The Effect of Phycological Supplementation of Dairy Cow Diets on Milk Quality in European Dairy Systems

Eric E. Newton, MSc

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Abstract

Interest in phycological feeding within the context of animal science has recently exploded in popularity due to an increasing awareness of a needed exploitation of aquatic resources to maintain output while increasing sustainability within the livestock sector. Animal-sourced foods, specifically milk and dairy products within Europe, represent a vector for essential nutrition for much of the world and increased or maintenance of quality within product is required for increased public health outcomes derived from consumption. This highlights a gap in research, wherein study of how the feeding of phycological matter affects the resulting quality of milk is needed to maintain or improve public nutrition and increase the sustainability of the European livestock sector in doing so. Four studies demonstrated the effect that differing amounts of diet supplementation (in the use of macroalgae) or protein replacement (in the use of microalgae) has upon the resulting milk quality, yield, pathogen resilience, and haematological indicators of the animal. Study 1 examined the effect of feeding Holstein cows 330 g/d *Ascophyllum nodosum* on DMI, milk yield and composition, and quantified the effect on animal haematological parameters. Studies 2 and 3 questioned the effect of feeding Icelandic cattle a mixture of *Laminara digitata* and *Ascophyllum nodosum* on milk yield and composition, and then performed microbiological analysis to judge product resiliency due to feeding seaweed. Finally, study 4 investigated the effect that replacing diets of Finnish Ayrshire cows with microalgae *Spirulina platensis* at varying rates had on milk composition along with mineral fate through analysis of biofluids included along with milk faeces and plasma. Overall, the feeding of phycological material to dairy cows at reported rates tends to slightly lower milk protein. There were no indications of abnormal rates of feed refusal, and thus there were no differences in milk yield. Microalgae also did not indicate any effect on milk mineral concentrations. Strikingly though, dairy cattle fed macroalgae had among other mineral concentrations that were raised, but not clinically relevant, vastly increased concentrations of iodine in their milk. This was shown to be dose-dependent, and therefore it would be reasonable to conclude that macroalgal feeding to dairy cattle could increase mineral milk quality in terms of iodine concentration in a controlled manner. High concentrations of iodine within the resulting milk due to phycological feeding was found to indicate possible pathogen resilience qualities that could be exploited for the production of milk within vulnerable contexts.

Declaration of Original Authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

ERIC E. NEWTON

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List of Abbreviations

18:1c9	oleic acid
ADF	acid detergent fibre
AI	adequate intake
ALG	*Spirulina platensis* diet
ANIBES	Anthropometric data, macronutrients and micronutrients intake, practice of physical activity, socioeconomic data and lifestyles in Spain
ANOVA	analysis of variance
As	arsenic
AURA	*aurantiochytrium limacinum*
AVG	average value
Ca	calcium
Cd	cadmium
Chao1	Chao richness estimator
CHP	conventional high-pasture
CLP	conventional low-pasture
CON	control diet
CP	crude protein
CRM	certified reference material
CSP	conventional standard-pasture
Cu	copper
DHA	docosahexaenoic acid
DIM	days in milk
DMI	dry matter intake
DNA	deoxyribonucleic acid
ECM	energy corrected milk yield
ECMY	energy corrected milk yield
EFSA	European Food Safety Authority
ESL	extended shelf-life
F:C	forage:concentrate
FFA	free fatty acids
G-	gram-negative
G+	gram-positive
GHG	greenhouse gas

Hg	mercury
HSW	high seaweed
HTST	high temperature, short time
I	iodine
ICP-MS	inductively coupled plasma mass spectrometry
iNDF	indigestible neutral detergent fibre
IQ	Intelligence Quotient
IRTA	Institute of Agrifood Research and Technology
K	potassium
LMIC	lower to middle income countries
LOQ	limits of quantification
LSW	low seaweed
LW	live weight
MAP	marine algae powder
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
Mg	magnesium
Mn	manganese
Mo	molybdenum
MUFA	monounsaturated fat
Na	sodium
NCDG	neutral detergent cellulase digestible organic matter
NDF	neutral detergent fibre
Ni	Nickel
NMKL	Nordic-Baltic Committee on Food Analysis
NNR	Nordic Nutritional Recommendations
OTU	operational taxonomic units
P	phosphorus
Pb	lead
PLT	platelet count
PMV	mean platelet volume
PUFA	polyunsaturated fat
RBC	red blood cells
RDI	recommended daily intake
RDM	raw dairy milk

RDW-CV	red blood cells distribution width
RNA	ribonucleic acid
RNI	recommended nutritional intake
RSAL	mixture of rapeseed and *Spirulina platensis* diet
RSS	pelleted rapeseed supplement diet
S	sulphur
SCC	somatic cell count
SCP	single cell protein
SD	standard deviation
SE	standard error
Se	selenium
SF	saturated fat
Sn	tin
SNF	solids-not-fat
SWD	seaweed diet
TF	trans fat
TMR	total mixed ration
UHMI	ultra-high matrix introduction
UHT	ultra-high temperature
UK	United Kingdom
UL	upper limit
USA	United States of America
WSC	water soluble carbohydrates
Zn	Zinc

Chapter 1: Introduction

Key findings from the United Nations Department of Economic and Social Affairs recently published in the World Population Prospects 2022 report that the world's population could reach 9.7 billion individuals in 2050 up from ~8 billion people at time of publication (UN, 2022). To add, half of this 2 billion person rise can be expected to take place in sub-Saharan Africa, and to further drive home this point, the countries that account for the 47 least developed will be expected to individually be the fastest growing, with many of their own populations projected to double in 2050 (UN, 2022). The rapid rise in more mouths to feed requires further land use, as in 3 different scenarios using 6 different models, within Stehfest et al. (2019) indicates a rise in cropland production, area, and in pasture utilization in some models with a "sustainable" solution being instituted. A rising amount of land needed as a function of an increasing population is compounded with and a contributor to the challenge of climate change. A loss of forests and the following degradation of soil and water quality to make room for pasture to continue to supply demand is a serious problem, as roughly 49% of the earth's ice-free land surface is used for intensive pasture, used savannahs and shrublands, extensive pasture, along with irrigated and non-irrigated croplands. As a result, it is estimated that 23% of all total anthropogenic greenhouse gas (GHG) emissions can be attributed to agriculture, forestry, and other land use (IPCC, 2019). Furthermore, of all animals used at an industrial scale, cattle in both beef and milk commodities total the highest contributor to emissions within animal agriculture (5.0 gigatonnes CO_2-eq total, 62% of animal agriculture emissions), with cattle milk specifically accounting for 1.6 gigatonnes of CO_2-eq (FAO, 2021b). Of the largest total contributors regionally, cattle milk as a commodity makes up for a significant portion of emissions in Western Europe (~30%), Eastern Europe (~30%), and Russia (~45%). (FAO, 2021b). Therefore, it is imperative to search for feed alternatives in the process of animal agriculture, specifically within the dairy industry, to not only increase sustainability via a reduction in land use and subsequent carryover effects in terms of GHG emissions, but to ensure that dairy quality is either maintained or better, improved, in terms of milk composition, microbiological hygiene and resiliency, and nutritional content.

Modern cattle are a product of human guided evolution with evidence of domestication as far back as 8,800 BC, which played a crucial part in our agricultural developmental history (Ajmone-Marsan, Garcia, & Lenstra, 2010). Even today, recent estimates number the amount of cattle at 1.43 billion, with almost 1 billion people directly affected in terms of livelihoods and food security by global livestock (Robinson et al., 2014). Cattle are used in some developing countries as draft animals, but generally these animals are more valued for products of more widespread industrial agricultural applicability, mainly for their hide, meat, and milk. While current annual production of bovine hides and skins numbers at near 6.5 million tons, and current production of beef numbers at 68 million tons, this literature review will feature bovine milk due to its importance in economic value, source of livelihoods, and nutritional importance (Mascianà, 2015; Ritchie, 2017). Animal milk has been utilized since the Neolithic era in UK and Northern Europe as far back as 4,000 BC with evidence of a direct influence on genetic traits such as lactase persistence found in high frequency in humans with ancestry from these exact areas (Ségurel and Bon, 2017). With 150 million households in the world currently participating in some manner in milk production, recent production totals to around 843 million tons per year (FAO, 2021).

While the advent of dairy farming has been extremely advantageous to human agricultural and nutritional development, there are certainly logistical problems within the current paradigm that requires streamlining. Rapid intensification over the most recent 50 years has led to negative impacts on the environment such as climate change causing GHGs, biodiversity loss due to forestry removal to make way for pasture, and the fact that to support the entire infrastructure of dairy farming, this requires a large devotion of a finite resource, land (Clay, Garnett, & Lorimer, 2020). To reduce the environmental impact of dairy farming, increase production and the quality of the product, and improve animal health, more sustainable alternative feeds have been increasingly considered (Halmemies-Beauchet-Filleau et al., 2018). Potential marine-based organisms such as algae have been under investigation to serve as a potential feed alternative for dairy cattle given the to-be-mentioned application in a variety of benefits to reduce the drawbacks to industrial agricultural cow's milk production (Abbott et al., 2020; M. Lamminen et al., 2019). Landmark studies examining the supplementation of algae have recently been published such as Roque et al. (2021) which reduced methane emissions in beef cattle by a significant margin using macroalgae and Lamminen et al. (2019) which reports the ability to utilize microalgae as an alternative protein feed. Recent work has mainly pertained to the effect that feeding algae may have on the animal and associated output of methane – but this begs the question, if the future holds the supplementation or outright replacement of animal feeds towards more sustainable options such as algae, how does this then affect the safety and nutritiousness of the milk from these animals?

Given that milk is critical to delivering nutrition around the world, it can be used as a tool to deliver food security to at-risk populations. In order for this to be used as an effective tool that can be legislated, research is acquired to understand the implications of animal nutrition on the final animal-sourced product. This work aims to pursue the work of examining the viability of algae-based solutions for animal nutrition from a different perspective, one that takes into account the holistic nature of the dairy food chain, and one that hones in on public consumption and nutrition.

1.1 Milk nutritional quality and safety

Dairy, as previously mentioned, is relied upon by a significant proportion of the world for the achievement of finance and/or nutrition, as it is a large contributor to overall food security. Food security is defined as having physical and economic access to ample amounts of safe and nutritious food (FAO, 2002). Following are important characteristics of dairy that are relevant to either: (i) the compositional quality, which results in ample amounts of a food source that has a potential health benefit to the consumer; (ii) nutritional quality, to assist in fulfilling a population's dietary needs; (iii) microbiological quality, which is required for food safety, and thus overall food security.

1.1.1 Basic milk composition

Traits, such as fat, protein, lactose, and somatic cell count (SCC), are the key intrinsic properties to milk composition (Roy et al., 2020; Talukder & Ahmed, 2017).

Three types of fatty acids exist in the form of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Liu et al., 2017). These types of fats are defined by the chemical bonds that exist, or do not exist within their chemical structure. SFAs have no double bonds and therefore are saturated with hydrogen (H), and MUFAs have one double bond in their carbon chain while PUFAs contain two or more bonds.

