MASTER'S THESIS

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Macroalgae as an alternative ruminant feed ingredient:

Impacts of dietary supplementation of *Laminaria hyperborea* on feed intake, growth, iodine intake and excretion in sheep

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Preface

This thesis was written for my master's degree in Bioscience- specialisation in Livestock, at Nord University. Through these two years, I have immersed myself in the exciting field of sustainable animal nutrition. This topic piqued my interest during my bachelor's degree in Livestock- welfare and production, where we had the course Animal Nutrition. As we enter the era of increased global food and feed requirements, I want to pursue the need for research to meet these demands. One of the many ways is to look for alternative, sustainable ways of feeding our livestock. I am thankful to Dr Prabhat Khanal, principal supervisor, for guidance at every step of the way and always being present for help whenever needed. Partaking in the Animal Nutrition group at Klab has been a great experience. I have enjoyed our discussions and openness and learned much by listening to others' experiences and following their projects. I am grateful for the opportunity to join project manager Dr Vibeke Lind at NIBIO in the experiment regarding feeding sheep with macroalgae. I want to thank her for inviting me to their facilities at Tjøtta and giving valuable support as co-supervisor, with guidance in the experiment, reviewing the thesis, conversations about the big-picture, and everything in between. I want to thank Dr Michael Allen Patten for his support with data analysis in R and for meeting all the challenges with a big smile and the best humour. I also thank Hege Overrein, co-supervisor, for helpful discussions about ruminant feeding strategies and digestive physiology. Finally, I want to direct a big thanks to Torbjørn Tufte, my partner, for endless support during challenging times and for always motivating and reminding me of the goal.

Steinkjer, 16.05.2023

Jorid Thorvaldsen Sandvik

1 Abstract

To feed the growing human population and combat challenges posed by adverse climate change impacts, there is an urgent need to search for alternative livestock feed sources. Norway has an extensive coastline and a well-established aquaculture sector which provides access to substantial macroalgae, a potential alternative feed ingredient. Ruminants are an essential part of the Norwegian livestock industry due to their ability to utilise uncultivated fields and provide meat and milk. Traditionally, they have been fed with macroalgae in times of feed scarcity. However, macroalgae are known to accumulate iodine from their environment. If iodine concentrations in the animal diet exceed the upper limits, iodine intoxication can occur. One of the most harvested and abundant macroalgae species, *Laminaria hyperborea*, has an exceptionally high iodine concentration. Thus, it is necessary to investigate how feeding macroalgae to ruminants affects their health and welfare. This study aimed to investigate the uptake, distribution, and excretion of iodine in sheep fed with a concentrate supplemented with *L. hyperborea* by-products. During a period of 3 months, 12 male sheep (~6 months age, 37.8 ± 4.1 kg body weight) were fed a macroalgae diet (roughage *ad libitum* and 6% *L. hyperborea* in concentrate) or control diet (roughage *ad libitum* and conventional concentrate), while feed intake and body weights were measured daily and every second week, respectively. The experiment was split into two periods, based on the daily concentrate level in the diet (period 1=300 g concentrate, period 2=600 g concentrate). Animals were adapted to their respective diets for 26 days before 6 animals were placed in metabolism crates for 62 hours, while faeces and urine were sampled daily to determine iodine concentration. The six remaining animals were placed in metabolism crates with the same procedure immediately after the first six returned to the barn. The exact process regarding metabolism crates was repeated for period 2 (5 weeks later). There were no significant effects of macroalgae inclusion on feed intake or body weight gain between the diets. A significant difference in feed intake was found between periods, consistent with animal growth. Animals fed the macroalgae diet had the highest iodine intake and excretion. Based on visual observations and parameters examined in this study, the animals showed no signs of iodine intoxication. We conclude that *L. hyperborea* can be fed to sheep for up to three months without compromising animal health and performance. However, long-term impacts should be evaluated in the future.

Keywords:

Body weight, feed intake, iodine metabolism, lambs, ruminants, seaweed, sheep.

2 Sammendrag

Kraftig befolkningsvekst og klimaendringer stiller nye krav til husdyrproduksjon. Det er et økende behov for mat, men også bærekraftige, lokale råvarer. Drøvtyggere er en viktig del av norsk husdyrproduksjon, da de kan utnytte utmarka og samtidig gi oss tilgang på næringsrike, animalske produkter med høy matsikkerhet. Tradisjonelt sett har drøvtyggere, og spesielt sau, vært tilleggsfôra med makroalger når fôrtilgangen har vært dårlig. Norge har en ekstensiv kystlinje og en veletablert akvakultur sektor, som gir tilgang på store mengder makroalge. Makroalger er en mulig alternativ fôringrediens til drøvtyggere, og har potensiale til å møte fremtidens krav til fôrressurser, samtidig som de er lokale og kortreist. Likevel er det utfordringer knyttet til makroalger, da de akkumulerer jod fra sjøvann og mange arter har svært høye verdier. Hvis jod inntaket hos mennesker og dyr overstiger øvre grenseverdier, risikerer man jodforgiftning. *Laminaria hyperborea* er en av makroalgene det høstes mest av i Norge, samtidig er jodkonsentrasjonen blant de høyeste. Hvis denne arten skal brukes som tilleggsfôr til drøvtyggere i fremtiden må det forskes på hvordan høy jodkonsentrasjon påvirker helse og produksjon hos drøvtyggerne. Målet med studien var å undersøke opptak, fordeling og utskillelse av jod hos sau fôra med kraftfôr som inneholder 6% *L. hyperborea* biprodukt. Over en 3 måneders periode ble 12 værer (~6 måneder gamle, 37.8 kg ± 4.1 kg kroppsvekt) fôra 2 dietter; makroalge (grovfôr *ad libitum* og tilsatt *L. hyperborea* i kraftfôret) eller kontroll (grovfôr *ad libitum* og konvensjonelt kraftfôr). Forsøket ble oppdelt i to perioder basert på daglig kraftfôrinntak (periode 1=300 g kraftfôr, periode 2=600 kg kraftfôr). Fôropptak ble registrert daglig, og dyrene ble veid annenhver uke gjennom forsøket. Dyrene ble tilvendt diettene i 26 dager før eksperimentstart, før 6 dyr ble satt i metabolismebokser i 62 timer. I løpet av disse timene ble avføring og urin samlet inn daglig for å analysere jodinnhold. Når de første 6 dyrene ble tatt ut av metabolismeboksene og plassert i hovedfjøset, ble straks de 6 resterende satt inn og samme prosedyre ble gjentatt. Deretter sto alle dyrene i hovedfjøset frem til periode 2 (5 uker senere), der identisk prosedyre med metabolismebokser ble gjentatt. Resultatene viser ingen signifikant forskjell mellom diettene når det gjelder fôropptak og tilvekst. Det var derimot en signifikant forskjell på fôropptak mellom periode 1 og 2, noe som passer dyrenes vekstkurve. Dyrene som var fôra makroalge diett hadde høyest jodinntak og utskillelse. Videre viste dyrene ingen tegn på jodforgiftning. Vi konkluderer med at sau kan håndtere jodkonsentrasjonen i en rasjon supplementert ~2% *L. hyperborea* over 3 måneder, men langtidseffekter må undersøkes.

Søkeord:

Drøvtygger, fôropptak, jodmetabolisme, makroalge, tang, tilvekst.

3 Abbreviations

ADG - Average Daily Gain

CP - Crude Protein

D1 - Type 1 Deiodinase

D2 - Type 2 Deiodinase

D3- Type 3 Deiodinase

DM - Dry Matter

NDF - Neutral Detergent Fibre

NPN - Non-Protein Nitrogen

T1 - Mono-tyrosine

T2 - Diiodotyrosine

T3 - Triiodothyronine

T4 - Tetraiodothyronine/thyroxine

TPO - Thyroperoxidase

TSH – Thyroid-stimulating hormone

VFA - Volatile Fatty Acids

4 Introduction

To feed the growing human population and combat challenges posed by adverse climate change impacts, there is an urgent need to search for alternative livestock feed sources. A global increase in food demand is projected to increase by 60% in 2050 compared to 2005. The demand for livestock products is expected to rise even more due to a change in dietary preferences trending towards a higher proportion of animal-derived protein (Alexandratos & Bruinsma, 2012).

Ruminants are essential to the Norwegian livestock industry due to their ability to utilise uncultivated fields. However, the industry depends on importing feed ingredients like soybean, rapeseed and minerals to produce a concentrate with an optimal nutritional value, thus maintaining intensive meat- and milk production (Nysted et al., 2021). Identifying local, sustainable ingredients to replace or supplement imported concentrate ingredients may increase domestic feed supply and Norwegian feed security in the future.

Norway has an extensive coastline and a well-established aquaculture sector, which facilitates the utilisation of marine feed ingredients. Macroalgae represent an abundant, local feed source that does not compete with terrestrial feed sources while showing nutritional potential as a ruminant livestock feed. They are on a low trophic level, have high biomass, and are projected to be central in the future circular bioeconomy (Løvdal & Skipnes, 2022). The total number of macroalgae species in Norway is estimated to be ~700 (Husa et al., 2021).

Norway has an industry that harvests wild macroalgae for alginate and macroalgae meal, while *Saccharina latissima* and *Alaria esculenta* is commercially cultivated. Most biomass derives from wild-harvested brown sp. *Laminaria hyperborea* and *Ascophyllum nodosum*, and annual harvest is approximately 130-180 000 tonnes (Directorate of Fisheries, 2022c). The first cultivating licenses were awarded in 2014 (*n*=54), and numbers have increased until 2022 (*n*=539) (Directorate of Fisheries, 2023a). The number of harvesting sites in the sea has risen from 2018 (*n*=83) to 2022 (*n*=105), indicating a growing industry (Directorate of Fisheries, 2023b). The number of companies remained the same from 2018 to 2021 (*n*=23), while the number of employees in macroalgae production increased from 2015 (*n*=44) to 2022 (*n*=69) (Directorate of Fisheries, 2022a, 2022b).

Figure 1. Overview: Norwegian Macroalgae Industry 2021, 2023, by Jorid Sandvik. CC BY-NC-SA 2.0 NO.

After macroalgae processing, there remains a by-product that is considered waste today with the potential to be utilised for feed purposes. Despite macroalgae being rich in nutrients and bioactive compounds, there are challenges related to their high mineral content. Iodine levels

are high, especially in brown macroalgae species. There is a need to identify how these levels will affect animal health if included in ruminant diets.

This study aimed to investigate how supplementing *L. hyperborea* in sheep impacts animal performance and health. The main objectives were to measure feed intake, body weight gain and iodine concentration in faeces, urine and blood compared to sheep fed a control diet.

Figure 2. Harvesting Laminaria hyperborea. Reprinted with permission from DuPont Nutrition Norge AS.

5 Theory

Evaluating existing feeding schemes and exploiting alternative feed strategies requires knowledge of the ruminant's digestive anatomy and physiology and are therefore discussed in the following sections.

5.1 Ruminant Digestive Anatomy and Physiology

Herbivores differ from carnivores and omnivores due to their ability to utilise grass and plants as the sole energy source (Dehority, 2002). Herbivores have evolved to ferment plant material by pre-gastric or hindgut fermentation. Ruminants belong to the pre-gastric type and are characterised by stomachs divided into four distinct compartments: the rumen, reticulum, omasum, and abomasum. The fermentation process reduces the amount of dry matter (DM) entering the abomasum, thus making it possible to process the daily requirements of heavily digestive feed (*e.g.*, grass and silage). The fermentation process is driven by a symbiosis between the ruminant and micro-organisms, protozoa, and fungi that inhabit its rumen (Cronje, 2000). Microorganisms make energy available for the ruminant by digesting nutrients with their enzymes and producing volatile fatty acids (VFAs; acetate, propionate, and butyrate), which are the primary energy sources for the ruminant (Millen et al., 2016).

The ruminant's digestive tract is adapted to effectively break down heavily digestive plant polysaccharides such as cellulose. Firstly, the mouth is shaped to fit the plant material that matches the respective ruminant's diet. Secondly, their hypsodont teeth are durable and resistant to friction. An example is grazers *(e.g.,* cattle and sheep), which have a wide muzzle ideal for a high intake of grass of homogeneous character (Pérez-Barbería, 2020). Mature sheep typically produce about 10 litres of saliva per day to ease the digestion of fibre by dilution and act as a buffer in the rumen to maintain an ideal pH (McDonald et al., 2022).

A large oesophagus connects the mouth with the rumen, the first compartment of the ruminant stomach. Digesta (*i.e.*, feed undergoing digestion) moves through the reticulum, omasum, and abomasum before entering the small intestine (Figure 3). The four compartments of the ruminant stomach are defined by numerous pillars and the reticulo-ruminal fold. All compartments are built up by the same three layers from outer to inner, respectively: (1) the Serous membrane, (2) the muscular- and (3) the inner-epithelial membrane (Krehbiel, 2014).

5.1.1 Rumen

The largest stomach compartment is the rumen, which functions like a fermentation chamber. The rumen is located in the abdominal cavity on the left side of the animal. Liquids, solids,

and gases are always present in the rumen; gases are on top, while the largest particles are floating on the ground layer composed of the finest and most dense plant material. Various papillae cover the inside and increase surface area up to 30 times compared to a smooth rumen (Pérez-Barbería, 2020). The papillae allow efficient absorption of VFAs in the blood, into the portal vein and further to the liver before it ends in the systemic circulation (Sjaastad et al., 2016).

The rumen depends on strong peristaltic movements to mix, regurgitate and rechew ingested feed. The rumen wall has large muscles that can repeat these movements until the plant material has reached a particle size suited for moving on to the next compartment (Dufreneix et al., 2019; King & Moore, 1957). The threshold particle size is between 1.9-2.0 mm in sheep (Dehority, 2002).

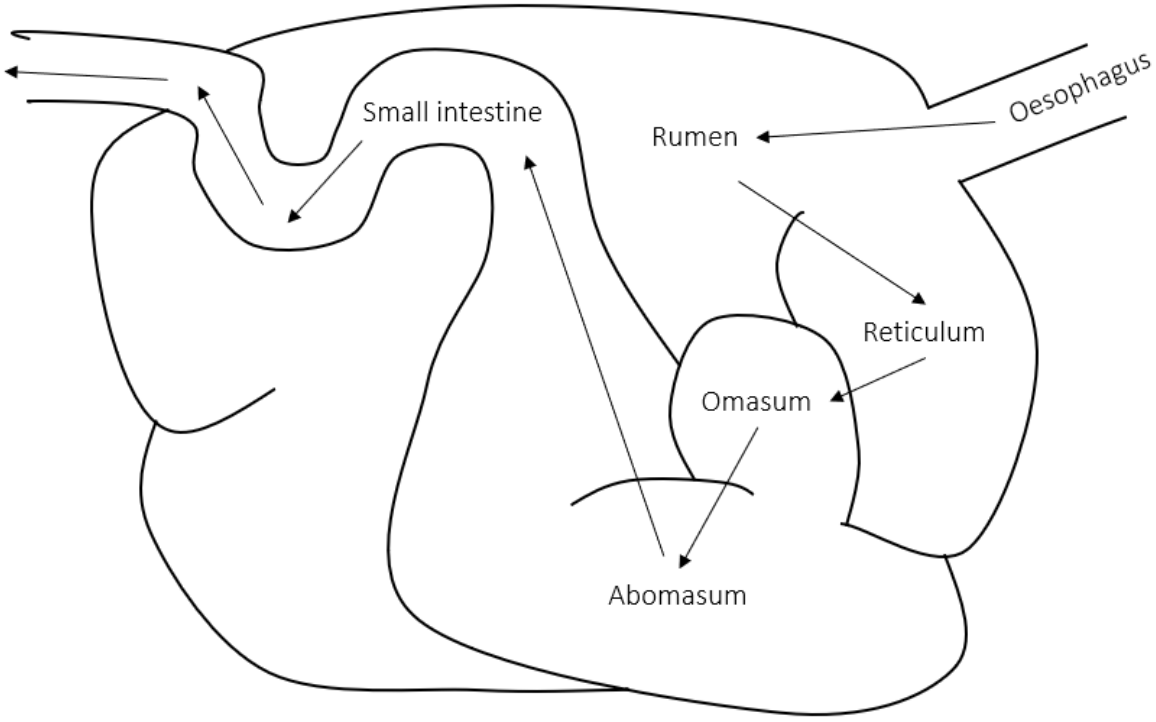

Figure 3. Illustration of ruminant stomach and direction of feed flow from the oesophagus to the small intestine. Seen from the right, 2022, by Jorid Sandvik

The rumen environment is strictly anaerobic, the temperature is stable at 39°C and DM content has an average of 10-13%. Ideally, pH is 6.5 to maintain a stable environment for the micro-organisms and facilitate fermentation, though it may vary between 5.5 - 7.0 based on the types of diets they consume. Variations in pH will affect the microbial flora and which VFAs are produced in relative proportions (Cholewińska et al., 2021; Krehbiel, 2014).

The VFAs alone can reduce rumen pH to 2.5 - 3.0, but phosphate and bicarbonate from the saliva act as a buffer and typically maintain pH at 6.5. Diet is the main factor affecting changes in pH. A diet dominated by concentrate will cause a drop in pH due to a stimulation of bacteria (*e.g.*, *Streptococcus bovis*) that produce lactate (Jans et al., 2015). Lactate is a short-chained fatty acid and is not present in considerable amounts in a healthy rumen. Concentrate generally contains high levels of starch, a nutrient that is easier to digest than cellulose due to its alfa 1-.4 bindings (McDonald et al., 2022). Cellulolytic bacteria (*e.g.*, *Bacteroides succinogenes* and *Ruminococcus flavefaciens*), which break down fibres, become inactive when pH reaches 5.2 or below, and ultimately, feed intake goes down (Hungate, 2013). Furthermore, microbes that use lactate as an energy source are vulnerable to changes in pH and become inactive at pH <5 (Sjaastad et al., 2016). All these mechanisms may lead to acidosis, a serious condition accompanied by an impairment in general animal health (Winkelmann et al., 2016). The acidity leads to a lack of peristaltic movements, and fluids are moving bloodstream to the rumen, causing a thickening of the blood. At the same time, acid levels rise in the bloodstream due to high lactate levels (McDonald et al., 2022). Methanogenic archaea are a type of microorganism that inhabitants the rumen. The archaea are reducing carbon dioxide (CO_2) and hydrogen (H_2) into methane (CH_4) as an end-product of carbohydrate fermentation. Gases are mainly removed from the ruminant through respiration and regurgitation, resulting in approximately 10% gross energy loss from feed (McDonald et al., 2022; Van Nevel & Demeyer, 1996).

