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Saccharina latissima in rations for dairy cows; effects on feed intake, milk yield and chemical composition of the milk

Saccharina latissima i rasjonen til mjølkekyr; effekt på fôrinntak, mjølkeyting og kjemisk samansetnad av mjølk

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Animal Science

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Forord

Denne oppgåva har vorte skriven i løpet av våren 2022 og markerer slutten av studietida mi på Institutt for husdyr- og akvakulturvitskap. Bakgrunnen av oppgåva er eit fôringsforsøk i regi av Foods of Norway og TINE, som eg var så heldig å få vera med å utføra ved Stoffskifteavdelinga ved NMBU.

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Ås, 14. juni 2022

Mary Ueland

Abstract

As the world's population is increasing, this sets demands to an increased food production. One way that Norwegian agriculture can meet these demands is to increase the use of naturally given resources in a sustainable way. Research on how to increase efficiency in livestock production is a part of this. The aim of the current research was to investigate if addition of seaweed in the diet can make dairy cows more efficient firstly in the milk production, and secondly in ensuring the iodine intake in the Norwegian population.

A cross-over milk production experiment with six Norwegian Red dairy cows was conducted, where total mixed rations of grass silage and concentrate with the addition of either 1 % sugar kelp (*Saccharina latissima*) on dry matter basis (SW diet) or no additive as control (CON diet) were used. The effect of the seaweed inclusion on feed intake, milk production and the chemical composition of the milk was studied. The results showed a significant increase in dry matter intake (DMI) for the SW diet than the CON diet (P = 0.008), which may have led to a significantly higher milk yield (MY; P = 0.024). There was a tendency towards an increased milk fat concentration of the SW diet (P = 0.06) which together with the increased MY resulted in greater yield of energy corrected milk (ECM) in the SW diet than in the CON diet (P = 0.037). Moreover, daily yield of fat and protein was higher for the SW diet than the CON diet ($P \leq$ 0.046). The milk iodine in the SW diet was almost seven times higher than in the CON diet (P = 0.0032). No effect was observed on rumen fermentation products or nutrient digestibility, except a tendency (P = 0.083) to a higher ash digestibility in the SW diet.

The results point towards a clear positive effect with the seaweed inclusion in the diet as it causes a higher DMI and MY, without affecting the rumen environment. A clear explanation as to why these results were obtained was not found, hence this is a topic that should be studied closer with e.g., other inclusion levels of the seaweed, or for a longer time period to study the long-term effects.

Samandrag

Ettersom befolkninga i verda aukar, set dette krav til ein auka matproduksjon. Ein måte norsk landbruk kan møta desse krava er å auka bruken av naturleg gjevne ressursar på ei berekraftig måte. Forskning på korleis å gjera husdyrproduksjonen meir effektiv er ein del av dette. Formålet med denne studien var å undersøka om tilsetning av sukkertare i fôret kan vera ein måte for å gjera mjølkekyr meir effektive først og fremst i mjølkeproduksjonen, og i andre rekke å sikra jodinntaket i den norske befolkninga.

Det vart utført eit krysningsforsøk for mjølkeproduksjon med seks kyr av rasen Norsk raudt fe, der det vart brukt total blanda rasjon av grassurfôr og kraftfôr med anten 1 % sukkertare (*Saccharina latissima*) tilsett på tørrstoffbasis (tarediett) eller med inga tilsetning som kontroll. Effekten sukkertare hadde på fôrinntak, mjølkeproduksjon og mjølkesamansetnad vart undersøkt. Resultata viste eit signifikant høgare tørrstoffinntak av taredietten enn kontrolldietten ($P = 0.008$), som kan ha bidrege til signifikant høgare mjølkeyting ($P = 0.024$). Det var ein tendens til auka konsentrasjon av mjølkefeitt for taredietten ($P = 0.06$) som saman med auken i mjølkeyting resulterte i auka yting av energikorrigert mjølk (EKM) i taredietten samanlikna med kontrolldietten ($P = 0.037$). Dessutan var dagleg yting av feitt og protein høgare for taredietten enn for kontrolldietten ($P \leq 0.046$). Jodinnhaldet i mjølk med taredietten var nesten sju gonger høgare enn frå kontrolldietten ($P = 0.0032$). Det vart ikkje observert nokon effekt på fermenteringsprodukt i vom eller på fordøyelse av næringsstoff, utanom ein tendens ($P = 0.083$) til høgare fordøyelse av oske i taredietten.

Resultata peikar på ein tydeleg positiv effekt ved tilsetning av sukkertare i fôret ettersom det gav ei auke i både fôrinntak og mjølkeyting, utan å påverka vommiljøet. Det vart ikkje funne nokon spesifikk forklaring på resultata, derfor er dette eit tema som bør studerast nærmare med t.d. andre mengder tare i fôret, eller over lenger tid for å kunna vurdera langvarige effektar.

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Abbreviations

AIA	Acid-insoluble ash
As	Arsenic
BW	Body weight
CF	Crude fat
CON	Control ration
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
FCM	Fat corrected milk
FFA	Free fatty acids
FTIR	Fourier-transform infrared spectroscopy
I	Iodine
LS	Least square
MY	Milk yield
NDF	Neutral detergent fiber
NDFom	Neutral detergent fiber corrected for ash
NPN	Non-protein nitrogen
OM	Organic matter
OMD	Organic matter digestibility
SEM	Standard error LSmeans
SCC	Somatic cell count
SCFA	Short chained fatty acids
SHF	Senter for husdyrforsøk (Livestock Production Research Centre)
SW	Seaweed ration
TMR	Total mixed ration
VFA	Volatile fatty acids
WSC	Water soluble carbohydrates

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1 Introduction

Over the past decades, the genetic potential for milk production in Norwegian Red dairy cows has increased rapidly. Today, one cow can produce more than 10 000 kg milk annually, with an average of just over 8000 kg. With the increase in potential for milk production, demands for digestible nutrients have also increased to meet the animal's requirements. These demands are mostly met by feeding locally grown forages and commercially obtained concentrates rich in energy and protein, containing mostly Norwegian produced raw materials, but also a significant proportion of imported ingredients (Landbruksdirektoratet, 2021). Being dependent on other countries' crop production is not sustainable in the long run. Thus, finding local feed ingredients that allow for efficient feed utilization at an acceptable cost is important in order to increase self-sufficiency and to maintain a profitable production.

In future, exploiting local resources will become more and more important, whether those be in mountainous pastures, forests or the ocean. Seaweeds have formerly been used for livestock feeding (Makkar et al., 2016), and an increased use of seaweed for animal feed might reduce the competition between food and feed in land-based plant production. Seaweed cultivation may be more stable and less vulnerable to climate changes, making agriculture less dependent on weather conditions in the harvest season (Newton et al., 2021). In the context of Norwegian agriculture, this can be highly advantageous due to the long coastline with open, cold waters giving rise to natural opportunities for exploring the use of marine resources in livestock production. Subsequently, local seaweed cultivation along the Norwegian coast can increase utilization of close surroundings.

Including different kinds of seaweed in the diet of dairy animals have shown promising results for increased feed utilization and production. *In vitro* studies have shown sufficient digestibility of seaweed in ruminants (de la Moneda et al., 2019; Morais et al., 2020), and *in vivo* studies presents potential to increase production and feed efficiency (Kidane et al., 2018; Lee et al., 2005; Singh et al., 2017), and elevating mineral content in the milk (Mišurcová, 2012; Newton et al., 2021).

After the year 2000 the milk iodine content has notably decreased in Norway. In the same period the inclusion of rape seed products in concentrate for ruminants have increased, which have shown to affect the iodine transfer from feed to milk (Trøan et al., 2018). Together with

a reduced intake of milk and seafood, this has contributed to a larger part of the Norwegian population being in danger of developing an iodine deficiency – especially children, young women and the elderly. Therefore, iodine enrichment of table salt and animal-derived products have been studied to increase the iodine intake in Norwegians (Abel et al., 2018; Dierick et al., 2009; Henjum et al., 2019). With a naturally high iodine content in seawater and seaweed's ability to take up and accumulate minerals from the surroundings, adding iodine rich seaweed in the animal diet might therefore be a tool for improving the intake in humans (Dierick et al., 2009).

The objective of the current study was to investigate if addition of 1 % sugar kelp (*S. latissima*) in the diet for Norwegian Red dairy cows would be valuable to utilize feed more efficiently, thereby increasing the amount of both fresh and energy corrected milk (ECM) per kilo dry matter (DM). It was hypothesized that inclusion of 1 % sugar kelp would increase milk production and iodine content in the milk.

This thesis is based on a feeding experiment, available literature on ruminant digestive physiology and the topic of seaweed as feed. To get an overview and better understanding, I will first present a literature review with relevant aspects, and afterwards my own study.

2 Background

2.1 Seaweed

Macroalgae is a term used for a wide range of marine plants known as seaweed due to their abundance in seawater and oceans (Mišurcová, 2012), including red (*Rhodophyceae*), brown (*Phaeophyceae*), and green (*Chlorophyceae*) seaweed species (Makkar et al., 2016; Øverland et al., 2019). The potential uses of seaweed are broad, as both the whole plant and extracted components are valuable for industrial exploitation within food, feed, fertilization, pharmaceutical, energy or fuel (Gjertsen et al., 2020). Seaweed has been used for livestock feeding for thousands of years, however, only a few of the about 10 000 known seaweed species are of interest for animal feed (Makkar et al., 2016). Some of the most common species are varieties of *Laminaria, Porphyra, Sargassum, S. latissima, Palmaria palmata* and *Ascophyllum nodosum* (Chaves Lopez et al., 2016; Makkar et al., 2016; Morais et al., 2020).

Cultivated seaweed represents the largest part of harvested seaweed, and in 2018 it made up 97.1 % of the global production. The global production of marine macroalgae has increased from 11 to 32 million tons in the period from 2000 to 2018 and is expected to increase further. Countries in Eastern and Southeastern Asia dominate the market, and Japanese kelp (*Laminaria japonica*) with its 11.4 thousand tons was quantitatively the largest in 2018 (FAO, 2020).

Sugar kelp (*S. latissima,* also called *Laminaria saccharina*), pictured in Figure 1, is a brown seaweed that grows naturally along the Atlantic coast of Europe, the eastern coast of America, the Pacific coasts and near Japan. Sugar kelp is suitable for cultivation by attaching small plants to long ropes instead of growing on the seabed or on rocks. The algae grow fast, with the most intensive growth being from late winter to spring, when both day length and available nutrients are at a desirable level (Broch & Slagstad, 2012).

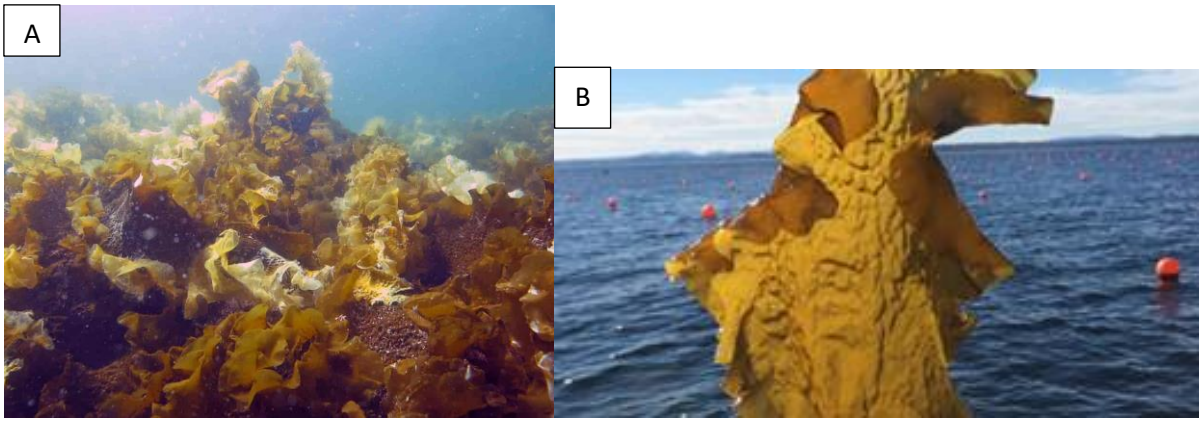

Figure 1: Sugar kelp on the seabed (A) (Andersen, 2021), and after harvest (B) (Seaweed Solutions, 2022).