On average milk contains about 33g/L total fat, with more than half associated with SFAs (roughly 19 g/L), MUFAs (roughly 8g/L and mostly 18:1c9 (oleic acid)), and the rest composed of PUFAs (around 2g/L) (Haug, Høstmark, & Harstad, 2007). Total fat concentrations of most milk products are controlled at the processor, meaning that by the time it has reached supermarket shelves, there will be little variation within the labelled group. This means though, that the value to the processor and thus the farmer is in higher overall fat concentrations, as higher fat amounts within milk mean a higher financial gain when sold to the processor as higher fat concentrations mean a higher variety in the different types that can be prepared (Zurborg, 1978). The average contribution of milk and milk products to British SFA intake is 28%, along with 13% for MUFAs (NDNS, 2020).

Two main types of protein that are relevant to milk exist in the form of casein and whey protein, with casein making up around 80% (29.5 g/L) and whey protein making up around 20% (6.3 g/L) of an average 36g/L of total protein (Davoodi et al., 2016). Protein is essential to delivering nutrition to the consumer, with milk contributing on average around 30-40% of the Recommended Nutritional Intake (RNI) (Haug et al., 2007). Due to the amino acid composition of these proteins, consumption of milk protein has been linked to improved metabolic health along with exercise (McGregor & Poppitt, 2013).

Lactose is the main carbohydrate within milk, and while approximately 65-70% of the population of the world will experience lactose maldigestion in their lifetime (commonly subclinical), consumption provides benefits such as a metabolic advantage due to its low glycaemic index, the support of immune function, and the facilitation of mineral absorption (Romero-Velarde et al., 2019; Vaskova & Buckova, 2016). Therefore, while accommodation of individuals with a lactose intolerance can be done with the preparation of lactose-free pasteurized milk, there still contains a benefit to the population that can digest milk without a problem (Johansson et al., 2013).

Finally, while SCC might not necessarily be considered a “milk compositional factor” per se, it is a value and qualitative trait of milk that must be considered. Somatic cells are a combination of milk-producing cells and immune cells that can be influenced by several traits of the animal such as productivity, parity, lactation stage, and breed – but it is primarily used to estimate the overall health of the animal (Alhussien & Dang, 2018). Somatic cells are primarily composed of leucocytes and epithelial cells around $\leq 100x10^3$ cells/ml milk in healthy animals (Cinar et al., 2015). A rise in SCC can lead to a reduction in the commercial value along with being an indicator that cows may have underlying mastitis (Dejyong et al., 2022). This can prove to be a compounding issue, as the processor will offer much less to the farmer in terms of financial reward. Additionally, it is an indicator of poor animal health that will generally result in lower yields and a requirement for veterinary assistance, which lowers the profitability of a dairy business. Raw milk upper limit standards within the UK for SCC cite ≤400,000/ml, with the general target being that of <150,000/ml (UK Government, 1995). Additionally, typically milk values of ≤100,000/ml does not indicate any sign of subclinical mastitis, ≤200,000 will indicate at least one quarter of the udder infected, and ≤300,000 generally indicates the presence of a significant number of problems for the animal, generally pathogenic in nature (AHDB, 2023b). As a rule of thumb, every 100,000 cells/ml increase for a dairy cow herd can be as much as a 10% increase in infected animals within that group (AHDB, 2023b).

1.1.2 Milk mineral concentrations

As for the nutritional value of milk, the product has been shown to provide important macro- and micro- nutrients that are required for human health. A nutritional literature review found that milk consumption is correlated with the facilitation of weight loss (along with energy restriction), reduced risk of type 2 diabetes, reduced risk of cardiac disease, bone density, inversely associated with various types of cancers, and there seems to be very few contraindications to its use as a foodstuff (Thorning et al., 2016).

Iodine (I), a crucial nutrient delivered by bovine milk, not only provides a major iodine source in industrialized countries, but the value for Lower to Middle Income Countries (LMIC) is even higher given the dependence on recommended intakes for physical development that is much more crucial to livelihoods (Flachowsky et al., 2014). Iodine deficiency, endemic to countries centralized in Africa and South/South-East Asia, can lead to infertility, cognitive impairment, and slowed physical growth leading to disability (Adu & Simpong, 2017). In addition to relevance within LMIC’s, recent literature describing the negative developmental effects of even mild-to-moderate I deficiency is reflected in lower intelligent quotient (IQ) scores in the children of mothers who were mildly deficient while pregnant (Bath et al., 2013). A recent study found that milk and dairy contributed to as high as 64% of recommended daily intake (RDI) for I in industrialized countries (van der Reijden, Zimmermann, &

Galetti, 2017). For countries without iodized salt programs, a larger portion of an individual's consumption of iodine comes from milk, reinforced with older studies indicating 40% and 39% of I coming from dairy for 11-18 year old males and females respectively within the UK (Payling et al., 2015). A more recent dietary survey has indicated milk and dairy products to contribute 32-64% of I intake for the population of the UK (NDNS, 2020). Milk could also supply even more of a countries' population RDI for areas in which there is not as much seafood consumption, as most I intake is linked to eating either fish or dairy (Nyström et al., 2016).

Zinc (Zn) contributes in a significant portion to an average person's diet by bovine milk. Zinc is important to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, replication, and cell proliferation along with correct neurobehavioral development and proper biochemical function (Bakhshizadeh et al., 2019; Brown, Wuehler, & Peerson, 2001; Li et al., 2022; Roohani et al., 2013). Dairy has been found to make up 16% of Zn in American diets for people aged 2 years and older (Cifelli et al., 2016), and 15-35% in average British diets (NDNS, 2020). In addition, Zn RNI has been covered by up to 18% in British children 1.5-3 years of age on average throughout the year (Newton et al., 2023).

Magnesium (Mg) functions as a major cofactor for hundreds of enzymes, regulating various bodily functions such as muscle contraction and blood pressure, transmembrane transport, and neuromuscular function (Al Alawi, Majoni, & Falhammar, 2018; Costello, Wallace, & Rosanoff, 2016; Schwalfenberg & Genuis, 2017). Dairy has been found to make up 13% of Mg in American diets for people aged 2 years and older, 10-20% of Mg in average Polish diets, and 10-25% in average British diets (Cifelli et al., 2016; Górska-Warsewicz et al., 2019b). In addition, Mg RNI has been covered by up to 26% in British children 1.5-3 years of age on average throughout the year (Newton et al., 2023).

Calcium (Ca) is crucial for the development of a strong skeletal system while also playing an important role in cardiovascular function such as the regulation of heart beat, and blood clotting, along with a large host of other benefits (Cormick & Belizán, 2019; Piste, Sayaji, & Avinash, 2012). Dairy provides 50% of daily calcium intake within European populations, specifically 55% in average Polish diets, and 34-59% in average British diets (Górska-Warsewicz et al., 2019a; Marangoni et al., 2018; NDNS, 2020). In addition, Ca RNI has been covered by up to 69% in British children 1.5-3 years of age on average throughout the year (Newton et al., 2023).

Potassium, (K) is contained in significant amounts within milk and dairy, and thus low consumption rates of milk contributes heavily to non-ideal total RDI of K for populations (McGill et al., 2008; Silva & Sant'Ana, 2016). It is required for optimal cell function and is beneficial in diets in the case of cardiovascular health and renal disease suppression (Kumssa, Joy, & Broadley, 2021; Stone, Martyn, & Weaver, 2016; Weaver, 2013). Dairy has been found to make up 10-20% of K in average Polish diets, and 11-30% in average British diets (NDNS, 2020). In addition, K RNI has been covered by up to 39% in British children 1.5-3 years of age on average throughout the year (Newton et al., 2023).

1.1.3 Milk microbiology

While beneficial microbes (bacteria or fungi) can be added to create products such as yogurt or sour cream, pathogens (disease-causing microbes) can be a significant problem. Some relevant pathogens found within occurrences of an outbreak are *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* (IASRI, 2012).

Total disease burden in countries without developed surveillance from consumption of dairy products can be hard to quantify. During 2007-2012, there were 81 outbreaks of disease from consumption of non-pasteurized, raw dairy milk (RDM), resulting in 979 illnesses and 73 hospitalizations within the United States of America (USA). Generally, the agents responsible were a majority of *Campylobacter* spp. but also by *E. coli*, *Salmonella*, and *Coxiella burnetii* in smaller proportions (Mungai, Behravesh, & Gould, 2015). In 2016 an outbreak within the UK led to 56 *Campylobacter* infections, all associated with drinking RDM (Willis et al., 2018). Outbreaks have an enormous effect on stakeholders as well. Velthuis, Meuwissen, and Huirne (2009) associated the cost of a recall of one batch of milk (150,000kg) to €100,000 within the first 24 hours. A *Listeria monocytogenes* outbreak in Canada were reasoned to have cost the economy $242 million, further emphasizing that while recalls can cause issues, some pathogens such as *Clostridium*, *Listeria*, and *Salmonella* have high rates of hospitalization, drawing upon finite resources (Scallan et al., 2011; Thomas et al., 2015). Disease because of drinking contaminated milk can occur from a number of sources. These include the consumption of raw milk, or as a result of environmental introduction at some point within the dairy food supply chain. For context, in 1938, just before the outbreak of World War II, 25% of all foodborne diseases were linked to dairy consumption, whereas today this has dropped to less than 1%; and in the US, tuberculosis, which was the cause of death of 65,000 individuals between 1912 and 1937 in England and Wales, has been largely forgotten of due to milk pasteurization programs (Lucey, 2015). Even with the implementation of milk heat-treatment programs and the known risks, individuals still choose to drink raw milk.

Various techniques are used to reduce or eradicate microbes within milk. These methods can include pasteurization, thermization, extended shelf-life (ESL), ultra-high temperature (UHT), and in-container sterilization (Deeth & Lewis, 2017). Pasteurization can be done with a high temperature for a short period (typically 72°C for 15 seconds), low temperature for a longer period of time (>63°C for 30 minutes), or somewhere in between with the benchmark being that of a negative result to alkaline phosphatase after the process is undergone (Ritota et al., 2017). Thermization is not as well defined, only that heat treatment of milk is undergone below 72° (generally temperature ranges are seen as 57°C to 68°C for 10-20 seconds) which does not lead to a negative result reaction to alkaline phosphatase. Instances of thermization use typically is due to the lower heat affecting ripening and aroma of cheese made from the milk among other benefits (Eugster, 2019). ESL milk is treated in a manner that is between high temperature, short time (HTST) pasteurization and UHT with the aim of extending shelf life from 21-45 days or more, and with the addition of aseptic packaging can mean the production of commercially sterile milk (Deeth, 2017). UHT processing generally goes above 130°C for 2-10 seconds and like ESL, packaged aseptically, which leads to all microbes that are able to grow under normal storage conditions to be destroyed (Datta et al., 2002). Finally, in-container pasteurization is the process in which the final containers of milk are autoclaved at 110-120°C for 10-20 minutes, this can cause a cooked flavour and change in colour (Robertson, 2011). Of all these methods, the general standard heat treatment strategy is either pasteurization or UHT (Fatih et al., 2021).