A balanced rumen environment is essential to maintain its function. Digesta will move on to the reticulum when processed into finer and more dense feed particles (Lund et al., 2006; Pérez-Barbería, 2020).

5.1.2 Reticulum

The inside of the reticulum has raised epithelia which looks like a honeycomb structure (Figure 4). Digesta can freely move between the rumen and the reticulum due to an opening between the two compartments. The function of the reticulum is to retain and further process particles >2mm before they move into the next compartment, the omasum (Sjaastad et al., 2016).

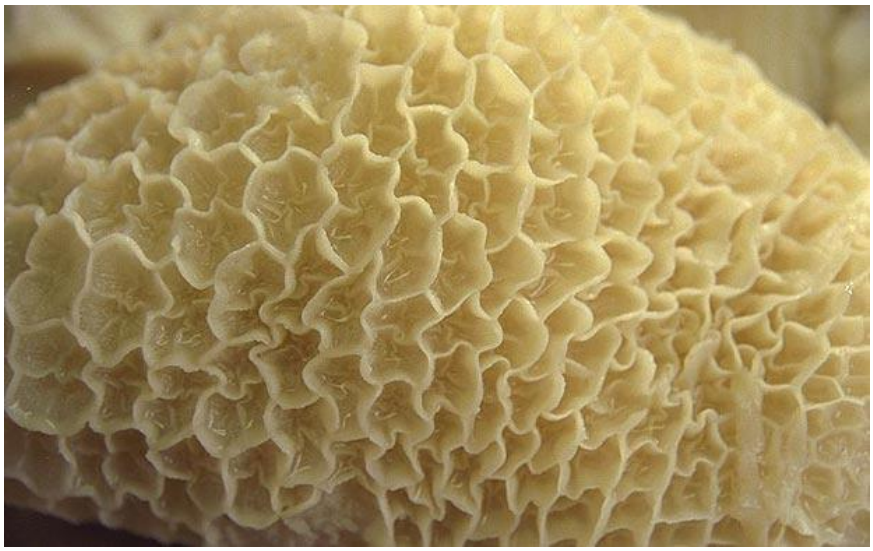

Figure 4. The inside surface of the reticulum has raised epithelia which looks like a honeycomb structure, 2014, by anon (https://en.wikipedia.org/wiki/Reticulum_(anatomy)#/media/File:Reticulum-honeycomb2.jpg). CC-BY-SA 3.0.

5.1.3 Omasum

The inside surface of the omasum is covered in leaf-looking folds connected to the smooth muscle that may alter its shape. These folds provide the omasum with an increased surface area which allows the absorption of water, VFAs, and ammonia into the blood. Fine feed particles that arrive from the reticulum get tightly trapped in these folds before entering the abomasum via the reticulo-omasal sphincter at a slow rate (Sjaastad et al., 2016). The main function of the omasum is to transport digesta between the reticulum and abomasum but also acts as a barrier between these two compartments (Hackmann & Spain, 2010). Furthermore, the morphology prevents large particles to enter the abomasum (Caushi & Martens, 2018).

5.1.4 Abomasum

The abomasum physiology and anatomy are similar to the ventricle in monogastric animals but slightly differ in function as it is not a storage organ. Abomasum receives digesta continuously from the forestomaches, and sheep receive 4-8 l per 24 hours, depending on life stage (*i.e.*, age, lactation) (Sjaastad et al., 2016). The pH of the abomasum is 3-4, which is low compared to the other compartments. The acidification is caused by hydrochloric acid (HCl) and pepsinogen, produced by glandular cells on the inside wall of the abomasum. Pepsinogens are cleaved into pepsin in contact with HCl. Pepsin is an enzyme that hydrolyses proteins. The secretion of HCl is also contributing to the further degrading of feed and the killing of microorganisms that may have entered the digestive system with the feed from the rumen (Pérez-Barbería, 2020). These processes facilitate further digestion and absorption of

nutrients in the small intestine. At the end of the abomasum, the pyloric muscle regulates the entry of digesta into the small intestine.

5.1.5 Intestine

The small intestine can be up to 30 times the ruminant's body length and is divided into three compartments: duodenum, jejunum, and ileum. Passage time depends on the length of the small intestine and diet. The longer the small intestine is, the longer the passage time, thus increasing absorption, enzyme and digestive secretion exposure to by-pass nutrients. Furthermore, an increased proportion of fibre in the diet will increase passage time.

Duodenum is the compartment with the highest activity regarding the absorption and digestion of nutrients. Enzymes, bile, and buffering solutions affect the digesta, breaking down nutrients into basic compounds such as amino acids, glucose, fatty acids and glycerol, which are then absorbed over the small intestine wall in both the duodenum and jejunum (Sjaastad et al., 2016). The primary function of the ileum is the absorption of water, vitamin B_{12} and the re-absorbing of bile into the liver, while digestive activity is low compared to the duodenum and jejunum (Guéant et al., 2022). Digesta moves on to the large intestine from the ileum, which is connected to the caecum. Micro-organisms contributes to further fermenting and digesting of remaining nutrients, in addition to vitamin synthesis. Excess water is absorbed; the remaining content is faeces, which exit the digestive system through the rectum. The proportion, amount and type of nutrients and feedstuffs affect health and efficiency in the ruminant digestive system and are therefore introduced in the following section.

5.2 Nutrients

Carbohydrates, proteins, and lipids are fermented in the rumen and reticulum. The ratio of hay and concentrate in the feedstuff greatly influences the ratio of VFA production (Krehbiel, 2014). Sheep fed a hay to concentrate in the ratio at 80:20 will have an individual VFA production of 0.61 acetate, 0.22 propionate, 0.09 butyrate, and 0.03 other VFAs, while a hay-to-concentrate ratio at 40:60 provide 0.52 acetate, 0.34 propionate, 0.15 butyrate, and 0.03 other VFAs (McDonald et al., 2022).

5.2.1 Carbohydrates

Carbohydrates are the main component of the ruminant diet, and the dominating types are cellulose, hemicelluloses, starch, and water-soluble carbohydrates, respectively.

Cellulose is the world's most abundant carbohydrate and is found in plant cell walls as a structural component. It is a chemically stable molecule and is classified as a homopolysaccharide. Cellulose consists of glucose units bound together by β-1.4 linkages. While no animal enzyme may break these linkages, the rumen microbiome is inhabited by microbes that produce extracellular enzymes that may break them. This benefits both the ruminant and the microbes; glucose from cellulose is made available as an energy source for the ruminal microbiome, which synthesises VFAs that provide energy for the ruminant. (Cherian, 2020; Hvelplund & Nørgaard, 2003). Similarly, hemicellulose consists of pentose and is also bound by β-1.4 linkages. Microbes that favour cellulose as their energy source typically have acetic acid and excess hydrogen and water as end-products of fermentation. Thus, a diet containing mostly cellulose results in a higher CH_4 production and, as discussed in previous sections, a healthy rumen environment (McDonald et al., 2022). On the other hand, a diet containing mostly starch results in higher H_2 production, thus leaving less H_2 available for CH_4 production, but may negatively impact the rumen environment. When providing the rumen microbes with starch, the microbes that favour glucose as their energy source will reproduce faster. These microbes produce lactate which will stimulate the growth of microbes favouring lactate as their energy source and producing propionate. This results in increased propionate production, and as H is an important component of propionate. Methane production will decrease when starch dominates the diet, due to reduced H availability (McDonald et al., 2022).

5.2.2 Protein

Most proteins in the feed are digested by microbes in the rumen and reticulum and converted into microbial protein. The microbial enzymes break down proteins into peptides that are taken up by bacteria before further broken down into amino acids. Amino acids are either utilized for microbial protein synthesis or deaminated into ammonia (NH_3) which ultimately is transported to the liver or stays in the rumen. Finally, the microbes synthesize proteins for further growth based on these end products (Sjaastad et al., 2016). The microbial protein synthesized in the rumen is digested in the small intestine together with by-pass protein which passed the forestomaches undigested.

Microorganisms are utilizing non-protein nitrogen (NPN) like ammonium (NH_4^+) to grow a larger population. Microorganisms are flushed from the rumen to the small intestine daily due to their short lifecycle. Thus, they are providing the ruminant with high-quality protein (Millen et al., 2016).

5.2.3 Lipids

Ingested lipids are hydrolysed in the rumen by bacterial lipases. Short-chained fatty acids are absorbed in the rumen, while long-chained lipids are hydrolysed and absorbed in the small intestine. A ruminant diet should not exceed 5% lipids due to the microorganisms limited ability to digest. Exceeding this threshold limit results in reduced microbial activity, especially related to the digestion of cellulose and hemicelluloses (McDonald et al., 2022).

5.2.4 Vitamins

Vitamins are defined as organic elements necessary for animals in small amounts to maintain a normal animal life and growth (McDonald et al., 2022). Classification of vitamins is usually done by dividing them into two groups: fat-soluble (*i.e.*, A, D_2, D_3, E, and K) and water-soluble (*i.e.*, the B complex; B_1, B_2, B_6, $B_{12,}$ and C). Vitamins differ from carbohydrates, protein, and fat in their function; they are not only providing energy or acting as building blocks but also mediate important biochemical pathways and act as antioxidants. The rumen microbiome is able to synthesize B and K vitamins, supporting the ruminant with sufficient concentrations under natural conditions (Dehority, 2002; Jiang et al., 2022). Although, animals in high production (*e.g.*, milking cows) may need additional supplementation due to increased requirements.

5.2.5 Minerals

Minerals are defined as inorganic elements that are essential for animals metabolic processes and physiological functions. (Underwood & Suttle, 1999). Classification of minerals is based on nutritional requirements, dividing them into two groups: major or macronutrients and trace elements or micronutrients. The nutritional requirement for trace elements is <0.01% of the diet, while macronutrients are >0.01%, making the upper, - and lower concentration limits relatively close and emphasizing the need for knowledge of the ruminant's diet to prevent intoxication or deficiency (McDonald et al., 2022). Furthermore, trace element requirements vary between animal species, life stages and state (*e.g.*, foetus, young animals, lactation stage, and gestation) (National Research Council, 2007). Trace element absorption and bioavailability differ between ruminants and monogastric animals due to the difference in their digestive system and diet. Interaction effects between trace elements are well-known to cause changes in absorption, excretion, function, and storage (Byrne & Murphy, 2022).

Selenium may be used as an example of the complexity of mineral absorption and bioavailability in ruminants variations and interaction effects. Selenium absorption in

ruminants is lower than in monogastric animals due to the rumen environment reducing dietary selenium to insoluble forms (Spears, 2003). Furthermore, selenium bioavailability is affected by sulphur concentrations; high dietary inclusion of sulphur may lead to selenium suppression due to their similar physical and chemical properties. Selenium are also an important component of enzymes which is converting thyroid hormones (T_4 and T_3) to its active form (Underwood & Suttle, 1999). Consequently, selenium deficiency results in an increased T_4:T_3 ratio. The feedback mechanism from T_4 is regulating D2 synthesis, thus will the mentioned ratio increase results in a lowered D2 production (Arthur et al., 1992; McDonald et al., 2022). Low levels of selenium are therefore interrupting thyroid hormone synthesis and can result in iodine deficiency symptoms.

The scope of this thesis is on dietary iodine intake and its further metabolism. Thus, in the following sections, different aspects of iodine metabolism in ruminants will be discussed.

5.2.6 Iodine

Iodine is an essential trace element in ruminants and was discovered in 1811 by Curtois. He exposed a seaweed extract to sulfuric acid, resulting in a form of a violet effluvium that condensed to dark crystals (Chapman, 1987). The primary function of iodine is the incorporation of the thyroid hormones, triiodothyronine (T_3) and tetraiodothyronine/thyroxine (T_4), which are made of mono-tyrosine (T_1) and diiodotyrosine (T_2). Most of the iodine in the body is found in the thyroid gland, where it is utilised for the biosynthesis of T_3, the active form of thyroid hormones, and T_4, the inactive transport form (Paulíková et al., 2002).

The function of T_3 is complex due to its role in metabolism. Gene transcription rate is influenced by T_3, and this affects protein synthesis and oxidation rate in all cells. Together, T_3 and T_4 are increasing both energy production and cellular respiration. These mechanisms will affect energy metabolism, muscle function, growth, circulation, and immune defense, thus there are several physiological areas of effect (*e.g.*, generation of insulin, growth hormones, and regulatory proteins). In addition, these effects play a role in determining the nutrient requirement and fertility cycles (Huszenicza et al., 2002; National Research Council, 2007).

Norwegian sheep are seasonal breeders and goes into heat during short days in the autumn. Lambing in spring increases the survivability of the lambs, due to the abundance of food and mild climate during spring and summer. Other factors (*e.g.*, reproduction hormones) are involved in inducing these specific breeding cycles, but T_3 concentration in the mediobasal

hypothalamus is important and is shown to be 10 times higher in sheep during short days compared to long days (Yoshimura, 2013).

5.2.6.2 Iodine Metabolism in Ruminants

Dietary iodine is easily absorbed (70 to 90%) in the rumen, reticulum and omasum (National Research Council, 2007). Recycling of iodine occurs in the abomasum, where the secretion to absorption rate is reported to be 18:1 in cattle (Miller et al., 1975). Iodine continues to be absorbed in the small intestine and enters the liver via the portal vein. From the liver, it enters the blood and circulates loosely bound to plasma proteins. Iodine in the form of T_4 is also entering the small intestine via the bile (Miller et al., 1975). The thyroid gland absorbs 80% of iodine, while the latter is stored in the extra-thyroideal iodine pool (*i.e.*, muscle and liver) and excess iodine is excreted through urine, faeces or milk (National Research Council, 2007).

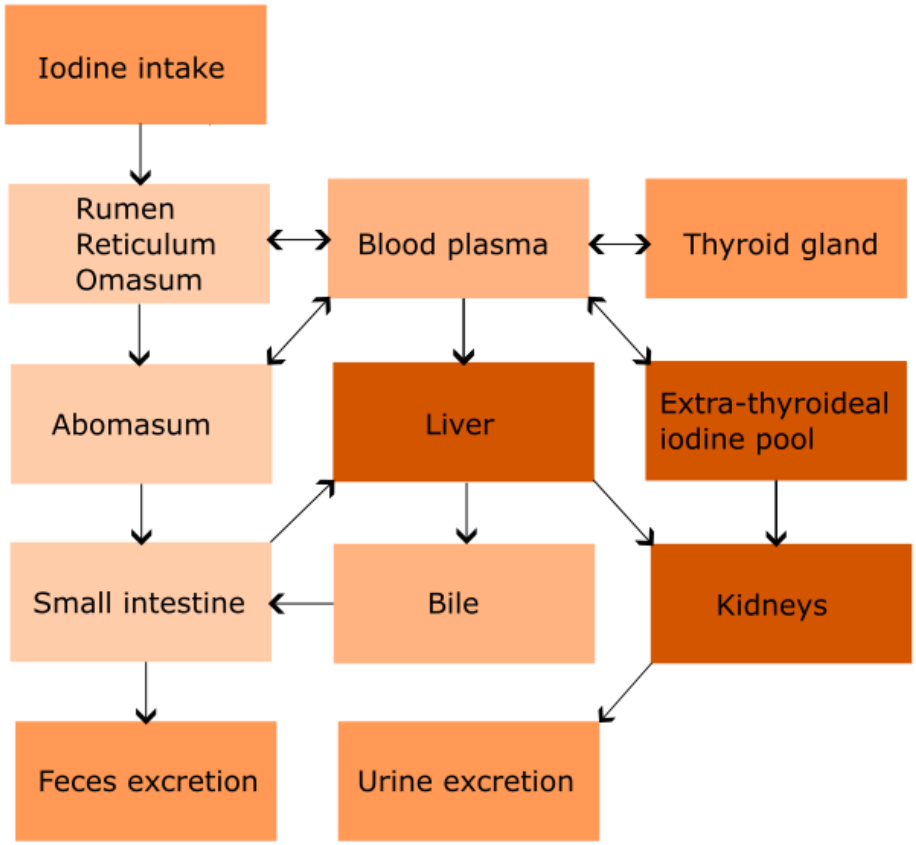

Figure 5. Overview of iodine metabolism in ruminants. Iodine enters the body via ingestion of feed or water containing iodine. When in the forestomaches, iodine can either get absorbed and enter the blood plasma or follow the gastrointestinal tract to the abomasum and further into the small intestine. Most part of the iodine that reaches the blood plasma will enter the thyroid gland, while the rest will enter the liver or be stored in the extra-thyroideal iodine pool (i.e., salivary glands, mammary glands, small intestine, mucous membrane of abomasum). From the extra-thyroidal iodine pool, it may enter the kidneys and get excreted through urine, while this

passageway is also used by iodine that has reached the liver. Furthermore, iodine from the liver can enter the bile and get excreted in faeces via the small intestine.

Iodide is transported into the thyroid gland by the sodium iodide symporter (NIS), which is the first step in thyroid hormone synthesis (Concilio et al., 2020). The NIS is located in the plasma membrane of the thyroid gland and mediates active transport of iodide into the thyroid follicular cells. Furthermore, NIS is found in extra-thyroideal tissues (*i.e.*, salivary glands, mammary glands, small intestine, and the mucous membrane of the stomach) where it serves the same purpose (Dohán et al., 2003; Nicola et al., 2012).