Sugar kelp could give a yield of 170 tons of biomass per hectare, which equates approximately to 25 tons of dry matter. For comparison, wheat could give a yield of three to five tons per hectare (Broch et al., 2013). Sugar kelp have shown a high *in vitro* organic matter digestibility (up to 97 %) in ruminants when measured with rumen fluid from seaweed-fed sheep or goats. Regarding adaptation of the rumen microbes for *in vivo* measurements, gradually increasing the amount of sugar kelp in a ruminant diet could enable microbes to adapt to a new feed for nutrient utilization (de la Moneda et al., 2019; Makkar et al., 2016).

2.1.1 Seaweed in Norway

With its over 100 000 km long coastline Norway has the second longest coastline in the world (Gjertsen et al., 2020), making the country a natural choice for marine industry. The Norwegian yearly production of seaweed is predicted to grow to 4 million tons by 2030 and 20 million tons by 2050, as 'the next coastal industry in Norway' (Gjertsen et al., 2020). Seaweed cultivation is an industry that will take up more space than fish farming, hence it counts for allocation of areas that could have been used for other purposes as the algae is dependent on the upper water masses to receive enough light. Estimations are that one acre is needed to produce 150-200 tons of algae (Gjertsen et al., 2020) in the most productive and suitable areas, but the industry should be studied closer to obtain more knowledge of consequences from the cultivation. There is no clear answer to the potential challenges or damage seaweed cultivation could inflict on marine ecosystems and coastal habitats, water quality, genetic diversity in natural algae populations, fisheries or tourism (Puente-Rodríguez et al., 2022).

2.1.2 Chemical composition of seaweed

The quality of the cultivated seaweed is influenced by factors in the surroundings. As mentioned, algae are plants that need light, hence they must be cultivated close to the water surface. The available nutrients, water salinity, temperature and water movements also affect their growth and nutritional content like carbohydrates, proteins and minerals (Mišurcová, 2012), which can vary a lot through seasons or between species (Morais et al., 2020). Currents and movements of the sea could be helpful for moving nutrients and organisms to the seaweed, but too much could break or tear off loose plants (Gjertsen et al., 2020).

Table 1 presents an overview of some average values of chemical composition of seaweed from various publications. Brown seaweeds generally contain more minerals and less crude protein than red and green seaweeds, but as seen in Table 1, the values have a wide range. The total carbohydrate fraction can be quite large and comparable to land-based plants, though seaweeds contain quite small amounts of cellulose (about 40 g/kg DM). Instead they contain large amounts of specific complex carbohydrates like alginate, laminarin and fucoidan (Figure 2); sulphated polysaccharides that are mainly found in brown seaweed and can exist in both water-soluble and insoluble forms (Broch & Slagstad, 2012; De Jesus Raposo et al., 2015; Halmemies-Beauchet-Filleau et al., 2018; Kadam et al., 2015a; Kadam et al., 2015b).

Table 1: Chemical composition of seaweeds (% of DM).

	Brown seaweed, *Phaecophyceae*	**Green seaweed, *Chlorophyceae***	**Red seaweed, *Rhodophyceae***
DM (% of wet biomass)	6-39	8-22	9-28
NDF[1]	10-69	5-66	12-59
Crude protein	2-23	4-35	6-47
Ether extract	0.1-4	0.3-4.2	0.3-3
Ash	9-40	7-55	8-42
Minerals	14-35		
Alginate	16-60		
Laminarin	0-35		
Fucoidan	6-26		

Source: Afonso et al. (2019), Makkar et al. (2016), Singh et al. (2017), Lee et al. (2005), Morais et al. (2020), Øverland et al. (2019).

[1]NDF = Neutral detergent fiber

Alginate, the salt of alginic acids, is the main polysaccharide in brown seaweeds, and count for 16-60 % of the DM (Afonso et al., 2019). Alginate is composed of (1→4)-linked α-L-guluronic acid and β-D-mannunoric acid residues arranged in hetero- or homopolymeric blocks. It is a cell wall polysaccharide partly responsible for the seaweed's flexibility; hence it can be found in larger quantities in seaweed grown in moving compared to calm waters. Alginate can work as a great prebiotic, as it significantly enhances the growth of several bacteria, and leads to an increase in acetate, propionate and several short chain fatty acids (SCFA) metabolites (Afonso et al., 2019).

Fucoidan is another polysaccharide in brown seaweed, and the content can vary from 6-26 % of the DM (Afonso et al., 2019). Fucoidan is mainly composed of fucose and sulphate, but can also include proteins, acetyl groups or monosaccharides. They have a high structural diversity and can mainly be divided in two types. The first kind being chains of alternating (1→3)- and (1→4)-linked α-L-fucopyranose residues, while the other type is long chains of (1→3)-linked α-L-fucopyranose residues, and this is the type mostly found in *S. latissima*.

The polysaccharide laminarin is a small molecule in the glucan family and is composed of β-(1-3)-linked glucose monomers. Laminarin is the main storage carbohydrate in brown seaweed mostly found in *Laminaria* and *Saccharina* sp. and is present in varying amounts through a year, as it is generally produced in the summer and autumn season. Species in the *Laminaria* family are known to contain the largest portion of laminarin, as the content in *L. Saccharina* (sugar kelp) and *Laminaria digitata* in particular can reach up to 35 % of the DM. Laminarin is considered as a fibre, hence it can be totally or partially fermented by the microbiota (Afonso et al., 2019).

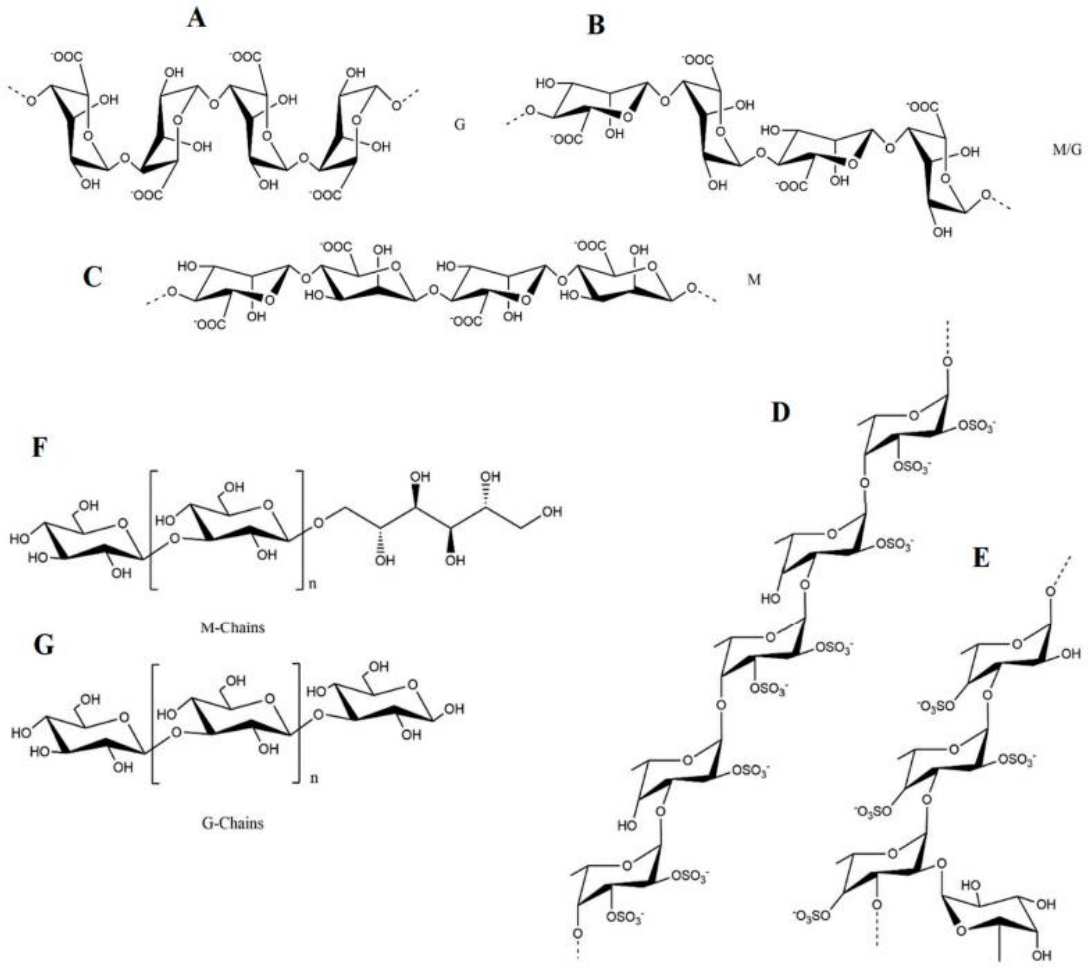

Figure 2: The structure of polysaccharides mostly found in brown seaweed: (A-C) alginic acids; (D-E) fucoidans from A. nodosum and S. latissima, respectively; (F-G) laminarins M and G chains. M chains have a terminal 1-O-substituted D-mannitol, and the G chains have a terminal glucose unit. From (Afonso et al., 2019).

Seaweed have the ability to absorb inorganic substances from their surroundings (Mišurcová, 2012), and this leads to a 10 to 20 times higher mineral content than in terrestrial plants (Gaillard et al., 2018; Tayyab et al., 2016). Brown algae in particular have a low protein and high ash content, resulting in a lower nutritional value than red and green algae (Biancarosa et al., 2018; Morais et al., 2020), and a potentially harmful quantity of minerals (Fleurence et al., 2012) stored in the cell wall polysaccharides and other tissues (Mišurcová, 2012).

Some seaweeds have been used to indicate metal contamination in waters (Melville & Pulkownik, 2006) due to their ability to take up substances like arsenic (As) from their environment. Uptake can happen in two ways; either a surface reaction where the metal is absorbed at the algal surface due to a difference in electrostatic charge, or by the slower process where metal ions are transported into the cytoplasm across the cell membrane (Sánchez et al., 2001).

Arsenic can be found in organic and inorganic species that differ in toxicity (Besada et al., 2009; Øygard et al., 1999). The inorganic species arsenite (AsO_3) and arsenate (AsO_4^{3-}) are embryotoxic, might be carcinogenic, and can impact biochemical processes like the oxidative phosphorylation or element binding in enzymes. A review from Phillips (1990) presents results that brown seaweed accumulate more total arsenic than others, but the amount is more dependent on specific species rather than the environment. It has been considered that the elevated arsenic content is a result of a competing uptake between phosphate and arsenic, where a higher phosphate concentration in brown algae is followed by an increased arsenic accumulation (Sanders, 1979). Although *S. latissima* contain relatively high levels of arsenic, only a small proportion of this (approx. 0.25-0.9 % of total arsenic) is inorganic arsenic (Biancarosa et al., 2018; Maulvault et al., 2015).

Marine species, brown seaweed in particular, contain high levels of iodine (I) (Biancarosa et al., 2018; Kylin, 1930), and have been used for increasing the iodine intake and preventing goiter in the population (Mišurcová, 2012; Norris et al., 1937). Almost all of the ingested inorganic forms of iodine is absorbed in the human digestive system, while the organic form is excreted (Fairweather-Tait & Hurrell, 1996). Iodine can be found in the environment as iodide (I^-) and iodate (IO^-_3), two anions that are easily dissolvable in water, which will follow rainwater to the sea after leaching from the soil, and contribute to the high concentration of iodine in seawater (Hudson, 2007). Brown seaweed have shown an iodine concentration up to 30 000 times higher than in seawater, which points to active transport processes rather than diffusion based on a concentration gradient (Küpper et al., 1998; Küpper & Carrano, 2019).

Iodine is a mineral contributing to the hormones triiodothyronine (T_3) and tetraiodothyronine (T_4, thyroxine). These play a big role as antioxidants, and in cell and fetus development, growth, oxygen consumption and the immune defense, and an iodine deficiency is the largest cause of preventable brain damage (Dierick et al., 2009; Dunn, 2003; McDonald, 2011). An insufficient intake of iodine may lead to a number of health problems, while an excessive intake as high as 10 to 20 times the recommendations might not even have an effect, but could in rare cases lead to hyperthyroidism (Dunn, 2003). Reports have shown that the median iodine intake for pregnant women in Norway is 89 µg/day, although WHO recommends 250 µg/day (Abel et al., 2018). Including iodine supplement to animal diets, and thereby enrich

animal-derived food like milk and eggs with iodine, have been studied as a way of increasing the iodine status in humans (Dierick et al., 2009).

A suitable daily iodine intake for an adult cow with a body weight (BW) of 600 kg is 10 mg, to assure synthesis of thyroid hormones (0.016 mg/kg BW) (Paulíková et al., 2002). Iodine cannot be synthesized in the body and must be included in the diet. Ruminants rarely experience iodine intoxication as they can handle large doses (Paulíková et al., 2002), but if the products are to be used for human consumption, the mineral amount in feed and animal products should be kept under control to avoid excessive intake for people. EU regulations are proposing a maximum of 2 mg/kg iodine in complete feeds for dairy ruminants (Makkar et al., 2016).