Therefore, any method in dairy procurement that leads to a safer or more resilient product would be beneficial to both raw milk consumers (despite a consensus among food safety authorities that this is detrimental to population health) and to heat-treated milk consumers, as environmental pathogens can always be a threat, whether this is on the processing floor, during shipment to the supermarket, or in the refrigerator at home.

1.2 Factors affecting milk nutritional quality and safety

1.2.1 Factors affecting milk basic composition and mineral concentrations

1.2.1.1 Animal diet

Milk quality is dictated largely by the feeding practices that are undertaken at dairying operations. Nutrition can bring about large changes in the concentrations of milk fat (Sutton, 1989), and affect mineral transfer efficiency (the proportion of mineral included in the animal diet that is then excreted in the milk), at various extents, being particularly relevant for Ca, P, Na, S, I, and Zn (Qin et al., 2023) The two main feed categories in dairy cows are forages and concentrates (Matsushima, 1979). The ratio between forage and concentrate (forage:concentrate; F:C) is typically between 40:60 and 60:40 (Mertens, 2009). Given the range of environmental conditions, different types of forages, external individual pressures on markets, world events, different breeds, quality metrics that are pursued and a large list of other factors, "popularity" of different species of forages are incredibly diverse (AHDB, 2023a; Loza et al., 2021). Thus, this generally comes down to individual choice, mainly affected by feed availability and cost (Greig, 2009; Paul et al., 2016).

1.2.1.1.1 Type of forage

Forages can be defined as whole crops that are used by the cows to graze freely, harvested and then fed to cows in a process known as "cut-and-carry", or preserved using different techniques (drying, ensiling, pelleting). There are many types of forage, such as herbage, hay, silage, browse (leaves/twigs), or straw – and can be more specifically tropical grasses, corn, cereal, root crops, kale, etc. (Rooke, 2001). "Cut-and-carry" methods will be employed during warm periods, where individuals will reap crops and feed them to animals housed indoors (Pincay-Figueroa et al., 2016). Alternatively, an allotment of hay (dried fodder) will be distributed typically during colder months, although this is not always the case (Flores & Tracy, 2012). Hay may be used throughout the year depending on farmer preference, but is used more frequently during the colder months, in which forage crops do not grow as much or at all (Flores & Tracy, 2012). Given that dairying is a business, a farmer must reduce the cost of the operation by limiting the expense of feed, whilst ensuring that the milk quality is deserving of a financial return and encouraging future profits such as maintaining animal health (Dynes, Henry, & Masters, 2003). Additionally, as forage tends to be the cheapest way of providing energy to the cow by cost per calorie, it can pressure the operation to maximize the amount of forage delivered while still maintaining beneficial aspects of dairying.

Feeding different types of forages to dairy cows can alter milk composition. Specifically, Coulon, Pradel, and Verdier (1995) found that the feeding of either ryegrass silage, cocksfoot hay, or corn silage had a significant effect on milk yield (17.0kg/d to 18.9kg/d), fat concentrations (39.7g/kg to 41.4g/kg), and protein concentrations (31.3g/kg to 32.2g/kg) when comparing the extreme measurements between treatments. Additionally, E. Manzocchi et al. (2020) found that feeding either hay, grass silage, or differing lengths of corn silage did not significantly affect milk yield, protein, or SCC, but did significantly affect milk fat concentrations (41.1g/kg to 43.2g/kg) and lactose concentrations (47.2g/kg to 47.9g/kg). Finally, Vanbergue, Peyraud, Ferlay, et al. (2018) highlighted the differences between not only corn or grass forage, but also at different amounts – showing a change in milk yield (27.8kg/d to 34.4kg/d), fat content (3.31% to 3.76%), protein content (2.89% to 3.07%), and lactose (4.91% to 5.01%) when comparing the extreme measurements between treatments. While there is considerable data behind the variation that a change in forage can offer in terms of milk composition, Sutton (1989) claims that fat concentrations within milk tend to be the most sensitive to dietary influences. These are just but a few individual studies examining the effect of differing forages on milk basic composition – as it is an enormous topic that is fundamental and relatively well-studied (National Research Council, 1988).

Feeding different types of forages to dairy cows can also alter milk mineral concentrations. As previously mentioned, some minerals can transcend through the feed into the milk. This transfer efficiency rate can be affected by; (i) the type and quantity of foodstuff consumed and (ii) physiological interactions between bodily processes and the content of the feed within the cow (Qin et al., 2021; Qin et al., 2023). The overall mineral content of forages can differ greatly (Wilson et al., 2011). Fahey and Spears (1994) show differences between four forages: legume forage, mixed and mainly legume, grass forage, and mixed mainly grass. There were notable differences in the forage mineral amounts, indicating concentrations of K (16.8g/kg DM to 22.6g/kg DM), Ca (4.9g/kg DM to 11.8g/kg DM), Mn (44.1mg/kg DM to 76.4mg/kg DM), Fe (184.4mg/kg DM to 221.7mg/kg DM), and Zn (18.1mg/kg DM to 27.6mg/kg DM) when comparing the extremes within the sample set (Adams, 1975). The concentrations of total plant minerals within tall fescue leaf compared to lucerne leaf, for example, shown in Whitehead, Goulden, and Hartley (1985) indicate a substantial difference found in Ca (0.9% vs 2.7%), S (0.4% vs 0.6%), and K (2.4% vs 1.3%), respectively. Finally, Stergiadis et al. (2021) analysed milk mineral concentrations when cows were fed high-pasture (CHP), standard-pasture (CSP), and low-pasture (CLP), finding Ca to differ from 966 mg/kg and 1016 mg/kg in CSP vs CHP, and P from 745mg/kg to 804mg/kg in CSP vs CLP.

1.2.1.1.2 Type of concentrate

Concentrates can be defined as feeds that are high in protein (proteinaceous), or high in energy (carbonaceous) and are much lower in dietary fibre (FAO, 2022a). They provide the second half to a well-rounded animal's diet and can be arguably more diverse than forages as farmers not only can target specific nutrients they want to maximize with a specific crop, but they may utilize by-products from operations adjacent to their dairying business to reduce expenses while still providing what is needed dietarily for the animal (Sandström et al., 2022). An arguable portion of what is fed as "concentrates" can be alternative supplementation material which can be region-specific (van Hal et al., 2019).

Feeding different types of concentrates can alter milk quality in terms of milk composition. McKay et al. (2019) showed that along with pasture as a control, and utilizing two different other treatments of barley or corn-based concentrate supplementation, milk yield (16.8kg/d to 18.2kg/d), fat (0.85kg/d to 0.93kg/d, protein (0.65kg/d to 0.73kg/d), milk solids (1.53kg/d to 1.68kg/d), and lactose

(0.75kg/d to 0.81kg/d) were significantly affected. Additionally, Huhtanen (1993) found that when cows were fed different energy sources of concentrates, either a barley or fibre-based diet, protein (31.9g/kg vs 31.0g/kg), and lactose yield (1208g vs 1285g) were significantly affected and fat (41.4g/kg vs 40.0g/kg) was near significance. Finally, Johansson et al. (2013) found that the feeding of two types of concentrate, one being based on protein supplements and cereals, and the other being based on only cereals significantly affected milk fat (3.77% vs 4.47%) and protein (3.03% vs 3.18%), as well as C18:2n-6 of total fatty acids (1.73% vs 1.39%), C18:3n-3 (0.85% vs 0.75%), and C18:2c9*t*11 (0.75% vs 0.49%) significantly. These are just but a few individual studies examining the effect of differing concentrates on milk basic composition – as it is an enormous topic that is fundamental and relatively well-studied (National Research Council, 1988).

Feeding different types of concentrates to dairy cows can also alter milk mineral concentrations, as found in Qin et al. (2023) which showed a reduction from 526 ug/kg to 382 ug/kg for I, and a reduction from 31.8 ug/kg to 28.2 ug/kg for Mn when cows were fed dark distillers' grains diets and rapeseed meal diets respectively. Additionally, Dunshea et al. (2019) indicates that the amount of dietary concentrates will affect milk mineral concentrations for Ca, Mg, P, and Se.

1.2.1.2 Diet as a function of dairying operation

As previously stated, the feeding of different types and amounts of forage and concentrate generally results in changes to milk composition. Intrinsic to bounds or criteria at which operations adhere to, multiple factors are functions of operations. Thus, a smallholder farm might choose to stock a certain breed which may result in a particular feeding strategy. Secondly, a standing of organic certified means that dairy production and thus dairy quality can be heavily altered. Thirdly, season generally dictates feeding pattern and animal intake, thus translating towards a change in composition for the resulting milk.

Cow breed can have a significant effect on milk basic composition – as Senbeta (2018) showed significant differences in fat, protein, and other factors such as casein when comparing differing cow breeds under the same management conditions. Additionally, Walsh et al. (2008) showed significant differences in fat, protein, and lactose when examining the breeds of across Holstein-Frisian, Montbéliarde, Normande, Norwegian Red and their crosses. Finally, Adesina (2012) showed differences in mean composition of milk from White Fulani, Red Bororo, and Muturu – which was significant for fat and protein.

Cow breed can have a significant effect on milk minerals – as Cerbulis and Farrell (1976) indicates numerical differences in the milk concentrations of Ca, Mg, and P between the breeds of Holstein, Jersey, Guernsey, Ayrshire, Brown Swiss, and Milking Shorthorns. Manuelian et al. (2018) indicated significant differences in the milk concentrations of Ca, Mg, K, P, and Na between the breeds of Holstein-Friesian, Brown Swiss, Jersey, Simmental, and Alpine Grey. Nantapo and Muchenje (2013) indicated significant differences in the milk concentrations of Ca, P, Mg, and Al between the breeds of Jersey, Friesian, and Jersey x Friesian. Qin et al. (2021) produced a redundancy analysis (RDA) depicting non-Holstein genetics directly negatively associated with milk concentrations of Al, Zn, Sn, Cu, and Fe to a slight degree. This was reinforced with an RDA from Stergiadis et al. (2021) also depicting non-Holstein genetics negatively associated with milk concentrations of Al, Sn, Cu, and Fe. Finally, Lim et al. (2020) indicated significant differences in the milk concentrations of K and Zn between the breeds of Jersey and Holstein.

Dairy production system can have a significant effect on milk basic composition – as Stergiadis et al. (2019) indicated an increase in organic systems compared to conventional or free-range systems for fat, but the opposite for lactose, wherein conventional and free-range systems were significantly higher than organic branded milk from retail outlets in the UK. Within-organic system variation depending on pastural intake also can lead to significant milk basic compositional differences, with fat and protein both higher within organic high-pasture feeding farms compared to organic low-pasture feeding farms (Stergiadis et al., 2021). Finally, Newton et al. (2023) indicated higher amounts of fat and lower amounts of lactose in retail milk from organic systems compared to conventional systems.

Dairy production system can have a significant effect on milk minerals – as Newton et al. (2023) indicated lower amounts of Fe and Mo in conventional systems compared to organic systems within retail milk as an average across the year. Additionally, Stergiadis et al. (2021) showed within-system differences in milk minerals, as standard-pasture feeding within conventional systems has been shown to have decreased Ca and P compared to high- or low-pasture feeding farms, yet within organic systems, P is no different and Ca is decreased within low-pasture feeding farms, and there tends to be higher Sn in high-pasture intake. Finally, Manuelian et al. (2022) indicated differences in system and month for milk minerals such as Fe, K, Mg, and S at varying points throughout the year.