5.2.6.2 Thyroid Hormone Synthesis

Iodine that has reached the thyroid gland will iodinate the amino acid tyrosine and form T_1 or T_2. Thyroperoxidase (TPO) is a helping enzyme stimulated by thyroid-stimulating hormone (TSH) and brings two T_2 molecules together to form T_4 (Carvalho & Dupuy, 2017). When reaching the thyroid gland, TSH acts on receptors that stimulate synthesis and release of T_4 into the blood based on feedback on levels of free T_3 and T_4 in blood and the iodide pool (Huszenicza et al., 2002).

Iodothyronine deiodinases are selenoenzymes necessary to activate or inactivate thyroid hormones. There are three types that differ in tissue distribution and how they act in the activation/deactivation process (Behne & Kyriakopoulos, 2001).

Type 1 deiodinase (D1) is providing T_3 to the plasma by removing one iodine atom from T_4, while it may also inactivate T_3 and eliminate reverse T_3 (*i.e.*, the inactive isomer of T_3 produced in the process of deactivating T_4) (Gomes-Lima et al., 2019). Type 2 deiodinase (D2) main biological function is to regulate thyroid hormones on the tissue level by activating T_4 to T_3 and inactivation of T_3 to T_2. Type 3 deiodinase (D3) is mainly located in the skin, placenta, and central nervous system. Its main biological function is to produce reverse T_3 from T_4 and T_2 from T_3, providing thyroid hormones to the fetus and assuring that the adult brain is not exposed to high concentrations of T_3 (Behne & Kyriakopoulos, 2001).

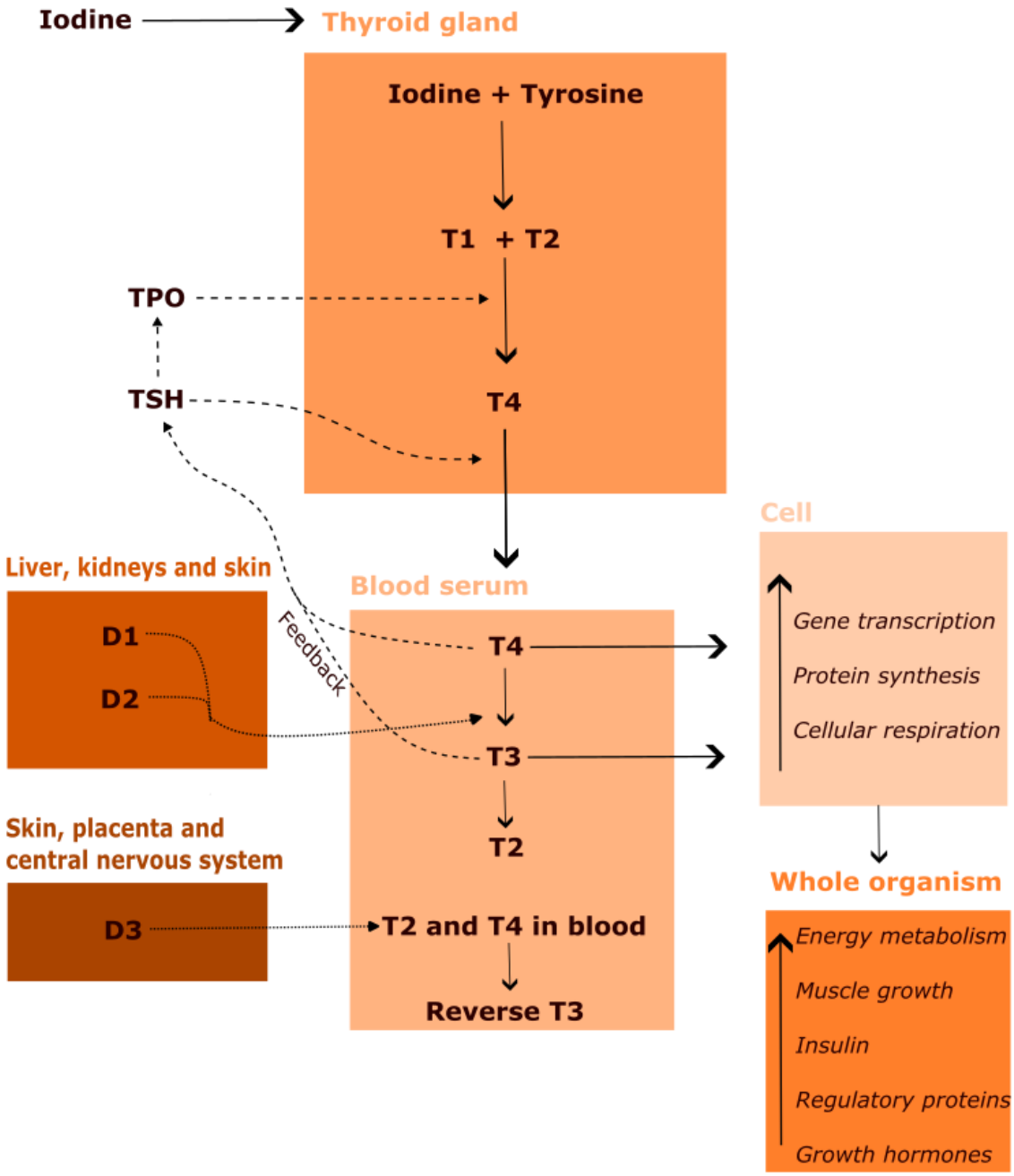

Figure 6. Overview of thyroid hormone synthesis and function. Iodine that reaches the thyroid gland will iodinate the amino acid tyrosine and form T_1 and T_2. The enzyme thyroperoxidase (TPO) is stimulated by thyroid-stimulating hormone (TSH) and brings two T_2 molecules together to form T_4. TSH both stimulates production and release of T_4 into the blood based on feedback levels of free T_3 and T_4 in the blood. Iodothyronine deiodinases are either activating or inactivating the thyroid hormones. Type 1 deiodinase (D1) is providing T_3 to the plasma by removing one iodine atom from T_4. Type 2 deiodinase (D2) is activating T_4 to T_3 and inactivates T_3 to T_2. Type 3 deiodinase (D3) is mainly located in the skin, placenta and nervous system, and produces reverse T_3 from T_4, and T_2 from T_3.

5.2.6.3 Iodine Requirements in Ruminants

As iodine is an essential trace element, the diet must provide sufficient concentrations. Iodine requirements are influenced by an array of animal parameters like lactation stage, growth, reproductive stage, and intake of goitrogens in the diet, in addition to environmental parameters like temperature.

In 2013, the EFSA Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) published a scientific opinion. The FEEDAP recommend a maximum iodine content in a complete feed to be 2 mg iodine/kg DM for dairy cows and minor dairy ruminants (EFSA, 2013). Given the absence of goitrogens, a concentration of 0.5 mg iodine/kg DM should be sufficient in pregnant and lactating sheep (EFSA, 2013). For non-lactating sheep, the requirement may be as low as 0.15 mg/kg DM. The FEEDAP do not present maximum limits for meat-producing ruminants, as the upper safe level could not be determined in this report or the previous one published in 2005 (EFSA, 2013; EFSA, 2005).

5.2.6.4 Iodine Deficiency

Iodine deficiency is a global health challenge, as humans and animals develop iodine deficiency disorders when the diet does not provide sufficient concentrations.

In nature, iodine is present in the lithosphere as iodide and iodate, which is accumulated in plants, soil, and marine organisms (Fuge & Johnson, 2015). Soils in mountainous regions are generally low in iodine due to its hydrophilic properties. Rain, wind, and erosion will quickly bring iodine into surface water, and it ends up in the sea, where it accumulates in marine organisms, particularly macroalgae. In terrestrial plants, iodine concentration is limited by soil concentration. Boulder clays and alluvial soils are examples of soils with high iodine concentration, while soil made of granite tends to have lower concentrations (Underwood & Suttle, 1999). Fundamentally, plants have low iodine levels as iodine is not an essential element for higher plants (Whitehead, 1984). Furthermore, geography in the plant growing site is an important factor. The marine environment is the major source of iodine; thus will, plants growing closer to the sea (50-80 km) have a higher iodine concentration due to marine deposition (Fuge & Johnson, 2015; Zimmermann, 2010).

Besides environmental deficiency, several other causes must be addressed to empemphasis the complexity of iodine deficiency. Goitrogens are substances that interrupt the synthesis of thyroid hormones and cause reduced levels of T_4 (Saleh et al., 1998). Ruminants can ingest goitrogens through feed sources like soybean meal, corn silage, and kale (Hemken et al.,

1971; López-Moreno et al., 2022). Thus, the levels of goitrogens in the diet need to be considered when addressing iodine requirements. In lactating sheep, an intake of 0.5 mg iodine/kg DM should be sufficient, but if goitrogens are present, this should instead be increased to 2 mg iodine/kg DM (EFSA, 2013).

Goitre is a main symptom of iodine deficiency (Wilkinson, 1997; Winkelmann et al., 2016). It appears as an enlargement of the thyroid gland as a result of an ongoing attempt to synthesize sufficient T_4. Goitre may also be induced by elevated levels of goitrogens in the diet. Plants from the cruciferous family (*e.g.*, rape and kale), raw soybean, and cyanogenic stains of white clover can contain goitrogen substances (Borucki Castro et al., 2011). Goitrogens interfere with the synthesis and uptake of thyroid hormones in several ways; (1) inhibits iodine uptake by the thyroid and mammary gland by interfering with NIS, (2) influence the oxidation rate of iodide to elemental iodine and thyroglobulin or (3) release of thyroid hormones (Flachowsky et al., 2014).

Perchlorate (ClO_4^-) is an inorganic anion that is stable in water, but its salts are highly soluble, thus, spreading rapidly as an environmental contaminant (Attanasio et al., 2011; Joint et al., 2011). The literature tends to refer to perchlorate and perchlorate salts as the same, due to the high solubility of the salts; the related health risks are equivalent (National Research Council, 2005). The sources of perchlorate in nature are both natural and anthropogenic due to its wide use in industry, for example in explosives, fireworks, and missile propellants (Trumbo, 2010; Zhang et al., 2022). Perchlorate has been detected in drinking water, seawater, soil, terrestrial plants, macroalgae, milk and food worldwide (Capuco et al., 2005; Jackson et al., 2005; Kirk et al., 2005). Martinelango et al. (2006) found perchlorate levels in seawater ranging from <0.07 - $0.34\ \mu g\ l^{-1}$. Furthermore, they investigated perchlorate levels in macroalgae and found that they can accumulate perchlorate 200-5000 times seawater levels. Perchlorate exposure is known to inhibit iodide transport in the thyroid gland by competitive inhibition of NIS, and act as a goitrogenic substance (Martinelango et al., 2006; Trumbo, 2010). This can potentially cause a decrease in thyroid hormone levels, thus perchlorate exposure should be considered when iodine requirements are determined (National Research Council, 2005).

Heat stress is known to influence iodine levels in ruminants. When exposed to high temperatures, the body tends to reduce thyroid hormone levels in the blood as a metabolic thermoregulation mechanism to lower basal heat production (Habibu et al., 2016; Macías-Cruz et al., 2018; Pratt & Wettemann, 1986). Sheep are handling heat stress better than cattle

due to their high sweat ratio, ability to conserve water and lower basal heat production (Henry et al., 2018). Although, lowered thyroid hormone levels in sheep exposed to heat stress have been observed (Koluman & Daskiran, 2011).

5.2.6.5 Iodine Intoxication in Ruminants

Although most literature focuses on iodine deficiency, the long-term consumption of a diet with high iodine concentration raises the need to address iodine's potential toxic properties. McCauley et al., (1973) conducted an experiment where lambs were given 94-784 mg of iodine over three weeks, and expressed clinical symptoms like coughing, hyperthermia, anorexia, and depression, while the highest doses caused death due to bronchopneumonia . The European Food Safety Authority (EFSA) concluded their report from 2005 that updated information on the upper safe levels of iodine in farm animals is required (EFSA, 2005). In EFSAs following report on the safety and efficacy of iodine compounds from 2013, they suggest the maximum iodine contents in a complete feed to be 2 mg/kg feed for dairy cows and minor dairy ruminants. However, EFSA point out that iodine intoxication is rare (EFSA, 2013). Macroalgae have a high iodine concentration due to their capacity to accumulate minerals from seawater; despite this, they have a history of long-term use as supplement feed in sheep (Makkar, 2018). When searching for novel feed ingredients suited for sheep, macroalgae are a local and potentially sustainable feed source that should be investigated further.

5.3 Macroalgae

Macroalgae are divided into three groups based on their pigmentation; brown macroalgae (*Phaeophyceae*), red macroalgae (*Rhodophyceae*), and green macroalgae (*Chlorophyceae*) (Charoensiddhi et al., 2017). Macroalgae are autotrophic organisms, meaning they can utilise dissolved carbon dioxide and simple nitrogen- and phosphorus compounds to synthesise nutrients and energy if exposed to sunlight (Stévant et al., 2017). Macroalgae accumulate minerals, heavy metals, and other environmental toxicants from seawater. There are about 10 000 unique species with vast variations in terms of chemical composition and content of bioactive compounds (*e.g.*, halogenated compounds, polyphenols, complex polysaccharides, and pigments) (Pandey et al., 2021). Macroalgae are cultivated and harvested for several purposes: food, feed, and the cosmetic and pharmaceutical industry (Makkar et al., 2016).

Macroalgae have a long history of use as supplement feed in ruminant nutrition, as mentioned in the Icelandic sagas and Ancient Greece (Newton, 1951). The use was mainly related to

periods lacking other feed sources, during winter or war. In the coastal lines of Iceland and Norway, sheep and cattle have been observed grazing macroalgae voluntarily, but it has also been conserved as silage or dried and stored as winter supplement feed (Bay-Larsen et al., 2018). World War 1 led to feed scarcity in many countries involved, and ruminants had macroalgae included in their diet for months. They seemed to maintain similar production and welfare compared to other animals on a conventional diet (Makkar, 2018).

Nutrient content, digestibility, and bioactive compounds vary between and within species and depend on extrinsic factors like growing season, water salinity, and post-harvest processing. Non-protein nitrogen levels are relatively high and result in overestimating protein content when using the conventional nitrogen-to-protein conversion factor of 6.25 (Biancarosa et al., 2017; Jones, 1931). More correct conversion factors suggested are 5.38 (brown macroalgae), 4.92 (red macroalgae), and 5.13 (green macroalgae) (Makkar et al., 2016; Øverland et al., 2019). Energy levels are generally low due to low lipid content, a high proportion of complex carbohydrates, and high ash content. Macroalgae have lower gross energy content than hay silage and concentrate, but some species have shown higher gross energy content than other forages such as lichen and winter rye (Makkar, 2018).

Despite low energy levels, macroalgae have properties that make them attractive as an alternative feed ingredient. They do not compete with terrestrial feed sources in terms of either land or fertilisation and have generally high growth rates, which are all valuable traits when looking for novel feed sources (Øverland et al., 2019).

5.3.1 Brown Macroalgae

Brown macroalgae grow globally and thrive in deep and cold waters. The highest regional species richness for kelp species is in the north-east Pacific (Fragkopoulou et al., 2022). The brown macroalgae species *L. hyperborea* and *S. latissima* are the most abundant in Norwegian kelp forests and cover more than 8000 km^2 (Evankow et al., 2019). Norway already has an established macroalgae industry, where brown species like *A. nodosum* and *L. hyperborea* are harvested to produce alginate, fertiliser components, and animal feed (Skjermo et al., 2014; Tayyab et al., 2016). They contain several bioactive components, while one specific polyphenol: phlorotannin, is found only in the brown type of macroalgae but is commonly found in terrestrial plants (Abbott et al., 2020; Pandey et al., 2021).

The function of phlorotannins is to protect against UV radiation and act as an herbivore deterrent (Abbott et al., 2020). They form complexes with proteins and fibre by non-covalent

bindings and making dietary CP and fibre less available for rumen fermentation (Vissers et al., 2018). Phlorotannins also tend to bind microbial digestive enzymes and inhibit the microbes ability to attach to fibre, thus decreasing both protein and fibre degradation. Pandey et al. (2022) investigated rumen fermentation of Nordic macroalgae *in vitro*, and the results indicate that polyphenol-rich brown macroalgae reduce feed degradation. These properties suggest that brown macroalgae inclusion rate should not be high in ruminant diets (Pandey et al., 2022). On the other hand, the protein binding properties have been shown to increase by-pass proteins in ruminants, thus increasing the proportion of proteins available for absorption in the small intestine (Mueller-Harvey, 2006). Although these protein-tannin complexes are not fully understood, as has been observed that some tannins reduce protein absorption in the small intestine in ruminants (Komolong et al., 2001).

Macroalgae chemical composition is highly variable among and within species, depending on external factors like season, location and water nutrient content (Makkar et al., 2016). An illustration of digestibility variation within brown macroalgae species was found by Greenwood et.al. (1983) who conducted an *in vitro* experiment with rumen fluid from seaweed-fed sheep. The results indicate high digestibility of brown macroalgae *L. digitata* (94%), *S. latissima* (97%), and *A. esculenta* (81%), while low digestibility for *A. nodosum* (33%), *Fucus serratus* (15%) and *F. vesicolosus* (26%). *In vivo* data on the rumen digestibility of brown macroalgae are limited. Tayyab et.al (2016) conducted an experiment on *Laminaria* and *Alaria* family, and the results indicated these species can supply high rumen degradable protein, but have low escape protein for the animal. Brown macroalgae have the lowest crude protein (CP) and lipid content among macroalgae types: 40-180 g/kg DM and 0.05-1.0 g/kg DM, respectively (Kolb et al., 2004)

Macroalgae tend to have high mineral levels due to accumulation from seawater. Brown macroalgae, especially the *Laminaria* sp., are currently known as the living system with the strongest iodine accumulation (Küpper & Carrano, 2019). The concentration range between 2000-6000 mg/kg in *Laminaria* and *Saccharina* spp., although drying, boiling and frying processes may reduce iodine content (Duinker et al., 2020; Leblanc et al., 2006; Martinelango et al., 2006). Like minerals, brown macroalgae tend to accumulate perchlorate from seawater. As shown in Martinelango et.al., (2006), various species may accumulate perchlorate at about 200 – 5000 fold seawater levels, but washing processes remove perchlorate even more effectively than iodine.