2.2 Digestion and milk production in ruminants

2.2.1 Ruminant digestive physiology

The main purpose of the digestive system can be divided into four functions. The food will go through mechanical processing by chewing and mixing, then through chemical processing by addition of enzyme-containing digestive juices, and thereby an enzymatic breakdown of organic nutrients before absorption of substances from the digestive tract (Sjaastad et al., 2016).

The digestive tract in ruminants can be divided into the forestomach, the true stomach, and the small and large intestines (Figure 3). The forestomach consists of rumen, reticulum and omasum, and contains many microorganisms that are responsible for much of the degradation and fermentation of nutrients in feed. The true stomach (abomasum) is the equivalent to the monogastric stomach, where the enzymatic and mechanical digestion occur (Sjaastad et al., 2016).

The rumen is the largest chamber and takes up almost the whole left side of the animal. The content in the rumen can generally be divided into liquid, particles and gas, and more specifically into a mix of ingested feed material, water, saliva and fermentation products like gases and microbial mass. The bottom part of the rumen content is the liquid phase with fermented particles, with a size so small that it is ready for further transport to the omasum through the reticulo-omasal orifice. The largest and least fermented particles are on the top, and contractions in the rumen wall are causing mixing of the content (Nørgaard & Hvelplund, 2003; Sjaastad et al., 2016).

The difference between the ruminant and monogastric digestive system is what is making it possible to utilize fiber rich plant material. When a ruminant ingest feed, the feed is not chewed to small pieces right away, but quite rapidly swallowed down through the esophagus to the reticulorumen. When the animal is resting, feed material will be regurgitated for rumination and mixing with HCO_3^--rich saliva that both adds water, and neutralizes about a third of the acids produced in the forestomach fermentation (Sjaastad et al., 2016).

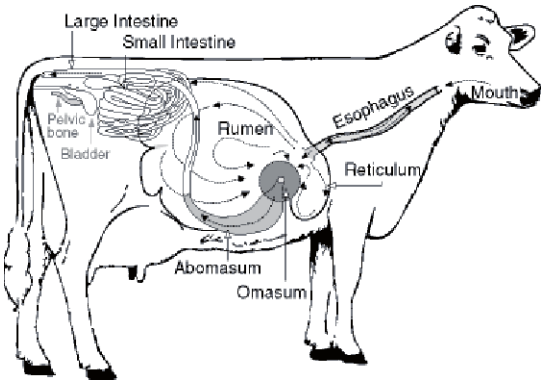

Figure 3: The digestive system in cattle. From Böhnlein (2007).

Ruminal microbes degrade nutrients to produce energy for their own growth (microbial protein) and volatile fatty acids (VFA) as by-product. Some microbes have very specialized demands for substrates, while others are more adaptable. Amylolytic and cellulolytic bacteria, as the name indicates, prefer the carbohydrates amylose (starch) and cellulose (fiber), respectively. Fungi have a large role in digestion of plant fibers, and protozoa mainly ingest bacteria (Sjaastad et al., 2016). The VFA are the animal's largest energy source, and the quantitatively most important are acetic acid, propionic acid and butyric acid. When the microbes die or follow rumen content further through the digestive tract, the microbes will be degraded and absorbed as amino acids and peptides in the small intestine like other bypass dietary protein.

The composition of the rumen content changes a lot throughout the day through feeding, fermentation, rumination and VFA absorption. Figure 4 illustrates the variation in ammonia (NH_4^+), pH and VFA after feeding. Nutrient fermentation happens constantly, and in order to keep the rumen environment stabile there have to be a constant absorption of VFA over the rumen wall, and a recycle of NH_4^+ in the body. The optimal pH in the rumen is above 5.8, and a prolonged period below this could afflict the ruminal microorganisms and make permanent destructions (Sjaastad et al., 2016).

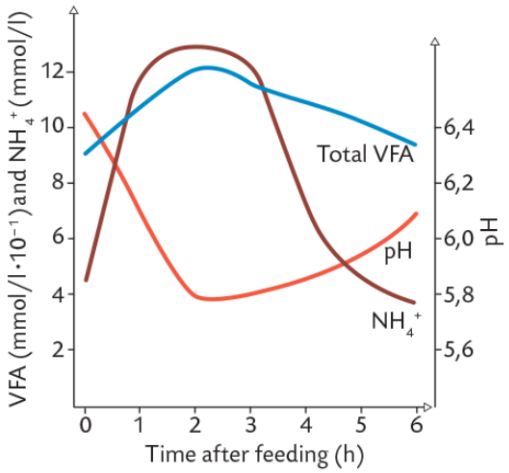

Figure 4: Change in pH and concentration of total VFA and ammonia (NH_4^+) in the rumen, 0 to 6 hours after feeding. From Sjaastad et al. (2016).

2.2.2 Nutrients in ruminant feed

Feedstuffs are generally divided into roughage and concentrates, where the particle length (6 mm) determines whether the feed is characterized as one or the other (Volden, 2011). The chemical composition of feed is determined by different analyzes, which fractionate dry matter (DM) into crude protein (CP), neutral detergent fiber (NDF), starch, crude fat (CF), residual carbohydrates, fermentation products and ash (Volden, 2011).

The quantitatively largest part of ruminant feed is carbohydrates – a large group of molecules with different structures, classified as sugars or non-sugars. The most abundant carbohydrates in a ruminant diet are starch from concentrate, and cellulose from roughage. Starch, mostly found in grass leaves and grains, is built by amylose and amylopectin, and is a carbohydrate which is mostly easily degradable due to the breakable α-1-4 and 1-6 bonds. Cellulose also consist of glucose molecules, but these are bound together by β-1-4 bonds which are harder to degrade (Weisbjerg et al., 2003). Cellulose and hemicellulose are structural carbohydrates found in the stem of plants, and cellulose is often bound to lignin in a tight bond, which makes it less digestible, or undigestible. Cellulose and hemicellulose along with lignin are together analyzed as the NDF fraction in feed (McDonald, 2011).

Proteins are complex molecules composed of chains of amino acids linked by peptide bonds. They contain carbon, hydrogen, oxygen, nitrogen and often also sulphur (McDonald, 2011). Protein in ruminant feed is referred to as CP which is estimated after analyzing amount of nitrogen in a sample and multiplying N by a factor of 6.25, based on the assumption that proteins contain 16 % N (Weisbjerg & Hvelplund, 2003). Even though over 200 amino acids

can be found in biological material, only 20 of them are found as protein components (McDonald, 2011). These can be divided in essential and non-essential amino acids depending on the animal's ability to synthesize them in the body, or they must be included in the feed. Although ruminants can synthesize amino acids from different nitrogen sources, the so-called essential ones must be ingested with the diet for better and efficient animal productivity. For a ruminant, the essential amino acids are lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, phenylalanine and valine (Hvelplund et al., 2003). These amino acids should be protected from rumen degradation to get maximum benefit out of them.

2.2.3 Feed intake

The voluntarily feed intake is the amount an animal eats when it has free access to feed (Campling, 1964), and is measured in dry matter intake (DMI) as the water content can differ a lot between different feeds. The feed intake varies a lot through an animal's life, and can be influenced by factors in the animal itself, the feed, by management and environment, or a combination of these (Ingvartsen & Kristensen, 2003). *Ad libitum* feeding where feed is available for at least 22 hours a day, and frequent servings of palatable feed is a good strategy for making the animals ingest more feed. An increase in feed intake is connected to a higher production but could also lead to a lower utilization of the feed as increased intake also increases passage through the gastrointestinal tract (Ingvartsen & Kristensen, 2003).

2.2.4 Milk production in dairy cows

The required energy and nutrients needed for growth, maintenance or milk production vary from production and feed level, and these requirements are based on established norms depending on factors like breed, age and life stage. An increase in feeding level or change in feed ration composition can increase milk yield and change the chemical composition of the milk. It can also reduce the feed efficiency, as it affects the distribution of the ingested energy between production and storage in body reserves (Kristensen et al., 2003).

Most of the synthesis of the different milk components takes place in the mammary gland and require energy and substrates like glucose, amino acids, and fatty acids. The disaccharide lactose is composed of glucose and galactose, and is synthesized from glucose in the Golgi apparatus in the mammary epithelial cells. Lactose is synthesized in an almost constant rate through a day and plays a large role in the osmolarity of milk, and therefore in the

determination of milk yield. The proportion of lactose in milk will not change notably, as the milk yield (MY) follows the lactose concentration (Sjaastad et al., 2016).

The milk fat originates from the diet as preformed fatty acids, or from *de novo* synthesis using smaller components like acetate or β-hydroxybutyrate (Sjaastad et al., 2016). Acetate is one of the VFA produced in the rumen by the microbial fiber digestion, hence an increase in NDF digestibility could lead to an increase in milk fat.

Synthesis of milk protein occur in the same way as in other cells. The essential amino acids that are included in the protein synthesis come from amino acids absorbed in the intestine (AAT) and transported by the blood (Madsen et al., 2003), while the non-essential can be produced in the mammary epithelial cells.

2.3 Seaweeds in ruminant diet

Cultivated and harvested seaweed have a large potential as animal feed, and studies have shown multiple advantages of dietary additions. A feed change could affect the microbial population, and in turn affect the fermentation of feed and VFA production. Changes in the microbial population after a feed change could impact feed efficiency (Hernandez-Sanabria et al., 2012), MY and chemical parameters (Jami et al., 2014; Lima et al., 2015). The addition of different brown algae like *L. digitata, Sargassum wightii* and *A. nodosum* have shown prebiotic effects on rumen function and health (Makkar et al., 2016), improved feed efficiency (Lee et al., 2005; Singh et al., 2017) and milk quality. They can also be suitable sources for dietary amino acids and minerals (Makkar et al., 2016; Mišurcová, 2012; Morais et al., 2020; Newton et al., 2021). In addition, some seaweeds like *Asparagopsis taxiformis* have shown a large mitigating effect on enteric methane emission (Abbott et al., 2020; Stefenoni et al., 2021).

The diet of Orkney sheep on the North Ronaldsay Island in Scotland consist of mainly seaweed (Morais et al., 2020). A feed study with brown seaweed of *Laminaria* species, evaluated degradation and digestibility of this diet in Orkney sheep compared with domesticated sheep not adapted to a seaweed diet. The results showed a high organic matter and rumen dry matter digestibility in both groups, and indicated that seaweed could offer an adequate nutrient supply (Hansen et al., 2003). Another study described by Gülzari et al. (2019) evaluated nutrient digestibility and rumen fermentation of some algae, including *S. latissima*,

in castrated rams. These results found a lower protein utilization and digestibility for the rams fed *S. latissima*, which could be explained by a low microbial activity.

Lee et al. (2005) performed a study with 14 Holstein dairy cows, feeding one group a basal diet, and included 4 % brown seaweed in the diet for the treatment group. The results showed no changes in DMI, but a significant increase in MY. The same results were obtained in the study from Singh et al. (2017) where one out of three groups of Sahiwal cows were fed 20 % *Sargassum wightii* in their concentrate, with a significant increase in MY, but no effect on DMI or chemical composition in milk.

A study supplementing a dairy cow ration with increasing amounts of seaweed (a mix of 91 % *A. nodosum* and 9 % *L. digitata* on DM basis) was conducted to evaluate changes in milk minerals (Newton et al., 2021). 37 Icelandic cows were split in three diet treatments; control (no seaweed added), low seaweed (0.75 % of concentrate DM) or high seaweed (1.5 % of concentrate DM). The results showed that the milk iodine content in the groups fed seaweed increased by 744 and 1649 µg/kg milk, compared to the control group, and the high seaweed treatment also gave an increase in arsenic concentration in milk (0.17 µg/kg milk). The dietary treatment had no significant effect on MY or milk protein content.

In 2018 a pilot production experiment with goats in late lactation was conducted at our department (Foods of Norway, unpublished data from Mydland, L. T., personal communication. Main results are partially shown in Supplementary table 1 in Appendix). Six goats in late lactation were divided in two groups, with two levels of treatment i.e. inclusion of 0 or 5 % sugar kelp. The experiment showed no significant effect in the rumen fermentation products, except a tendency for higher concentration of valerate in the seaweed group. The seaweed group had a significant higher daily MY both in fresh milk and ECM, and lower content of lactose and urea (g/kg). The milk DM was lower for the seaweed group, but due to the large increase in MY, the total daily production of fat, protein and lactose was slightly higher, but not significant. The iodine and arsenic intake were a lot higher in the seaweed group, and results for iodine excretion showed that 70 % ended up in feces and urine and 20 % in milk. 90 % of the ingested arsenic ended up in feces and urine, and < 1 % in the milk. The content of iodine and arsenic in milk was significantly higher in the seaweed group than the group fed the basal diet.

