discussion above has assumed the control feed contained an optimal mineral concentration for a dairy calf. This is not necessarily the case, therefore, when evaluating the seaweed feed as a mineral supplement, a comparison with the NRC (2001) mineral recommendations and requirements for 700 g growth per day needs to be performed. The NRC (2001) requirements have been chosen, as they are internationally used including in the Danish NorFor system (except for the Ca requirement, which is based on Pehrson et al. (1975)).

Table 20 shows the requirements from the NRC (2001) along with the retention of the control and seaweed group. When comparing them, it becomes evident, that both feeds resulted in a greater retention of Mg, K, Na, Fe, Mn, Zn, Cu and I and smaller retention of Ca and P than the requirements.

*Table 20 The average mineral retention in the two groups compared to the NRC (2001) requirements.*

| Macrominerals | NRC (2001) requirement, g/day | Retention of control group, g/day | Retention of seaweed group, g/day |
|---|---|---|---|
| **Ca** | 12.10 | 8.40 | 9.10 |
| **P** | 8.74 | 6.31 | 5.71 |
| **Mg** | 0.62 | 3.16 | 3.71 |
| **K** | 13.76 | 40.32 | 53.39 |
| **Na** | 2.48 | 5.48 | 13.10 |
| **S** | NA | 3.60 | 4.85 |
| **Trace elements** | mg/day | mg/day | mg/day |
| **Fe** | 23.80 | 262.35 | 187.00 |
| **Mn** | 0.69 | 58.37 | 73.92 |
| **Zn** | 21.30 | 53.35 | 52.31 |
| **Cu** | 1.52 | 11.33 | 7.67 |
| **I** | 0.60 | 2.62 | 900.43 |

The smaller P and Ca retention compared to the requirements could lead to deficiency of the minerals, however, it does not seem to be the case, as there was an excretion of Ca in the faeces in both groups and P in the urine from the seaweed group. If the requirements of Ca and P were not fulfilled, then it would not be expected to see any excretion of Ca in the faeces and P in the urine, as both minerals retention are hormonally regulated based on the calf's current status (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010; Sjaastad et al. 2016b). Calcium is absorbed according to the need of the calves; a low concentration of Ca would therefore be expected in the faeces if the animal is deficient in Ca, but this was not the case. In the same manner, the P concentration in the urine would be very low, as seen in the control group. However, as there was no significant difference in the P retention between the two groups, it can be assumed that both feeds met the calves' requirements. In addition, the feed content of P in both feeds were just greater than the reported content of 0.25% of DM at which deficiency occurs according to the NRC (2001) and the Ca retention corresponds to the Ca requirement from Pehrson et al. (1975) (see Table 11). This indicates that a 5% DM inclusion of *S. latissima* should cover calves need for Ca and P.

The ratio between Ca and P need to be considered as the regulation of the absorption and deposition in bones are correlated (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010). The Ca:P ratio in the seaweed feed was 1.46:1, which is within the recommended range of 1:1 to 2:1 reported in McDonald et al. 2010. Therefore, no negative interacting effects are assumed.

The higher retention of the essential metals (Fe, Mn, Zn and Cu) in both groups compared to the requirements might, in part, be caused by the baseline feeding. During the baseline feeding the calves were fed without mineral supplement. Since the absorption of the metals are regulated by the concentration in the feed and the calves' needs (NRC 2005; Suttle 2010), the low concentration of them in the baseline feed could have increased the number of transporters in the intestine. Depending on the time required to achieve a new homeostasis, the baseline feeding strategy could have increased the absorption of the metals during the feeding trial. Furthermore, the metal storages might have been emptied, which could have increased the calves' requirement compared to the NRC (2001). If this was the case, then the high retention would not be a concern, as it would decrease when the storages were filled, and a new homeostasis has been reached. The equal retentions and the excretion of Mn, Zn and Cu supports this theory. The faecal excretion was greater in the control group compared to the seaweed group and not significantly different in the urine (see Table 15 and 16). This means the total absorption was the same between the two groups even though the concentration was significantly greater in the control feed. A lower total absorption would be expected in the seaweed group, as the feed content was lower, unless the absorption capacities were not yet fully down regulated from the baseline feeding or the concentration in the feed was so high the intestine was not able to lower the absorption capacity further.

Despite a lower intake of Zn and Cu in the seaweed group (compared to the recommended intake per day from Table 11), the retention of them were greater than the requirements from the NRC (2001) (see Table 20). As discussed above, it could be due to the baseline feeding strategy, which supports the argument that the feed is not harmful for the calves, as a new homeostasis is expected to occur, if the experiment continued. In addition, it could be explained by a higher availability or another absorption mechanism of the minerals in the seaweed compared to the minerals in the mineral mix, grass silage and wheat. This is supported by a greater absorption coefficient in the seaweed group than the control group (see Appendix 9.1.). The recommendations from the NRC (2001) are based on absorption mechanisms from terrestrial plant sources and commercial mineral mixtures, which may not correspond to absorption coefficients of seaweed feeds, as the bioavailability depends on the state of the mineral as well as their structural binding (NRC 2005).

The greater retention of Mg compared to the requirements despite the similarity to the feed recommendations, could likewise be explained by availability and absorption differences. This is supported by the greater absorption coefficients of the two groups compared to the NRC (2001) coefficients (see Appendix 9.1.). The mechanisms seem to be the case for the retention

of S as well. According to the results, the S concentration in the seaweed feed tended to be greater than the control feed, whereas there was a significantly greater retention of it. This could be explained by a greater availability of the S in the seaweed compared to the S in the control feed. Unfortunately, it is not possible to compare the retention or absorption coefficient with the NRC (2001), as it does not include any requirements nor an absorption coefficient, but only a recommended concentration in the feed.

Both the retention and the intake of Na, K and I in the seaweed group were greater than the requirements and recommended intakes, respectively. As the recommendation is surpassed for all three minerals, it is logical, that the retention is too. However, retention should be regulated by urinal excretion, which means either the kidneys were not able to excrete enough of it or the greater retentions than requirements were not problematic for the calves. For the K and Na retention both explanations seem possible. The most likely explanation for the greater I retention is the inability to compensate for the I content in the feed, as the I intake in the seaweed group exceeded the recommended intake with 99.93%.

Both the recommendations and the requirements of the minerals were based on a growth of 700 g/day, which does not correspond to the growth of the seaweed group. The average growth of the control group was 0.71 kg/day, while the growth of the seaweed group was 0.24 kg/day. Therefore, the recommendations and requirements might be overestimated for the calves in the seaweed group, except for the S and I as they are not based on growth. This would suggest that the mineral retentions, which already exceeded the requirements at 700 g/day (Mg, K, Na, Fe, Mn, Zn and Cu), might have been fed in even more unnecessarily high concentrations or had a potentially greater risk of toxicity than first expected. However, when evaluating the feed, it is important to look at a weight gain of approximately 700 g/day, as this is a standard weight gain goal for dairy calves of this breed.