5.3.2 Red Macroalgae

Red macroalgae are characterized by their bright pink colour and occur in several habitats, but most species thrive in temperate oceans from low tide marks to 100 meters depth. They are cultivated on a large scale for food in China, Japan, and South Korea and are commonly used in sushi.

Red macroalgae tend to have the highest CP content among macroalgae, but variations between species occur. *Palmaria* sp., *Pyropia* sp., and *Porphyra* sp. are reported to contain up to 200-500 mg CP/kg DM (Pandey et al., 2021). Lind et.al. (2020) did a feeding trial in sheep fed either clover silage, soybean meal or *Porphyra* sp. As a protein supplement. No differences in VFA production were detected *in vitro*, while the *in vivo* trial showed no difference in average daily body weight gain. They conclude that *Porphyra* sp. And soybean meal has similar protein values (Lind et al., 2020). Similar to the brown group, red macroalgae also have low lipid levels (< 5 g/kg DM) (Kolb et al., 2004).

5.3.3 Green macroalgae

Green macroalgae are recognizable by their distinctive green colour provided by chlorophyll. They are dependent on the abundance of sunlight and therefore thrive in tide pools and shallow waters worldwide. Green macroalgae growth is boosted by high nutrient concentration and water temperature, which may cause eutrophication and secondary ecosystem challenges and are considered a major environmental challenge in several countries (Green-Gavrielidis et al., 2018).

Green macroalgae are edible and the most abundant *Ulva* and *Acrosiphonia* spp. have a CP content of 25% and 31% on a DM basis, respectively (Biancarosa et al., 2017; Peña-Rodríguez et al., 2011). However, studies show that CP in *Ulva* sp. has low rumen digestibility, and thus is better suited as a source of by-pass protein (Tayyab et al., 2016).

Like red and brown, green macroalgae have low fat content. Levels of phlorotannins are low when compared to brown, but are more similar to red. Although, the levels will vary depending on factors like macroalgae species and harvesting time. Green macroalgae have less potential for binding metals, thus tend to have lower levels than red and brown macroalgae (Besada et al., 2009)

Despite the qualities of red and green macroalgae, this thesis will focus on brown macroalgae due to their high iodine content, accessibility, and cultivation potential in Norway. *Laminaria*

spp. has shown particularly high iodine content, while also being one of the most abundant species.

5.3.4 Laminaria hyperborea

Laminaria hyperborea thrives in depths of 8-30 meters in cold waters and can be sighted on rocky shores at low tides. They are characterized by their flat, long laminated blades. The structural carbohydrates in *L. hyperborea* are alginates and cellulose. Alginates are reported to account for 40% of DM, while cellulose content is reported up to 8% (Schiener et al., 2015). Storage carbohydrates are mainly mannitol and laminarin. Alginates are a polysaccharide with low digestibility, thus *L. hyperborea* has a low energy content compared to terrestrial plants that store carbohydrates as starch (Duinker et al., 2016).

In 2020, the Norwegian Sea Institute published a report on knowledge updates on macroalgae food and feed security (Duinker et al., 2020). The *Laminaria* sp. is known to have some of the highest iodine concentrations among macroalgae, as shown in Duinker et.al. (2020) with *L. hyperborea* (*n*=4) scoring second highest in iodine concentration (mean=4200 mg/kg DM). Lead and mercury concentrations are generally low. Furthermore, *L. hyperborea* showed a low concentration of inorganic arsenic (*mean*=0.036 mg/kg DM) compared to its relative *L. digitata* (*n*=40, *mean*=24 mg/kg DM).

Mære et.al. (2014) analysed common Norwegian seaweeds to investigate their potential as food and feed. *Laminaria hyperborea* (*n*=2) had the highest iodine concentration (mean=3500 mg/kg DM). Arsenic levels were higher in these samples (*mean*=55 mg/kg DM), while mercury levels were low, and lead was not analysed. Despite *L. hyperborea* having a high iodine content, it remains an alternative as a novel feed source available in Norway. Abundance and availability provide a cultivation potential, while its nutritional value includes high rumen degradable protein, minerals, and bioactive compounds which may benefit the ruminant. Although, it is necessary to investigate how the high iodine content may affect animals in the long term.

Figure 7. Stortare (Laminaria hyperborea). Reprinted with permission from DuPont Nutrition Norge AS.

6 Materials and Methods

6.1 Background

Searching for novel feed ingredients is important to support the increased future food and feed demand. Sheep are utilizing grass and providing high-quality protein for human feed. Although, the livestock industry is reliant on supplement feed to maintain a high growth rate. Feed ingredients (*e.g.,* soy and wheat) are often directly competing with human terrestrial food sources, thus we must look to the ocean to find alternative feed ingredients. *L. hyperborea* may be suitable as a Norwegian source of alternative feed ingredients in ruminants, but there is a knowledge gap related to how the high iodine content is affecting animals long-term. Based on this knowledge gap, an experiment was conducted to investigate growth performance, feed intake and iodine accumulation in sheep fed a conventional diet including *L. hyperborea* by-product in the concentrate. The results will provide information on *L. hyperborea* as a future alternative feed ingredient.

6.2 Ethics

The *in vivo* trial was conducted in accordance with the regulation for the use of animals in experiments, adopted by the Norwegian Ministry of Agriculture and Food, and approved by the Ethics Commission on Animal Use by the Norwegian Food and Safety Authority, application number FOTS ID 19314. It complies with the EU Directive 2010/63/EU on the use of experimental animals, which was incorporated into the European Economic Area Agreement in May 2015.

6.3 In vivo Feeding Trial

The experimental study was a feeding trial with randomized block design ongoing at NIBIO (Norwegian Institute of Bioeconomy Research) in Tjøtta, Nordland County, Norway (65°49′22 N 12°25′37 E). Male sheep (*n*=12) of the breed NKS (Norwegian white sheep) born in spring 2021 were selected from a local herd based on average body weight (37.8 ± 4.1 kg body weight). Animals were housed in a traditional, uninsulated sheep house with slatted floors and individual pens (3 m^2 size).

Sheep were placed in 6 blocks with 2 animals per block, whereas 1 control and 1 macroalgae per block. Animals with similar initial weights were placed in the same block, while the diet was randomly assigned within the block. The two lightest sheep were placed in block 1, with weights increasing with a block number, and the heaviest sheep were in block 6.

The *in vivo* feeding trial was ongoing from September to November 2021 and split into two periods (P1 01/09 - 17/10 and P2 18/10 - 29/11). Animals were fed daily, twice a day (08:30 h and 16:00 h) and had *ad libitum* access to water. Animal weights were registered every two weeks during the experiment.

Six animals from their respective 3 blocks (1-3) were moved to individual metabolism crates for 72 hours. The same feeding and water regime was maintained while the daily collection of urine, faeces, and feed refusal was done at 08:00 h. Animals in block 1-3 were returned to the sheep house, while animals in block 4-6 went through the exact procedure in metabolism crates. The same routines continued in the sheep house for five weeks, before they entered metabolism crates for a second time in P2, with exact procedures as described above.

A control diet based on a conventional feeding regime including roughage *ad libitum* and concentrate was compared with a macroalgae diet containing roughage *ad libitum* and 6% *L. hyperborea* in the concentrate. During P1 the daily ration consisted of 4-6 kg silage and 400 g concentrate/animal, while in P2 the daily amount of concentrate was increased to 600 g/animal consistent with animals in growth. Daily feed intake was registered based on daily supply and refusal. All animals were fed their respective diet 26 days prior to the experiment start, providing the rumen environment time to adapt.

6.4 Collection and Storage of Samples

Roughage was produced from 1st cut mixed pasture with 25% inclusion of white and red clover. During the experiment, roughage samples were collected once a week. One handful was taken out, weighed and oven dried for 48 h at 60°C to measure DM before storing at -18°C until further processing.

Individual faeces samples (2 x per animal) were collected daily from metabolism crates in the morning (08:00 h). Total amounts were weighed and registered, and then 10% were taken out and stored at -18 °C until further processing. Excessive faeces were discarded.

Individual urine samples (3 x 50 ml per animal) were collected daily from buckets under metabolism crates (08:00 h). The total amount of urine was measured, and a pH indicator strip non-bleeding pH 0 - 6.0 (Teststrips, pH, MColorpHast™, product number: 1.09531.0001, VWR International, Merck KGaA, Darmstadt, Germany) was used to measure the acidity of every urine sample. Sulphuric acid (100 ml) was added to each empty collection bucket to

ensure the binding of N and avoid loss of NH_3. Samples were stored at -18 °C shortly after collection. Excessive urine was discarded.

Individual blood samples were collected on day 2 of P1 and P2. The procedure was done by a veterinary 4 h after morning feeding. Blood samples were taken from the jugular vein to Vacuette® EDTA tubes (product number: 454021, Greiner Bio-One, GmbH, Kremsmünster, Austria). Plasma was separated by centrifugation at 4,000 rpm for 15 min at 4 °C, after which aliquots of two samples per animal were removed and pooled into a glass vial to give one sample per animal per period. Plasma was stored at -80 °C until further chemical analyses.

6.5 Preparation of Macroalgae and Concentrate

A sample of two tons of *Laminaria hyperborea* by-products from industrial alginate production was provided by IFF/Dupont Nutrition Norge AS, Haugesund, Norway. The by-products originally contained ca. 3% DM and were first pressed (twin screw press, Fjell Technology, Straume, Norway) to ca. 8% DM, before drying in a disc dryer (Fjell Technology, Straume, Norway). The drying temperatures were 80 - 90 °C for 4 h, followed by 11 h at 60 - 65 °C with vacuum, and finally 2 h at 80 °C, to final DM 93 %. The dried biomass was ground in a spice grinder (Multi Grinder, Model RCMZ-1000, Royal Catering, Berlin, Germany), and transported to a commercial factory of Felleskjøpet Agri (Rindsem Mølle, Verdal, Norway). At the factory, two concentrate feeds were produced, a control without macroalgae and an experimental feed including macroalgae (Table 1). Ingredients were ground in a hammer mill (Tietjen Verfahrenstechnik GmbH, Hemdingen, The Netherlands) with dimensions 5 and 6 mm and mixed in a Forberg mixer at 77 °C (Forberg, Oslo, Norway). To produce the experimental concentrate, the macroalgae mass was weighed and mixed with the same ingredients as used in the control feed (Table 1). After mixing, the concentrates were expanded in a Kahl expander (Amandus Kahl GmbH&Co.KG, Reinbek, Germany) at 110 - 115 °C before pelleting (Van Aarsen, Heel, Netherland) and cooling. The pellet sizes were on average 4.8 mm in diameter. Both concentrates were added iodine as part of the mineral premix as a standard.

6.6 Chemical Analyses of Macroalgae, Feed, and Animal Samples

6.6.1 Dried Laminaria hyperborea By-products

Carbon and nitrogen in the dried, ground biomass was determined using a Vario EL cube Elemental Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Total amino acids were quantified as described by Forbord et al. (2020), and total lipids by the

Bligh and Dyer method, using the same protocol as Jakobsen et al. (2008). Minerals, including iodine, were determined using ICP-MS as described in section 6.6.4.

6.6.2 Roughage and Concentrate

Feed samples were ground to pass a 1-mm screen using a Tecator Cyclotec Sample Mill (Foss Analytical Co., Ltd., Suzhou, China). Dry matter, ash, CP, and NDF content of the roughage were analysed using NIRs by Eurofins Norway performed at Eurofins Agro Testing Wageningen (Binnenhaven 5, NL-6709 PD, Wageningen ISO/IEC 17025:2005 RvA L122). The same components of the concentrates were determined by Felleskjøpet Agri using the same NIRS method. Iodine was quantified as described in section 5.6.4.

6.6.3 Preparation of Animal Samples

Frozen faecal samples were course ground and freeze-dried for 48 h, using a Labconco FreeZone 4.5 Plus® (Kansas City, Missouri, USA) freeze-drier at a temperature and vacuum ranging between −80°C and −86°C, and 0.52 and 0.97 mbar, respectively. The freeze-dried samples were weighed immediately, and again after 24 h storage at ambient temperature. The samples were then ground to pass a 1-mm screen using a Tecator Cyclotec Sample Mill® (Foss Analytical Co., Ltd., Suzhou, China). The final weight of the samples was measured, and subsamples were taken and stored in plastic zipped bags at -18°C until further analyses. Urine and blood plasma were analysed without any further processing.

6.6.4 Minerals in Macroalgae, Feed, and Animal Samples

The analysis of minerals in macroalgae, feed and animal samples were performed at SINTEF Industry, Norway. Halogens (*i.e.*, brom and iodine) were extracted with 5 ml 20% (v/v) TMAH at 80°C in a bead bath overnight and diluted to 1% (v/v) upon analysis, according to Norwegian standard NS-EN 15111:2007. For analyses of additional elements in the seaweed by-products (K, Na, Mg, Ca, P, S, Fe, Cu, Se, As, Cd, Pb), samples were extracted with HNO_3 in an UltraWAVE microwave oven (Milestone S.r.l., Sorisole, Italy) at 250 °C for 10 min in accordance with NS-EN 15763_2009.

The prepared samples were analysed on an 8800 Triple Quadropole ICP-MS (ICPQQQ) with SPS 4 autosampler (Agilent Technologies, Santa Clara, USA). Samples were quantified by use of standards from Inorganic Ventures (JRC Plankton BCR-414), with Te as an internal standard for the halogens and 115In for the non-halogens. All extractions were performed once, with analysis using two analytical replicates.

6.7 Statistical Analyses and Calculations

Data were analysed using Rstudio, version 4.2.2 (2022-10-21 ucrt) “Innocent and Trusting”. R script are available in appendix.

Significance levels were set to 0.05. Analyses used were Wilcoxon-Mann-Whitney test and the Kruskal Wallis test. Diet and Period are independent variables, while feed intake parameters, body weight gain and iodine parameters are dependent variables.

Feed intake was calculated based on the respective animals daily feed intake, refusal, and feed analyses of roughage and concentrate. Weight gains were calculated based on body weights registered on day one, and then every two weeks during the experiment.

6.8 Model Assumptions

To perform parametric statistics tests like t-test and ANOVA, the assumption of normality must be met. When the data does not follow a normal distribution, one may use non-parametric tests (Kim & Park, 2019). In the present study, the Shapiro-Wilks test did not approve normality and therefore, non-parametric tests were used for data analyses.

6.8.1 The Wilcoxon-Mann-Whitney test

The Wilcoxon-Mann-Whitney test is the nonparametric counterpart to the t-test. The test compares the mean values for two groups and tests whether they differ significantly from each other (Bonnini, 2014). The assumptions are (1) repeated measurements, (2) natural couples and (3) independence. The data meets all the assumptions.

6.8.2 The Kruskal-Wallis test

The Kruskal-Wallis’ test is, like the Wilcoxon-Mann-Whitney, a non-parametric test. It is considered the counterpart to ANOVA, and may be applied if normality cannot be verifiable (Bonnini, 2014). Assumptions are like those for Wilcoxon-Mann-Whitney, and the data meets all the assumptions, as described in 6.8.1.

7 Results

7.1 Diet Ingredients

Table 1 presents ingredient composition in the two types of concentrate feeds. The chemical composition of *L. hyperborea* by-product that was used in the concentrate is presented in table 2. In table 3, the average chemical composition of roughage from the two periods is presented.

Table 1 Ingredient composition (g/100 g) in concentrate feeds

Ingredient	Control concentrate	Macroalgae concentrate
Soybean meal	13.7	11.2
Barley	20.6	19.9
Oat	39.9	39.9
Wheat	10.7	10.4
Molasses	5.0	5.0
Lucerne	5.0	3.0
Salt	1.7	1.4
Vitamin and mineral mixture	3.4	3.2
Laminaria hyperborea	-	6.0

Table 2 Chemical composition of Laminaria hyperborea by-product. Ash, carbon, nitrogen, total amino acids, and total lipids are presented as % of dry matter (DM) while the content on iodine and bromine is presented in mg/kg dry matter.

Content	% of DM
Ash	11.8
Carbon	39.3
Nitrogen	3.74
Total Amino Acid	15.4
Total Lipids	4.4
Iodine, mg/g DM	8.6
Bromine, mg/g DM	2.5

Table 3 Average chemical composition (g/kg DM) of diet ingredients. Where no other units are listed in the table, the content is g/kg dry matter.

	Roughage P1*	Roughage P2*	Control concentrate	Macroalgae concentrate
Dry matter, g/kg	349	473	881	881
Energy, MJ/kg	5.7	5.6	9.0	9.1
Crude protein	114.0	113.5	135.7	138.3
Fat	27.5	24.5	35.2	34.4
Ash	63.5	53.5	75.8	74.0
NDF	497	507	165	163
Sugar	60	124	43.2	40.5
Calcium	6.7	5.8	0.97	0.97
Phosphor	2.6	2.0	0.35	0.35
Selenium, mg/kg DM	0.01	0.01	0.35	0.35
Iodine, mg/kg DM	0.45	0.40	4.1	476

P1 Period 1 01/09/2021 to 17/10/2021*
P2 Period 2 18/10/2021 to 29/11/2021*

7.2 Animal Performance

Feed intake increased in all animals from P1 to P2, with significant differences in all nutrient parameters. There was no observed effect of diet on feed intake in any groups ($P > 0.05$). Total DM intake increased significantly with period ($P < 0.05$) consistent with growth rate and increased concentrate intake (Figure 4).

Table 4. Average animal body weight (BW) and average daily nutrient intake for control and macroalgae groups in periods 1 and period 2, ± standard deviation.