The high content of minerals restricts the potential use of brown algae as feed ingredient in ruminants. However, processing of the brown algae could contribute to lower mineral content, allowing higher intake and usage. Rinsing and boiling *S. latissima* cultivated on different depths have been evaluated, and Blikra et al. (2021) have reported average reduction of 85 % in iodine and 43 % in arsenic content after this type of processing.

3 Materials and method

The current research work was a collaboration between Foods of Norway and Tine. The experiment was performed at the Norwegian University of Life Sciences (NMBU) in Ås, Norway, following laws and regulations controlling experiments on live animals under the surveillance of the Norwegian Animal Research Authority (FOTS ID: 28737). The main focus was to study effects of seaweed inclusion in dairy cow's diet on milk production; however, samples of feces, urine and rumen fluid were also collected to ensure enough parameters for explaining the results. The results are based on registrations and samples from day 11 to 26 in the experimental period unless stated otherwise.

3.1 Animals and housing

Six dairy cows of the breed Norwegian Red (NR) were selected by health status, lactation number, days in lactation (DIM) and milk yield (MY). An overview of the animals is shown in Table 2. Parity ranged 2 – 4 and lactation stage in days ranged 45 to 58, with an average at 50 DIM at the start of Period 1. The cows were housed individually in tie stalls with rubber mats and had *ad libitum* access to freshwater and feed via an individual water bowl and feed bunk.

Table 2: Overview of experimental animals with respect to age, parity and days in milk (DIM) at the start of the experiment.

Cow	Birth date	Last calving	Parity	DIM
6436	27.12.2015	09.10.2021	4	47
6517	18.10.2016	02.10.2021	4	54
6610	05.09.2017	12.10.2021	3	44
6678	18.11.2017	28.09.2021	3	58
6679	20.11.2017	06.10.2021	3	50
6770	09.09.2018	11.10.2021	2	45

3.2 Experimental design and diets

The cows were divided into two groups of three, which were balanced according to DIM, milk production, and average parity. Cows were then fed either a control diet (CON) or a seaweed diet (SW) in a crossover design with 28 days periods as shown in Figure 5. Period 1 lasted from 25th of November to 23rd of December 2021, and Period 2 from 6th of January until 3rd of February 2022. Between the two experimental periods, there was a transition period from 23rd of December to 6th of January, where the cows were moved to the free-stall barn at the Livestock Production Research Centre (Senter for Husdyrforsøk, SHF) and fed a basal diet

consisting of separately fed grass silage and concentrate. In each experimental period, first ten days were used for adaptation, and days 11 to 26 used for sampling.

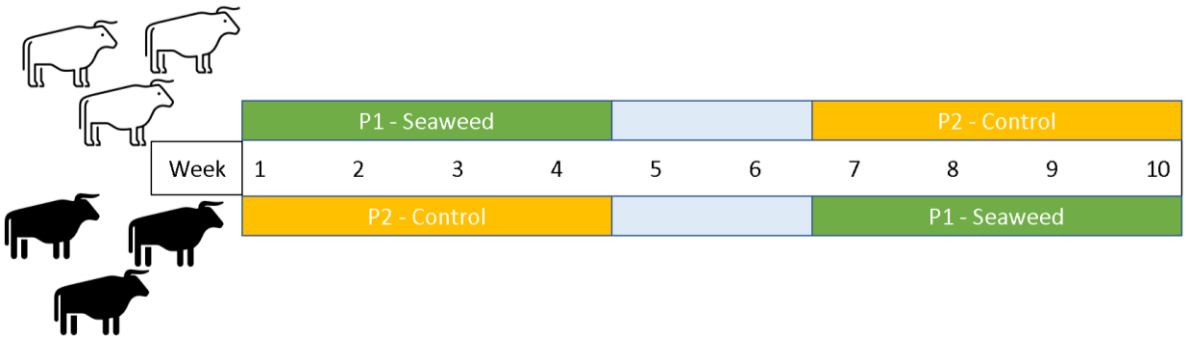

Figure 5: Graphical representation of experimental design explaining groups of animals, number of weeks and diets in each experimental period. Light blue area in the middle was the transition period.

Both experimental diets were prepared as a total mixed ration (TMR) of conserved grass silage and concentrate with a ratio of 65:35 (on DM basis) using a mobile TMR-mixer (Kverneland Silo King Duo 1814, Bryne, Norway). Diets were optimized according to NorFôr plan using TINE OptiFôr to ensure an adequate nutritional supply for high producing dairy cows.

For preparation of the CON diet, grass silage (1st cut) was weighed and precut in the mixer for 15 minutes, and a sample was withdrawn for a quick DM determination using a microwave oven. When the DM content in the silage was known, concentrate (FORMEL Favør 80, Felleskjøpet Agri, Lillestrøm, Norge), water and a propionic acid-based preservative (GrasAAT® Feed, Addcon Nordic AS; 3 L/ton TMR) were calculated and added to the mixer. All ingredients were mixed for an additional 15 minutes. The SW diet was prepared in the same way as the control diet, but 1 % of the grass silage on DM basis was replaced with sugar kelp (*S. latissima*). The blanched sugar kelp (Figure 6) was cultivated and harvested outside Frøya by Seaweed Solutions (Trondheim, Norway), and added in the same step as the concentrate, water and TMR-preservative. Due to a high water content in kelp, extra water was only added in the CON diet to balance the DM with the SW diet. Both experimental diets were prepared in five batches that were used throughout the experiment.

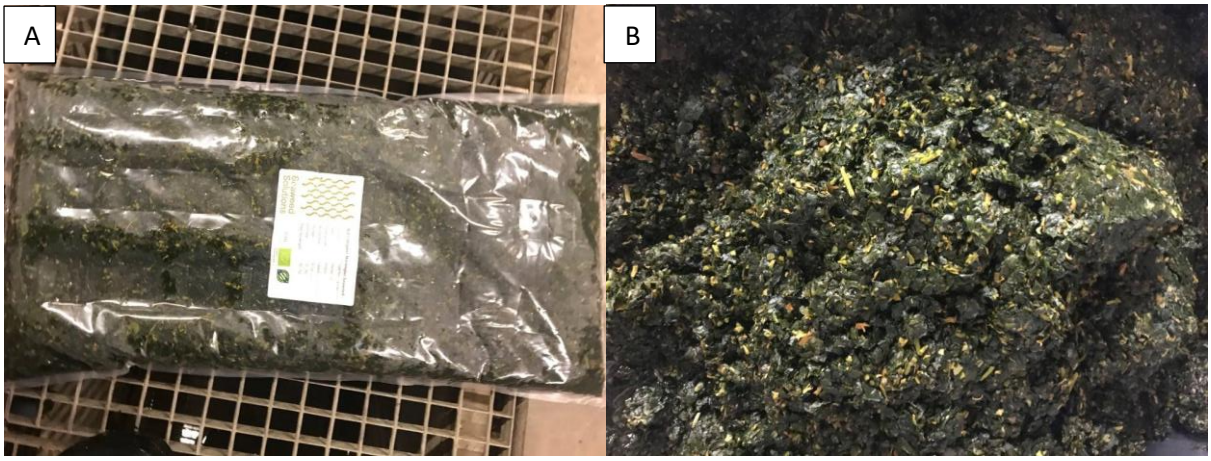

Figure 6: Seaweed used in experiment. A: Bag of sugar kelp. B: Sugar kelp before adding to the TMR.

After taking samples for chemical analysis, the experimental diets were stored in boxes in a freezer room until suitable time for thawing to preserve the best quality as possible before feeding. Samples for chemical analysis were kept stored in the freezer at -20 °C until the end of the experiment. At the end of the experiment representative samples of each diet were dried for 24 hours at 60 °C as described by Mertens (ISO 16472:2006 IDT) (Volden, 2011). After drying, all samples were ground with a Retsch cutting mill SM 200 (Retsch, GmbH, Haan, Germany) using a 1 mm screen. After milling, 3 g of material was taken from each batch sample and pooled to get a representative sample for each diet. The samples were then analyzed for chemical composition.

Apart from taking sample for experimental diets, samples of grass silage and seaweed was also taken to do chemical analysis. For concentrate, chemical composition provided by the factory was used. The chemical composition of the experimental diets and individual ingredients is presented in Table 3. The concentration of NDFom was higher in the CON diet than the SW diet (460 vs. 432 g/kg DM, respectively), whereas the concentration of starch was highest in the SW diet (113 vs. 124 g/kg DM, respectively). The concentration of iodine and arsenic was considerably higher in the SW diet than the CON diet.

Table 3: Components of experimental diets (TMRs) along with chemical composition of experimental diets and individual components (grass silage, concentrate and sugar kelp).

	Experimental diets[1]		Individual components		
	CON	**SW**	**Grass silage[2]**	**Concentrate[3]**	**Sugar kelp[4]**
Components, kg/ton TMR					
Grass silage	847	822			
Concentrate	138	137			
Sugar kelp	0	38			
TMR-preservative[5]	3	3			
Water[6]	12	0			
Chemical composition, g/kg DM if not stated otherwise					
Dry matter (g/kg)	305.1	306.1	293	877	
Organic matter	923.4	919.2	929	924	664.5
NDFom[7]	459.5	432.3	505	209	511.2
Crude protein	151.9	155.3	131	177	108.2
Starch	112.9	124.1	-	440	-
Crude fat	27.9	27.5	38	41	6.72
Ash	76.6	80.8	71	76	335.5
AIA[8]	4.74	4.16	-	-	-
WSC[9]			-	-	0.67
Minerals, mg/kg DM					
Iodine	1.67	15.97	-	-	1120.82
Arsenic	0.048	0.67	-	-	48.20

[1]CON = control diet; SW = seaweed diet.

[2]First cut grass silage, *in vitro* digestible organic matter, 769 g/kg OM (Eurofins Agro Testing Norway AS, NO-1538 Moss).

[3]FORMEL Favør 80 (Felleskjøpet Agri, Lillestrøm, Norge).

[4]*S. latissima* (Seaweed Solutions, Trondheim, Norway), average values for triplicate analyses of two separate samples from two harvest days.

[5]GrasAAT® Feed, ADDCON, Porsgrunn, Norway.

[6]Water added to reach the same DM content as the SW diet.

[7]NDFom = neutral detergent fiber corrected for ash. Content of NDFom in seaweed was corrected for protein content in the residue.

[8]AIA = acid-insoluble ash.

[9]WSC = water soluble carbohydrates

3.3 Data registration and sampling

Scheme of different samplings with respect to days is presented in Figure 7.

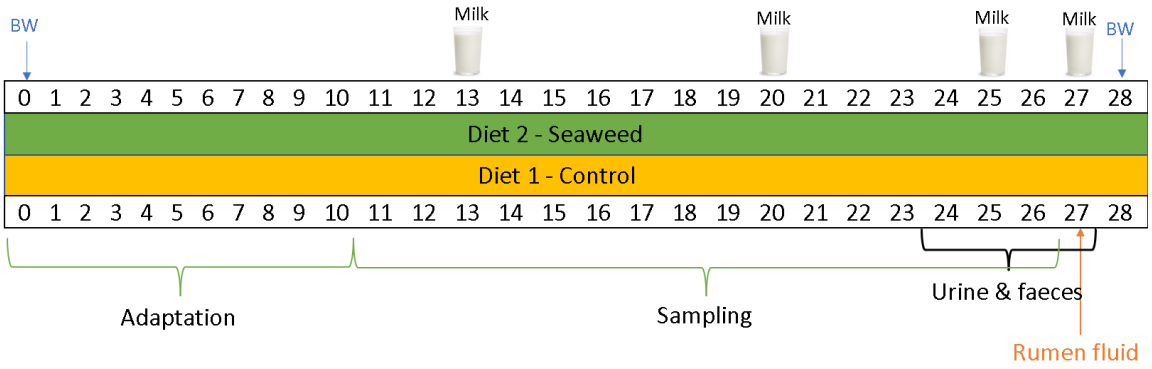

Figure 7: Presentation of the experimental periods with days of adaptation and collection of different samples.

3.3.1 Feeding of animals and feed intake

The experimental diets were fed *ad libitum* with ten percent refusals where 2/3 of daily ration were fed at 7:00, and the last 1/3 at 19:00. Feed refusals were removed before the morning feeding, and daily feed intake was calculated.