### 5.2.6. Potentially toxic retention

Since there was a greater retention of the minerals; K, Na, S, I and As in the seaweed group and the retention of the same minerals and Mg, Fe, Mn, Zn and Cu were greater than the daily requirement, the possibility of toxic effects of these minerals must be discussed. In addition, the risk of toxicity of Cd will also be discussed.

The feed content of K and Na does not exceed the MTL (see Table 12). Therefore, the greater retention than the NRC (2001) requirements should not pose a problem. However, the absorption coefficient of the minerals in the seaweed might, as explained earlier, be different from the coefficient reported in the NRC (2001). The coefficient for Na and K absorption in the

seaweed group (see Appendix 9.1.) was close to the 90% reported in the NRC (2001), whereby it should not change the expected MTL. Even when considering the worst-case scenario of an absorption coefficient of 100%, this would only slightly increase the absorption since the coefficient used for Na and K was 90% (NRC 2001). Therefore, the estimated MTL should be applicable to the Na and K contents and a 5% DM inclusion of *S. latissima* should not result in K or Na toxicity but still cover calves' requirements.

The retention of Mg was not different between the two groups and the daily intake was very close to the NRC (2001) recommendation. This means that the greater Mg retention compared to the requirement was most likely not a problem. In addition, the urinal excretion of Mg was also quite low compared to the K and Na excretion, which support this assumption. The feed content of Mg did not exceed the MTL reported in Table 12. Though, when considering the greater absorption coefficients than the coefficient from the NRC (2001), the feeds' Mg content could have exceeded the MTL, as it is overestimated by the lower absorption coefficient. However, the Mg concentration in the seaweed feed was 87,5% smaller than the MTL. Therefore, a toxic effect of Mg, when including 5% *S. latissima* on DM basis in calves' diet, is unlikely.

It is not expected that the greater S retention in the seaweed group will cause toxicity as the S content in the seaweed feed did not exceed the NRC (2001) and Strudsholm et al. (1999) recommendation (see Table 11). Furthermore, it was 50% lower than the MTL from the NRC (2005). The first effect of S toxicity is loss of appetite (NRC 2001; Suttle 2010) and the feed intake was not different between the two groups, which supports the assumption that inclusion of 5% *S. latissima* on DM basis does not result in a toxic level of S.

The retention of Fe, Mn, Cu and Zn was not significantly different between the groups, but above the NRC (2001) requirements. The content in both feeds were below the MTL from the NRC (2005) (see Table 12) and the tolerance to the metals is quite high and variable as the absorption is heavily regulated by the calves' needs (NRC 2005; Suttle 2010). In addition, the Mn retention is regulated by urinal excretion (NRC 2001, 2005), which was not detected indicating the retention was not at a toxic level. According to Suttle (2010), Zn toxicity only occurs, when the feed content is so high, that the passive absorption results in a toxic absorption. As the feed content of Zn was below the recommendation, this is not likely to have occurred. Based on this, and the earlier discussion of the metals, a 5% inclusion of *S. latissima* om DM basis should not result in toxicity of Fe, Mn, Cu and Zn.

The content of Cd in both feeds did not exceed the maximum permitted level (MPL) for complete feed of 0.57 mg/kg DM (Commission Regulation 2019/1869 amending Annex I to

Directive 2002/32/EC, 2019). However, the absorption of Cd was very high (99%; see Appendix 9.1.) for both groups compared to the expected low absorption of 1% reported by the NRC (2001). The observed absorption coefficient could be overestimated due to intestinal retention or detection failure. Cadmium absorption might be prevented by metallothionein binding it in the intestinal mucosa (Squibb et al. 1976; Min et al 1991; Kimura et al. 1998). Therefore, the low Cd concentration in the faeces samples, could be caused by Cd either being trapped in the intestinal mucosa or indetectable in the samples, as it could be bound to metallothionein. As the effect of metallothionein on Cd in the intestine and importance hereof is contradicted by Klaassen et al. (2009), the calculated absorption might be correct. If so, the MTL would be less than first expected, which means the feeds might cause Cd toxicity. The maximum tolerable absorption is 0.01 μg/kg DM, when assuming an absorption coefficient of 1% and using the MTL of 0.001% of DM. The expected absorption would be 0.0074 μg/kg DM, which is smaller, when using the absorption coefficient of 99% and the feed concentration of 0.0075 μg/kg DM from the seaweed feed. Therefore, an inclusion of 5% *S. latissima* on DM basis in the feed to calves does not exceed the MTL even if the absorption is greater than expected.

The greater retention of As in the seaweed group compared to the control group should not pose a problem. According to the NRC (2001) >50 mg iAs/kg feed will result in toxicity. As mentioned above the seaweed feed contained 1.8 mg As/kg DM, which is 0.93 mg As/kg feed. The feed should therefore not result in toxic effects of As since it is below both the iAs MTL from the NRC (2001) and the reported MTL from the NRC (2005) in Table 12. In addition, the majority of the As in seaweeds are arsenosugars (Llorente-Mirandes et al. 2011), which is oAs and should be less toxic than iAs (EFSA 2005; NRC 2005). When evaluating the As content, the MPL should also be considered as well. The ensiled *S. latissima*, as a feedstuff, contained 47 mg/kg DM As, which exceeds the MPL of 45.45 mg/kg DM with a maximal concentration of 2 mg/kg iAs from the EU Directive (Commission Regulation 2019/1869 amending Annex I to Directive 2002/32/EC, 2019). However, when including 5% *S. latissima* in the diet, the ration's As concentration of 1.8 mg/kg DM did not exceed the 2.27 mg/kg DM allowed for complete feed in the same EU Directive. This means, *S. latissima* might not be approved as an individual feedstuff, but feed containing 5% *S. latissima* could be approved. This leads to the assumption, that a 5% inclusion of *S. latissima* on DM basis should be possible without causing As toxicity or exceeding the MPL of As.

The I concentration in the seaweed feed was greater than the MTL reported by both the NRC (2005) and Strudsholm et al. (1999). The I concentration in the plasma was on average at

12.33 ppm. According to research with preweaned calves, I toxicity will occur at an I plasma concentration of 1.1 ppm (Jenkins and Hidiroglou 1990). Furthermore, Newton et al. (1974) found significant negative effects on the feed intake and growth on 10-14 weeks old calves, when they were fed with an I concentration of 50, 100 and 200 mg/kg. In addition, concentrations of 100 and 200 mg I/kg resulted in toxicity symptoms such as coughing and nasal discharge. The I concentration in the seaweed feed was 300 mg/kg DM. In the trial, the calves in the seaweed group may have exhibited signs of I toxicity; all calves were coughing, and multiple calves had nasal discharge (personal observation). As mentioned earlier, the calves also had a tendency for a lower growth than the control group, which could be caused by I toxicity. Though other factors could be co-contributing. For instance, the coughing and nasal discharge could be due to pneumonia, where the greater prevalence in the seaweed group could be explained by excessive I intake, as it can cause decreased immune function (Haggard et al. 1980; Hillman & Curtis 1980). In addition, the I concentration in the seaweed feed was 155 mg/kg (300 mg/kg DM), which exceeds the MPL of 2 mg/kg for complete feeds to minor dairy ruminants (EFSA 2013). Including 5% *S. latissima* in the feed seems to result in I concentrations, which leads to I toxicity and possibly decreased growth.