Farming season can have a significant effect on milk basic composition – as Li, Ye, & Singh (2019) indicated differences in milk fat, protein, lactose, and SCC within-years, and for protein and SCC there was significances in difference between years. Additionally, Yang et al. (2013) indicated differences in milk fat, protein, lactose, and SCC depending on the month. Finally, Newton et al. (2023) indicated significances in fat, protein, casein, whey protein, lactose, and SCC across the entirety of a year in retail milk samples.

Farming season can have a significant effect on milk minerals – as Newton et al. (2023) indicated significances in Cu, Fe, I, Mn, Mo, and Zn across the year within milk samples. Additionally, Qin et al. (2021) showed a significance for all minerals that were analysed on farm, specifically, Ca, K, Mg, Na, P, Cu, Fe, I, Mn, Mo, Zn, Al, and Sn. Finally, Christophe et al. (2021), in development of methods to accurately predict the mineral concentrations of Na, Ca, Mg, P, and K, showed that there is substantial variation for these macrominerals depending on DIM.

1.2.2 Factors affecting milk microbiology

Feeding different types of feed to dairy cows can also alter milk quality in terms of the milk microbial concentrations. The differences in season largely dictates the types of forages that are consumed (Qin et al., 2021). Celano et al. (2022) shows that significant differences in the cell densities of total mesophilic aerobic, mesophilic, and thermophilic lactobacilli, as well as *Enterobacteriaceae* were found in the milk analysed by cows within the winter and summer. Coates et al. (2022) by proxy details the association of cow diets with increased fibre content leading to higher milk fat content which can be associated with higher microbial richness, but that being said – this research does indicate that the mechanisms between the feed-milk microbiota axis is poorly understood and unclear. Additionally, toxic secondary metabolites excreted by contaminated silage can permeate animal feed, undergo metabolization, and result in a harmful component of cow's milk (Newton, 2019). Finally, while some bacterial populations may have been found to be influenced by diet, Gagnon et al. (2020) found no difference in specifically lactic acid bacteria (LAB) when fed different types of forages.

1.3 Sustainability of current feeding practices in European dairy systems

System sustainability is a massive factor in the feeding of any farming system, as dairying collectives and even individual operations should seek to ensure the least amount of harm to the environment and subsequent drawback effects from their work while still maintaining output to feed nations. In particular, three factors that must be addressed when examining system sustainability are the efficiency of terrestrial land use, aquacultural/oceanic biodiversity maintenance, and greenhouse gas emissions from farming.

1.3.1 Efficiency in terrestrial land use

Within Europe, 125 million hectares of land are used in livestock grazing and production of animal feed (Levitt, 2019). Strikingly, the production sustainability of Europe is poor, with the EU importing most of its soybean requirements from megaproducers in Brazil (which exports 75 million tonnes per year (Rauw et al., 2023)). Additionally problematic is the fact that this very international production of feedstuff has untold negative consequences upon forestry and thus biodiversity along with societal implications (Fearnside, 2001). Additionally problematic is that while 75-82% of current EU animal production could be sustained without these soybean imports, there would be chiefly reduced EU pork and poultry production and lead to an additional problem, an increase in palm oil demand (Karlsson et al., 2021). This exemplifies a systemic issue in that the plant protein used in Europe is imported across large distances, leading to further emissions (Leip et al., 2010).

1.3.2 Farming greenhouse gas emissions

As clear from the introduction, the contribution of agricultural systems is large, with nearly 400Mt CO_2eq per year as recently as 2022, with 80% of that agricultural GHG emission sourced from CH_4 (enteric fermentation), and N_2O (related to soils) (EEA, 2022). Comparably even to other sectors, food production accounts for 15% of net GHG emissions within Europe (EEB, 2023). Due to the nature of the dairying industry utilising ruminants, enteric fermentation – which must take place, as it is how digestion occurs – is sourced primarily from cattle, buffaloes, goats, and sheep (Chang et al., 2019). Unfortunately, due to the rising amount of mouths to feed, and thus a push for increased production, the amount of methane emitted by livestock is directly related to levels of feed intake, which, understandably, is directly related to milk production (Oldenbroek, 1988; Ramin & Huhtanen, 2013; Winders et al., 2020). Therefore, if a more sustainable goal is to be reached, an alternative feed such as phycological matter should either maintain or better yet, reduce enteric fermentation intrinsically, as limitation of feed intake would not have to take place, leading to sustained production while reducing the environmental impact of the agricultural sector.

1.3.3 The role of alternative feeds in sustainability

Regarding these four farming sustainability points, alternative feed options such as algae production indicates viability in terms of serving as a solution to increasing or at least leading to maintenance of sustainability, and specifically, across Europe.

Algae serves as a positive path in the development of more efficiency ways to utilise terrestrial land, and improve sustainability, as this would serve as a local feed additive or whole protein replacement that lessens dependence on international feeds such as soybean. Additionally common is the non-utilisation of land that while not arable within Europe, and not useable for livestock grazing, exists in abandonment (Keenleyside & Tucker, 2010). Microalgal farming can utilise this land to be more efficient, sourcing primary production from area that wouldn't necessarily be used more efficiently.

Algae also could serve as a catalyst for increased oceanic biodiversity improvement, as mainly macroalgae has been found to serve as a strong backbone for the oceanic ecosystem in several ways. The creation of coastal habitats for a diverse home for marine creatures, along with increase manageability of seaweed farms, are some of the benefits of the protective structures of seaweed canopies that dampen wave energy (Duarte et al., 2017). Additionally important is maintenance of a stable environment for biodiversity improvement which can be jeopardised by increased ocean acidity; generally caused by increased emissions and the proclivity for the ocean to absorb mass amounts of CO_2 (Kinnby et al., 2021). Therefore, seaweeds tend to be known as a buffer for communities such as shellfish aquaculture by ameliorating eutrophication and ocean acidification, providing an additional opportunity for increased farm production and health of additional aquacultural products (Hamilton et al., 2022). Current European marine diversity is under threat, with almost all marine species groups labelled as "in bad condition" (EEA, 2021). According to a joint paper by 21 EU conservation agencies, irreversible biodiversity loss, which includes that which takes place in water bodies and coastal areas, lists agricultural intensification has a driving force (EHF, 2015). Finally, for much of Europe, estimated global diversity loss is projected to reach 34-40% of species threatened or extinct (Isbell et al., 2023). That being said, high biodiversity and species richness has been shown to enhance ecosystem processes, ultimately increasing the stability of the region (Cochrane et al., 2016). Thus, conservation of biodiversity is critical to system sustainability – and agricultural paradigms should be shifted to incorporate aspects of sustainability by considering alternative forms of supplementation and/or feed.

Additionally, microalgae itself – while generally grown within circuit ponds at a large scale, provides an opportunity in its ability to reduce water eutrophication, leading to water purification in freshwater ecosystems – perhaps an opportunity for the creation of synergistic aquacultural production (Zhou, Li, & He, 2023).

In regards to a reduction of emissions that an alternative feed must lead to for increased sustainability, *in vitro* studies have shown that supplementation of *Asparagopsis taxiformis*, a red seaweed, can reduce methane production by 95% at just 5% organic matter rate (Roque et al., 2019). Additionally, *in vivo* studies have also presented promising results, as *A. taxiformis* has been shown to reduce methane production between 40-98% (Kinley et al., 2020). Finally, *Spirulina*, a microalgae, has been encouraged recently due to its possible role in the reduction of methane production via manipulation of the ruminal microbiome (Wang et al., 2023).

An often-overlooked aspect of algal farming is the benefits of economic growth, as coastal communities would experience increased investment, European countries could channel this into an economic boon with investment in aquaculture, and additionally, for non-coastal communities, microalgae offers a solution to a need for increased investment as well; The phycological market would look to spur on increased European opportunities for jobs and investment. Seaweed itself representing a €9.3B potential market, and a creation of 115,000 jobs (Europe, 2020). Already, Europe produces microalgae/*Spirulina* across 23 countries, with most production centred within France, Italy, Germany, and Spain. According to Araújo et al. (2021), algae production limitations are sourced from a lack of technological, regulatory, and market-related barriers; but given this, an increase in research could reduce these barriers to realize the increase in market potentiality and ultimately a higher standard of living for European citizens.

Finally, it must be mentioned that animal health has been the focus of multiple phycological feeding-based studies, as indicated in Newton et al. (2021), work has been done examining haematological parameters in dairy cows fed seaweed – and Karatzia et al. (2012) showed increased blood glucose and decreased sorbitol dehydrogenase – indicating a possible hepatoprotective effect. In addition, Saadaoui et al. (2021) indicates that due to the diverse nutritional profile of microalgae, feeding this to livestock can improve animal health, such as lowering cholesterol, improving immune response, and offering resistance to disease.

1.4 Macroalgae

Macroalgae (also known as seaweed) is split into three groups, easily remembered by their colour-association as green seaweed (Chlorophyceae), red seaweed (Rhodophyceae), and brown seaweed (Phaeophyceae) (Chapman, 2013). Worldwide, *Phaeophyceae* makes up ~48%, Rhodophyceae ~51%, and Chlorophyceae ~0.1% of total global production (Figure 1.1).These are commonly associated with marine vegetables consumed as a staple in East Asia such as *Laminaria japonica*, better known as kombu, or *Undaria pinnatifida*, better known as wakame (Buschmann et al., 2017). Additional uses for seaweed can be found for *Eucheuma* and *Gracilaria*, in their ability to contain compounds such as carrageenan – used in gelling and thickening in a wide array of industrial applications, agar, and alginate – used in scientific application and cooking (Noor, 2018; Tseng, 1944). Literature concerning current seaweed use mentions TASCO being relatively common, an *Aschophyllum nodosum* extract that has been cited as having beneficial properties for ruminants such as increased tolerability to increased heat stress (Williams et al., 2009). *Ascophyllum*, a brown seaweed, tends to be the main seaweed fed to animals, as in many European countries, there is a long history of livestock animals eating *Ascophyllum* that has washed upon the shore and is featured in use within Europe as it is so plentiful and accessible (FAO, 2001).