DM content in the different batches was determined after drying a sample for 24 hours at 103 °C, and these values were used to estimate DMI for the days with the corresponding TMR batch.

3.3.2 Milk

The cows were milked twice daily (7:45 and 19:45) and the daily MY was registered individually with a DeLaval milk meter MM6 (DeLaval Inc., Tumba, Sweden). Milk samples were taken on day 13, 20, 25 and 27 with separate morning and evening samples, though day 27 was excluded from the data due to deviating values caused by stress from collecting other samples. Each sample was divided into four parts; two 15 mL centrifugation tubes and one 40 mL container were filled and frozen at -20 °C, and one 40 mL container was preserved with bronopol (2-bromo-2-nitro-1.3-propanediol, Broad Spectrum Microtabs®) and stored at 4 °C until analysis. An additional analysis of the somatic cell count was done on triplicates of the fresh samples from Period 1 using a DeLaval cell counter DCC (DeLaval Inc., Tumba, Sweden), to compare this simple on-farm technique with the laboratory results from TINE.

The frozen reserve samples taken at day 25 and 27 were thawed, and 6 mL from each glass were merged to make a pooled sample for mineral analysis.

On day 26 in period 2, two containers of 40 mL milk from each cow were sent to sensory analysis. This test was done by a panel of four authorized judges, and the classification for raw milk 'MA 540' was used, evaluating the samples with a score from 1-5, where 1 and 2 is given to abnormal milk with large deviations in taste and smell, and 3 to 5 points are given to normal milk.

3.3.3 Rumen samples

Samples of rumen fluid were taken on day 27 in each period by an authorized veterinarian from the Faculty of Veterinary Science at NMBU. A tube was lead through the esophagus, and to a sampling point 175-185 cm from the mouth opening. A handheld pump was connected to the tube, and one person used this pump to evacuate a sample of approximately 3 dL rumen fluid in a bucket, after discarding the first 200 mL to avoid contamination. The pH was measured, and samples were taken for VFA and ammonia analysis (2 separate samples of 9.5 mL mixed with 0.5 mL formic acid). The samples for VFA and ammonia were stored at 4 °C until analysis.

3.3.4 Feces and urine

Samples of feces and urine were taken at six different timepoints (9:00, 15:00, 19:00, 10:00, 17:00 and 22:00) from day 24 to 27 in each period. Fecal samples of approximately 250 g were taken by rectal examination or when the cow was defecating, and the samples were placed in aluminum trays (Figure 8). Urine was collected in a bucket while the cow was urinating or by stimulating the area below the vulva, and a sample of 50 mL was poured in a plastic container. All samples were stored frozen at -20 °C until the end of the experiment.

All fecal samples were freeze dried and weighed directly from the freeze dryer and at equilibrium. After equilibrium all samples were broken in two parts, where one part of the sample was put in a plastic bag as a reserve sample, and the other was milled using a Retsch cutting mill SM 200 (Retsch GmbH, Haan, Germany) with a 1 mm screen and put in a sample container. All six samples per cow from each period was thereafter pooled into one sample container, using a scale to weigh out 3 g material from each sample, leaving twelve pooled samples for analysis. The pooled samples were sent for analysis.

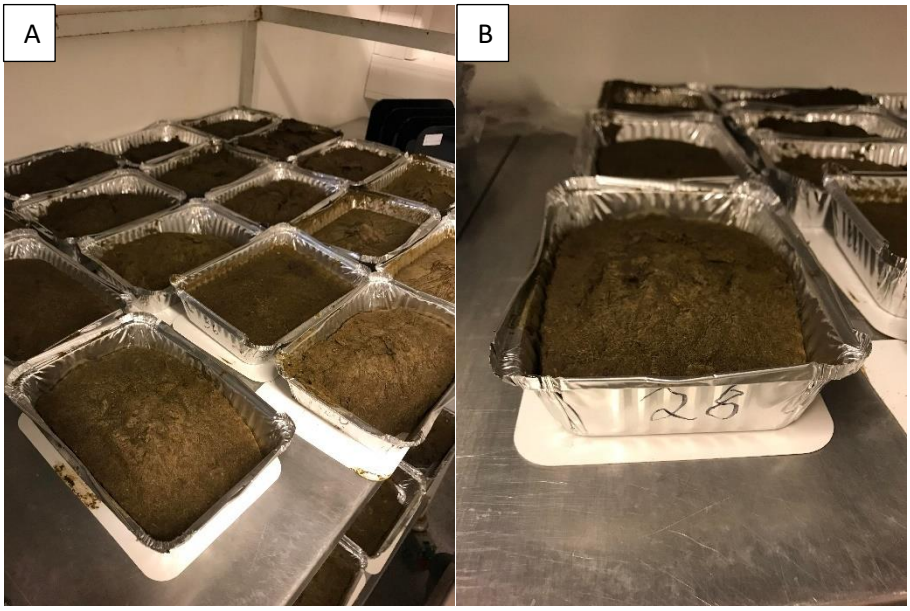

Figure 8A & B: Aluminum trays with fecal samples after freeze drying.

The urine samples were thawed after the experiment, and an amount of 6 mL was taken from each sample and merged to make one sample for every cow from each period, before mineral analysis.

3.4 Chemical analyses

The samples of feed and feces were analyzed for chemical content at LabTek at the Department of Animal and Aquacultural Sciences at NMBU. Determination of dry matter content was done after the NorFor procedure (Åkerlind et al., 2011) by drying at 103 °C ± 2 °C for at least 4 hours, or until constant weight. Determination of crude protein was done after measuring nitrogen content by the Kjeldahl-N method with a Kjeltec TM 8400 (Foss, Denmark) after the AOAC Official method 2001.11 as described by Thiex et al. (2002). CP was estimated as 5 * N for seaweed (Angell et al., 2016), and as 6.25 * N for others. Analysis of crude fat was done after the Randall modification of the Soxhlet method with a Soxtec™ 8000, as described by Manirakiza et al. (2001).

Ash content was determined after complete combustion of a sample at 550 °C for at least 4 hours, according to the method ISO 5984 (*Animal feeding stuffs - Determination of crude ash*, 2002). The acid-insoluble ash (AIA) was determined by boiling the inorganic content with HCl, filtering and drying the solution (Johnsen, 2020). The NDF was determined with an ANKOM220 fiber analyzer (ANKOM Technology, Fairport, NY, USA) according to Mertens et al. (2002) using

sodium sulfite and heat-stable α-amylase. The samples were corrected for residual ash, and the results are presented as aNDFom (Berg, 2018).

Concentrations of total and individual VFA in rumen fluid were analyzed on a TRACE 1300 Gas Chromatograph, equipped with Stabilwax-DA column from Thermo Fischer Scientific S.p.A. (Milan, Italy) (Johnsen, 2016), whereas the ammonia nitrogen (NH_3-N) in the rumen fluid concentration was analyzed on a Kjeltec 8400 after the same procedure as for Kjeldahl-N and CP (Berg, 2013; Thiex et al., 2002).

Milk samples were analyzed by TINE SA (TINE Råmelklaboratoriet, Heimdal) for fat, protein, lactose, urea and free fatty acid (FFA) concentration using fourier-transform infrared spectroscopy (FTIR) (Bentley FTS/FCM, Bentley Instruments Inc., Chaska MN, USA), and for somatic cell count (SCC) by flow cytometry using a BactoCount IBC (Bentley Instruments Inc.).

The analyzes for mineral concentration in feed, feces, urine and milk were done at the Faculty of Environmental Sciences and Natural Resource Management (MINA), NMBU. Iodine was analyzed by inductively coupled plasma spectrometry with mass spectrometric detection (ICO-MS) (PerkinElmer, MA, USA). Dry samples (feed and faeces) were extracted with concentrated 25 % (w/w) tetramethylammonium hydroxide (TMAH) at 90 °C, centrifugated and diluted using the alkaline BENT solution (a mixture of 1-Butanol, H_4EDTA, NH_4OH and Triton X-100) before analysis. For analysis of the other minerals, the samples were decomposed with concentrated HNO_3 in UltraClave (MLS Milestone, Italy) at 260 °C and diluted with water before analysis using ICP-MS (Perkin Elmer).

The mineral analyses were validated using a range of certified reference materials. For dry samples (feed and faeces), the following reference materials were used: 8415 Whole Egg Powder, 1515 Apple Leaves, 1570a Spinach Leaves (National Institute of Standards and Technology, MD, USA), BCR-129 Hay Powder (Joint Research Centre of the European Commission), and NCS DC 73349 Bush Branches and Leaves (National Analysis Center for Iron and Steel, Beijing, China). For milk samples, 1549a Whole Milk Powder (National Institute of Standards and Technology) and ERM-BD150 Skimmed Milk Powder (Joint Research Centre of the European Commission) were used. For urine samples, Seronorm™ Trace Elements Urine L-1 and L-2 (SERO AS, Billingstad, Norway) were used.

3.5 Calculations

3.5.1 Feed intake and digestibility

Organic matter in samples was determined indirectly after ash analysis (Equation 1).

Equation 1

$$OM\ \left(\frac{g}{kg}\right) = 1000 - ash\ (\frac{g}{kg})$$

Daily DMI was estimated from DM in separate batches and daily feed intake of the corresponding batch (Equation 2).

Equation 2

$$DMI\ (kg) = DM_{feed}(\frac{g}{kg}) * feed\ intake\ (kg)$$

Daily intake of NDFom, CP, CF and ash was estimated from chemical content of the diets and daily DMI (Equation 3).

Equation 3

$$nutrient\ intake\ (g) = diet\ DMI\ (kg) *\ nutrient_{diet}\left(\frac{g}{kg\ DM}\right)$$

Daily undigested DM in faeces was calculated from the AIA content in the diet and faeces (Equation 4).

Equation 4

$$undigested\ DM = \frac{total\ AIA\ intake\ \left(\frac{g}{kg\ DM}\right)}{AIA\ in\ faeces\ \left(\frac{g}{kg\ DM}\right)}$$

Afterwards, nutrients in faeces were estimated from the daily undigested DM fraction (Equation 5).

Equation 5

$$nutrients\ in\ faeces\ (g) = undigested\ DM\ (kg)\ x\ nutrient\ in\ faeces\ \left(\frac{g}{kg\ DM}\right)$$

Total tract digestibility of DM, OM, NDFom, CP and ash were estimated using the formula given in Equation 6.

Equation 6

$$digestibility\ of\ nutrient\ (\%) = \frac{nutrient\ in\ feeds - nutrient\ in\ faeces}{nutrient\ in\ feeds}\ x\ 100$$

where nutrient in feeds and faeces were given in g/day, and digestibility in %.

3.5.2 Milk production

Energy corrected milk was calculated from daily MY and chemical composition, using Equation 7 from Sjaunja et al. (1990).

Equation 7

$$ECM\ (kg) = MY\ (kg)\ *\ (0.01 + 0.122\ *\ fat\ content\ (\%)\ +\ 0.077\ *\ protein\ content\ (\%) + 0.053\ *\ lactose\ content\ (\%))$$

Daily yield of the different components (fat, lactose, protein) was calculated from MY in the morning (MY_M) and evening (MY_E) and respective chemical analysis as illustrated for fat in Equation 8.

Equation 8

$$daily\ fat\ yield\ \left(\frac{kg}{d}\right) = MY_M\ (kg) * \frac{fat\ content_M\ (\%)}{100} + MY_E\ (kg) * \frac{fat\ content_E\ (\%)}{100}$$

Iodine and arsenic transfer to milk and faeces were given in percentage using Equation 9 as described by Trøan et al. (2018). Here illustrated with iodine.

Equation 9

$$iodine\ transfer = \frac{daily\ MY\ (kg) * milk\ I\ concentration\ (\frac{mg}{kg})}{daily\ DMI\ (kg) * feed\ I\ concentration\ (\frac{mg}{kg})} * 100$$

3.6 Statistics

The data collected during and after the experiment were registered and sorted in excel (Microsoft Office Excel, 2016). The statistical analyzes were done in SAS (SAS Institute Inc. 2002-2012, SAS for Windows 9.4; Cary, 237 NC, USA). Feed intake, digestibility of nutrients, rumen parameters and mineral partitioning data were analyzed using a linear mixed model (PROC MIXED) with diet and period as fixed effects, and cow as a random effect.

Milk parameters were statistical analyzed with mixed procedure of SAS for repeated measurements using a model with diet, day, period and interaction term (Day*Diet) as fixed effects, and cow as a random effect. Kenward-Roger method was used to calculate denominator degrees, and day within Cow*Period was considered repeated measurement using the spatial power covariance structure called autoregressive order 1 (AR1).