### 5.2.7. Potential negative consequences of mineral interactions

Even though the elevated concentrations of K and S did not seem to lead to toxicity, other negative effects could occur. One of the negative effects of increased mineral content could be interactions with other minerals. These will be discussed in the following.

A decrease in the absorption of Mn (NRC 2001) and Mg (NRC 2001, 2005; McDonald et al. 2010; Suttle 2010) has been reported when high levels of K are fed. However, as both the Mn and Mg contents in the feed were greater than the daily recommended intakes and the retentions were higher than the requirements, the high content of K does not seem to have had negative interacting effects on the absorption of either Mn nor Mg. Furthermore, there was no difference between the two groups' retention of Mn and Mg despite of the greater K content in the seaweed feed. The interacting effect of K was, therefore, either too small to be significant or just not present.

A high concentration of S can decrease the absorption of Cu (Bird 1970) and Zn (Suttle 2010). This does not seem to cause a problem in this trial; the Cu and Zn retentions in the seaweed group were greater than the requirements even though the feed concentrations were lower than the recommended daily intakes. There was no difference between the control and seaweed group's retention, which means that if there were any interacting effect of S, they were

not significant. A high S content can also decrease Cd absorption by forming cadmium sulphide (Suttle 2010), which could be a positive side-effect. However, the effect was indetectable in the retention of Cd and the seaweed content of Cd was not different from the control feed. Therefore, the beneficial effect of the interaction does not seem to improve the seaweed supplemented feed when using an inclusion level of 5%. It might be beneficial if a higher inclusion level of *S. latissima* were used since the seaweed has a greater Cd concentration than the other feedstuffs.

### 5.2.8. The effect of the *Saccharina latissima* production

In Table 21 the mineral concentrations in the *S. latissima,* used in this trial, are compared to the interval of mineral concentrations from the literature reviewed in Section 2.1. From the table, it becomes evident, that the mineral concentrations are within the normal range, except for Mg and Na, which are greater than the concentrations found in the earlier studies. There is a high variation in the mineral concentrations between studies. Thus, the greater concentrations of Mg and Na might not be unlikely or unrealistic. However, if the concentrations of Na and Mg are unusually high in the specific seaweed used, a lower concentration of both Mg and Na should not be problematic, as the retention of both minerals were greater than the calves' requirements. Despite the greater concentrations of Mg and Na, the order of the concentrations from highest to lowest are almost identical between the seaweed used in this trial which was K>Na>**Mg**=S>**Ca**>I>P>Fe>As>Zn>Mn?>Cu?>Cd>Hg and the literature which was K>Na>S>**Ca>Mg**>I>P>Fe>As>Zn>Mn>Cu>Cd>Hg. The relative position of Cu and Mn is uncertain as the concentration was below the detection limit, but they are assumed to be greater than Cd and Hg. The only exception is the Mg concentration was greater than the Ca concentration in the seaweed used in the trial (indicated by bold text). Therefore, the *S. latissima* used in the trial should be representative for the seaweed species under the conditions of varying concentrations.

*Table 21 The mineral content of S. latissima compared to the interval of mineral concentrations from the reviewed literature.*

| **Source** | ***S. latissima* used in the trial** | | | **Literature** | |
|---|---|---|---|---|---|
| **Mineral** | **Content** | Upper CI | Lower CI | **Lower content** | **Upper content** |
| **Ca g/kg** | **7.79** | 8.7248 | 6.8552 | 1.29 | 17.8 |
| **P g/kg** | **1.29** | 1.4448 | 1.1352 | 1 | 17 |
| **Mg g/kg** | **11.3** | 12.656 | 9.944 | 5.31 | 9.5 |
| **K g/kg** | **96.3** | 107.856 | 84.744 | 25 | 120 |
| **Na g/kg** | **67.8** | 75.936 | 59.664 | 12.26 | 48.1 |
| **S g/kg** | **11** | 13.2 | 8.8 | 8.4 | 34 |
| **Fe mg/kg** | **36.8** | 43.424 | 30.176 | 21 | 1854 |
| **Mn mg/kg** | **<5** | | | 3 | 35 |
| **Zn mg/kg** | **16.5** | 19.47 | 13.53 | 7.5 | 68.9 |
| **Cu mg/kg** | **<2** | | | 0.29 | 38.6 |
| **I mg/kg** | **6100** | 7320 | 4880 | 958 | 6568 |
| **As mg/kg** | **47** | 56.4 | 37.6 | 28 | 99.11 |
| **Cd mg/kg** | **0.22** | 0.264 | 0.176 | 0.07 | 4.6 |
| **Hg mg/kg** | **0.015** | 0.0195 | 0.0105 | 0.0009 | 0.1054 |

Even though the *S. latissima* used in the trial was representative for the seaweed species, the applicability of the results of the feeding trial can be questioned, as the mineral concentration in *S. latissima* is variable. Therefore, repeating the trial with seaweed from another production site might not lead to the same conclusions. The trial could, however, be representative for certain productions of seaweed for example IMTA seaweed grown in Nordic seawater or only for the specific production by Lerøy Ocean Harvest. This should be tested by analysing more samples from different years and locations before certain conclusions can be made.

The I content in the seaweed feed exceeded the MTL and seems to have resulted in toxicity in the calves. Therefore, the I concentration in the seaweed must be lowered before it can be used as a mineral or feed supplement to calves. When looking at the literature in Section 2.1. it becomes evident, that the I concentration is dependent on different environmental factors. For instance, lower temperature (Roleda et al. 2018) and salinity (Nitschke & Stengel 2014), stress by oxygen exposure (Küpper et al. 2008) and lower water turnover or I concentration in the water (Lüning & Mortensen 2015) could decrease the I content in the seaweed. As most of these factors are not changeable in nature, it might be possible to produce *S. latissima* with a more favourable I content by changing the production site. However, this seems less practical and realistic, as farmers need to choose the area based on multiple production factors. In addition, the changes could result in concentration changes in other minerals or nutrients, which could decrease the value of the seaweed. Another method could be to discard the holdfasts and

new blades, as they have a greater I content compared to the older plant parts (Nitschke & Stengel 2015; Roleda et al. 2018).

The As content of *S. latissima* exceeded the European legislations limit for As content in animal feedstuffs (45.45 mg/kg) (Commission Regulation 2019/1869 amending Annex I to Directive 2002/32/EC, 2019). It is either necessary to find a method to decrease the content or the legislation needs alterations, wherein the organic and inorganic As are considered separately, before *S. latissima* can be approved as a feedstuff. Using the older plant parts, to ensure lower I content, could unfortunately result in greater As content, as the content increased with frond length (Nielsen et al 2016), which indicates it increases with age. However, the salinity tended to correlate with the frond length as well (Nielsen et al. 2016), which means the increased As could be due to the effect of salinity instead of age. If so, removing the younger blades would not increase the As content. In addition, harvesting in the spring might lower the As content, as it seems to be lowest in the spring (Omette et al. 2018), though this is uncertain.