Nutrient intake and BW	Control P1*	Control P2*	Macroalgae P1*	Macroalgae P2*	P value diet	P value period
BW, kg	37±4.3	61.3±4.7	38.5±4.2	59±4.9	0.52	<0.05
DMI roughage, g	1006±190	1383 ±70	988±183	1280±183	0.35	<0.05
DMI concentrate, g	348±0	564±0	352±0	571±0	0.91	<0.05
DMI total, g	*1354±191*	*1947±70*	*1340±183*	*1851±163*	*0.40*	*<0.05*
CP, g/kg DM	167±29	230±19	165±24	211±49	0.44	<0.05
NDF, g/kg DM	556±95	817±94	526±95	768±96	0.84	<0.05
Ash, g/kg DM	90±12	118±8	89±11	113±9	0.63	<0.05

P1 Period 1 01/09/2021 to 17/10/2021*

P2 Period 2 18/10/2021 to 29/11/2021*

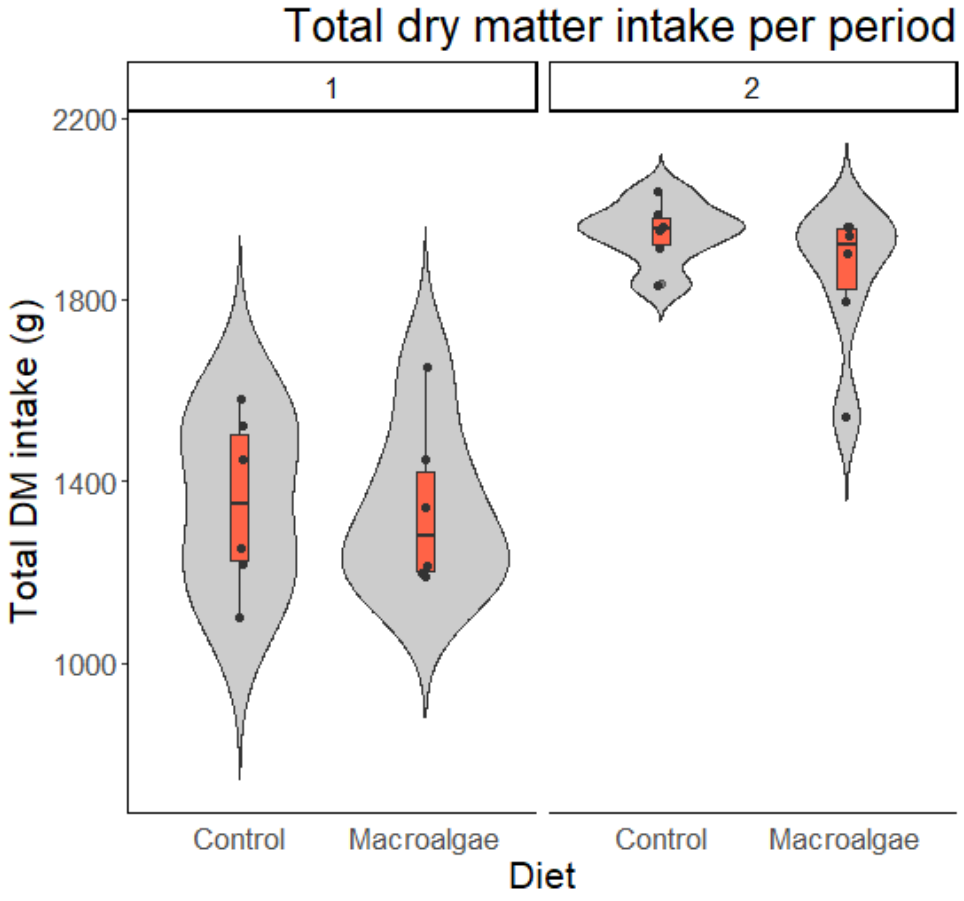

Figure 8. Total daily dry matter intake (g) per period and diet

Sheep were in a growing stage during the experiment. Average animal live weight increased from 48.2 kg (control) and 45.6 kg (macroalgae) in period 1 to 57.6 kg (control) and 61.3 kg (macroalgae) in period 2 kg (Table 4). The total body weight gain did not differ between sheep assigned with the control diet or the macroalgae diet ($W = 9$, $P = 0.17$).

7.3 Iodine

Total iodine intake increased significantly from P1 to P2 consistent with increased feed intake and increased concentrate intake ($P = < 0.05$) (Table 5). Statistical analysis indicates a significant difference in iodine excretion in faeces between diet and period ($P = < 0.05$) (Table 5, Figure 8). The proportion of iodine excreted in faeces was 67% in average for P1 and P2 in animals fed the macroalgae diet. While the animals fed the control diet had a proportion of 37% of iodine excretion in faeces. Iodine excretion in urine differed between diet and period (Table 5, Figure 9).

Table 5 Average daily iodine intake and excretion (mg/day) for male sheep fed control and macroalgae diets in period 1 and 2 (± standard deviation).

	Control P1*	Control P2*	Macroalgae P1*	Macroalgae P2*	P value diet	P value period
Iodine intake roughage	1.1±0.20	1.2±0.05	1.0±0.19	1.1±0.14	0.18	0.29
Iodine intake concentrate	1.4	2.3	167.7	271.9	<0.05	<0.05
Total iodine intake	*2.5±0.2*	*3.5±0.06*	*168.7±0.2*	*273±0.1*	*<0.05*	*<0.05*
Iodine excretion faeces	5.9±7.9	1.6±1.2	92.5±16.7	165.4±28.4	<0.05	<0.05
Iodine excretion urine	4.9±2.4	2.9±0.8	43.3±9.2	87.3±18.6	<0.05	<0.05
Total iodine excretion	*10.8±9.7*	*4.5±1.8*	*135.8±19.3*	*252.6±25.2*	*<0.05*	*0.56*
Intake – excretion	-8.3	-1.0	32.9	20.4	<0.05	0.86

P1 Period 1 01/09/2021 to 17/10/2021*

P2 Period 2 18/10/2021 to 29/11/2021*

Iodine retention (*i.e.*, iodine intake – excretion) was significantly different between groups (P = <0.05). Animals fed the macroalgae diet had an iodine excretion at 83% of the total iodine intake in P1, and 91% in P2, with an average of 87%, while the excretion was closely to balance with intake for the control group.

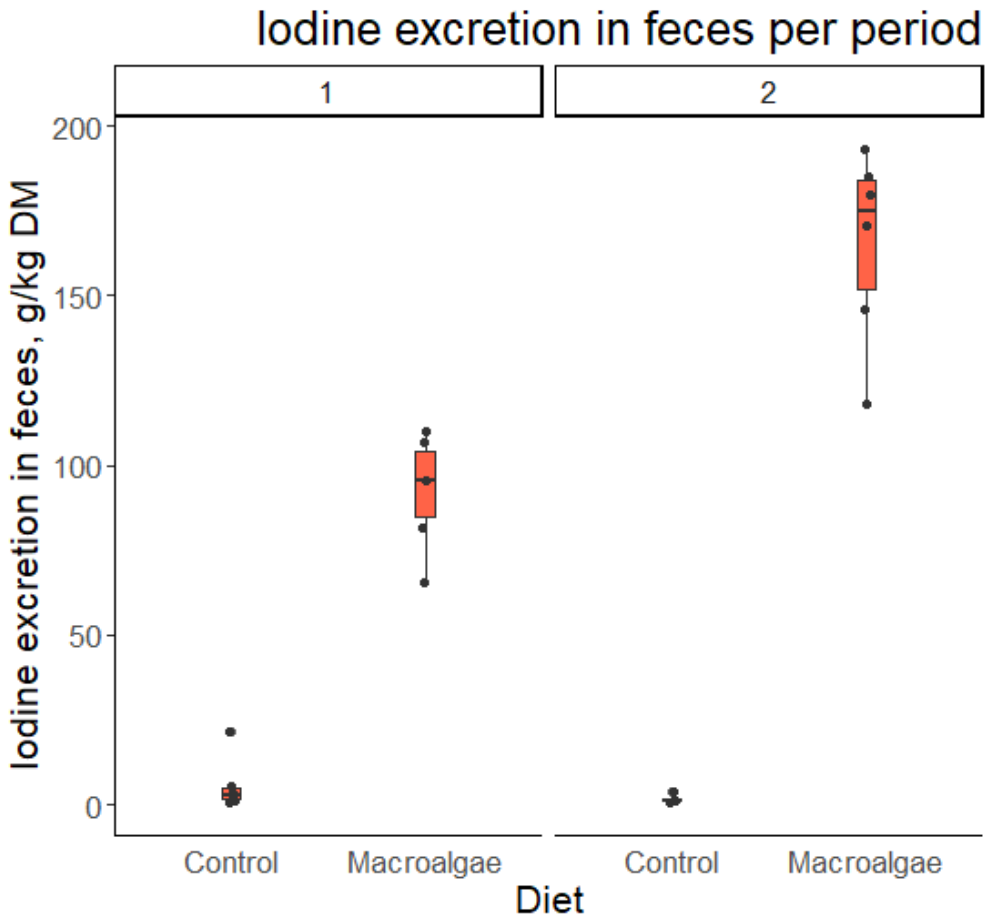

Figure 9. Iodine excretion in faeces per period, g/kg DM.

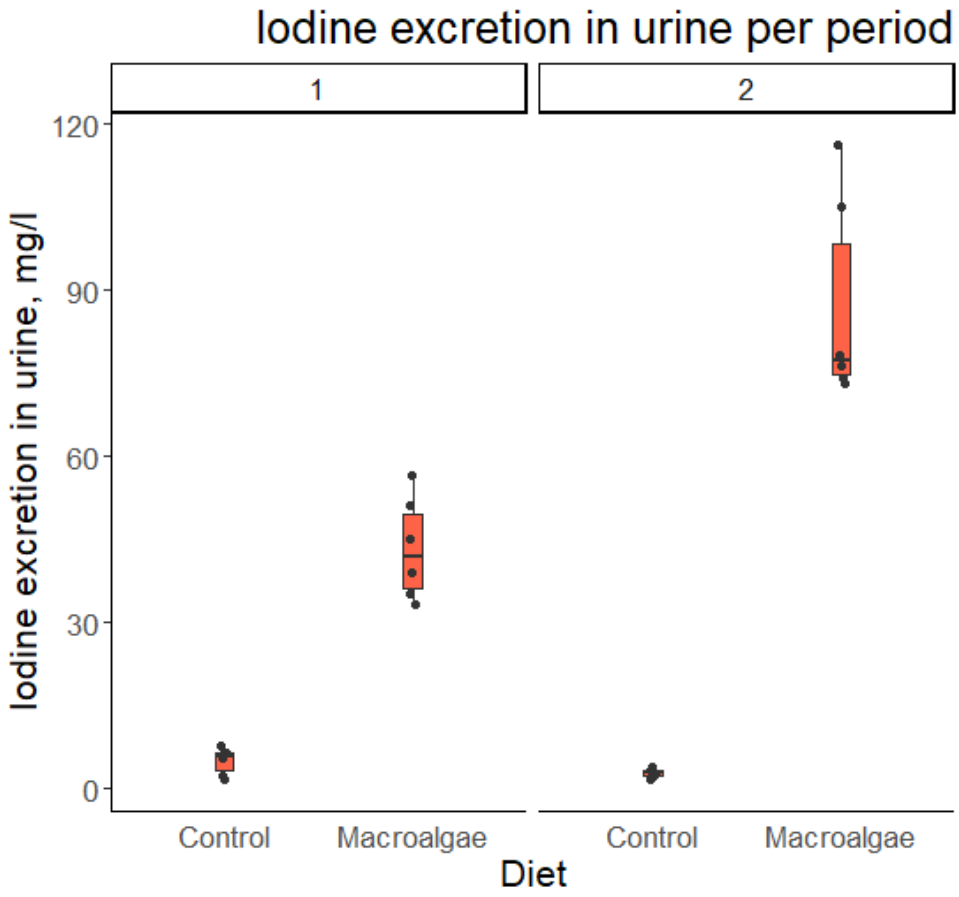

Figure 10. Iodine excretion in urine per period, mg/l

Iodine analysis of blood samples indicate a significant difference between diet and period (*P* = < 0.05) (Figure 7). The average values in P1 were 3.44 (macroalgae) and 0.88 (control) mg iodine per ml blood, while in P2 5.53 (macroalgae) and 0.20 (control). The iodine content in blood in macroalgae group increased by 85% from P1 to P2.

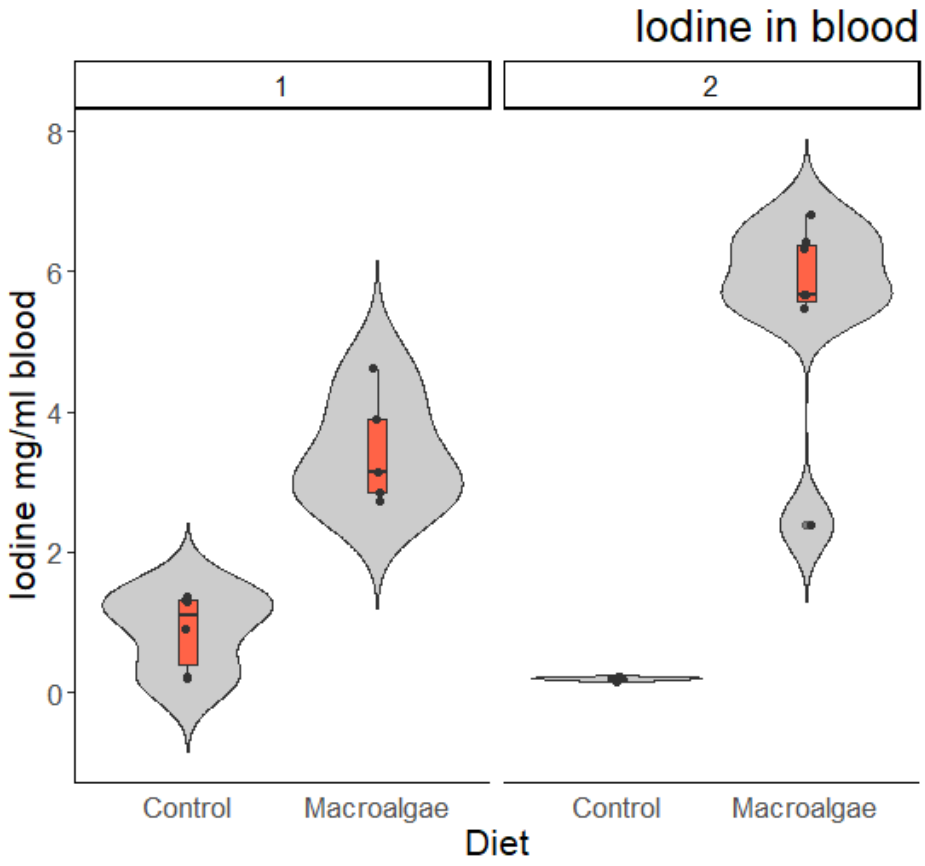

Figure 11. Iodine concentration in blood (mg/ml) in sheep fed macroalgae and control diet, period 1 and period 2.

8 Discussion

Laminaria hyperborea have been identified as a potential alternative feed ingredient in ruminants. However, their high iodine concentration may negatively impact animal performance and welfare. To the best of my knowledge, there have been no studies on the effect of supplementing *L. hyperborea* by-products in sheep. Therefore, the results are mostly discussed in context with previous feeding trials in ruminants where the diet is supplemented with macroalgae. These experiments differ from the present in terms of objectives, macroalgae species, animal species and iodine intake. However, they bring interesting perspectives to the case.

Overall objectives of the study were to assess the animals feed intake, body weight gain and iodine concentration in blood, faeces, and urine.

Major findings of this study include:

1) No significant difference in animal performance and welfare between control and macroalgae diet.
2) Significant effect of macroalgae diet on iodine concentration in blood, faeces and urine.

8.1 Laminaria hyperborea Supplementation did not affect Animal Performance

First and foremost, it is necessary to ensure supplementing macroalgae in the animal diet does not negatively affect animal performance. Thus, it is important to address animal performance and welfare parameters like feed intake and body weight gain.

8.1.1 Feed Intake

Feed intake increased in all animals in both groups from P1 to P2, consistent with growth rate and increased concentrate intake. These results indicate satisfactory palatability of the macroalgae-supplemented concentrate, which is important to maintain sufficient animal performance. Furthermore, feed intake is considered an animal welfare parameter; a normal feed intake is part of being a healthy animal (Stamp Dawkins, 2021). The sheep had no hesitation in eating the concentrate, they seemed to enjoy the taste. This also provides security and strength concerning the experimental design; we are sure the sheep ingested all macroalgae.

Costa et al. (2021) did a review on the use of macroalgae in livestock production and have summarised main findings on the effect of dietary supplementation on feed intake. Bach et al. (2008) conducted an experiment on lambs (*n*=40) fed sun-dried *A. nodosum* with an inclusion rate of either 1% of feed for 14 or 28 days or 2% of feed for 7 or 14 days. Macroalgae supplementation was provided to the animals as a top-dress on concentrate pellets, and feed intake was measured weekly. The chemical composition of *A. nodosum* was not provided, but the species is known to have a high concentration of iodine, like *L. hyperborea* (Antaya et al., 2019). Their results show no effect of diet on feed intake.

Al-Shorepy et al. (2001) investigated weight gain and carcass characteristics of indigenous lambs (*n*=18) in the United Arab Emirates. The macroalgae inclusion rate was 1% of feed for 35 days, although macroalgae species are not specified. Their results show an increase in DM intake in the macroalgae group. However, since macroalgae species and type are unknown and the sheep breed presumably differ from Norwegian White Sheep; caution must be taken when comparing results to the present study.

Ueland et al. (2022) conducted a feeding trial where Norwegian Red dairy cows (*n*=6) were fed a total mixed ration diet supplemented with 1% (DM) *S. latissima* within a 28 day period. Results show an effect of diet on feed intake, indicating that supplementing *S. latissima* in dairy cows may have a positive impact.

These studies suggest that macroalgae inclusion in ruminant diets does not negatively impact feed intake.

8.1.2 Body Weight Gain

As the effect of dietary supplementation of *L. hyperborea* on body weight gain in ruminants has yet to be considered in the literature, there is no literature available to compare results directly. However, the review by Costa et al. (2021) summarised the main findings on the effect of macroalgae dietary supplementation on body weight. Fike et al. (2005) investigated *A. nodosum* extract as a feed ingredient in lambs for 5 weeks at an inclusion rate of 1-2% of the feed while also inducing heat stress. No effect of *A. nodosum* on body weight gain was observed. Bach et al. (2008) found an effect on weight gain in their study mentioned in section 8.1.1.