The results are reported as least square (LS) means with standard error of the mean (SEM). Dietary effects were judged using the PDIFF statement with TUKEY adjustments, and significance was claimed when $P \leq 0.05$, whereas tendencies were considered at $0.05 < P \leq 0.10$.

4 Results

4.1 Feed intake and digestibility

Dry matter intake (DMI) of individual animals in experimental Period 1 and 2 is presented in Figure 9. The highest intake was observed for cow 6610 in both periods. The variation among animals and between days were larger in Period 1 than in Period 2.

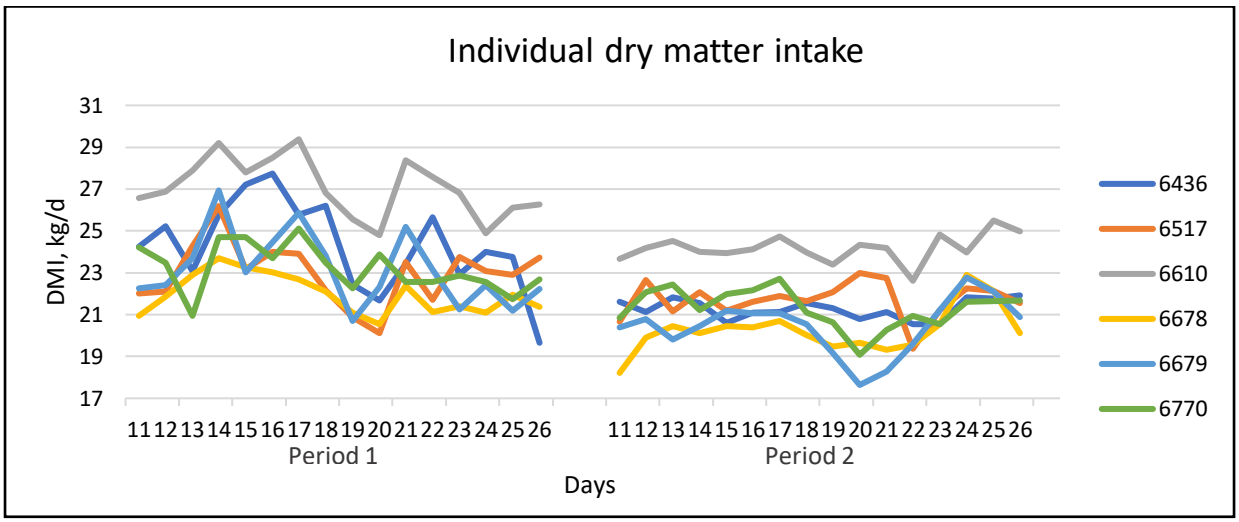

Figure 9: Dry matter intake (DMI, kg/d) of individual animals in experimental period 1 and 2 for day 11 to 26.

Daily DMI of the experimental diets and in the periods is presented in Figure 10A and B.

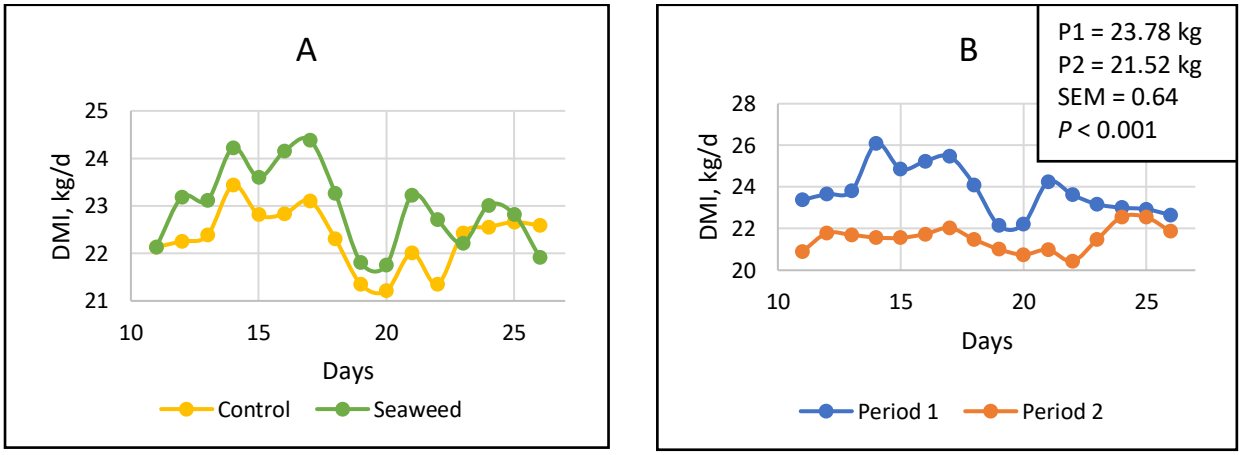

Figure 10: Daily dry matter intake (DMI, kg/d) for experimental diets (A) and periods (B) from day 11 to 26.

The intake of the SW diet was significantly higher than of the CON diet (P = 0.008; Table 4). Dry matter intake between the two periods was significantly different (P < 0.001; Figure 10B), being highest in Period 1.

Table 4: Intake and total tract digestibility of nutrients.

	Experimental diets[1]		SEM	*P*-value
	CON	**SW**		
Intake, kg/d				
Dry matter	22.34	22.97	0.65	0.008
Organic matter	20.63	21.12	0.59	0.0062
NDFom[2]	10.27	9.93	0.28	0.0019
Crude protein	3.39	3.57	0.099	<0.001
Starch	2.52	2.85	0.078	<0.001
Ash	1.71	1.86	0.051	<0.001
Digestibility, %				
Dry matter	81.97	83.38	0.80	0.245
Organic matter	82.64	83.94	0.81	0.283
NDFom	80.76	81.30	1.36	0.788
Crude protein	78.67	80.69	1.01	0.188
Ash	73.96	76.90	1.11	0.083

[1]CON = control diet; SW = seaweed diet.

[2]NDFom = neutral detergent fibre corrected for ash.

Intake of OM, CP, starch and ash was significantly higher ($P \leq 0.0062$; Table 4) when feeding the SW diet compared to the control. In contrast, intake of NDFom was significantly higher for the control diet ($P = 0.0019$) compared to the SW diet.

No significant differences were observed between experimental diets for digestibility of any nutrient, except that there was a tendency of increased ash digestibility ($P = 0.083$) for the SW diet.

4.2 Rumen variables

The pH and analysis of fermentation products in rumen fluid are shown in Table 5.

Table 5: Rumen fermentation products and pH in rumen fluid samples.

	Experimental diets[1]		SEM	*P*-value
	CON	**SW**		
pH	6.82	6.73	0.078	0.314
VFA[2] total (mmol/L)	83.5	85.0	5.12	0.752
VFA proportion (molar % of total VFA)				
Acetate	61.45	61.44	0.71	0.979
Propionate	20.77	20.78	0.60	0.983
Iso-butyrate	0.88	0.90	0.034	0.573
Butyrate	14.49	14.47	0.19	0.923
Iso-valerate	1.09	1.12	0.062	0.748
Valerate	1.33	1.29	0.047	0.298
Acetate:propionate	2.97	2.98	0.13	0.915
Ammonium-N (mg/L)	99.17	118.17	14.37	0.256

[1]CON = control diet; SW = seaweed diet.

[2]VFA = volatile fatty acids.

No significant effects of dietary treatments on pH, VFA production, ammonium-N or the acetate to propionate ratio were observed.

4.3 Milk parameters

4.3.1 Milk production and chemical composition

Mean milk yield, ECM and milk composition are presented in Table 6.

Table 6: Milk yield, milk parameters and feed efficiency in the experimental diets.

	Experimental diets[1]		SEM	*P*-value		
	CON	**SW**		**Diet**	**Day**	**Diet*day**
Production, kg/day						
Milk yield[2]	32.9	33.4	1.53	0.024	0.0005	0.943
ECM[3]	33.9	35.8	1.296	0.037	0.406	0.489
Composition of milk						
Protein, %	3.47	3.48	0.083	0.870	0.499	0.414
Fat, %	4.33	4.47	0.131	0.060	0.488	0.360
Lactose, %	4.80	4.78	0.054	0.464	0.697	0.726
Milk urea, mmol/L	4.21	4.24	0.250	0.960	0.080	0.825
FFA[4], mmol/L	0.637	0.655	0.126	0.914	0.310	0.378
SCC[5], 1000/mL	50.88	86.63	33.47	0.444	0.366	0.317
Milk component yields, kg/d						
Protein	1.11	1.16	0.035	0.046	0.149	0.602
Fat	1.39	1.49	0.053	0.043	0.711	0.425
Lactose	1.55	1.60	0.079	0.227	0.114	0.852
Feed efficiency						
Milk yield/DMI, kg/kg	1.48	1.47	0.094	0.652	0.0009	0.646
ECM/DMI, kg/kg	1.56	1.59	0.089	0.377	0.034	0.719

[1]CON = control diet; SW = seaweed diet.

[2]Milk yield based on daily registrations.

[3]ECM = energy corrected milk based on three milk sampling days and corresponding yields.

[4]FFA = free fatty acids

[5]SCC = somatic cell count

The SW diet resulted in a significant increase in daily milk yield (P = 0.024; Figure 11A) compared to the CON diet. As seen in Figure 11B, daily milk yield was significantly higher (P < 0.001) in Period 1 than Period 2. Energy corrected milk yield was higher for the SW diet than the CON diet (P = 0.037; Table 6; Figure 13).

Fat concentration in milk tended to be higher (P = 0.06; Table 6; Figure 12) and the fat production was higher (P = 0.043) in the SW diet than in the CON diet. There was no effect of diet on protein concentration in milk, but the higher milk yield resulted in a highest protein production for the SW diet (P = 0.046).

No significant effect on feed efficiency was observed.

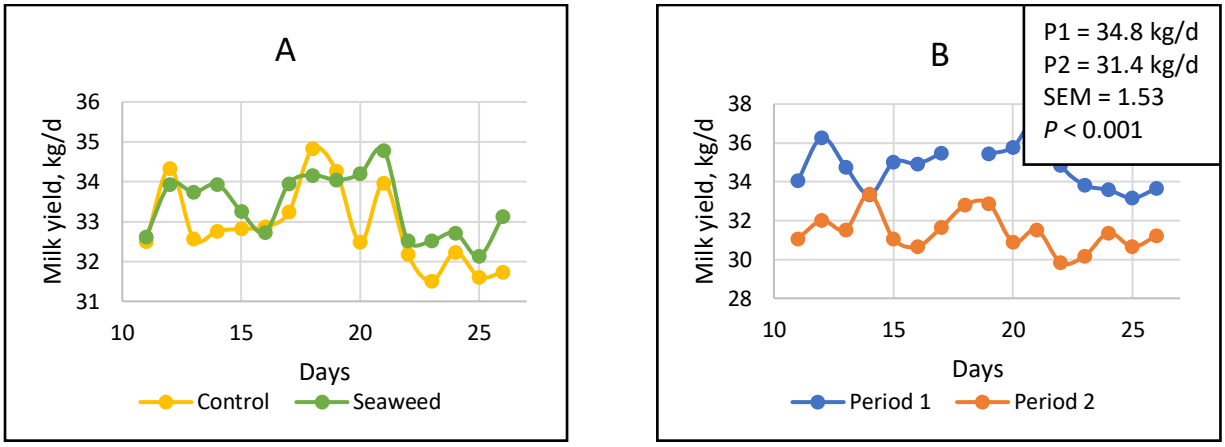

Figure 11: Daily milk yield (kg/d) for experimental diets (A) and periods (B).

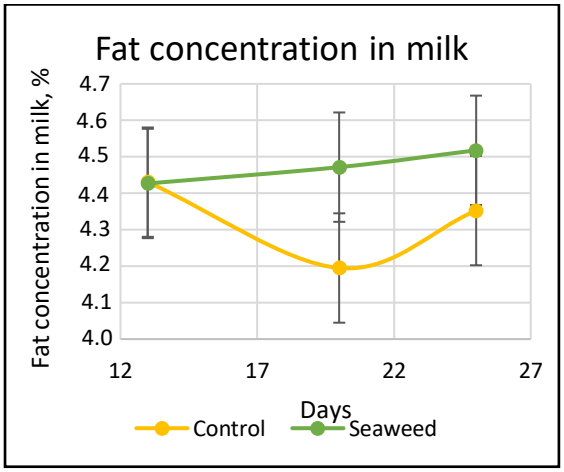

Figure 12: Concentration of fat in milk for experimental diets on sampling days 13, 20 and 25.