Instead of changing the production method to decrease the I content of the seaweed, other methods for removing I from the seaweed have previously been investigated. Boiling the seaweed for five minutes can reduce the I content with approx. 70% (Lüning & Mortensen 2015).Soaking it in warm water (32℃) for an hour has also shown to reduce the I content with approx. 85% (Stévant et al. 2018). According to Lüning & Mortensen (2015) the inorganic I, which is not bound in compounds, is the fraction, that can be removed by boiling. It seems the inorganic I is the more bioavailable compared to organic I in humans (Alexander et al. 1967; Hurrell 1997) and maybe also in cattle. If true, boiling or soaking could help reduce the total absorption of I even further. These methods should be investigated further while focussing on the cheapest and most environmentally friendly approach.

The fermentation of the seaweed could also have affected the mineral content or availability. In a recent study, fermentation of *S. latissima* resulted in a significantly decreased concentration of Mn, Fe and Zn. While no effects of ensiling were found on the same macro-minerals as investigated in this trial nor in the trace elements; Cu, I and As (Campbell et al. 2020). The effect of ensiling *S. latissima* on the mineral availability was not tested and no literature has been found. Based on this study, ensiling *S. latissima* does not seem to affect the problematic minerals (I and As). Ensiling the seaweed could therefore be one appropriate mean of preservation, when only considering the mineral profile. However, more research is needed to fully understand the effects of ensiling seaweed on the mineral content and availability.

### 5.2.9. Previous studies feeding seaweed as a mineral supplement

None of the studies supplementing seaweed to dairy cows have reported any negative effects of the seaweed as opposed to the presumed toxic effects of I in this trial. There could be multiple reasons for this: Firstly, the other studies used dairy cows, which have a greater daily requirement than calves (NRC 2001). Secondly, the studies were conducted in different areas of the world, where the I status of the cows might be different from the status expected in Nordic or European farms. In fact, the dairy farm used in Rey-Crespo et al. (2014) was chosen based on a low I status. Lastly, they used different inclusion levels of other types of seaweed, which might have different absorption qualities and concentrations of I. The concentrations will be discussed in the following paragraph.

The feeds used in Hong et al. (2010) had an I content of 0.000065% of DM and 0.00013% of DM, whereas the seaweed feed in this trial had an I content of 0.03% of DM. This could explain the insignificant increase in T3 and T4 when they were analysed together in Hong et al. (2010). Lee et al. (2005) included a greater percent of the same seaweed in the feed, which then had an I content of 0.00026% of DM. This resulted in an elevated I retention reflected by an increase in the plasma concentration of T3 and T4. However, no negative effects were reported, which could be explained by the I content of the feeds being lower than the MTL of 0.005% of DM from the NRC (2005). The study of Rey-Crespo et al. (2014) fed dairy cows 100 g seaweed/day, which resulted in an assumed I content in the feed of 0.00014% of DM, which is also below the MTL. The I balance was not investigated in the study by Singh et al. (2016), which supplemented 20% *S. wightii* in the feed to dairy cows. Indian *S. wightii* seems to have an I content between 0.05% and 0.35%, depending on the season (Thomas & Subbaramaiah 1991). Assuming this I content, the feed in Singh et al. (2016) would have an I concentration of 0.01-0.07% of DM, which are both above the MTL (NRC 2005). Despite this, there were no reports of I toxicity or symptoms of sickness. The I toxicity may either have occurred unknowingly or would have occurred if the trial had continued. This is, however, unlikely, as the study had a duration of 18 weeks and I toxicity has previously been reported after three to four weeks of supplementation in calves (Newton et al. 1974; Hillman & Curtis 1980). All though, dairy cows might have a higher resistance to I toxicity, as they can excrete it through the milk (Suttle 2010).

The lower intake of Zn and Cu in the feeding trial is in accordance with the findings of Singh et al. (2016), that seaweed (*S. wightii*) is a poor source of Zn and Cu . However, the low intake of the two minerals did not decrease the absorption and both had greater retention than the requirement of the calves in this study. This might not be the case for dairy cows, as they

have a greater requirement than calves (NRC 2001). Singh et al. (2016) does not compare the retentions to the dairy cow requirements. Therefore, the seaweed might fulfil the requirements despite the lower retention compared to the positive control group. At last, the different conclusions could also be caused by different contents or bioavailability of Zn and Cu in *S. latissima* and *S. wightii*.

### 5.2.10. Methodological effects

Pooling the samples in both groups decreased the number of samples in the trial, which decreases the validity of the results. In addition, the number of samples and calves were already limited, which means the trial's results can only be used for proof of concept. Furthermore, pooling the samples removed the opportunity to evaluate the individual differences and the possibility to remove potential outliers in the group. If any outliers were present, they will instead have affected the results and could, in the worst case scenario, have caused significant results, where none were present or concealed significant results by increasing or decreasing the measured concentration in the sample, respectively. However, pooling the samples have given the overall effect, which is usually the focus on a dairy farm, as the individual animal is rarely considered.

The uncertainty of the method used by Eurofins Agro was not considered in the T-tests. Including the uncertainty of the method in the statistical analysis would have decreased the residual variance by explaining part of the unexplained variation in the model. When more information is considered in the model, it becomes stronger, which decreases the variance. This means there might be significant differences between the two groups, which were not discovered. However, it does not affect the significant differences found in the analysis, as they would only be strengthened, when including the methodical variance in the model.

The calculation of the mineral retention was based on the largest possible value when the content was below the analysis' detection limit. This was the case for the As content in the control feed, the As and P content in the control urine and the Ca, Fe, Mn, Cu, Zn and Cd content in both groups' urine samples. This could have resulted in an overestimation of the retention of the As in the control group. The overestimation would be decreased because the As loss in the urine was overestimated as well. As there was already a significant difference between the control and seaweed group, this will not change the results. There will have been an underestimation of the retention of P in the control group and an underestimation of the retention of Ca, Fe, Mn, Cu, Zn and Cd in both groups. As the underestimation was present in both groups for Ca, Fe, Mn, Cu, Zn and Cd it has most likely not affected the results. In addition,

the detection limit was <0.1, meaning even though the underestimations are not necessarily the same between the two groups, the result would probably not change if the accurate concentrations were used. The underestimation of the P retention in the control group could have changed the results. However, the detection limit for the P concentration in urine was <0.05, which means the estimated loss in urine was very small compared to the retention and would most likely not have affected the results.