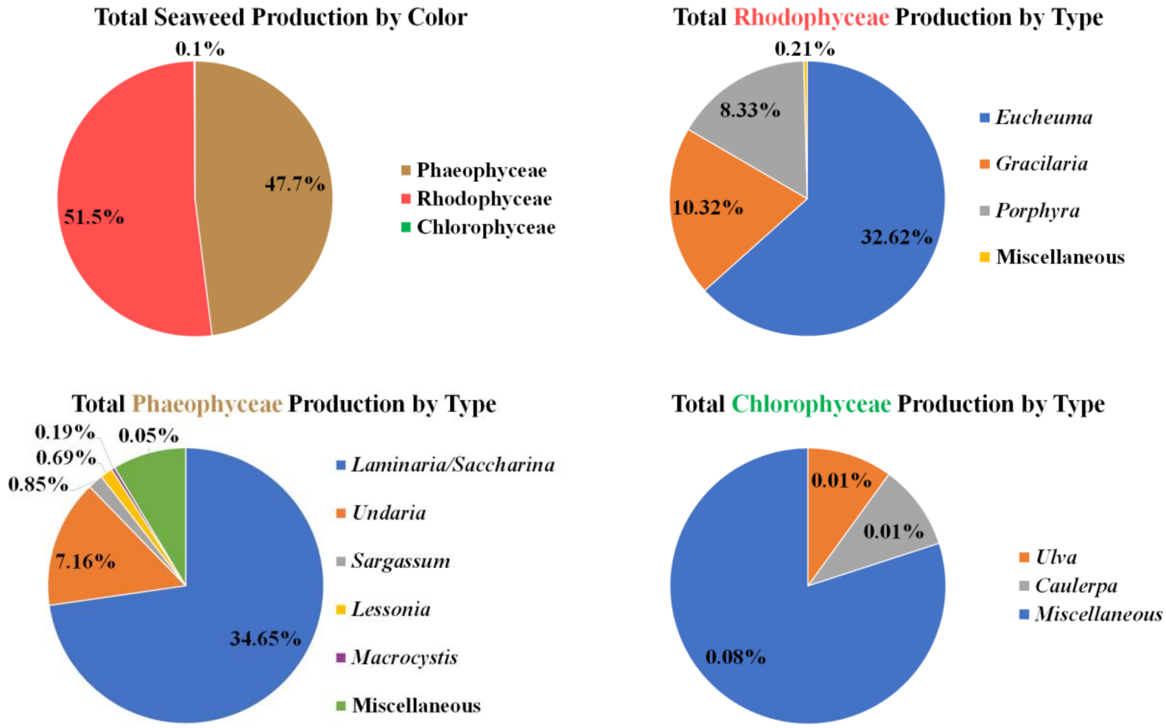

Figure 1.1 Global seaweed production by color and color seaweed production by type as a % share of the world total in 2019 (FAO, 2021a).

Total seaweed production in 2020 amounted to ~35 million tons, with most being from culture farming rather than wild collection which is attributed to a value of more than $6B in 2018, with a value of exports totalling $1.1B in 2020 alone (FAO, 2022b; Ferdouse et al., 2018). Most of the macroalgae produced in the world is by China (62.8%), Indonesia (13.7%), and Philippines (10.6%), with the rest by other Asian countries (Ghadiryanfar et al., 2016). While global production figures may indicate farmed seaweed to be vastly more popular than wild-caught (Figure 1.2), regions of the world have individual breakdowns that may deviate from this in terms of production type prevalence. Europe represents just 0.8% of world production (Figure 1.3), yet Norway, the highest contributor to European seaweed production, produces the third highest tonnage in the world of wild-caught seaweed at 163,080 tons (FAO, 2021a). Among the main algal species that are exploited in Europe are *Laminaria digitata*, *Laminaria hyperborea*, and *Ascophyllum nodosum* (Cai et al., 2021). Given the increase in the human population (expected to reach ~10B by 2050 from 8B in 2022; UN (2022)), forewarned threats upon terrestrial crop growth, and subsequent need for increased agricultural productivity, algae could serve as an alternative supplement and exploitable resource to continue to power human demand for food ("FAO's Director-General on How to Feed the World in 2050," 2009)

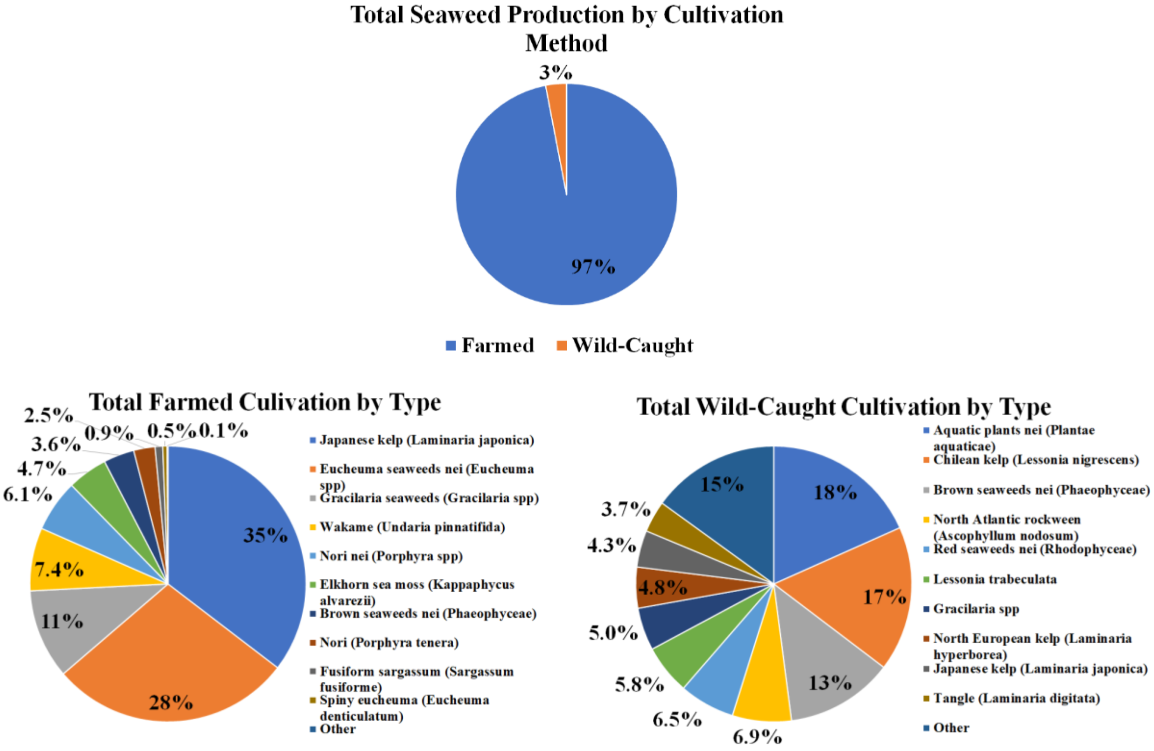

Figure 1.2 Global seaweed production by cultivation method and cultivation method by % type (FAO, 2021a).

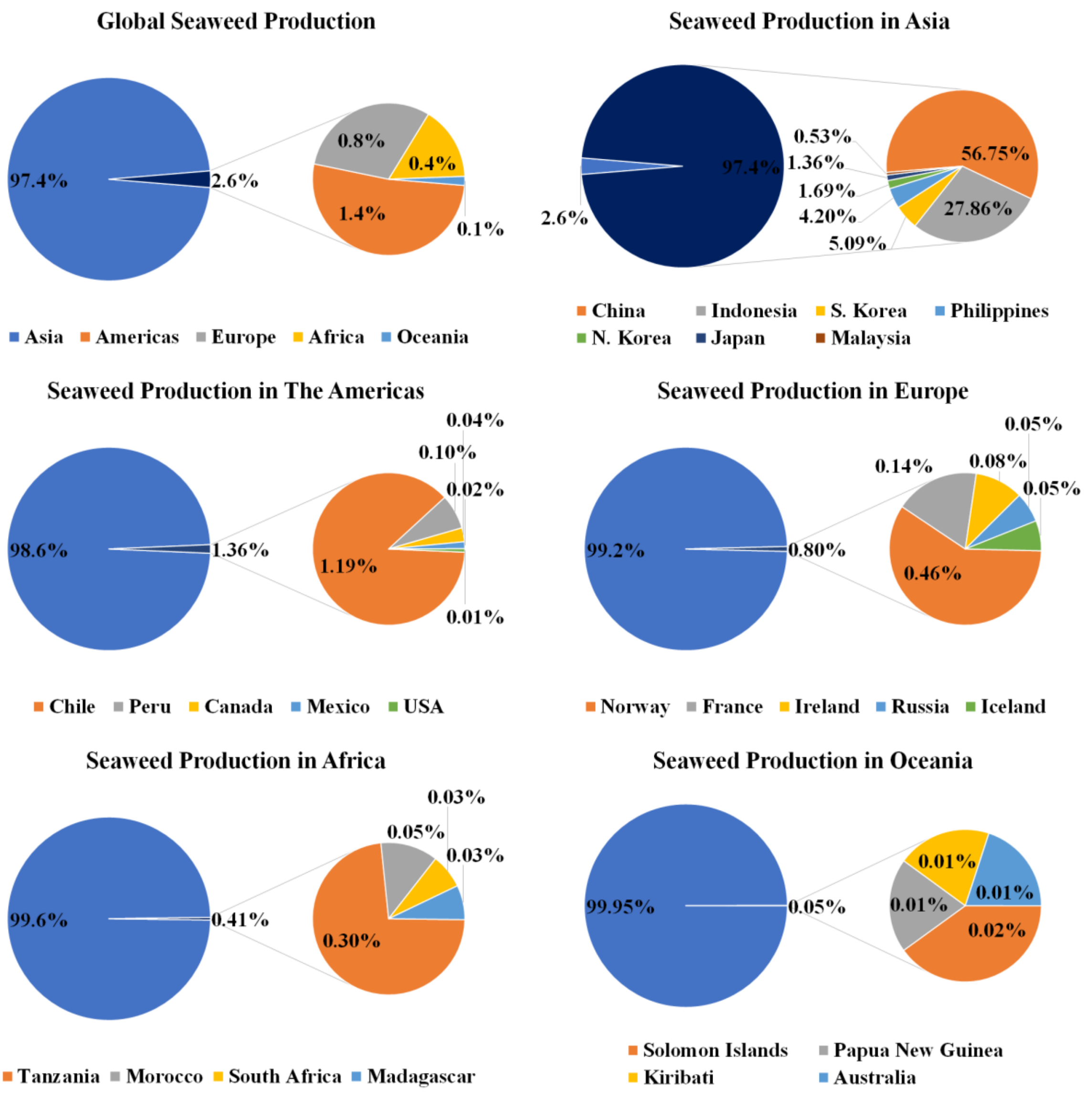

Figure 1.3 Global seaweed production in 2019 as a % share of the world total by area and area seaweed production by the top producing countries as a % share of the world total (FAO, 2021a).

1.4.1 Macroalgal supplementation in dairy cows' diet

Historically macroalgae have been a supplement in livestock diets since human writing was observable, with the Greeks having been recorded to collect and feed to their cattle in as far back as 45 BCE (Evans & Critchley, 2014). Icelandic sagas feature their use in feeding livestock and more recently reports of seaweed supplementation in Celtic lands and Scandinavia have been recorded in the 19th and early 20th centuries (Makkar et al., 2015). Nowadays, a focus has been set on the effects of feeding seaweed on methane emissions within cattle, but the effect this might have on milk quality is still under-researched.