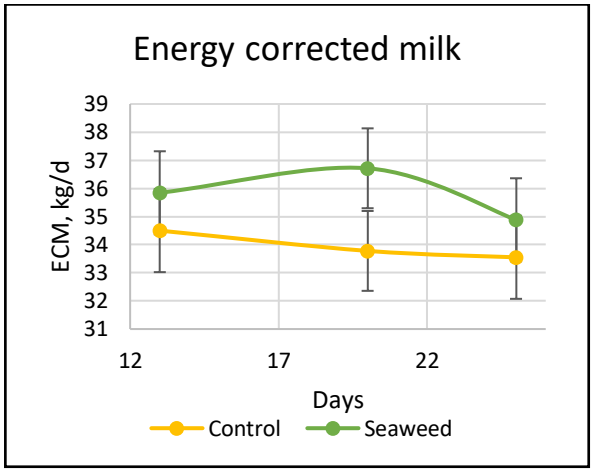

Figure 13: Yield of energy corrected milk (ECM, kg/d) for the experimental diets on sampling days 13, 20 and 25.

4.3.2 Cell count

The somatic cell count (SCC) was not different between treatments (P = 0.444; Table 6). The relationship of SCC analysis done by a DeLaval cell counter (DCC) and TINE FTIR is presented in Figure 14.

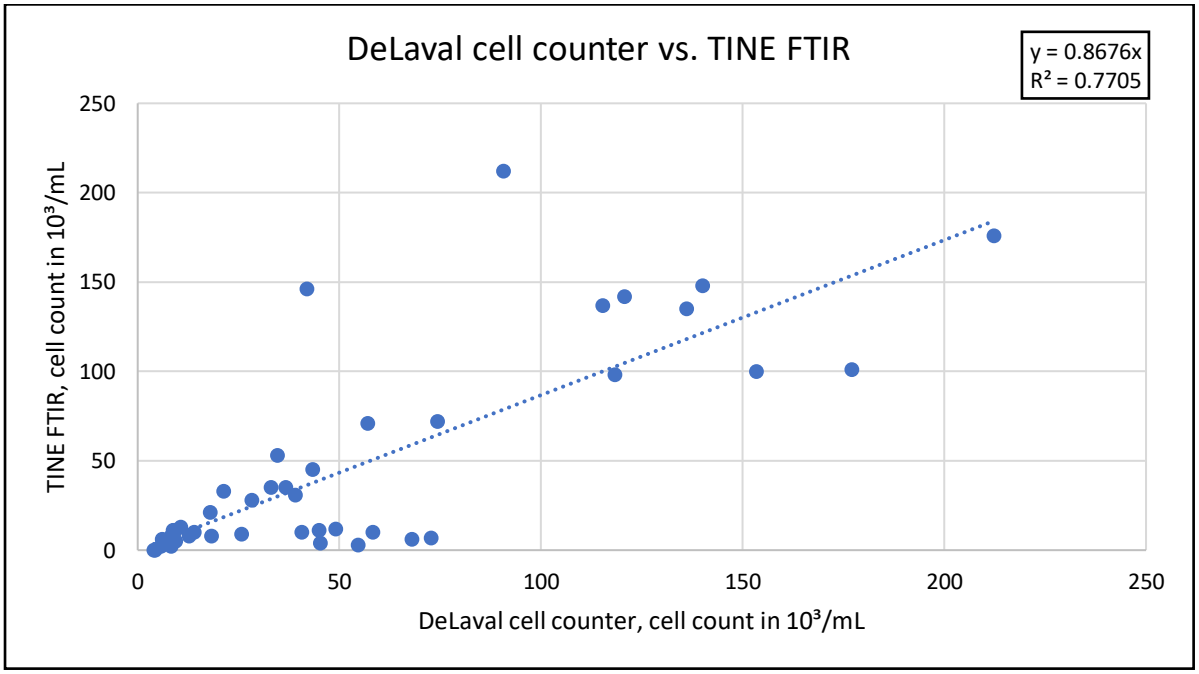

Figure 14: Plotted values of somatic cell count determined by TINE FTIR and DeLaval cell counter.

The coefficient of determination (R^2) is 0.77 for these results and shows a quite good accuracy for the low values, but a larger variance in the higher values.

4.3.3 Sensory analysis

The results from the sensory analysis revealed all samples as normal milk with no deviations in either smell or taste. Samples from cow 6610 were commented as a little salty, although they received the same score as the other samples.

4.4 Partitioning of iodine and arsenic

The results of the mineral analysis of feed, faeces, urine and milk are illustrated in Table 7 and Figure 4.9.

Table 7: Intake and excretion of iodine and arsenic for the experimental diets.

	Experimental diets[1]		SEM	*P*-value
	CON	**SW**		
Intake (mg/d)				
Iodine	37.3	366.86	9.33	<0.001
Arsenic	1.07	15.42	0.39	<0.001
Excreted in faeces				
I (mg/kg)[3]	3.85	32.56	1.32	<0.001
As (mg/kg)[3]	0.092	1.11	0.024	<0.001
Iodine (%)[2]	45.29	36.86	3.38	0.0797
Arsenic (%)[2]	37.57	29.83	1.89	0.0351
I (mg/d)	17.06	133.81	5.3	<0.001
As (mg/d)	0.41	4.56	0.11	<0.001
Secreted in milk				
I (mg/kg)[3]	0.136	0.903	0.11	0.0032
As (µg/kg)[3]	0.20	0.78	0.025	<0.001
Iodine (%)[2]	11.62	8.92	4.33	0.0065
Arsenic (%)[2]	0.57	0.18	0.032	0.0004
Iodine (mg/d)	4.52	30.2	4.33	0.0065
Arsenic (µg/d)	6.45	25.4	0.64	<0.001
Residual[4] (%)				
Iodine[2]	43.09	54.22	4.06	0.0174
Arsenic[2]	61.86	69.99	1.89	0.0299
Urine				
Iodine (mg/kg)[3]	0.35	2.55	0.22	0.0007
Arsenic (mg/kg)[3]	0.0056	0.127	0.0053	<0.001

[1]CON = control diet; SW = seaweed diet.

[2]Expressed as % of ingested iodine and arsenic.

[3]Analyzed results.

[4]Proportion of ingested mineral not recovered in faeces, milk or urine.

Daily intake of I and As was significantly higher for the SW diet than the CON diet ($P < 0.001$; Table 7). Similarly, the excreted amounts of I and As in milk and faeces were higher for the SW than the CON diet ($P \leq 0.0065$). However, the percentage of amount ingested minerals secreted in milk and feces were either significantly higher or tended to be higher in CON than SW ($P \leq 0.0797$; Table 7; Figure 15). The residual part (assumed to end up in urine or absorbed in body tissue) of I and As were significantly higher for the SW diet than the CON diet ($P \leq 0.0299$). The concentration of these minerals in urine was higher for SW than CON diet ($P < 0.001$).

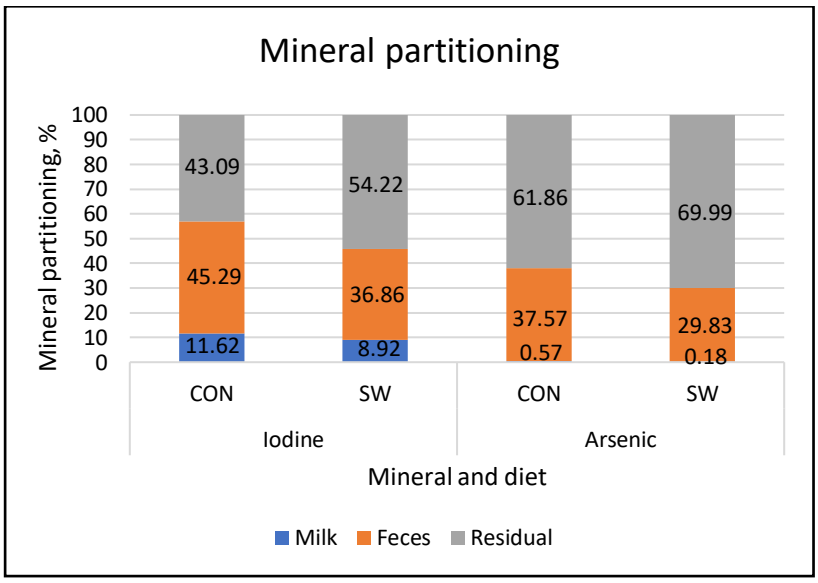

Figure 15: Mineral partitioning of iodine and arsenic from experimental diets (CON = control diet; SW = seaweed diet).

Less than 1 % of the arsenic intake was excreted in milk, and a large amount was found in urine.

5 Discussion

This study was conducted to investigate the effects of replacing 1 % of grass silage DM in a dairy cow ration with the brown macroalgae *S. latissima* on milk production, feed intake, nutrient digestibility, and mineral partitioning.

5.1 Effects on milk parameters

Inclusion of 1 % *S. latissima* resulted in a significant increased yield of both daily milk and ECM. When comparing the daily MY for the two experimental periods, the yield in Period 1 was significantly higher than in Period 2. At the start of Period 1 and 2, the cows were averaging at 50 and 119 DIM, respectively. According to a standard lactation curve (McDonald, 2011), cows are expected to be in top lactation around 35 DIM, which means that these cows had already reached the maximum yield. Lee et al. (2005) described that cows fed 4 % brown seaweed had an increased MY of 11.1 % during the 90 days of experiment while cows in the control group had a decreased MY, indicating that the seaweed diet stimulated MY. This counteracted the formerly described decline in MY related to DIM. This was also observed in dairy cows by Singh et al. (2017) and in a previous pilot experiment with lactating goats at our department (Foods of Norway (FoN), unpublished results, Mydland L. T., personal communication. Main results are partially shown in supplementary Table 1 in Appendix; hereafter referred to as 'FoN experiment, 2018'), where a significantly higher MY was achieved with the addition of seaweed in the diet for both species.

An increased fat concentration and no effects in milk protein and lactose concentration in the SW diet contradicts Lee et al. (2005) who observed a reduction in fat and protein contents but obtained a slight increase in lactose content for Holstein cows fed 4 % seaweed diet. Similarly, in the FoN experiment on goats in 2018, a numerically lower concentration of milk fat and protein, and significantly lower lactose was observed for the seaweed diet compared to the basal diet (Supplementary table 1 in Appendix). However, due to the increase in milk yield, the total daily yield of fat, protein and lactose was higher for the seaweed diet, which is partially in agreement with my study where daily yield of fat and protein was greater for the SW diet than for the CON diet. In another study with Icelandic cows, Newton et al. (2021) observed a decrease in milk protein concentration for the group fed seaweed compared to the control.

The increased ECM yield for the SW diet in my experiment can be attributed to increased milk yield and fat concentration as milk fat contributes with the highest factor in calculating ECM. Since acetate is the main driver of *de novo* fat synthesis, the increased concentration of milk fat in the SW diet could be explained by an increased NDF digestibility and acetate production in the rumen (Sjaastad et al., 2016). However, NDF digestibility and rumen fermentation products were not shown to be affected differently between the experimental diets. The effects of dietary treatments on rumen fermentation patterns are discussed more in detail in section 5.3.

The sensory analysis of the milk did not reveal any deviating taste for the SW diet, which is in agreement with Lee et al. (2005). A salty flavor in two milk samples from cow nr. 6610 was found, which could be attributed to the high DMI and the low MY of this cow. According to McDonald (2011) a reduced milk yield could lead to changes in the chemical composition of the milk with lowered levels of potassium and lactose, and raised concentrations of sodium and chloride. This was supported by the milk analysis for cow nr. 6610, showing the lowest concentrations of milk lactose and the highest concentration of sodium in milk ranging from 0.48 to 0.57 g/kg compared to 0.31 to 0.42 g/kg in the other cows (data not shown).

The milk samples used for analysis were samples from days 13, 20 and 25 in the experimental period. Originally results from day 27 were also analyzed, but these were excluded from the data set due to large deviations in MY and DMI in several cows, affecting the results for the whole experiment. This may have been due to sampling disturbances and stress from the rumen and blood (data not shown) sampling on day 27.

5.2 Effects on feed intake and digestibility

In this study, a significant increase in DMI was observed when the cows were fed a diet including seaweed. The increase in DMI partially concurs with Antaya et al. (2019), observing a tendency for increased DMI without any change in MY for cows fed a brown seaweed diet. However, it contradicts observations by Lee et al. (2005), Singh et al. (2017) and the FoN experiment (2018) of no change in DMI but a significant increase in milk yield for the seaweed diet, which indicates that animals increased their feed efficiency with seaweed inclusion. The present study, on the other hand, saw cows producing the same amount of milk per kg ingested DM, indicating no change in feed efficiency.