The decision of not mixing the daily collected faeces before sampling, will have overestimated the DM content, as it was not possible to take samples from the looser and more fluid faeces. This method was chosen to decrease the contamination with sawdust, which would have overestimated the DM of the faeces even further. Even though care was taken to avoid sawdust in the smaller samples, some contamination was unavoidable, and this will also have resulted in an overestimation of the DM of the faeces samples. This overestimation will in turn have resulted in an underestimation of the retention, as the calculation of minerals in the faeces was overestimated. This would probably not have changed the results, as all significant retention results were far below a p-value of 5%. Furthermore, a similar underestimation is expected on both groups as the same method was used throughout the trial. Therefore, it should not affect the comparison of the groups. A more exact measurement could have been achieved by attaching bags onto the calves for faeces and urine collection or using metabolic cages instead of individual boxes (Mondal et al. 2008; Kumar et al. 2018; Amado et al. 2019). By applying one of these methods, the use of creatinine concentration in the urine to estimate the total urine excretion, could then have been avoided as well, which would also have increased the accuracy of the mineral retention. On the other hand, these methods would probably have affected the behaviour of the calves and perhaps their feed intake. Furthermore, there was no budget for a metabolic experiment.

The water at the farm was not analysed for mineral content. This could have affected the results, as the mineral uptake through the water was not included in the retention. As the water intake of the control group was significantly greater than the seaweed group, this might have affected the results. However, earlier analysis of the water at the farm showed a low relative concentration of minerals compared to the feed (GEUS 2020). Therefore, the water would, most likely, have a very small impact on the retention. Furthermore, the MTL reported in the NRC (2005) are concentrations in the feed and does not include the mineral intake through the water. Thus, including the mineral uptake from the water would most likely not change the results, tough inclusion would have been more accurate.

# 6. Conclusion

From the feeding trial it can be concluded that calves will not reduce their daily DM feed intake when offered 5% *Saccharina latissima* of the total ration DM. However, the supplementation might have decreased their water intake, as the seaweed group had a significantly lower water intake compared to the control group. Despite the similar DM intake and a similar energy content of the two feeds, the seaweed group had a tendency for a lower growth compared to the control group. This was most likely a result of iodine toxicity, but additional and longer-lasting studies are needed to confirm it.

When comparing the mineral content of the seaweed feed and the mineral retention of the seaweed group to the NRC (2001) recommendations and requirements of the minerals, it seemed the 5% inclusion of ensiled *S. latissima* could replace the mineral supplement. However, the iodine concentration exceeded the maximum tolerable level from the NRC (2005), which led to calves showing symptoms related to iodine toxicity. Furthermore, the iodine was supplemented and retained at concentrations above threshold values for feed and plasma concentrations from earlier studies investigating iodine toxicity in calves.

The seaweed supplementation caused an elevated feed concentration and retention of arsenic, but not cadmium, compared to the control group. However, the concentration of arsenic in the feed, did not exceed the maximum tolerable level from the NRC (2001). In addition, the seaweed feed concentration of arsenic did not exceed the maximum permitted level from the EU Directive. Though, the concentration of arsenic in the seaweed did exceed the maximum permitted level from the same EU Directive for feedstuffs, which could cause a problem if the seaweed needs approval as an individual feedstuff.

In conclusion, it is not possible to replace mineral supplements with a 5% inclusion of *Saccharina latissima* as the iodine content of the seaweed is too high and will most likely result in iodine toxicity. If the iodine content of the seaweed could be reduced, then inclusion of *Saccharina latissima* in the feed could have potential for fulfilling calves' mineral requirements without resulting in toxicity of arsenic and cadmium.

# 7. Perspectives

The level of I fed to the seaweed group was above the MTL and solutions for future application should be investigated. The effects of boiling or soaking the seaweed for removing the I should be explored, where the effect on the other minerals are monitored as well as the methane reducing effect. Other options for avoiding I toxicity in the animals could be inhibiting the I absorption directly by adding inhibiting substances to the feed. The effect of feeding goitrogens

on the I retention in the muscles and on the I toxicity should be investigated, as they already show potential for reducing the I concentration in milk (Flachowsky et al. 2014) and increasing the I excretion in the faeces and urine (Franke et al. 2009). As for the removal of I, it is important to investigate the substances effect on absorption and digestion of other minerals and nutritional compounds in addition to the methane reducing effect.

The I intake could also be reduced by decreasing the inclusion level of *Saccharina latissima* in the feed. According to the results, it should be possible to reduce the inclusion level without harming the supplementation of most of the other minerals, whereas P and Ca might need to be supplied additionally. However, the maximal inclusion level with regards to the I concentration and the retention of all minerals need to be investigated.

The findings in this feeding trial could be improved or confirmed by performing more trials with a greater number of calves and for a longer duration. In addition, individual sampling could give a better understanding of the physiological mechanisms and the seaweed's effect on the calves. A higher certainty on the risk of toxicity could be obtained by analysing the plasma for all minerals and taking biopsies. The metals are usually stored in the liver and muscles (Suttle 2010). Therefore, it could be beneficial to analyse for the metal concentration in liver and muscles tissue as well as all minerals in the blood both before and after the trial. This could help in gaining a better understanding of, and evaluate, the seaweed's effect on the metal storage and be useful when evaluating whether the animal is exposed to toxicity of the minerals.

In addition to investigating the effects on the storage and toxicity, analysis of the animal products (i.e. meat and milk) should also be performed before an optimal or safe inclusion level of *S. latissima* can be estimated with regards to human nutrition. Especially the I and As concentration in the muscles could have given information about optimal inclusion level in the beef cattle production. A feeding trial with lactating dairy cows and mineral analysis of milk samples, need to be performed before it is possible to include it into their ration. The mineral requirements of dairy cows in lactation are different from the growth requirements of calves (NRC 2001), where especially the phosphorus and calcium content could be a problem based on the low concentrations in the seaweed feed. Therefore, trials with dairy cows should be performed, as the production potentially could benefit greater from an inclusion of seaweed in the dairy cow ration compared to the calf ration, since they are in this stage of production for a longer period of time.

# 8. References


Alexander, W.D., R.M. Harden, M.T. Harrison & J. Shimmins (1967): Some aspects of the absorption and concentration of iodide by the alimentary tract in man. *Proc. Nutrition Soc.*, Vol 26:1, pp. 62-66.

Almeida, M., S. Filipe, M. Humanes, M.F. Maia, R. Melo, N. Severino, J.A.L. da Silva, J.J.R. Fraústo da Silva & R. Wever (2001): Vanadium haloperoxidases from brown algae of the Laminariaceae family. *Phytochemistry*, Vol. 57:5, pp. 633–642.

Amado, L., H. Berends, L.N. Leal, J. Wilms, H. Van Laar, W. J. J. Gerrits & J. Martín-Tereso (2019): Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. *Journal of Dairy Science*, Vol. 102:5, pp. 3994–4001.

Baek, I.K., W.J. Maeng, S.H. Lee, H.G. Lee, S.R. Lee & J.K. Ha (2004): Effects of the Brown Seaweed Residues Supplementation on *In Vitro* Fermentation and Milk Production and Composition of Lactating Dairy Cows. *Journal of Animal Science and Technology*, Vol. 46:3, pp. 373–386. (Abstract).