Furthermore, Lind et al. (2020) investigated *Porphyra sp.* as a protein source compared to diets including soybean meal or clover silage. Inclusion levels were 59% of feed for white

clover, while 10% of feed for *Porphyra sp*. and soybean meal and the feeding trial lasted for 6 weeks. They found an effect of *Porphyra sp.* on growth rate in lambs; ADG increased compared to the control diet, while the effect was insignificant compared to soybean meal.

The present study aimed at approximately a 2% inclusion rate on total DM intake basis in the total diet. However, variations occurred due to the changes in concentrate level and feed intake. The previous literature agrees with our study, where macroalgae inclusion had no negative impact on body weight gain. It seems like animal body weight gain is not negatively affected by macroalgae inclusion at these inclusion rates and experiment durations. In the present experiment, this is expected, as the diets were aimed to be isonitrogenous and isocaloric, and there was no significant difference in feed intake between diets.

8.2 Laminaria hyperborea Increased Iodine Levels in Blood, Faeces and Urine

The present study shows an effect of diet on iodine concentration in blood, faeces and urine, as the levels in macroalgae and control group are significantly different. These results indicate a high level of iodine in the animal system and need to be seen in the context of the upper limits. As mentioned in section 4.5.3, iodine requirements in non-lactating sheep may be as low as 0.15 mg/kg DM. A safe upper level could not be determined by FEEDAP, although a recommendation of <10 mg/animal/day is set to ensure the safety of the animals. However, in the present study, animals in the macroalgae group had an average daily iodine intake exceeding this recommendation by 17 times in P1 and 27 times in P2. The highest daily iodine dose in the present study was found in the macroalgae group in P2 (5.2 mg iodine/kg BW). In the present study, no animals showed any clinical symptoms of iodine intoxication (*e.g.*, coughing, hyperthermia, anorexia, depression or death), suggesting that the animals can tolerate these iodine levels.

Paulíková et al. (2002) suggested sheep are less sensitive to iodine than cattle. In their review, the authors report clinical symptoms of iodine intoxication in dairy cows when provided 0.12 to 1.0 mg iodine/kg BW daily. On the other hand, lambs had clinical symptoms when provided 13 to 19 mg iodine/kg BW daily. Özkan-Gülzari et al. (2019) compared the protein digestibility of *Porphyra spp.* and *Saccharina latissima* in NKS sheep under Norwegian conditions. For 8 weeks, the daily iodine intake was 18 times higher than recommended levels, but no clinical symptoms of iodine intoxication were observed. These findings indicate that sheep can tolerate an iodine intake that exceeds present upper limit recommendations, although the mechanisms are unknown. It is also important to note the duration of these

studies, as the response to high iodine doses may express differently at longer durations and should be investigated in the future. However, the sheep used in this study show normal growth and health status today (1.5 years after the study) regardless of high iodine exposure earlier.

8.2.1 High Iodine Levels in Blood serum, Urine and Faeces

8.2.1.1 Blood Serum Iodine:

In the present study, blood serum levels of iodine were significantly higher in the macroalgae group than in the control group.

Sorge et al. (2016) investigated serum levels of iodine in dairy cows fed 56 g *A. nodosum* per day for 30 days. Their results indicate elevated iodine levels in blood serum in the macroalgae group, which correlated with iodine concentration in milk and tears. Silva et al. (2022) investigated iodine metabolism in lactating dairy cows supplemented with *A. nodosum* meal for 28 days. They operated with 5 different macroalgae inclusion rates (0, 5, 113 or 170 g *A. nodosum* meal/day). The blood serum concentration of iodine increased linearly with supplementation of *A. nodosum* meal, in agreement with the present study.

8.2.1.2 Faeces and Urine Iodine

Iodine excretion in faeces and urine differed in the control diet and macroalgae diet, with a significantly higher excretion in the macroalgae group. In the macroalgae group, 67% of iodine was excreted in faeces, while 37% in the control group. In the mentioned study by Silva et al. (2022), iodine excretion in faeces increased linearly with the supplementation of *A. nodosum*, and 63% of total iodine intake was excreted in faeces. Miller et al. (1975) investigated iodine metabolism in lactating dairy cows and found that 20% of intravenous radioiodine was excreted in faeces. Their result indicates that ingested iodine is extensively recycled in the digestive system.

When addressing faeces excretion of iodine, it may be appropriate to consider both short-term and long-term perspectives. In the short-term, faeces excretion is unproblematic, if even favourable to the animal. Iodine is prevented from entering the blood plasma and potentially interrupting thyroid hormone balance or causing iodine intoxication. While long term, the high proportion of excretion in faeces may lead to iodine fertilisation of fields. Iodine content in soil may increase, which also interferes with iodine content in feedstuffs. If macroalgae with these iodine levels will be implemented in Norwegian feed regimes in the future, there is

a need to monitor iodine content in soil and feedstuffs and, if necessary, intervene to ensure that iodine content does not exceed upper limits.

8.2.1.3 Iodine Retention

The iodine retention was significantly higher in the control group relative to the macroalgae group. However, the control group had an average daily iodine intake of 1.4 mg (P1) and 2.3 mg (P2), compared to 169 (P1) and 273 (P2) in the macroalgae group. The results for retention indicate that the control group iodine excretion exceeds iodine intake. The excessive iodine could derive from the extra-thyroideal iodine pool. Silva et al. (2022) had similar results with lower intake than excretion. They propose that iodine excretion may have been overestimated due to the method of analysis and recommends caution when interpreting absolute values of faeces and urine iodine excretion. Furthermore, Ueland (2022) found similar trends, with the amount of ingested minerals secreted in milk and faeces being higher in the control than the macroalgae group. On the other hand, the SD for iodine excretion in faeces in the control group is higher than the mean (5.9 ± 7.9). The data is indicating that this is caused by one outlier, which could be caused by random errors. Removing the outlier did not change the statistical results.

8.3 Future Perspectives

Under the circumstances of the present experiment, the inclusion level of *L. hyperborea* does not seem to affect health in sheep. However, this experiment has brought certain elements to attention, which may be improved or further investigated in future experiments.

8.3.1 Thyroid Hormone Analysis

It seems that high iodine intake leads to high iodine concentration in blood serum. This could promote changes in levels of T_3 and T_4, but this was not investigated in the present study. However, several studies have investigated T_3 and T_4 concentrations.

Antaya et al. (2019) investigated serum levels of T_3 and T_4 in lactating Jersey cows supplemented *A. nodosum* meal for 84 days (*i.e.*, 3 periods lasting 28 days). Their results indicate no difference in T_4 concentration in either period or diet, while T_3 concentration was significantly lower for macroalgae group in period 3. A lowered T_3 concentration during the last period may indicate excess iodine intake during an extensive period may influence thyroid function. On the other hand, Antaya et al. (2015) and Sorge et al. (2016) investigated serum levels of TSH, T_3 and T_4 and did not find a difference when supplemented *A. nodosum*

meal. Antaya had an inclusion rate of 57, 113 or 170 g per day for 21 days in early lactation dairy cows, and found a significant increase of iodine in milk, while animal performance was not affected. Sorge et al. had an inclusion rate of 56 g per day for 30 days. It should be noted that maximum daily iodine intake from in the compared studies was 41 mg, 69 mg, 147 mg, and 273 mg in Sorge et al. (2016), Silva et al. (2022) Antaya et al. (2015), and the present study, respectively.

Given that the maximum daily iodine intake is considerably higher in the present study, it is not possible to draw conclusions from these other studies. However, it could be interesting to investigate these parameters when supplementing *L. hyperborea,* as relative thyroid hormone concentration is affecting the animal physiological function, as previously mentioned in chapter 4.5.

8.3.2 Meat Mineral Profile

In the present study, the meat mineral profile was not investigated. However, as the sheep are raised for meat production purposes, this parameter needs to be investigated in the future.

Like ruminants, humans also have a narrow therapeutic window for iodine; the range between minimum requirements and upper limits are between 1:2.5 and 3 (Flachowsky et al., 2014). Although meat is not normally a considerable source of iodine, feeding macroalgae may lead to iodine accumulation in the meat. While this poses a potential risk to the consumer, it may also be an opportunity of obtaining a novel iodine source in human nutrition given that iodine concentration is suitable (Grabež et al., 2022). The risk of iodine deficiency is well-known, and iodine-rich meat may contribute to improving daily iodine intake in human. Therefore, it is interesting to investigate the mineral profile in meat from ruminants fed with macroalgae in the future.

There have been some studies of meat in animals fed with macroalgae. He et al. (2002) investigated iodine content in pigs supplemented *L. digitata* for 3 months with inclusion rates on 1 – 2 % of DM. Findings show increased iodine content in fresh muscle (45%), adipose tissue (213%), heart (124%), liver (207%) and kidneys (127%) compared to control.

Grabež et al. (2022) investigated micronutrient content and flavour-related compunds of raw meat in Norwegian White female lambs fed macroalgae. Lambs were finished during 35 days supplemented with dried *S. latissima* at 5% of DM inclusion rate. Their results indicate a significant increase of iodine content in both raw meat and dry-cured leg in lambs

supplemented macroalgae. Average iodine content was < 1.9 (control) and 56 (macroalgae) µg/100 g raw meat (Grabež et al., 2022). The iodine content in 100 g dry-cured leg represents 60% of recommended daily intake in human (150 µg/day) (EFSA, 2017). However, iodine is known to transfer easier to milk than meat (Borucki Castro et al., 2011; Franke et al., 2009), and macroalgae with high iodine concentration should be used with caution when fed to dairy ruminants in particular.

8.3.3 Perchlorate Analyses

The literature describes perchlorate as a goitrogen that interrupts the uptake of iodine by competitive inhibition of NIS and macroalgae tend to accumulate perchlorate from seawater. In the future, it may be interesting to analyse perchlorate levels in *L. hyperborea*, but also study the mixed effect of perchlorate and iodine in macroalgae and identify mode of action. Based on the literature, perchlorate may inhibit the NIS and cause iodine in macroalgae to become by-pass iodine in ruminants, thus making the macroalgae more suitable as a feed ingredient.

8.3.4 Macroalgae Processing

Despite the high mineral levels, macroalgae remains a potential alternative feed ingredient based on their high growth rate, abundancy, bioactive components and, in some species, protein content (Holdt & Kraan, 2011; Lind et al., 2020; Makkar, 2018). Processing methods to reduce mineral levels have been investigated. Iodine content have been reduced by 85% by rinsing and boiling *S. latissima* for 15 minutes, although the processing did not reduce content of cadmium, mercury or lead (Blikra et al., 2021). Pandey (2023) investigated how hot water blanching affects chemical composition and in vitro digestibility of *A. nodosum* and *F. vesicolous*. Ash content was significantly reduced in both species. The results also indicate reduced digestibility, and hot water blanching should be further investigated in vivo to determine how it affects animal health and welfare in addition to feed utilization.

9 Conclusion

There are no significant differences in feed intake or body weight gain between diets. Animals fed the macroalgae diet had the highest iodine intake and excretion. We conclude that sheep can handle the iodine concentration in a diet supplemented ~2% *L. hyperborea*. For future experiments, meat analyses could explain whether the iodine accumulates in the meat over time or if the animal adapts and increases iodine excretion. In that case, macroalgae fed sheep could become an iodine-enriching food source in human nutrition. Moreover, it is necessary to investigate the ratio of thyroid hormones in the blood to obtain knowledge about thyroid gland health. However, long-term effects of high iodine concentrations in the diet should be investigated.

10 Acknowledgement and Funding

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I want to thank Trond Helgerud at IFF/DuPont Nutrition Norge AS, for providing the *L. hyperborea* by-product for the experiment, *L. hyperborea* photographs for this thesis and for helping out with questions related to the Norwegian algae industry.

11 Appendix

11.1 R script

```
#### Data exploration and library loading ####
library(car)
library(ggplot2)
library(effects)
library(doBy)
library(lattice)
library(ggthemes)
library(forcats)
library(tidyverse)
library(dplyr)
library(vctrs)
library(ggpubr)
d <- Sheep_and_feed_samples

#### Wilcox test BW-Diet ####
# Checking T.test model assumptions
shapiro.test(d$bw_gain)                          #Significant deviation from normality
ggdensity(d$bw_gain)
qqPlot(d$bw_gain)
#Deciding to use Wilcox.test due to deviation from normality
wilcox.test(data = d, bw_gain
        ~ diet, paired = FALSE, exact = FALSE)     #No significant difference, p>0.05

#### DM roughage analysis ####
# Checking model assumptions for ANOVA
shapiro.test(d$dm_roughage)                      #Assumption of normality not met
leveneTest(d$dm_roughage~d$diet)                 #Assumption of equality of variances met

#Deciding to use Kruskal Wallace from here in replacement of ANOVA
kruskal.test(dm_roughage~diet, data=d)           #DM roughage~diet, p>0.05
kruskal.test(dm_roughage~period, data=d)         #DM roughage~period, p<0.05
```

```
summaryBy(dm_roughage~diet+period, data=d,   #Means and SDs for each combination of
the treatment level
        FUN=c(mean,sd,length))

#### DM concentrate analysis ####
kruskal.test(dm_concentrate~diet, data=d)          #DM concentrate~diet, p<0.05
kruskal.test(dm_concentrate~period, data=d)        #DM concentrate~period, p<0.05
summaryBy(dm_concentrate~diet+period, data=d,
FUN =c(mean,sd,length))                            #Means and SDs for each combination
of the treatment level

#### Total DM analysis ####
kruskal.test(total_dm~diet, data=d)                #Total DM~diet, p>0.05
kruskal.test(total_dm~period, data=d)              #Total DM~period, p<0.05
summaryBy(total_dm~diet+period, data=d,
        FUN=c(mean,sd,length))                     #Means and SDs for each combination of
the treatment level

####Protein analysis ####
kruskal.test(protein~diet, data=d)                 #Protein~diet, p>0.05
kruskal.test(protein~period, data=d)               #Protein~period, p<0.05
summaryBy(protein~diet+period, data=d
         FUN=c(mean,sd,length))                    #Means and SDs for each combination of
the treatment level

####NDF analysis ####
kruskal.test(NDF~period, data=d)                   #NDF~diet, p>0.05
kruskal.test(NDF~diet, data=d)                     #NDF~period, p<0.05
summaryBy(NDF~diet+period, data=d,
          FUN=c(mean,sd,length))                   #Means and SDs for each combination of
the treatment level

#### Ash analysis ####
kruskal.test(ash~diet, data=d)                     #Ash~diet, p>0.05
kruskal.test(ash~period, data=d)                   #Ash~period, p<0.05
```

```
summaryBy(ash~diet+period, data=d,
        FUN=c(mean,sd,length))                    #Means and SDs for each combination of
the treatment level

#### Iodine analysis ####

#Iodine intake from roughage

kruskal.test(i_roughage~diet, data=d)            #Iodine roughage~diet, p<0.05

kruskal.test(i_roughage~period, data=d)          #Iodine roughage~period, p<0.05


summaryBy(i_roughage~diet+period, data=d,
        FUN=c(mean,sd,length))


#Iodine in blood

kruskal.test(i_blood~diet, data=d)               #Iodine blood~diet, p<0.05

kruskal.test(i_blood~period, data=d)             #Iodine blood~period, p<0.05


#Iodine in concentrate

kruskal.test(i_concentrate~diet, data=d          #Iodine concentrate~diet, p<0.05

kruskal.test(i_concentrate~period, data=d)       #Iodine concentrate~period, p<0.05


#Iodine in faeces

kruskal.test(i_faeces~diet, data=d)              #iodine faeces~diet, p<0.05

kruskal.test(i_faeces~period, data=d)            #iodine in faeces~period, p<0.0.5

summaryBy(i_faeces~diet+period, data=d,
         FUN=c(mean,sd,length))


#Iodine in urine

kruskal.test(i_urine~diet, data=d)               #Iodine urine~diet, p<0.05

kruskal.test(i_urine~period, data=d)             #Iodine urine~period, p<0.05

summaryBy(i_urine~diet+period, data=d,
        FUN=c(mean,sd,length))


#Total iodine excretion

kruskal.test(total_i~diet, data=d)               #Total iodine excretion~diet, p<0.05

kruskal.test(total_i~period, data=d)             #Total iodine excretion~period, p>0.05
```

```
summaryBy(total_i~diet+period, data=d,
          FUN=c(mean,sd,length))

#Iodine intake-excretion
kruskal.test(i_in_per_i_out~diet, data=d)            #Iodine i-e~diet, p<0.05
kruskal.test(i_in_per_i_out~period, data=d)          #Iodine i-e~period, p>0.05
summaryBy(Jod_ut_per_jod_inn~Diet+Period, data=d,
          FUN=c(mean,sd,length))

#Body weight analysis
D1P1 <- c(31,34,36,38.5,40,43)
sd(D1P1)
mean(D1P1)
D2P1 <- c(33.5,36,36,39,43,44)
sd(D2P1)
mean(D2P1)
D1P2 <- c(55.8,60.2,58.6,59,67.8,66.6)
sd(D1P2)
mean(D1P2)
D2P2 <- c(54.5,65.7,52,60,60.7,61.2)
sd(D2P2)
mean(D2P2)

#### Data visualization ####
#I Blood
O <- ggplot(i_blood, aes(diet, i_blood)) +
  geom_violin(trim = FALSE, fill = "grey80", scale = "width") +
  geom_boxplot(width = 0.1, fill = "tomato", outlier.alpha = 0.5) +
  theme_classic() +
  geom_jitter(width = 0.02, colour = "grey20") +
  facet_wrap(~Period) +
  labs(y = "Iodine mg/ml blood", title = "Iodine in blood") +
  theme(plot.title = element_text(hjust = 1), text=element_text(size=16), axis.ticks.x
=element_blank()
```

```
  )
#DM intake
C <- ggplot(d, aes(diet, total_dm)) +
  geom_violin(trim = FALSE, fill = "grey80") +
  geom_boxplot(width = 0.1, fill = "tomato", outlier.alpha = 0.5) +
  theme_classic() +
  geom_jitter(width = 0.02, colour = "grey20") +
  facet_wrap(~period) +
  labs(y = "Total DM intake (g)", title = "Total dry matter intake per period") +
  theme(plot.title = element_text(hjust = 1), text=element_text(size=16), axis.ticks.x
=element_blank()
  )
#iodine excretion in faeces
B <- ggplot(d, aes(diet, i_faeces)) +
  geom_boxplot(width = 0.1, fill = "tomato", outlier.alpha = 0.5) +
  theme_classic() +
  geom_jitter(width = 0.02, colour = "grey20") +
  facet_wrap(~period) +
  labs(y = "Iodine excretion in faeces, g/kg DM", title = "Iodine excretion in faeces per period")
+
  theme(plot.title = element_text(hjust = 1), text=element_text(size=16), axis.ticks.x
=element_blank()
  )
#total iodine excretion in urine
B <- ggplot(d, aes(diet, i_urine)) +
  geom_boxplot(width = 0.1, fill = "tomato", outlier.alpha = 0.5) +
  theme_classic() +
  geom_jitter(width = 0.02, colour = "grey20") +
  facet_wrap(~period) +
  labs(y = "Iodine excretion in urine, mg/l", title = "Iodine excretion in urine per period") +
  theme(plot.title = element_text(hjust = 1), text=element_text(size=16), axis.ticks.x
=element_blank()
  )
```