The significantly higher DMI in Period 1 compared to Period 2 could have been due to higher energy requirement in early lactation. The feed intake capacity after calving is lower than the energy requirement, and the capacity is not at its maximum until ten weeks into lactation (Volden et al., 2011; Zom et al., 2012). At the beginning of Period 1, the cows were averaging 50 DIM and had a high MY. A high energy demand in early lactation could explain the significantly higher DMI in Period 1 compared to Period 2.

The varying DMI in Period 1 (Figure 10B) may be explained by the methane measurements conducted with an Automated Head-Chamber System (GreenFeed, C-lock, Inc., Rapid City, SD) at day 18 and 19 in Period 1. Here, small portions of concentrate were offered, and this seems to have affected the intake of TMR, as a clear reduction is visible in the graph for DMI even when corrected for the concentrate intake. Concentrate is more energy dense than TMR and could lead to a substituting effect on the feed intake. Although the methane measurements do not take very long time, the cows are disturbed in their daily feeding and resting regimens. Thus, these measurements might have affected DMI mean estimates at these days for both diets, as the reduction in Period 1 is reflected in DMI in both diets.

The differences in nutrient intake, and observations of total tract digestibility were not reported in other studies. Singh et al. (2017) observed no significant change in nutrient intake or digestibility when the animals were fed a control or a seaweed diet ($P > 0.05$), but Antaya et al. (2019) observed a tendency to a higher intake of OM, NDF and CP for cows fed TMR with kelp meal (*A. nodosum*) compared to the control diet, although there was no change in total tract digestibility.

The digestibility of organic matter (OMD) and NDFom for both diets were above 80 %, which is a quite high digestibility degree. However, the grass silage was an early first cut, and the inclusion of concentrate in the TMR was quite high. Garmo et al. (2008) studied the digestibility of grass silage consisting of timothy, meadow fescue and red clover at the ratio 64:11:25, cut at an early and late stage at different lengths, and the early cut had an OMD of around 80 %, for all cutting lengths. A combination of a possibly increased palatability and a high digestibility of the feed could be reasons for the increase in DMI. Another factor which could influence the digestibility results is the use of AIA as a marker. When using AIA from grab samples as a marker for digestibility, AIA in feed is suggested to be over 7.5 g/kg DM (Sales & Janssens, 2003). In this study, the AIA was 4.74 and 4.16 g/kg DM for the CON and

the SW diet, respectively. This could contribute to inaccurate measurements of digestibility with this method compared to total collection. The review from Sales and Janssens (2003) pointed out that grab samples should be taken over several days, especially when the diet included a low AIA content, and this was secured by sampling at different time points over four days.

The chemical composition of the diets seems to be slightly different (Table 3). The five different TMR batches per diet were mixed before analyses. However, there is a possibility of incomplete mixing of concentrate into the silage for certain batches, which can cause unrepresentative samplings for analyses. The analyzed triplicate samples of the CON diet had a lower starch content and significantly higher NDFom content (P = 0.0024) than the SW diet. This points towards a different TMR composition, as the chemical composition would not be that different just from including 1 % of sugar kelp. The iodine and arsenic content in the SW diet was as expected significantly higher than in the CON diet, although the analyzed result of arsenic seems a little higher (0.67 mg/kg DM) than expected when only including 1 % sugar kelp. However, both TMRs were well below the maximum arsenic content of 2 mg/kg feed (relative to a feed ingredient with a DM of 88 %) (EU, 2013).

According to former studies, the usual nitrogen-to-protein factor of 6.25 could lead to an overestimation of the protein content in almost all seaweeds, because a large proportion of the N originates from non-protein nitrogen (NPN)(Gaillard et al., 2018; Mišurcová, 2012). Lourenço et al. (2002) presents the conversion factor of 5.38 for nitrogen-to-protein in brown algae, based on analysis of the true amino acid composition in 19 seaweeds, while Angell et al. (2016) suggests a factor of five after reviewing 103 species across 44 studies on this exact topic. The NDFom analysis result was very high for *S. latissima,* and it was suspected to be a problem in this analysis due to gel formation of alginate inside the NDF bags that might trap proteins inside the bags. Thus, the NDF residue was also analyzed for nitrogen, and the value of NDFom value in Table 3.2 was corrected for CP using the recommended nitrogen-to-protein factor for seaweed of 5 (Angell et al., 2016).

5.3 Effects on rumen parameters

The analyzed rumen samples showed no significant differences for rumen fermentation products between the two diets, which differs from Antaya et al. (2019) who observed a

reduction in butyrate concentration, and the FoN experiment (2018) which obtained a significant higher concentration of valerate in the goats fed seaweed.

The pH values in the rumen fluid from my experiment appeared to be high. Rumen fluid samples were taken about four hours post feeding, although in order to get a good picture of the rumen environment throughout a day, multiple samples should have been taken with set intervals over 24 hours. When feeding a TMR diet twice a day, the rumen environment will not be affected as much as by separate feeding with grass silage and concentrate, and one sample five to eight hours after feeding should be enough as long as it is taken at a representative position for the whole rumen environment (Nordlund & Garrett, 1994). However, the rumen fermentation is most intense two to four hours after feeding where usually rumen pH is the lowest and VFA production is the highest (Sjaastad et al., 2016).

Moreover, as described in section 5.1, a high fat concentration in milk in the SW diet can often be explained by a high acetate concentration in the rumen fluid, but this was not observed in the SW group in the present study.

The exact reason for above discrepancies and deviations from previous studies is unknown, however this could be linked to the method used to take rumen fluid samples. Due to the hard fiber mat and variable tube positioning in the rumen, rumen samples taken with an esophageal tube may not be representable for the rumen environment, as the rumen fermentation is usually higher in medial rumen (Schären et al., 2016). In addition, as the top layer contains mostly large particles and saliva, the rumen samples could be contaminated with buffer substances when taken with an esophageal tube rather than through a rumen fistula or by rumenocentesis. Steiner et al. (2015) discussed and reviewed these sampling methods using different kinds of stomach tubes and rumen fistula sampling. These authors reported that the contamination with saliva was low, and that pH values of esophageal tubes samples tended to be higher than the rumen fistula samples (mean differences ranging between -0.02 and +0.09 compared to samples taken from rumen fistulas). A comparison of the rumen sampling methods with a stomach tube or by rumenocentesis done by Nordlund and Garrett (1994) showed on average 1.1 pH unit higher when using a stomach tube. Thus, rumen fluid sampling could have been more accurate for observing dietary effects in rumen fermentation if rumen fistulated animals had been used in the present study.

5.4 Effects on iodine and arsenic partitioning

Due to the high mineral content in seaweed, arsenic in particular, the inclusion of *S. latissima* in the SW diet was set to 1 % on DM basis. As the EU regulations have a legal threshold of 40 mg/kg As per 88 % DM of seaweed (EU, 2013), the analyzed content at 43.8 mg was just within acceptable values when taken the uncertainty of the analysis (± 10 %) into account.

A large amount of the ingested minerals ended up in the residual part, which is intake corrected for feces and milk, as the amount assumed to end up in urine or absorbed in body tissue. The concentration of I and As in urine was significantly higher for the SW diet, indicating that a major part of an excessive mineral intake is excreted in urine. The iodine secretion to milk was 11.6 and 8.9 % of intake for the CON and the SW diet, respectively, which is considerable lower than reported by Newton et al. (2021), with 59 and 38 % for the control and seaweed diet. The proportion of arsenic secreted to milk for the CON diet was higher, but for the SW diet it was in line with Newton et al. (2021) (0.19 %).

The results of this study were comparable to the results from the FoN experiment (2018) with < 1 % of arsenic ending up in the milk, though a lot less iodine was secreted to milk in our study. The concentrate used in this study (FORMEL Favør 80, FKA) contained 15-18 % rapeseed and rapeseed-based products (FKF, personal communication), and this could reduce the iodine transfer to milk (Trøan et al., 2018). However, the animals may also regulate mineral partitioning by themselves i.e., absorbing the necessary amount in the body and excreting the excessive amounts if intake is high (Hudson, 2007).

The inclusion of rape seed in ruminant diets have increased in the last years, resulting in a linear decrease of milk iodine content (Trøan et al., 2018). This have led to a large part of the Norwegian population, particularly young women that eat less fish and drink less milk, having an iodine deficiency (Abel et al., 2018; Dierick et al., 2009). One thing to consider if seaweed is included in a ruminant diet, iodine should be reduced or removed from the concentrate being used, in order to avoid an excessive amount ingested. If the iodine content is too high, rapeseed could be added to reduce the transfer to milk (Trøan et al., 2018).

There may be other negative effects of the high mineral content, and the high secretion rate in milk. A lot of the minerals from the feed ends up in the feces or urine, but a significant high amount in the feed will also give an effect on the animal products that end up for human

consumption. The amount excreted in feces and urine is a short-term favorable effect as it is not retained in the animal body, but this will in time end up as a fertilizer in the field and may thereafter accumulate in animal feed or in crops and animal-derived food for human consumption.

6 Conclusion and future perspectives

This study confirms that inclusion of 1 % of the brown seaweed *S. latissima* in the ration of dairy cows can increase the milk yield and energy corrected milk. However, the seaweed inclusion gave a higher DMI than the control diet resulting in no difference in the feed efficiency between the two diets. The concentration of fat in milk and daily yield of fat and protein did also increase for the seaweed diet, but as the experimental period only lasted for four weeks, these results may not reflect the whole lactation period. Indeed, findings in this thesis need further investigations.

This thesis indicates that inclusion of *S. latissima* have given positive effects. My MSc study is a part of a larger project, where samples of blood were also taken, but not analyzed due to time constraints. These samples will be analyzed for e.g., plasma biochemistry parameters, immunoglobulins, cytokines, chemokines, acute phase proteins, and hormones such as the thyroid hormones T3 and T4, growth hormones and insulin growth (factor). This might give some explanation to the observed effects. In addition, partitioning of total tract digestion into rumen and intestinal digestion by using duodenal fistulated animals could have given more insight into dynamics of nutrient digestion and absorption along the gastrointestinal tract. Moreover, the use of AIA as a marker for digestibility and oral stomach tube for rumen fluid sampling have some limitations. Future studies should take these into consideration.

In present form, including 1-2 % seaweed in a total mixed ration could create problems when it comes to toxic substances. Thus, processing of the raw material to remove toxic substances (e.g., blanching) is important. In addition, challenges related to availability, transport and storage must be solved. The easiest and most convenient way for a farmer to include seaweed in the ration would probably be to include this in a concentrate.

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Appendix

Supplementary table 1: Main results from the lactating goat pilot experiment, unpublished results from Foods of Norway[1].

	Experimental diets		SEM	*P*-value
	Control	5 % seaweed		Diet
Milk parameters				
Milk production, kg/d				
Milk yield	1.81	2.39	0.170	0.019
ECM[2]	2.17	2.60	0.05	0.004
Milk composition, %				
Fat	5.05	4.63	0.496	0.577
Protein	4.43	3.90	0.336	0.329
Lactose	4.54	4.30	0.026	0.0026
Milk component yield, g/d				
Fat	90.1	105.9	10.75	0.145
Protein	79.3	91.5	10.68	0.235
Lactose	81.9	103.2	12.41	0.103
Rumen parameters				
NH_3-N	315.0	349.9	14.37	0.232
Total VFA[3], mmol/L	94.6	89.5	3.25	0.357
Molar proportions of VFAs, %				
Acetate	63.2	62.7	1.60	0.848
Propionate	23.2	22.8	0.41	0.603
Butyrate	9.2	9.6	0.26	0.342
Iso-butyrate	2.2	2.3	1.25	0.952
Valerate	1.1	0.7	0.10	0.028
Iso-valerate	1.2	1.3	0.17	0.595
Acetate:propionate	2.7	2.8	0.06	0.755
Mineral partitioning, % of daily intake				
Iodine				
Milk	43.3	20.1		
Feces	7.5	14.1		
Urine	22.8	54.5		
Residual	26.4	11.2		
Arsenic				
Milk	0.23	0.52		
Feces	32.4	31.3		
Urine	18.2	59.9		
Residual	49.2	8.3		

[1]Kidane, Øverland & Mydland, Seaweed to lactating goats. 2018. Foods of Norway, unpublished results (FOTS-ID: 16405).

[2]ECM = energy corrected milk

[3]VFA = volatile fatty acids

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