Beresford, N.A., N.M.J. Crout & R.W. Mayes (2001): The transfer of arsenic to sheep tissues. *Journal of Agricultural Science*, Vol. 136:3, pp. 331–344.

Berg, J.M., J.L. Tymoczko, G.J. Gatto Jr. & L. Stryer (2015a): Cap. 16: Glycolysis and Gluconeogenesis in *Biochemistry*. 8th Ed. W. H. Freeman & Company, New York. pp. 449-495.

Berg, J.M., J.L. Tymoczko, G.J. Gatto Jr. & L. Stryer (2015b): Cap. 18: Oxidative Phosphorylation in *Biochemistry*. 8th Ed. W. H. Freeman & Company, New York. pp. 523-565.

Bird, P.R. (1970): Sulfur metabolism and excretion studies in ruminants. III. The effect of sulphur intake on the availability of copper in sheep. Proceedings of the Australian Society of Animal Production 8:212-218.

Cabrita, A.R.J., M.R.G. Maia, H.M. Oliveira, I. Sousa-Pinto, A.A. Almeida, E. Pinto & A.J.M. Fonseca (2016): Tracing seaweeds as mineral sources for farm-animals. *Journal of Applied Phycology*, Journal of Applied Phycology, Vol. 28:5, pp. 3135–3150.

Campbell, M., J. Ortuño, L. Ford, D.R. Davies, A. Koidis, P.J. Walsh & K. Theodoridou (2020): The effect of ensiling on the nutritional composition and fermentation characteristics of brown seaweeds as a ruminant feed ingredient. *Animals*, Vol. 10:6, pp. 1–17.

Chizzotti, M.L., S. de C. Valadares Filho, R.F.D. Valadares, F.H.M. Chizzotti & L.O. Tedeschi (2008): Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livestock Science*, Vol. 113:2–3, pp. 218–225.

Dalley, D.E., P. Isherwood, A.R. Sykes & A.B. Robson (1997): Effect of *in vitro* manipulation of pH on magnesium solubility in ruminal and caecal digesta in sheep. *Journal of Agricultural Science*, Vol. 129:1, pp. 107–111.

European Food Safety Authority (2005): Opinion on the scientific panel on contaminants in the food chain on a request from the Commission related to arsenic as undesirable substance in animal feed. Adopted on 31 January 2005. *The EFSA Journal*. Vol 180:, pp. 1–35. doi:10.2903/j.efsa.2005.180.

European Food Safety Authority (2013): Scientific Opinion on the safety and efficacy of iodine compounds (E2) as feed additives for all species: calcium iodate anhydrous and potassium iodide, based on a dossier submitted by HELM AG. *The EFSA Journal*. Vol 11:2, pp. 1–34.

Felsman, R.J., M.B. Wise, R.W. Harvey & E.R. Barrick (1973): *Effect of graded levels of copper sulfate and antibiotic on performance and certain blood constituents of calves*. Journal of Animal Science, Vol 36;, pp. 157–160.

Fish, R.E. & E.W. Swanson (1982): Effects of Excessive Intakes of Iodine upon Growth and Thyroid Function of Growing Holstein Heifers. *Journal of Dairy Science*, Vol. 65:4, pp. 605–610.

Flachowsky, G., K. Franke, U. Meyer, M. Leiterer & F. Schöne (2014): Influencing factors on iodine content of cow milk. *European Journal of Nutrition*, Vol. 53:2, pp. 351–365.

Franke, K., U. Meyer, H. Wagner, H.O. Hoppen & G. Flachowsky (2009): Effect of various iodine supplementations, rapeseed meal application and two different iodine species on the iodine status and iodine excretion of dairy cows. *Livestock Science*, Elsevier B.V., Vol. 125:2–3, pp. 223–231.

Geological Survey of Denmark and Greenland (2020): Grundvandskemi for boring [Online]. [Citet 5th of November 2020]. Available at https://data.geus.dk/JupiterWWW/proeve.jsp?borid=186776&indtagsid=1

Haggard, D.L., H.D. Stowe, G.H. Conner & D.W. Johnson (1980): Immunologic effects of experimental iodine toxicosis in young cattle. American Journal of Veterinary Research. Vol 41(4):539-543.

Hillman, D. & A.R. Curtis (1980): Chronic Iodine Toxicity in Dairy Cattle: Blood Chemistry, Leukocytes, and Milk Iodide. *Journal of Dairy Science*, Vol. 63:1, pp. 55–63.

Hong, Z.-S., Z.-H. Lee, C.-X. Xu, J.-L. Yin, Y.-C. Jin, H.-J. Lee, S.-B. Lee, Y.-J. Choi & H.-G. Lee (2010): Effect of Fermented Brown Seaweed Waste (FBSW) on Milk Production, Composition and Physiological Responses in Holstein Dairy Cows. *Journal of Animal Science and Technology*, Vol. 52:4, pp. 287–296. (Abstract).

Hurrell, R.F. (1997): Bioavailability of Iodine. *European Journal of Clinical Nutrition*. Vol. 51:, pp. 9-12.

Indergaard, M., G. Skjåk-Bræk & A. Jensen (1990): Studies on the Influence of Nutrients on the Composition and Structure of Alginate in *Laminaria saccharina* (L.) Lamour. (Laminariales, Phaeophyceae). *Botanica Marina*, Vol. 33:3, pp. 277–288.

Jenkins, K.J. & M. Hidiroglou (1990): Effects of Elevated Iodine in Milk Replacer on Calf Performance. *Journal of Dairy Science*, Elsevier, Vol. 73:3, pp. 804–807.

Kandylis, K. & A.C. Bray (1987): Effects of Variation of Dietary Sulfur on Movement of Sulfur in Sheep Rumen. *Journal of Dairy Science*, Vol. 70:1, pp. 40–49.

Khorasani, G.R., R.A. Janzen, W.B. McGill & J.J. Kennelly (1997): Site and Extent of Mineral Absorption in Lactating Cows Fed Whole-Crop Cereal Grain Silage or Alfalfa Silage. *Journal of Animal Science*, Vol. 75:1, pp. 239–248.

Kimura, T., N. Itoh, K.S. Min, I. Fujita, N. Muto & K. Tanaka (1998): Tissue accumulation of cadmium following oral administration to metallothionein-null mice. *Toxicology Letters*, Vol. 99:2, pp. 85–90.

Klaassen, C.D., J. Liu & B.A. Diwan (2009): Metallothionein protection of cadmium toxicity. *Toxicology and Applied Pharmacology*, Vol. 238:3, pp. 215–220.

Kumar, D.R., N. Kewalramani, V. Mani, S. Gupta, D. Parihar & A.S.T. Kujur (2018): Evaluation of bioavailability of selected minerals from Maize germ oilcake in crossbred male calves. *Indian Journal of Animal Research*, Vol. 52:5, pp. 730–734.

Küpper, F.C., L.J. Carpenter, G.B. McFiggans, C.J. Palmer, T.J. Waite, E.-M. Boneberg, S. Woitsch, M. Weiller, R. Abela, D. Grolimund, P. Potin, A. Butler, G.W. Luther III, P.M.H. Kroneck, W. Meyer-Klauckel & M.C. Feiters (2008): Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 105:19, pp. 6954–6958.