12 References

Abbott, D. W., Aasen, I. M., Beauchemin, K. A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D. A., Krizsan, S. J., Kirwan, S. F., Lind, V., Meyer, U., Ramin, M., Theodoridou, K., Dirk von, S., Walsh, P. J., Waters, S., & Xing, X. (2020). Seaweed and Seaweed Bioactives for Mitigation of Enteric Methane: Challenges and Opportunities. *Animals*, *10*(12), 2432. https://doi.org/http://dx.doi.org/10.3390/ani10122432

Al-Shorepy, S. A., Alhadrami, G. A., & Jamali, I. A. (2001). Effect of feeding diets containing seaweed on weight gain and carcass characteristics of indigenous lambs in the United Arab Emirates. *Small ruminant research*, *41*(3), 283-287. https://doi.org/https://doi.org/10.1016/S0921-4488(01)00204-8

Alexandratos, N., & Bruinsma, J. (2012). *World Agriculture Towards 2030/2050: the 2012 revision*. FAO. Retrieved 16.03.2022 from https://www.fao.org/fileadmin/templates/esa/Global_persepctives/world_ag_2030_50_2012_rev.pdf

Antaya, N. T., Ghelichkhan, M., Pereira, A. B. D., Soder, K. J., & Brito, A. F. (2019). Production, milk iodine, and nutrient utilization in Jersey cows supplemented with the brown seaweed Ascophyllum nodosum (kelp meal) during the grazing season. *Journal of Dairy Science*, *102*(9), 8040-8058. https://doi.org/https://doi.org/10.3168/jds.2019-16478

Antaya, N. T., Soder, K. J., Kraft, J., Whitehouse, N. L., Guindon, N. E., Erickson, P. S., Conroy, A. B., & Brito, A. F. (2015). Incremental amounts of Ascophyllum nodosum meal do not improve animal performance but do increase milk iodine output in early lactation dairy cows fed high-forage diets1. *Journal of Dairy Science*, *98*(3), 1991-2004. https://doi.org/https://doi.org/10.3168/jds.2014-8851

Arthur, J. R., Nicol, F., & Beckett, G. J. (1992). The role of selenium in thyroid hormone metabolism and effects of selenium deficiency on thyroid hormone and iodine metabolism. *Biol Trace Elem Res*, *34*(3), 321-325,326. https://doi.org/10.1007/BF02783686

Attanasio, R., Scinicariello, F., Blount, B. C., Valentin-Blasini, L., Rogers, K. A., Nguyen, D. C., & Murray, H. E. (2011). Pendrin mediates uptake of perchlorate in a mammalian

in vitro system. *Chemosphere*, *84*(10), 1484-1488. https://doi.org/https://doi.org/10.1016/j.chemosphere.2011.04.038

Bach, S. J., Wang, Y., & McAllister, T. A. (2008). Effect of feeding sun-dried seaweed (Ascophyllum nodosum) on fecal shedding of Escherichia coli O157:H7 by feedlot cattle and on growth performance of lambs. *Animal Feed Science and Technology*, *142*(1), 17-32. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.05.033

Bay-Larsen, I., Risvoll, C., Vestrum, I., & Bjørkhaug, H. (2018). Local protein sources in animal feed - Perceptions among arctic sheep farmers. *Journal of Rural Studies*, *59*, 98-110. https://doi.org/https://doi.org/10.1016/j.jrurstud.2018.02.004

Behne, D., & Kyriakopoulos, A. (2001). Mammalian selenium-containing proteins. *Annual Review of Nutrition*, *21*, 453-473. https://login.ezproxy.nord.no/login?url=https://www.proquest.com/scholarly-journals/mammalian-selenium-containing-proteins/docview/204179280/se-2?accountid=26469

Besada, V., Andrade, J. M., Schultze, F., & González, J. J. (2009). Heavy metals in edible seaweeds commercialised for human consumption. *Journal of Marine Systems*, *75*(1), 305-313. https://doi.org/https://doi.org/10.1016/j.jmarsys.2008.10.010

Biancarosa, I., Espe, M., Bruckner, C. G., Heesch, S., Liland, N., Waagbø, R., Torstensen, B., & Lock, E. J. (2017). Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. *Journal of Applied Phycology*, *29*(2), 1001-1009. https://doi.org/https://doi.org/10.1007/s10811-016-0984-3

Blikra, M. J., Wang, X., James, P., & Skipnes, D. (2021). Saccharina latissima Cultivated in Northern Norway: Reduction of Potentially Toxic Elements during Processing in Relation to Cultivation Depth. *Foods*, *10*(6), 1290. https://doi.org/https://doi.org/10.3390/foods10061290

Bonnini, S. (2014). *Nonparametric hypothesis testing : rank and permutation methods with applications in R*. Wiley.

Borucki Castro, S. I., Lacasse, P., Fouquet, A., Beraldin, F., Robichaud, A., & Berthiaume, R. (2011). Short communication: Feed iodine concentrations on farms with contrasting levels of iodine in milk. *Journal of Dairy Science*, *94*(9), 4684-4689. https://doi.org/https://doi.org/10.3168/jds.2010-3714

Byrne, L., & Murphy, R. A. (2022). Relative Bioavailability of Trace Minerals in Production Animal Nutrition: A Review. *Animals*, *12*(15), 1981. https://doi.org/https://doi.org/10.3390/ani12151981

Capuco, A. V., Rice, C. P., Baldwin Vi, R. L., Bannerman, D. D., Paape, M. J., Hare, W. R., Kauf, A. C. W., McCarty, G. W., Hapeman, C. J., Sadeghi, A. M., Starr, J. L., McConnell, L. L., & Van Tassell, C. P. (2005). Fate of dietary perchlorate in lactatinq dairy cows: Relevance to animal health and levels in the milk supply [Article]. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(45), 16152-16157. https://doi.org/10.1073/pnas.0508337102

Carvalho, D. P., & Dupuy, C. (2017). Thyroid hormone biosynthesis and release. *Molecular and Cellular Endocrinology*, *458*, 6-15. https://doi.org/https://doi.org/10.1016/j.mce.2017.01.038

Caushi, D., & Martens, H. (2018). Absorption of bicarbonate in sheep omasum. *Research in Veterinary Science*, *118*, 324-330. https://doi.org/https://doi.org/10.1016/j.rvsc.2018.03.008

Chapman, E. M. (1987). The history of the discovery of iodine and its many uses [Article]. *The Alabama journal of medical sciences*, *24*(2), 216-219. https://www.scopus.com/inward/record.uri?eid=2-s2.0-0023320211&partnerID=40&md5=3dc9e106edc83e39bf25174d93107560

Charoensiddhi, S., Conlon, M. A., Franco, C. M. M., & Zhang, W. (2017). The development of seaweed-derived bioactive compounds for use as prebiotics and nutraceuticals using enzyme technologies. *Trends in Food Science & Technology*, *70*, 20-33. https://doi.org/https://doi.org/10.1016/j.tifs.2017.10.002

Cherian, G. (2020). *A guide to the principles of animal nutrition*. Oregon State University ;.

Cholewińska, P., Górniak, W., & Wojnarowski, K. (2021). Impact of selected environmental factors on microbiome of the digestive tract of ruminants. *BMC Veterinary Research*, *17*(1), 25. https://doi.org/10.1186/s12917-021-02742-y

Concilio, S. C., Zhekova, H. R., Noskov, S. Y., & Russell, S. J. (2020). Inter-species variation in monovalent anion substrate selectivity and inhibitor sensitivity in the sodium iodide symporter (NIS). *PLoS One*, *15*(2). https://doi.org/https://doi.org/10.1371/journal.pone.0229085

Costa, M., Cardoso, C., Afonso, C., Bandarra, N. M., & Prates, J. A. M. (2021). Current knowledge and future perspectives of the use of seaweeds for livestock production and

meat quality: a systematic review. *Journal of Animal Physiology and Animal Nutrition*, *105*(6), 1075-1102. https://doi.org/https://doi.org/10.1111/jpn.13509

Cronje, P. (2000). *Ruminant Physiology : Digestion, Metabolism, Growth and Reproduction*. CABI. http://ebookcentral.proquest.com/lib/nord/detail.action?docID=293865

Dehority, B. A. (2002). Gastrointestinal Tracts of Herbivores, Particularly the Ruminant: Anatomy, Physiology and Microbial Digestion of Plants. *Journal of Applied Animal Research*, *21*(2), 145-160. https://doi.org/10.1080/09712119.2002.9706367

Directorate of Fisheries. (2022a, 13.10.2022). *Number of companies and licenses with production by county* Directory of Fisheries. Retrieved 19.04.2023 from https://www.fiskeridir.no/English/Aquaculture/Statistics/Algae

Directorate of Fisheries. (2022b, 13.10.2022). *Number of men and women employed in algae production* Directorate of Fisheries. Retrieved 19.04.2023 from https://www.fiskeridir.no/English/Aquaculture/Statistics/Algae

Directorate of Fisheries. (2022c). *Tarehøsting*. Retrieved 10.03.2023 from https://www.fiskeridir.no/Yrkesfiske/Havmiljoe/Tarehoesting

Directorate of Fisheries. (2023a, 26.01.2023). *Number of Licenses 2014-2022*. Directorate of Fisheries. Retrieved 19.04.2023 from https://www.fiskeridir.no/English/Aquaculture/Statistics/Algae

Directorate of Fisheries. (2023b). *Number of sites in sea water 2006- 2021*. Directorate of Fisheries. Retrieved 19.04.2023 from https://www.fiskeridir.no/English/Aquaculture/Statistics/Algae

Dohán, O., De la Vieja, A., Paroder, V., Riedel, C., Artani, M., Reed, M., Ginter, C. S., & Carrasco, N. (2003). The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical Significance. *Endocrine Reviews*, *24*(1), 48-77. https://doi.org/10.1210/er.2001-0029

Dufreneix, F., Faverdin, P., & Peyraud, J. L. (2019). Influence of particle size and density on mean retention time in the rumen of dairy cows. *Journal of Dairy Science*, *102*(4), 3010-3022. https://doi.org/https://doi.org/10.3168/jds.2018-15926

Duinker, A., Kleppe, M., Fjære, E., Biancarosa, I., Hldal, H. E., Dahl, L., & Lunestad, B. T. (2020). *Knowledge update on macroalgae food and feed safety* https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-en-2020-44

Duinker, A., Roiha S., I., Amlund, H., Dahl, L., Lock, J., Kögel, T., Måge, A., & Lunestad, B. (2016). *Potential risks posed by macroalgae for application as feed and food - a*

Norwegian perspective https://issuu.com/nifes-pdf/docs/rapport-makroalger-27-june-final?utm_medium=referral&utm_source=www.hi.no

EFSA. (2013). Scientific Opinion on the safety and efficacy of iodine compounds (E2) as feed additives for all animal species: calcium iodate anhydrous, based on a dossier submitted by Calibre Europe SPRL/BVBA. *EFSA Journal*, *11*(2), 3100. https://doi.org/https://doi.org/10.2903/j.efsa.2013.3100

EFSA. (2017). Dietary Reference Values for nutrients Summary report. *EFSA Supporting Publications*, *14*(12), e15121E. https://doi.org/https://doi.org/10.2903/sp.efsa.2017.e15121

EFSA, E. F. S. A. (2005). Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the use of iodine in feedingstuffs [https://doi.org/10.2903/j.efsa.2005.168]. *EFSA Journal*, *3*(2), 168. https://doi.org/https://doi.org/10.2903/j.efsa.2005.168

Evankow, A., Christie, H., Hancke, K., Brysting, A. K., Junge, C., Fredriksen, S., & Thaulow, J. (2019). Genetic heterogeneity of two bioeconomically important kelp species along the Norwegian coast. *Conservation genetics*, *20*(3), 615-628. https://doi.org/10.1007/s10592-019-01162-8

Fike, J. H., Saker, K. E., O'Keefe, S. F., Marriott, N. G., Ward, D. L., Fontenot, J. P., & Veit, H. P. (2005). Effects of Tasco (a seaweed extract) and heat stress on N metabolism and meat fatty acids in wether lambs fed hays containing endophyte-infected fescue. *Small ruminant research*, *60*(3), 237-245. https://doi.org/https://doi.org/10.1016/j.smallrumres.2004.12.007

Flachowsky, G., Franke, K., Meyer, U., Leiterer, M., & Schöne, F. (2014). Influencing factors on iodine content of cow milk. *Eur J Nutr*, *53*(2), 351-365. https://doi.org/10.1007/s00394-013-0597-4

Forbord, S., Matsson, S., Brodahl, G. E., Bluhm, B. A., Broch, O. J., Handå, A., Metaxas, A., Skjermo, J., Steinhovden, K. B., & Olsen, Y. (2020). Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated Saccharina latissima (Phaeophyceae) along the Norwegian coast. *Journal of Applied Phycology*, *32*(4), 2215-2232. https://doi.org/10.1007/s10811-020-02038-y

Fragkopoulou, E., Serrão, E. A., De Clerck, O., Costello, M. J., Araújo, M. B., Duarte, C. M., Krause-Jensen, D., & Assis, J. (2022). Global biodiversity patterns of marine forests of brown macroalgae. *Global Ecology and Biogeography*, *31*(4), 636-648. https://doi.org/https://doi.org/10.1111/geb.13450

Franke, K., Meyer, U., Wagner, H., & Flachowsky, G. (2009). Influence of various iodine supplementation levels and two different iodine species on the iodine content of the milk of cows fed rapeseed meal or distillers dried grains with solubles as the protein source. *Journal of Dairy Science*, *92*(9), 4514-4523. https://doi.org/https://doi.org/10.3168/jds.2009-2027

Fuge, R., & Johnson, C. C. (2015). Iodine and human health, the role of environmental geochemistry and diet, a review. *Applied Geochemistry*, *63*, 282-302. https://doi.org/https://doi.org/10.1016/j.apgeochem.2015.09.013

Gomes-Lima, C., Wartofsky, L., & Burman, K. (2019). Can Reverse T3 Assay Be Employed to Guide T4 vs. T4/T3 Therapy in Hypothyroidism? *Front Endocrinol (Lausanne)*, *10*, 856-856. https://doi.org/10.3389/fendo.2019.00856

Grabež, V., Coll-Brasas, E., Fulladosa, E., Hallenstvedt, E., Torunn Thauland, H., Øverland, M., Berg, P., & Egelandsdal, B. (2022). Seaweed Inclusion in Finishing Lamb Diet Promotes Changes in Micronutrient Content and Flavour-Related Compounds of Raw Meat and Dry-Cured Leg (Fenalår). *Foods*, *11*(7), 1043. https://doi.org/https://doi.org/10.3390/foods11071043

Green-Gavrielidis, L. A., MacKechnie, F., Thornber, C. S., & Gomez-Chiarri, M. (2018). Bloom-forming macroalgae (Ulva spp.) inhibit the growth of co-occurring macroalgae and decrease eastern oyster larval survival. *Marine Ecology Progress Series*, *595*, 27-37. https://www.jstor.org/stable/26503017

Greenwood, Y., Orpin, C., & Paterson, I. (1983). Digestibility of seaweeds in Orkney sheep. Journal of Physiology-London,

Guéant, J.-L., Guéant-Rodriguez, R.-M., & Alpers, D. H. (2022). Chapter Nine - Vitamin B12 absorption and malabsorption. In G. Litwack (Ed.), *Vitamins and Hormones* (Vol. 119, pp. 241-274). Academic Press. https://doi.org/https://doi.org/10.1016/bs.vh.2022.01.016

Habibu, B., Kawu, M. U., Makun, H. J., Aluwong, T., & Yaqub, L. S. (2016). Seasonal variation in body mass index, cardinal physiological variables and serum thyroid hormones profiles in relation to susceptibility to thermal stress in goat kids. *Small ruminant research*, *145*, 20-27. https://doi.org/https://doi.org/10.1016/j.smallrumres.2016.10.023

Hackmann, T. J., & Spain, J. N. (2010). Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. *Journal of Dairy Science*, *93*(4), 1320-1334. https://doi.org/https://doi.org/10.3168/jds.2009-2071

HE, M. L., HOLLWICH, W., & RAMBECK, W. A. (2002). Supplementation of algae to the diet of pigs: a new possibility to improve the iodine content in the meat. *Journal of Animal Physiology and Animal Nutrition*, *86*(3-4), 97-104. https://doi.org/https://doi.org/10.1046/j.1439-0396.2002.00363.x

Hemken, R. W., Vandersall, J. H., Sass, B. A., & Hibbs, J. W. (1971). Goitrogenic Effects of a Corn Silage-Soybean Meal Supplemented Ration [Article]. *Journal of Dairy Science*, *54*(1), 85-88. https://doi.org/10.3168/jds.S0022-0302(71)85782-X

Henry, B. K., Eckard, R. J., & Beauchemin, K. A. (2018). Review: Adaptation of ruminant livestock production systems to climate changes. *Animal : an international journal of animal bioscience*, *12*, s445-s456. https://doi.org/https://doi.org/10.1017/S1751731118001301

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, *23*(3), 543-597. https://doi.org/10.1007/s10811-010-9632-5

Hungate, R. E. (2013). *The rumen and its microbes*. Elsevier.