1.4.2 Effects on milk composition

Table 1.1 Available average, minimum, and maximum values for basic composition of *Ascophyllum nodosum* (dried) sourced from Feedipedia (2023b)

Analyte	Unit	Avg	SD	Min	Max
Dry Matter	% as fed	88	2.7	85	92
Crude Protein	% DM	8	2.7	6	12
Crude Fibre	% DM	5.5		4.1	6.8
NDF	% DM	20.9		19.8	22.0
ADF	% DM	13.1			
EE	% DM	4	1.6	3	6
Ash	% DM	23	2.1	19	25
Gross Energy	MJ/kg DM	14.7		14.5	14.7

Table 1.1 indicates a certain amount of basic compositional analytes associated with a common seaweed, *A.* nodosum, and as such preliminary research regarding the effect that feeding this nutrient rich feed has on milk composition is plentiful but shows generally suppressed results. Studies have found that protein, fat, lactose, and solids-not-fat (SNF) were unaffected by brown seaweed by-product from *U. pinnatifida* (Hong et al., 2015). A 8.5-week study with Holstein Friesian cows was also conducted using *A. nodosum* at 100g per day per cow, and compositional components such as fat, protein, lactose, casein, and urea were similarly unaffected (Chaves Lopez et al., 2016). Even in previously described papers using large amounts of supplementation with *S. wightii*, the researchers did not find any significant chances to milk composition (Singh et al., 2015). Roque et al. (2019) observed that an inclusion rate of 1% *A. armata* on an organic matter basis, caused a significant reduction in lactose from 4.74% (control) to 4.69%, while all other factors such as fat %, protein %, SnF %, and SCC were unaffected. Regarding somatic cell count, an indicator of cow mastitis which is heavily penalized financially by processing plants, *A. nodosum* has been found to reduce SCC by 45% in some studies (Chaves Lopez et al., 2016). A study with Friesian dairy cows also found that 50g per day with *A. nodosum* reduced SCC by almost 18% between control and experimental groups (Bendary et al., 2013).

1.4.3 Effects on milk mineral concentrations

As described earlier, milk is generally a large contributor to specific minerals in a human diet such as I, Mo, Zn, Mg, Ca, and K (Newton et al., 2023). Supplementing ruminants with macroalgae has been shown to affect milk mineral concentrations.

I, one of the most obvious choices for focus and experimentation due to the generally high levels found within macroalgae has been shown to increase in milk when cows are fed algae. Using a 100g per animal per day dose of a mix of 80% *Ulva rigida* flakes, 17.5% *Sargasum muticum* flakes, and 2.5% *Saccorhiza polyschides* powder more than doubled the amount of milk iodine, with supplemented vs control cows producing milk with 290 ug/L vs 136 ug/L, respectively (Rey-Crespo, López-Alonso, & Miranda, 2014). In another study I has been more than doubled from 920 ug/L to 1,960 ug/L by including *A. nodosum* in dairy cow diets at that resulted in 65mg I consumed/cow/day (Chaves Lopez et al., 2016); while other studies showed an increase from 517 ug/L to 660 ug/L when 56g per day of *A. nodosum* was fed (Sorge et al., 2016). A study using *Thallus laminariae* in powder form at 5% substitution for forage saw a statistically significant increase from 70 ug/L to 120 ug/L of iodine in the milk produced from Chinese Holstein cows (Xue et al., 2019).

Mo, a cofactor for multiple essential enzymes within the body, also has been found to fluctuate in milk when ruminants are fed algae. It has previously been shown that when ruminants ingest large amounts of Cu or S, an increased amount of the ingested Mo is excreted through urine (Suttle, 2010). Due to the fact that sulphate tends to be a component of algal polysaccharides, typically levels of Mo will be reduced within supplemented groups of high Cu or S which was shown in such study resulting in 61.9 ug/L compared to the control 101 ug/L (Rey-Crespo et al., 2014).

Zn concentrations seem to be relatively unaffected by the increase of supplementation of seaweed to dairy cows. Singh et al. (2014) indicated no difference in Zn concentrations within the milk after feeding 20% seaweed in concentrate (*S. wightii*) between the control group and the seaweed group.

Ca, of which milk is known well for containing large amounts, has been shown to be reduced when cows were fed *Saccharina latissimi* from 1080mg/kg to 1031mg/kg of milk (Qin et al., 2023). Yet, Newton et al. (2021) found no change in calcium concentrations within the milk when cows were fed a brown seaweed mix. Overall, literature suggests that Ca may be affected by seaweed supplementation, but not necessarily in a manner that is tangible for consumers of said milk in terms of nutritional sufficiency.

K, another key mineral that is provided in sizeable amounts by milk and dairy products was found not to be affected in Qin et al. (2023).

Arsenic (As), an element that can lead to arsenicosis and multiple types of cancers via chronic ingestion of contaminated drink, should be reduced as much as possible, as it is both non-essential, and relevant given the nature of consumption of milk (Chowdhury, Krause, & Zimmermann, 2016). Typically, As levels have been found to be slightly higher within supplemented groups, but given the relatively low amount within studies, the concentrations are generally negligible (EFSA, 2014). For example, in a study using a mix of seaweed, As peaked at 0.857 ug/L, raised from 0.615ug/L in the control group (Rey-Crespo et al., 2014). Mercury (Hg), a heavy metal, is a toxic element that can be found in aquatic organisms as some species are known to be mineral accumulators which can translate into higher intake within the cow, thus making its way into the milk to be consumed by humans (Morais et al., 2020). Generally Hg concentrations are too low and below quantification limits in studies that have attempted to analyse them (Qin et al., 2023; Rey-Crespo et al., 2014). Seaweed can bioaccumulate heavy metals and trace elements that can be harmful to the consumer, in particular As and Hg (Chen et al., 2018; Filippini et al., 2021; Sadhasivam et al., 2012). Therefore, essential minerals as well as heavy metals and non-essential trace elements should be considered.

There is currently very little literature regarding the effect that macroalgal supplementation of dairy cows would have upon the milk mineral profile and a standardized laboratory method delivering large amounts of data is required to correctly analyse milk mineral concentrations and transference from feed to milk.

1.4.4 Effects on milk microbiology

While milk, when synthesized within the mammary gland, is considered theoretically sterile - a host of factors such as milking technique and procedure, environment, teat health, and udder sanitation can influence the microbiome then found within the product (Anderson et al., 2011). Feeding seaweed to cows poses a unique solution to possible rates of microbial contamination, as algal supplementation has been shown to lead to improved immune function in ruminants and therefore possibly aiding in the prevention of mastitis (Saker et al., 2001). Potential bactericidal and bacteriostatic properties of, for example, iodine, which is typically found within algal species and can pass on to milk, can also be hypothesized to then limit harmful bacterial species in raw and processed milk at different stages of the supply chain including transport, storage, packaging, or use by the consumer (Selvaggi et al., 2003).

Previous studies have indicated a substantial and statistically significant increase in the mineral I when cows are fed seaweed which has been used as an antimicrobial agent for more than 150 years in the form of a variety of different preparations (Selvaggi et al., 2003; Vasudevan & Tandon, 2010). Elemental iodine in vapor form has been used to disinfect liquid, and while this specific technique was ineffective in mediums with high turbidity, gram-negative (G-) bacteria such as *Escherichia* and *Salmonella*, along with gram-positive (G+) bacteria such as *Enterococcus*, have been shown to be vulnerable to this type of intervention (Hove et al., 2020). Disinfection efficacy has also been analysed to vary by elemental speciation which is dictated in a large part to the pH of the liquid, therefore within certain pH ranges, certain classifications of microbial entities will be affected at different efficiencies (Bevan, 2018; Taylor & Butler, 1982).

There is currently no literature regarding the effect that macroalgal supplementation of cows would have upon the milk microbiome, and therefore a need for research is high to address the potential value-additions or reductions with the introduction of seaweed supplementation to dairy cows.

1.5 Microalgae

Microalgae (also known as phytoplankton), are a diverse grouping of unicellular photosynthetic organisms, with major groups being Bacillariophyceae (diatoms), Pyrrophyceae (dinoflagelates), Chrysophyceae (golden algae), and Cyanophyceae (blue-green algae or cyanobacteria) (Hemaiswarya et al., 2013; Madeira et al., 2017). Uses for microalgae are wide-ranging, with a majority used in health supplements, a sustainable feed, cosmetics, and biofuels (Dragone et al., 2010; Khan, Shin, & Kim, 2018). Given the sophisticated technology required for mass production of microalgae, estimates in 2007 saw the total world production to be around 10,000 tons (Becker, 2007). Persistence Market Research recently depicts the industry with a market value worth more than $2.76 billion (Mohit, 2021). *Arthrospira* (*Spirulina*) makes up ~97% of global microalgae that are produced, with China making up 97% of all produced biomass, followed by Chile (1.6%), France, Greece, Tunisia, Burkina Faso, Central African Republic, Chad, Bulgaria, and Spain contributing much less than 1% (Ashour, 2022).

1.5.1 Microalgal supplementation in dairy cows

For microalgae, prevalence in animal feed in much less documented compared to macroalgae. The viability of its use within agricultural systems came from an interest in its potential as a biofuel – but when analysed for chemical composition was found to contain protein with high digestibility for livestock and large amounts of long-chain omega-3 fatty acids, particularly relevant to animal health and nutrition (Benemann, 2013). This has spawned a push for research regarding the effects of microalgal supplementation in dairy cows on milk quality.

1.5.2 Effects on milk composition

Table 1.2 Available average, minimum, and maximum values for basic composition of *Spirulina* (dried) sourced from (Feedipedia, 2023a)

Analyte	Unit	Avg	SD	Min	Max
Dry Matter	% as fed	92	2.2	89	96
Crude Protein	% DM	64	5.6	54	73
Crude Fibre	% DM	3	2.8	0.1	6
NDF	% DM	0.1			
ADF	% DM	0.0			
EE	% DM	6.1		1.2	10.9
Ash	% DM	9	2.6	4	14
Gross Energy	MJ/kg DM	21	0.7	20	23

Table 1.2 indicates a certain amount of basic compositional analytes associated with a common microalgae, *Spirulina*, and as such preliminary research regarding the effect that feeding this nutrient rich feed has on milk composition is plentiful but, as in macroalgae, shows differing results. Milk basic

composition seems to be relatively unaffected by microalgal supplementation by some studies, such as a long-term animal trial looking at the effect of *Schizochytrium limacinum sp.* on fat content or another examining algae meal partially replacing corn meal, yet as phytoplankton diversity is vast, some studies do see differences (da Silva et al., 2016; Till et al., 2020). In a study using marine algae powder (MAP) fed at 1.5% dry matter intake (DMI), there was indicated a 22% loss in cow's milk fat content compared to control, and in another, DHA gold supplementation fed at 1.8% DM saw, again, fat content lowered close to 50% and reduced fat globule size (Fougère & Bernard, 2019; Vanbergue, Peyraud, & Hurtaud, 2018). This is reinforced with a study indicating a marine algae diet (Docosahexaenoic acid (DHA) gold) increasing yield, but decreasing fat yield and content, and another feeding *Schizochytrium spp.* providing an improve fatty acid composition by way of increasing the proportion of polyunsaturated fatty acids, and decreasing the proportion of saturated milk fat, but lower fat overall (Franklin et al., 1999; Hostens et al., 2011). On the other side of the coin, DHA-rich microalgae (10% DHA content) fed at 100 g/cow/day in one study, and 50g, 100g, and 150g in another, have been found to increase milk fat concentrations, compared with a control diet without microalgae, in dairy cows (Sinedino et al., 2017; Till et al., 2019). Fatty acid profile improvement tends to be a theme within the confines of supplementing dairy cows' diets with DHA-rich microalgae, as 100g of *Aurantiochytrium limacinum* (AURA) is seen to lower saturated fatty acids and increase omega-3 fatty acids, while however it has been shown to reduce milk fat concentration (Moran, Morlacchini, & Fusconi, 2017; Moran et al., 2018). Other studies have described the ability of DHA-rich microalgae to lead to the production of DHA-enriched milk (Liu et al., 2020).