Lee, H.G., Z.S. Hong, Z.H. Li, C.X. Xu, X. Jin, M.G. Jin, H.J. Lee & N.J. Choi (2005): Effect of Brown Seaweed Waste Supplementation on Lactational Performance and Endocrine Physiology in Holstein Lactating Cows. *Journal of Animal Science and Technology*, Vol. 47:4, pp. 573–582. (Abstract).

Lennon, H.D. & J.P. Mixner (1956): Some Factors Affecting the Determination of Plasma Protein-bound Iodine, Using The Alkaline Fusion-ceric Sulfate Method. *New Jersey Agricultural Experiment Station*, pp. 351–355.

Llorente-Mirandes, T., M.J. Ruiz-Chancho, M. Barbero, R. Rubio & J.F. López-Sánchez (2011): Determination of water-soluble arsenic compounds in commercial edible seaweed by LC-ICPMS. *Journal of Agricultural and Food Chemistry*, Vol. 59:24, pp. 12963–12968.

Lüning, K. & L. Mortensen (2015): European aquaculture of sugar kelp (*Saccharina latissima*) for food industries: Iodine content and epiphytic animals as major problems. *Botanica Marina*, Vol. 58:6, pp. 449–455.

Maia, M.R.G., A.J.M. Fonseca, H.M. Oliveira, C. Mendonça & A.R.J. Cabrita (2016): The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. *Scientific Reports*, Vol. 6:, pp. 1–10.

Makkar, H.P.S., G. Tran, V. Heuzé, S. Giger-Reverdin, M. Lessire, F. Lebas & P. Ankers (2016): Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, Vol. 212:, pp. 1–17.

Manns, D., A.L. Deutschle, B. Saake & A.S. Meyer (2014): Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (Laminariaceae). *RSC Advances*, Vol. 4:49, pp. 25736–25746.

Manns, D., M.M. Nielsen, A. Bruhn, B. Saake & A.S. Meyer (2017): Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. *Journal of Applied Phycology*, Vol. 29:3, pp. 1493–1506.

Marinho, G.S., S.L. Holdt, M.J. Birkeland & I. Angelidaki (2015): Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. *Journal of Applied Phycology*, Vol. 27:5, pp. 1963–1973.

Martens, H. & M. Schweigel (2000): Pathophysiology of grass tetany and other hypomagnesemias: Implications for clinical management. *The Veterinary Clinics of North America: Food Animal Practice*, Vol. 16:2, pp. 339–368.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh, C.A. Morgan, L.A. Sinclair & R.G. Wilkinson (2010): Cap. 6: Minerals in *Animal nutrition*. 7. ed., Prentice Hall Press. pp. 103-139

Miller, J.K., E.W. Swanson & G.E. Spalding (1975): Iodine Absorption, Excretion, Recycling, and Tissue Distribution in the Dairy Cow. *Journal of Dairy Science*, Vol. 58:10, pp. 1578–1593.

Min, K.S., Y. Fujita, S. Onosaka & K. Tanaka (1991): Role of intestinal metallothionein in absorption and distribution of orally administered cadmium. *Toxicology and Applied Pharmacology*, Vol. 109:1, pp. 7–16.

Mondal, S., S.K. Paul, B. Bairagi, M.C. Pakhira & P. Biswas (2008): Comparative studies of reducing level of organic with inorganic trace minerals supplementation on the performance, nutrient digestibility and mineral balance in cross-bred male calves. *Livestock Research for Rural Development*, Vol. 20:7, pp. 1–10.

Møller, J., R. Thøgersen, M.E. Helleshøj, M.R. Weisbjerg, K. Søegaard & T. Hvelplund (2005): Fodermiddel tabel. Report no. 112. Dansk Kvæg.

National Research Council (2001): *Nutrient requirements of dairy cattle*. 7th rev. ed. Washington, D.C.: National Academy Press.

National Research Council (2005): *Mineral Tolerance of Domestic Animals*. 2nd rev. ed. Washington, D.C.: National Academy Press.

Neto, R.T., C. Marçal, A.S. Queirós, H. Abreu, A.M.S. Silva & S.M. Cardoso (2018): Screening of *Ulva rigida*, G*racilaria* sp., *Fucus vesiculosus* and *Saccharina latissima* as Functional Ingredients. *International Journal of Molecular Sciences*, Vol. 19:10.

Newton, G.L., E.R. Barrick, R.W. Harvey & M.B. Wise (1974): Iodine Toxicity. Physiological Effects Of Elevated Dietary Iodine On Calves. *Journal of Animal Science*, Vol. 38:2, pp. 449–455.

Nielsen, M.M., D. Manns, M. D'Este, D. Krause-Jensen, M.B. Rasmussen, M.M. Larsen, M. Alvarado-Morales, I. Angelidaki & A. Bruhn (2016): Variation in biochemical composition of Saccharina latissima and Laminaria digitata along an estuarine salinity gradient in inner Danish waters. *Algal Research*, Elsevier B.V., Vol. 13:, pp. 235–245.

Nielsen, N.I. & H. Volden (2011): Cap. 9: Animal requirements and recommendations in Volden, H. (2011): *NorFor – The Nordic feed evaluation system*. EAAP publication No. 130. Wageningen Academic Publishers, Wageningen. pp. 105-112.

Nitschke, U. & D.B. Stengel (2014): Iodine contributes to osmotic acclimatisation in the kelp *Laminaria digitata* (Phaeophyceae). *Planta*, Vol. 239:2, pp. 521–530.

Nitschke, U. & D.B. Stengel (2015): A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products. *Food Chemistry*, Elsevier Ltd, Vol. 172:, pp. 326–334.

Ometto, F., K.B. Steinhovden, H. Kuci, J. Lunnbäck, A. Berg, a. Karlsson, A. Handå, H. Wollan & J. Ejlertsson (2018): Seasonal variation of elements composition and biomethane in brown macroalgae. *Biomass and Bioenergy*, Elsevier Ltd, Vol. 109:, pp. 31–38.

Pehrson, B., V. Kossila, J.T. Hansen & N.E. Hansen (1975): Förslag til normer för makro- och mikromineraler till nötkreatur och svin. *Foderjournalen* Vol. 14:, pp. 55-106. (In Swedish).

Pompeu, L.B., J.E. Williams, D.E. Spiers, R.L. Weaber, M.R. Ellersieck, K.M. Sargent, N.P. Feyerabend, H.L. Vellios & F. Evans (2011): Effect of *Ascophyllum nodosum* on alleviation of heat stress in dairy cows. *Professional Animal Scientist*, Vol. 27:3, pp. 181–189.

Preus, N., M. Gregersen & C. Nielsen (2017): *FORBRUGER- OG FØDEVARERTRENDS*. Landbrug & Fødevarer. Axelborg, Axeltorv 3, 1609 København V.