Husa, V., Schneider, S., Langangen, A., Steen, H., & Eilertsen, M. (2021). *Artsgruppeomtale Alger (Cyanophyta, Rhodophyta, Chlorophyta og Ochrophyta). Norsk rødliste for arter 2021.* . Artsdatabanken. Retrieved 25.04.2023 from https://artsdatabanken.no/rodlisteforarter2021/Artsgruppene/alger

Huszenicza, G., Kulcsar, M., & Rudas, P. (2002). Clinical endocrinology of thyroid gland function in ruminants. *Veterinární medicína*, *47*(7), 199-210. https://doi.org/10.17221/5824-VETMED

Hvelplund, T., & Nørgaard, P. (2003). *Kvægets ernæring og fysiologi : Bind 1 : Næringsstofomsætning og fodervurdering* (Vol. nr 53). Danmarks JordbrugsForskning.

Jackson, W. A., Joseph, P., Laxman, P., Tan, K., Smith, P. N., Yu, L., & Anderson, T. A. (2005). Perchlorate accumulation in forage and edible vegetation [Article]. *Journal of Agricultural and Food Chemistry*, *53*(2), 369-373. https://doi.org/10.1021/jf0493021

Jakobsen, A. N., Aasen, I. M., Josefsen, K. D., & Strøm, A. R. (2008). Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid Aurantiochytrium sp. strain T66: effects of N and P starvation and O2 limitation. *Appl Microbiol Biotechnol*, *80*(2), 297-306. https://doi.org/10.1007/s00253-008-1537-8

Jans, C., Meile, L., Lacroix, C., & Stevens, M. J. A. (2015). Genomics, evolution, and molecular epidemiology of the Streptococcus bovis/Streptococcus equinus complex

(SBSEC). *Infection, Genetics and Evolution*, *33*, 419-436. https://doi.org/https://doi.org/10.1016/j.meegid.2014.09.017

Jiang, Q., Lin, L., Xie, F., Jin, W., Zhu, W., Wang, M., Qiu, Q., Li, Z., Liu, J., & Mao, S. (2022). Metagenomic insights into the microbe-mediated B and K2 vitamin biosynthesis in the gastrointestinal microbiome of ruminants. *Microbiome*, *10*(1), 1-109. https://doi.org/10.1186/s40168-022-01298-9

Joint, F., Additives, W. E. C. o. F., & Organization, W. H. (2011). *Safety evaluation of certain contaminants in food: prepared by the Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*. World Health Organization.

Jones, D. B. (1931). *Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins*. US Department of Agriculture.

Kim, T. K., & Park, J. H. (2019). More about the basic assumptions of t-test: normality and sample size. *Korean J Anesthesiol*, *72*(4), 331-335. https://doi.org/10.4097/kja.d.18.00292

King, K. W., & Moore, W. E. C. (1957). Density and Size as Factors Affecting Passage Rate of Ingesta in the Bovine and Human Digestive Tracts1. *Journal of Dairy Science*, *40*(5), 528-536. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(57)94516-2

Kirk, A. B., Martinelango, P. K., Tian, K., Dutta, A., Smith, E. E., & Dasgupta, P. K. (2005). Perchlorate and Iodide in Dairy and Breast Milk. *Environmental Science & Technology*, *39*(7), 2011-2017. https://doi.org/10.1021/es048118t

Kolb, N., Vallorani, L., Milanovic, N., & Stocchi, V. (2004). Evaluation of marine algae wakame (Undaria pinnatifida) and kombu (Laminaria digitata japonica) as food supplements. *Food Technology and Biotechnology*, *42*(1), 57-62.

Koluman, N., & Daskiran, I. (2011). Effects of ventilation of the sheep house on heat stress, growth and thyroid hormones of lambs. *Tropical Animal Health and Production*, *43*(6), 1123-1127. https://doi.org/https://doi.org/10.1007/s11250-011-9811-7

Komolong, M. K., Barber, D. G., & McNeill, D. M. (2001). Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucerne hay (Medicago sativa) with increasing levels of quebracho tannins. *Animal Feed Science and Technology*, *92*(1), 59-72. https://doi.org/https://doi.org/10.1016/S0377-8401(01)00246-2

Krehbiel, C. R. P. (2014). INVITED REVIEW: Applied nutrition of ruminants: Fermentation and digestive physiology. *Professional Animal Scientist*, *30*(2), 129-139. https://login.ezproxy.nord.no/login?url=https://www.proquest.com/trade-

journals/invited-review-applied-nutrition-ruminants/docview/1564036683/se-2?accountid=26469

Küpper, F. C., & Carrano, C. J. (2019). Key aspects of the iodine metabolism in brown algae: a brief critical review. *Metallomics*, *11*(4), 756-764. https://doi.org/10.1039/c8mt00327k

Leblanc, C., Colin, C., Cosse, A., Delage, L., La Barre, S., Morin, P., Fiévet, B., Voiseux, C., Ambroise, Y., Verhaeghe, E., Amouroux, D., Donard, O., Tessier, E., & Potin, P. (2006). Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases. *Biochimie*, *88*(11), 1773-1785. https://doi.org/https://doi.org/10.1016/j.biochi.2006.09.001

Lind, V., Weisbjerg, M. R., Jørgensen, G. M., Fernandez-Yepes, J. E., Arbesú, L., & Molina-Alcaide, E. (2020). Ruminal Fermentation, Growth Rate and Methane Production in Sheep Fed Diets Including White Clover, Soybean Meal or Porphyra sp. *Animals (Basel)*, *10*(1). https://doi.org/10.3390/ani10010079

López-Moreno, M., Garcés-Rimón, M., & Miguel, M. (2022). Antinutrients: Lectins, goitrogens, phytates and oxalates, friends or foe? *Journal of Functional Foods*, *89*, 104938. https://doi.org/https://doi.org/10.1016/j.jff.2022.104938

Lund, P., Weisbjerg, M. R., & Hvelplund, T. (2006). Passage kinetics of fibre in dairy cows obtained from duodenal and faecal ytterbium excretion: Effect of forage type. *Animal Feed Science and Technology*, *128*(3), 229-252. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2005.10.009

Løvdal, T., & Skipnes, D. (2022). Assessment of Food Quality and Safety of Cultivated Macroalgae. *Foods*, *11*(1), 83. https://doi.org/https://doi.org/10.3390/foods11010083

Macías-Cruz, U., Correa-Calderón, A., Mellado, M., Meza-Herrera, C. A., Aréchiga, C. F., & Avendaño-Reyes, L. (2018). Thermoregulatory response to outdoor heat stress of hair sheep females at different physiological state. *International Journal of Biometeorology*, *62*(12), 2151-2160. https://doi.org/https://doi.org/10.1007/s00484-018-1615-2

Makkar, H. P. S. (2018). Review: Feed demand landscape and implications of food-not feed strategy for food security and climate change. *Animal*, *12*(8), 1744-1754. https://doi.org/https://doi.org/10.1017/S175173111700324X

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, *212*, 1-17. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.09.018

Martinelango, P. K., Tian, K., & Dasgupta, P. K. (2006). Perchlorate in seawater: Bioconcentration of iodide and perchlorate by various seaweed species. *Analytica Chimica Acta*, *567*(1), 100-107. https://doi.org/https://doi.org/10.1016/j.aca.2006.02.015

McCauley, E. H., Linn, J. G., & Goodrich, R. D. (1973). Experimentally induced iodide toxicosis in lambs. *American journal of veterinary research*, *34*(1), 65-70. http://europepmc.org/abstract/MED/4683976

McDonald, P., McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., & Wilkinson, R. G. (2022). *Animal nutrition* (Eighth edition. ed.). Pearson Education.

Millen, D. D., De Beni Arrigoni, M., & Lauritano Pacheco, R. D. (2016). *Rumenology* (1st 2016. ed.). Springer International Publishing : Imprint: Springer.

Miller, J. K., Swanson, E. W., & Spalding, G. E. (1975). Iodine Absorption, Excretion, Recycling, and Tissue Distribution in the Dairy Cow1. *Journal of Dairy Science*, *58*(10), 1578-1593. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(75)84753-9

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health [https://doi.org/10.1002/jsfa.2577]. *Journal of the Science of Food and Agriculture*, *86*(13), 2010-2037. https://doi.org/https://doi.org/10.1002/jsfa.2577

Mæhre, H. K., Malde, M. K., Eilertsen, K. E., & Elvevoll, E. O. (2014). Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *J. Sci. Food Agric*, *94*(15), 3281-3290. https://doi.org/10.1002/jsfa.6681

National Research Council. (2005). *Health implications of perchlorate ingestion*. National Academies Press.

National Research Council. (2007). *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. The National Academies Press. https://doi.org/doi:10.17226/11654

Newton, L. (1951). Animal and human nutrition from seaweed resources in Europe: uses and potential. *Seaweed utilization. Sampson Low, London*, 387-393.

Nicola, J. P., Reyna-Neyra, A., Carrasco, N., & Masini-Repiso, A. M. (2012). Dietary iodide controls its own absorption through post-transcriptional regulation of the intestinal

Na+/I- symporter. *J Physiol*, *590*(23), 6013-6026. https://doi.org/10.1113/jphysiol.2012.241307

Nysted, E. T., Uldal, S. H., & Vakse, I. (2021). *Norskandel i husdyrfôret* (Kjøttets tilstand 2020, Issue. https://www.animalia.no/contentassets/f0776ffffc914b32a7f5d1bb9e6d6f1a/kt20-forartikkel-02web.pdf

Pandey, D., Hansen, H. H., Dhakal, R., Aryal, N., Rai, S. P., Sapkota, R., Nielsen, M. O., Margarita, N.-G., & Khanal, P. (2022). Interspecies and seasonal variations in macroalgae from the Nordic region : Chemical composition and impacts on rumen fermentation and microbiome assembly. *Interspecies and seasonal variations in macroalgae from the Nordic region: Chemical composition and impacts on rumen fermentation and microbiome assembly*. https://doi.org/https://doi.org/10.1016/j.jclepro.2022.132456

Pandey, D., Mansouryar, M., Novoa-Garrido, M., Næss, G., Kiron, V., Hansen, H. H., Nielsen, M. O., & Khanal, P. (2021). Nutritional and anti-methanogenic potentials of macroalgae for ruminants.

Pandey, D., Pandey, D., & Nord universitet Fakultet for biovitenskap og, a. (2023). *Marine macroalgae as an alternative, environment-friendly, and bioactive feeding resource for animals* Nord universitet, Faculty of Biosciences and Aquaculture]. Bodø.

Paulíková, I., Kováč, G., Bíreš, J., Paulík, Š., Seidel, H., & Nagy, O. (2002). Iodine toxicity in ruminants. *Veterinární Medicína*, *47*(12), 343-350. https://doi.org/https://doi.org/10.17221/5845-VETMED

Peña-Rodríguez, A., Mawhinney, T. P., Ricque-Marie, D., & Cruz-Suárez, L. E. (2011). Chemical composition of cultivated seaweed Ulva clathrata (Roth) C. Agardh. *Food Chemistry*, *129*(2), 491-498. https://doi.org/https://doi.org/10.1016/j.foodchem.2011.04.104

Pérez-Barbería, F. J. (2020). The Ruminant: Life History and Digestive Physiology of a Symbiotic Animal. In S. García-Yuste (Ed.), *Sustainable and Environmentally Friendly Dairy Farms* (pp. 19-45). Springer International Publishing. https://doi.org/10.1007/978-3-030-46060-0_2

Pratt, B. R., & Wettemann, R. P. (1986). The Effect of Environmental Temperature on Concentrations of Thyroxine and Triiodothyronine After Thyrotropin Releasing Hormone in Steers. *Journal of Animal Science*, *62*(5), 1346-1352. https://doi.org/10.2527/jas1986.6251346x

Saleh, D. M., Barrell, G. K., Bailey, C. I., & Frampton, C. M. A. (1998). Effects of exogenous triiodothyronine (T3) and a goitrogen, methylthiouracil (MTU), on thyroid gland function in sheep. *Small Ruminant Research*, *30*(1), 49-56. https://doi.org/https://doi.org/10.1016/S0921-4488(98)00083-2

Schiener, P., Black, K. D., Stanley, M. S., & Green, D. H. (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*, *27*(1), 363-373. https://doi.org/10.1007/s10811-014-0327-1

Silva, L. H. P., Reis, S. F., Melo, A. T. O., Jackson, B. P., & Brito, A. F. (2022). Supplementation of Ascophyllum nodosum meal and monensin: Effects on diversity and relative abundance of ruminal bacterial taxa and the metabolism of iodine and arsenic in lactating dairy cows. *Journal of Dairy Science*, *105*(5), 4083-4098. https://doi.org/https://doi.org/10.3168/jds.2021-21107

Sjaastad, Ø. V., Hove, K., & Sand, O. (2016). *Physiology of domestic animals* (3rd ed.). Scandinavian Veterinary Press.

Skjermo, J., Aasen, I. M., Arff, J., Broch, O. J., Carvajal, A. K., Christie, H. C., Forbord, S., Olsen, Y., Reitan, K. I., Rustad, T., Sandquist, J., Solbakken, R., Steinhovden, K., Wittgens, B., Wolff, R., & Handå, A. (2014). *A new Norwegian bioeconomy based on cultivation and processing of seaweeds: Opportunities and R&D needs*.

Sorge, U. S., Henriksen, M., Bastan, A., Cremers, N., Olsen, K., & Crooker, B. A. (2016). Short communication: Iodine concentrations in serum, milk, and tears after feeding Ascophyllum nodosum to dairy cows—A pilot study. *J Dairy Sci*, *99*(10), 8472-8476. https://doi.org/10.3168/jds.2015-10810

Spears, J. W. (2003). Trace Mineral Bioavailability in Ruminants. *J Nutr*, *133*(5), S1506-S1509. https://doi.org/10.1093/jn/133.5.1506S

Stamp Dawkins, M. (2021). *The Science of Animal Welfare: Understanding What Animals Want*. Oxford: Oxford University Press. https://doi.org/10.1093/oso/9780198848981.001.0001

Stévant, P., Rebours, C., & Chapman, A. (2017). Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International*, *25*(4), 1373-1390. https://doi.org/10.1007/s10499-017-0120-7

Tayyab, U., Novoa-Garrido, M., Roleda, M. Y., Lind, V., & Weisbjerg, M. R. (2016). Ruminal and intestinal protein degradability of various seaweed species measured in

situ in dairy cows. *Animal Feed Science and Technology*, *213*, 44-54. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.01.003

Trumbo, P. R. (2010). Perchlorate consumption, iodine status, and thyroid function [https://doi.org/10.1111/j.1753-4887.2009.00260.x]. *Nutrition Reviews*, *68*(1), 62-66. https://doi.org/https://doi.org/10.1111/j.1753-4887.2009.00260.x

Ueland, M. (2022). *Saccharina latissim in rations for dairy cows : effects on feed intake, milk yield and chemical composition of the milk* Norwegian University of Life Sciences, Ås].

Underwood, E. J., & Suttle, N. (1999). *Mineral Nutrition of Livestock*. Wallingford: CABI.

Van Nevel, C. J., & Demeyer, D. I. (1996). Control of rumen methanogenesis. *Environmental Monitoring and Assessment*, *42*(1), 73-97. https://doi.org/10.1007/BF00394043

Vissers, A. M., Pellikaan, W. F., Bouwhuis, A., Vincken, J.-P., Gruppen, H., & Hendriks, W. H. (2018). Laminaria digitata phlorotannins decrease protein degradation and methanogenesis during in vitro ruminal fermentation. *Journal of the Science of Food and Agriculture*, *98*(10), 3644-3650. https://doi.org/https://doi.org/10.1002/jsfa.8842

Whitehead, D. C. (1984). The distribution and transformations of iodine in the environment. *Environment International*, *10*(4), 321-339. https://doi.org/https://doi.org/10.1016/0160-4120(84)90139-9

Wilkinson, A. C. (1997). Color atlas of diseases and disorders of the sheep and goat. *Small ruminant research*, *24*(2), 147. https://doi.org/10.1016/S0921-4488(96)00883-8

Winkelmann, J., Winkelmann, J., & Anderson, S. (2016). *Sheep and goat diseases : veterinary book for farmers and smallholders* (4th edition. ed.). 5m Publishing.

Yoshimura, T. (2013). Thyroid hormone and seasonal regulation of reproduction. *Frontiers in Neuroendocrinology*, *34*(3), 157-166. https://doi.org/https://doi.org/10.1016/j.yfrne.2013.04.002

Zhang, B., An, W., Shi, Y., & Yang, M. (2022). Perchlorate occurrence, sub-basin contribution and risk hotspots for drinking water sources in China based on industrial agglomeration method. *Environment International*, *158*, 106995. https://doi.org/https://doi.org/10.1016/j.envint.2021.106995

Zimmermann, M. B. (2010). Symposium on 'Geographical and geological influences on nutrition' Iodine deficiency in industrialised countries: Conference on 'Over- and undernutrition: challenges and approaches'. *The Proceedings of the Nutrition Society*, *69*(1), 133-143. https://doi.org/https://doi.org/10.1017/S0029665109991819

Øverland, M., Mydland, L. T., & Skrede, A. (2019). *Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals* [13-24]. Chichester ; New York, NY :.

Özkan Gülzari, Ş., Lind, V., Aasen, I. M., & Steinshamn, H. (2019). Effect of supplementing sheep diets with macroalgae species on in vivo nutrient digestibility, rumen fermentation and blood amino acid profile. *animal*, *13*(12), 2792-2801. https://doi.org/10.1017/S1751731119001502