1.5.3 Effects on milk mineral concentrations and microbiology

To the best of our knowledge, there is no literature that concerns the effect of microalgal supplementation of dairy cows and the effect on milk mineral concentrations or microbiology. That being said, there is a relatively large amount of iron (560 mg/kg DM) found within *Spirulina*, and this could possibly transfer into the milk (Feedipedia, 2023a).

1.6 Thesis aims

There has been a recent increased interest in macro- and micro-algae as an animal feed due to potential auxiliary effects such as increased animal health outcomes, product nutrition, and reduced association with reduced emissions but mainly due to the raw supply potential that these phycological items can provide as a protein source. However, altering the nutrition of cattle often has downstream effects on milk production, quality, and safety. This can be particular in the case of seaweed and microalgae because some are rich in certain minerals that can pass on to milk and alter its nutritional, safety and technological properties. This has particular importance for the farmers, dairy industry and consumers due to much of Europe's reliance on milk and dairy products for core public nutrition. However, there is very limited literature investigating the implications of feeding seaweed and microalgae in milk mineral profile, and therefore this study aimed to investigate the effect of including different species of seaweeds and microalgae (among the most prevalent and with high potential for use in Europe) in dairy cows' diets on (i) milk production, basic composition and mineral concentrations, and (ii) model the potential impact that the differences in milk minerals concentrations would have to the provision of minerals in different consumer demographics.

Focusing on agricultural science applicability, this thesis aims to examine samples from multiple algae-fed dairy cows, and to analyse mainly the milk for primarily nutritional content, basic composition, and added positive qualities. This will elucidate the viability of more sustainable dairy cow feeding strategies in terms of providing dairy-based food nutrition. Since dairy farming cannot be viewed as a single process - as management practices, feed, and human consumption among other aspects vary widely across the world – each animal trial's nutritional results will be theorized to explain how each country wherein the experiment occurred might be affected in terms nutritional intake. This is done to keep this work as applicable as possible, as each country maintains different practices that will affect product nutritional composition, along with differing consumption rates and legislative regulations, relying on dairy for a certain amount of mineral intake at various degrees.

Farmers are not incentivized for the level of mineral concentrations found within milk, but rather basic compositional aspects – and therefore much more research is associated with investigating the effects on production and milk basic compositional changes as a result of a changes in animal diets. This could be considered a disconnect between food production and consumer nutrition , as milk is a huge contributor to of certain macrominerals and trace elements across the world. Therefore, large gaps exist in the effect of supplementation of seaweeds and microalgae on the milk mineral concentrations and this should be explored to identify the potential effects and nutritionally model the impact that this may have to certain populations and demographics.

The end product's aim will be to provide detailed agricultural and food science papers so that various stakeholders, whether it be consumers, lawmakers, private business owners, etc, can be more informed as to the benefits and drawbacks that might come with a shift in the current paradigm for dairy cow supplementation. It is contributions like this that will help, in a small part, to maintaining and/or improving food security, while still supplying the increasing demand by a rising population in a more sustainable and less-environmentally harmful manner.

1.7 References

Adams, R. S. (1975). Variability in Mineral and Trace Element Content of Dairy Cattle Feeds. *Journal of Dairy Science, 58*(10), 1538-1548. doi:https://doi.org/10.3168/jds.S0022-0302(75)84750-3

Adesina, K. (2012). Effect of Breed on the Composition of Cow Milk under Traditional Management Practices in Ado-Ekiti, Nigeria. *Journal of Applied Sciences and Environmental Management, 16*.

Adu, P., & Simpong, D. (2017). Addressing the Challenge of Iodine Deficiency in Developing Countries. *Medcraveonline journal Public Health, 5*, 00129. doi:10.15406/mojph.2017.05.00129

Aftab, R., Dodhia, V. H., Jeanes, C., & Wade, R. G. (2023). Bacterial sensitivity to chlorhexidine and povidone-iodine antiseptics over time: a systematic review and meta-analysis of human-derived data. *Scientific reports, 13*(1), 347. doi:10.1038/s41598-022-26658-1

AHDB. (2023a). Crop choice for livestock grazing brassica and forage crops. Retrieved from https://ahdb.org.uk/knowledge-library/crop-choice-livestock-grazing-brassica-forage-crops

AHDB. (2023b). Somatic Cell Count, an indicator of milk quality. Retrieved from https://ahdb.org.uk/somatic-cell-count-milk-quality-indicator#:~:text=The%20SCC%20is%20quantified%20as,cells%20per%20ml%20of%20milk.&text=An%20individual%20cow%20SCC%20of,cow%20is%20infected%20with%20mastitis.

Al Alawi, A. M., Majoni, S. W., & Falhammar, H. (2018). Magnesium and Human Health: Perspectives and Research Directions. *International journal of endocrinology, 2018*, 9041694-9041694. doi:10.1155/2018/9041694

Alhussien, M. N., & Dang, A. K. (2018). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. *Vet World, 11*(5), 562-577. doi:10.14202/vetworld.2018.562-577

Anderson, M., Hinds, P., Hurditt, S., Miller, P., McGrowder, D., & Alexander-Lindo, R. (2011). The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. *Asian Pacific journal of tropical biomedicine, 1*(3), 205-211. doi:10.1016/S2221-1691(11)60028-2

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. doi:https://doi.org/10.3168/jds.2014-8851

Araújo, R., Vázquez Calderón, F., Sánchez López, J., Azevedo, I. C., Bruhn, A., Fluch, S., Garcia Tasende, M., Ghaderiardakani, F., Ilmjärv, T., Laurans, M., Mac Monagail, M., Mangini, S., Peteiro, C., Rebours, C., Stefansson, T., & Ullmann, J. (2021). Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy. *Frontiers in Marine Science, 7*. doi:10.3389/fmars.2020.626389

Ashour, M. (2022). Recent Advances in Marine Microalgae Production: Highlighting Human Health Products from Microalgae in View of the Coronavirus Pandemic (COVID-19). *Fermentation, 8*, 466. doi:10.3390/fermentation8090466

Bakhshizadeh, S., Mirzaei Aghjehgheshlagh, F., Taghizadeh, A., Seifdavati, J., & Navidshad, B. (2019). Effect of zinc sources on milk yield, milk composition and plasma concentration of metabolites in dairy cows. *South African Journal of Animal Science, 49*, 882. doi:10.4314/sajas.v49i5.11

Bath, S. C., Steer, C. D., Golding, J., Emmett, P., & Rayman, M. P. (2013). Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Lancet, 382*(9889), 331-337. doi:10.1016/s0140-6736(13)60436-5

Bath, S. C., Verkaik-Kloosterman, J., Sabatier, M., Ter Borg, S., Eilander, A., Hora, K., Aksoy, B., Hristozova, N., van Lieshout, L., Tanju Besler, H., & Lazarus, J. H. (2022). A systematic review of iodine intake in children, adults, and pregnant women in Europe-comparison against dietary recommendations and evaluation of dietary iodine sources. *Nutrition reviews, 80*(11), 2154-2177. doi:10.1093/nutrit/nuac032

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnol Adv, 25*(2), 207-210. doi:10.1016/j.biotechadv.2006.11.002

Bendary, M. M., Bassiouni, M. I., Ali, M. F., Gaafar, H., & Shamas, A. (2013). Effect of premix and seaweed additives on productive performance of lactating Friesian cows. *Int. Res. J. Agric. Sci. Soil Sci., 3*, 174-181.

Benemann, J. (2013). Microalgae for Biofuels and Animal Feeds. *Energies, 6*, 5869-5886. doi:10.3390/en6115869

Besle, J. M., Viala, D., Martin, B., Pradel, P., Meunier, B., Berdagué, J. L., Fraisse, D., Lamaison, J. L., & Coulon, J. B. (2010). Ultraviolet-absorbing compounds in milk are related to forage polyphenols. *J. Dairy Sci., 93*(7), 2846-2856. doi:https://doi.org/10.3168/jds.2009-2939

Bevan, R. N., Andreas. (2018). *Iodine as a drinking-water disinfectant*. Retrieved from

Boubendir, A., Serrazanetti, D. I., Hamidechi, M. A., Vannini, L., & Guerzoni, M. E. (2016). Changes in bacterial populations in refrigerated raw milk collected from a semi-arid area of Algeria. *Annals of Microbiology, 66*(2), 777-783. doi:10.1007/s13213-015-1163-5

Brooke, J. (2021). Advances in the Microbiology of Stenotrophomonas maltophilia. *Clinical microbiology reviews, 34*, e0003019. doi:10.1128/CMR.00030-19

Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The Importance of Zinc in Human Nutrition and Estimation of the Global Prevalence of Zinc Deficiency. *Food and Nutrition Bulletin, 22*(2), 113-125. doi:10.1177/156482650102200201

Buschmann, A., Camus, C., Infante Rosselot, J., Neori, A., Israel, A., Hernández-González, M., Pereda, S., Gomez Pinchetti, J. L., Golberg, A., Tadmor Shalev, N., & Critchley, A. (2017). Seaweed production:

overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology, 52*, 391-406. doi:10.1080/09670262.2017.1365175

Butler, S., Curran, F., & Gleeson, D. (2017). Purple Patch: TEAGASC research has contributed to recommendations on iodine supplementation in Irish dairy industry. In TEAGASC (Ed.).

Cai, J., Lovatelli, A., Garrido Gamarro, E., Geehan, J., Lucente, D., Mair, G., Weimin, M., Bondad-Reantaso, M., & Roubach, R. (2021). *Seaweeds and Microalgae: An Overview for Unlocking Their Potential in Global Aquaculture Development FAO Fisheries and Aquaculture Circular NFIA/C1229 (En)*.