Rey-Crespo, F., M. López-Alonso & M. Miranda (2014): The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. *Animal*, Vol. 8:4, pp. 580–586.

Roleda, M.Y., H. Marfaing, N. Desnica, R. Jónsdóttir, J. Skjermo, C. Reboursa & U. Nitschke (2019): Variations in polyphenol and heavy metal contents of wild-harvested and cultivated seaweed bulk biomass: Health risk assessment and implication for food applications. *Food Control*, Elsevier, Vol. 95:, pp. 121–134.

Roleda, M.Y., J. Skjermo, H. Marfaing, R. Jónsdóttir, C. Rebours, A. Gietl, D.B. Stengel & U. Nitschke (2018): Iodine content in bulk biomass of wild-harvested and cultivated edible seaweeds: Inherent variations determine species-specific daily allowable consumption. *Food Chemistry*, Vol. 254:, pp. 333–339.

Rupérez, P. (2002): Mineral content of edible marine seaweeds. *Food Chemistry*, Vol. 79:1, pp. 23–26.

Santesa, G. D., Hansen, H. H., Dhakal, R., & Nielsen, M. O. (2018). Effects of intact and extracted seaweed products on methane formation during rumen fermentation. 1. Poster session presented at 8th Nordic Seaweed conference, Grenaa, Denmark.

Schiener, P., K.D. Black, M.S. Stanley & D.H. Green (2015): The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *Journal of Applied Phycology*, Vol. 27:, pp. 363–373.

Scrutton, M.C., M.F. Utter & A.S. Mildvan (1966): Pyruvate Carboxylase. *The Journal of Biological Chemistry*, Vol. 241:15, pp. 1480–3487.

Sharma, S., L. Neves, J. Funderud, L.T. Mydland, M. Øverland & S.J. Horn (2018): Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. *Algal Research*, Elsevier, Vol. 32:, pp. 107–112.

Singh, B.K., R.C. Chopra, S.N. Rai, M.P. Verma & R.K. Mohanta (2016): Effect of Feeding Seaweed as Mineral Source on Mineral Metabolism, Blood and Milk Mineral Profile in Cows. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, Springer India, Vol. 86:1, pp. 89–95.

Singh, B.K., R.C. Chopra, S.N. Rai, M.P. Verma & R.K. Mohanta (2017): Nutritional Evaluation of Seaweed on Nutrient Digestibility, Nitrogen Balance, Milk Production and Composition in Sahiwal Cows. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, Springer India, Vol. 87:2, pp. 437–443.

Sjaastad, Ø.V., O. Sand & K. Hove (2016a): Cap. 6: The endocrine system in *Physiology of domestic animals*. 3rd ed. Scandinavian Veterinary Press, Oslo. pp. 241-294.

Sjaastad, Ø.V., O. Sand, & K. Hove (2016b): Cap. 7: Bone tissue and metabolism of calcium and phosphate in *Physiology of domestic animals*. 3rd ed. Scandinavian Veterinary Press, Oslo. pp. 295-323.

Sjaastad, Ø.V., O. Sand, & K. Hove (2016c): Cap. 15: The digestive system in *Physiology of domestic animals*. 3rd ed. Scandinavian Veterinary Press, Oslo. pp. 629-724.

Statistikbanken (2019): Animalsk production: Mælkeproduktion og anvendelse. [Online]. Denmark's statistics. [accessed 2nd of March 2020] available at: https://www.dst.dk/da/Statistik/emner/erhvervslivets-sektorer/landbrug-gartneri-og-skovbrug/animalsk-produktion

Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales & C. de Haan (2006): *Livestock's Long Shadow: Environmental Issues and Options*. Food and Agriculture Organization of the United Nations, Rome.

Strudsholm, F., J. Madsen, V.F. Kristensen, H.R. Andersen, T. Hvelpund & S. Østergaard (1999): Danske fodernormer til kvæg. Report no. 84. Landsudvalget for Kvæg.

Squibb, K.S., R.J. Cousins & B.L. Silbon (1976): Liver and Intestinal Metallothionein: Function in Acute Cadmium Toxicity. *Experimental and Molecular Pathology*, Vol. 25:, pp. 163–171.

Stévant, P., H. Marfaing, A. Duinker, J. Fleurence, T. Rustad, I. Sandbakken & A. Chapman (2018): Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) and health risk estimation for human consumption. *Journal of Applied Phycology*, Vol. 30:3, pp. 2047–2060.

Suttle, N.F. (2010): *Mineral Nutrition of Livestock*. 4th ed. CABI, Oxfordshire.

Suttle, N.F., H. Lloyd-Davies & A.C. Field (1982): A model for zinc metabolism in sheep given a diet of hay. *British Journal of Nutrition*, Vol. 47:1, pp. 105–112.

Thomas, P.C. & K. Subbaramaiah (1991): Seasonal variations in growth, reproduction, alginic acid, mannitol, iodine and ash contents of brown alga *Sargassum wightii*. *Indian Journal of Marine Sciences*, Vol. 20:, pp. 169–175.

Tibbetts, S.M., J.E. Milley & S.P. Lall (2016): Nutritional quality of some wild and cultivated seaweeds: Nutrient composition, total phenolic content and in vitro digestibility. *Journal of Applied Phycology*, Vol. 28:6, pp. 3575–3585.

Valadares, R.F.D., G.A. Broderick, S.C. Valadares Filho & M.K. Clayton (1999): Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *Journal of Dairy Science*, Elsevier, Vol. 82:12, pp. 2686–2696.

# 9. Appendix

## 9.1. Absorption coefficients

*Table 1. Calculated average mineral absorption coefficients in the two groups compared to the reported ceofficients from the NRC (2001).*

| Mineral | Control | | Seaweed | | NRC (2001) |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| **Ca g/kg** | 57.85 | 6.65 | 73.43 | 2.46 | 42.9 |
| **P g/kg** | 68.08 | 2.17 | 67.54 | 5.33 | 78 |
| **Mg g/kg** | 58.38 | 2.49 | 64.03 | 5.89 | 16 |
| **K g/kg** | 92.45 | 1.45 | 92.91 | 0.98 | 90 |
| **Na g/kg** | 89.23 | 0.38 | 93.18 | 0.44 | 90 |
| **S g/kg** | 68.70 | 2.94 | 74.07 | 5.11 | NA |
| **Fe mg/kg** | 47.99 | 2.84 | 44.54 | 13.52 | 50 |
| **Mn mg/kg** | 27.45 | 9.47 | 51.60 | 10.14 | 0.75 |
| **Zn mg/kg** | 26.28 | 10.65 | 56.81 | 6.26 | 15 |
| **Cu mg/kg** | 22.94 | 10.20 | 55.13 | 9.36 | 4.6 |
| **I mg/kg** | 56.60 | 11.66 | 93.61 | 0.80 | 85 |
| **As mg/kg** | 99.97 | 0.01 | 99.96 | 0.01 | 0.45 |
| **Cd mg/kg** | 99.95 | 0.00 | 99.95 | 0.01 | >1 |