Celano, G., Calasso, M., Costantino, G., Vacca, M., Ressa, A., Nikoloudaki, O., Palo, P. D., Calabrese, F. M., Gobbetti, M., & Angelis, M. D. (2022). Effect of Seasonality on Microbiological Variability of Raw Cow Milk from Apulian Dairy Farms in Italy. *Microbiology Spectrum, 10*(5), e00514-00522. doi:doi:10.1128/spectrum.00514-22

Cerbulis, J., & Farrell, J. H. (1976). Composition of the Milks of Dairy Cattle. II. Ash, Calcium, Magnesium, and Phosphorus. *Journal of Dairy Science, 59*, 589-593. doi:10.3168/jds.S0022-0302(76)84245-2

Chang, J., Peng, S., Ciais, P., Saunois, M., Dangal, S. R. S., Herrero, M., Havlík, P., Tian, H., & Bousquet, P. (2019). Revisiting enteric methane emissions from domestic ruminants and their δ13CCH4 source signature. *Nature Communications, 10*(1), 3420. doi:10.1038/s41467-019-11066-3

Chapman, R. L. (2013). Algae: the world's most important "plants"—an introduction. *Mitigation and Adaptation Strategies for Global Change, 18*(1), 5-12. doi:10.1007/s11027-010-9255-9

Chaves Lopez, C., Serio, A., Rossi, C., Mazzarrino, G., Marchetti, S., Castellani, F., Grotta, L., Fiorentino, F. P., Paparella, A., & Martino, G. (2016). Effect of diet supplementation with Ascophyllum nodosum on cow milk composition and microbiota. *Journal of Dairy Science, 99*(8), 6285-6297. doi:10.3168/jds.2015-10837

Chen, Q., Pan, X.-D., Huang, B.-F., & Han, J.-L. (2018). Distribution of metals and metalloids in dried seaweeds and health risk to population in southeastern China. *Scientific reports, 8*(1), 3578. doi:10.1038/s41598-018-21732-z

Chowdhury, S., Krause, A., & Zimmermann, K. (2016). Arsenic Contamination of Drinking Water and Mental Health. *SSRN Electronic Journal*. doi:10.2139/ssrn.2811583

Christophe, O. S., Grelet, C., Bertozzi, C., Veselko, D., Lecomte, C., Höeckels, P., Werner, A., Auer, F. J., Gengler, N., Dehareng, F., & Soyeurt, H. (2021). Multiple Breeds and Countries' Predictions of Mineral Contents in Milk from Milk Mid-Infrared Spectrometry. *Foods, 10*(9). doi:10.3390/foods10092235

Cifelli, C. J., Houchins, J. A., Demmer, E., & Fulgoni, V. L. (2016). Increasing Plant Based Foods or Dairy Foods Differentially Affects Nutrient Intakes: Dietary Scenarios Using NHANES 2007–2010. *Nutrients, 8*(7). doi:10.3390/nu8070422

Cinar, M., Serbester, U., Ceyhan, A., & Gorgulu, M. (2015). Effect of Somatic Cell Count on Milk Yield and Composition of First and Second Lactation Dairy Cows. *Italian Journal of Animal Science, 14*(1), 3646. doi:10.4081/ijas.2015.3646

Coates, L. C., Storms, D., Finley, J. W., Fukagawa, N. K., Lemay, D. G., Kalscheur, K. F., & Kable, M. E. (2022). A Low-Starch and High-Fiber Diet Intervention Impacts the Microbial Community of Raw Bovine Milk. *Current Developments in Nutrition, 6*(6), nzac086. doi:10.1093/cdn/nzac086

Cochrane, S. K. J., Andersen, J. H., Berg, T., Blanchet, H., Borja, A., Carstensen, J., Elliott, M., Hummel, H., Niquil, N., & Renaud, P. E. (2016). What Is Marine Biodiversity? Towards Common Concepts and Their Implications for Assessing Biodiversity Status. *Frontiers in Marine Science, 3*. doi:10.3389/fmars.2016.00248

Cormick, G., & Belizán, J. M. (2019). Calcium Intake and Health. *Nutrients, 11*(7). doi:10.3390/nu11071606

Costello, R., Wallace, T. C., & Rosanoff, A. (2016). Magnesium. *Advances in Nutrition, 7*(1), 199-201. doi:10.3945/an.115.008524

Coulon, J., B., Pradel, P., & Verdier, I. (1995). Effect of forage type on milk yield, Chemical composition and clotting properties of milk. *Lait, 75*(6), 513-521. Retrieved from https://doi.org/10.1051/lait:1995640

da Silva, G. G., Ferreira de Jesus, E., Takiya, C. S., Del Valle, T. A., da Silva, T. H., Vendramini, T. H. A., & Rennó, F. P. (2016). Short communication: Partial replacement of ground corn with algae meal in a dairy cow diet: Milk yield and composition, nutrient digestibility, and metabolic profile. *J Dairy Sci, 99*(11), 8880-8884. doi:10.3168/jds.2016-11542

Datta, N., Elliott, A., Perkins, M., & Deeth, H. (2002). Ultra-high-temperature (UHT) treatment of milk: Comparison of direct and indirect modes of heating. *Australian Journal of Dairy Technology, 57*.

Davoodi, S. H., Shahbazi, R., Esmaeili, S., Sohrabvandi, S., Mortazavian, A., Jazayeri, S., & Taslimi, A. (2016). Health-Related Aspects of Milk Proteins. *Iran J Pharm Res, 15*(3), 573-591.

Deeth, H. (2017). Optimum Thermal Processing for Extended Shelf-Life (ESL) Milk. *Foods (Basel, Switzerland), 6*(11), 102. doi:10.3390/foods6110102

Deeth, H., & Lewis, M. J. (2017). Heat Treatments of Milk – Thermisation and Pasteurisation. In *High Temperature Processing of Milk and Milk Products* (pp. 15-39).

Dejyong, T., Chanachai, K., Immak, N., Prarakamawongsa, T., Rukkwamsuk, T., Tago Pacheco, D., & Phimpraphai, W. (2022). An economic analysis of high milk somatic cell counts in dairy cattle in Chiang Mai, Thailand. *Frontiers in Veterinary Science, 9*. doi:10.3389/fvets.2022.958163

Dragone, G., Fernandes, B., Vicente, A., & Teixeira, J. (2010). Third generation biofuels from microalgae. In (Vol. 2, pp. 1355-1366).

Duarte, C. M., Wu, J., Xiao, X., Bruhn, A., & Krause-Jensen, D. (2017). Can Seaweed Farming Play a Role in Climate Change Mitigation and Adaptation? *Frontiers in Marine Science, 4*. doi:10.3389/fmars.2017.00100

Dunshea, F., Walker, G., Williams, R., & Doyle, P. (2019). Mineral and Citrate Concentrations in Milk Are Affected by Seasons, Stage of Lactation and Management Practices. *Agriculture, 9*, 25. doi:10.3390/agriculture9020025

Dynes, R., Henry, D., & Masters, D. (2003). Characterising Forages for Ruminant Feeding. *Asian-Australasian journal of animal sciences, 16*, 116-123. doi:10.5713/ajas.2003.116

EEA. (2021). *Europe's marine biodiversity remains under pressure*. Retrieved from https://www.eea.europa.eu/publications/europes-marine-biodiversity-remains-under-pressure

EEA. (2022). *Greenhouse gas emissions from agriculture in Europe*. Retrieved from https://www.eea.europa.eu/ims/greenhouse-gas-emissions-from-agriculture

EEB. (2023). An unavoidable step after Paris: Cutting emissions from farming. Retrieved from https://eeb.org/cutting-emissions-from-farming-project/

EFSA. (2014). Dietary exposure to inorganic arsenic in the European population. *EFSA Journal, 12*. doi:10.2903/j.efsa.2014.3597

EHF. (2015). *Joint paper on the Action for Biodiversity in the EU and the Fitness Check of the Birds and Habitats Directives*. Retrieved from Brussels: https://rewildingeurope.com/wp-content/uploads/2015/11/EHF-Joint-Paper-20151.pdf

Eugster, E. J., Ernst. (2019). Pre-treatments of Milk and their Effect on the Food Safety of Cheese Milk Science International: journal of nutrition research and food science. *AVA-Agrar Verlag Allgaeu, 72*(8), 45-52.

Europe, S. f. (2020). *Hidden champion of the ocean: Seaweed as a growth engine for a sustainable European future*. Retrieved from

Evans, F. D., & Critchley, A. T. (2014). Seaweeds for animal production use. *Journal of Applied Phycology, 26*(2), 891-899. doi:10.1007/s10811-013-0162-9

Fahey, G., & Spears, J. (1994). Minerals in Forages. In.

FAO's Director-General on How to Feed the World in 2050. (2009). *Population and Development Review, 35*(4), 837-839. Retrieved from http://www.jstor.org/stable/25593700

FAO. (2001). Other Uses of Seaweeds. Retrieved from https://www.fao.org/3/y4765e/y4765e0c.htm

FAO. (2002). Chapter 2. Food security: concepts and measurement. Retrieved from https://www.fao.org/3/y4671e/y4671e06.htm#TopOfPage

FAO. (2021a). *FAO Global Fishery and Aquaculture Production Statistics (FishStatJ)*. Retrieved from: https://www.fao.org/fishery/en/global-search?q=statistics%20software%20fishstatj%20en&lang=en

FAO. (2021b). GLEAM.

FAO. (2022a). *Homemade Animal Feed Concentrates*. Retrieved from https://pdf.usaid.gov/pdf_docs/PNADT827.pdf

FAO. (2022b). *The State of World Fisheries and Aquaculture 2022*. Retrieved from https://www.fao.org/documents/card/en/c/cc0461en

Fatih, M., Barnett, M. P. G., Gillies, N. A., & Milan, A. M. (2021). Heat Treatment of Milk: A Rapid Review of the Impacts on Postprandial Protein and Lipid Kinetics in Human Adults. *Frontiers in Nutrition, 8*(186). doi:10.3389/fnut.2021.643350

Fearnside, P. M. (2001). Soybean cultivation as a threat to the environment in Brazil. *Environmental Conservation, 28*(1), 23-38. Retrieved from http://www.jstor.org/stable/44521676

Feedipedia. (2023a). Microalgae. Retrieved from https://www.feedipedia.org/node/67

Feedipedia. (2023b). Seaweeds (marine macroalgae). Retrieved from https://www.feedipedia.org/node/78

Ferdouse, F., Yang, Z., Holdt, S. L., Murúa, P., & Smith, R. (2018). *The global status of seaweed production, trade and utilization*.

Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., Valpiani, G., Buzzi, R., Manfredini, S., & Vertuani, S. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere, 263*, 127983. doi:10.1016/j.chemosphere.2020.127983

Fiordalisi, S. A. L., Honorato, L. A., Loiko, M. R., Avancini, C. A. M., Veleirinho, M. B. R., Filho, L., & Kuhnen, S. (2016). The effects of Brazilian propolis on etiological agents of mastitis and the viability of bovine mammary gland explants. *J Dairy Sci, 99*(3), 2308-2318. doi:10.3168/jds.2015-9777

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. doi:10.1007/s00394-013-0597-4

Flores, J. P., & Tracy, B. (2012). Impacts of winter hay feeding on pasture soils and plants. *Agriculture, Ecosystems & Environment, 149*, 30-36. doi:https://doi.org/10.1016/j.agee.2011.12.009

Fougère, H., & Bernard, L. (2019). Effect of diets supplemented with starch and corn oil, marine algae, or hydrogenated palm oil on mammary lipogenic gene expression in cows and goats: A comparative study. *J Dairy Sci, 102*(1), 768-779. doi:10.3168/jds.2018-15288

Franke, K., Meyer, U., Wagner, H., Hoppen, H. O., & Flachowsky, G. (2009). Effect of various iodine supplementations, rapeseed meal application and two different iodine species on the iodine status and iodine excretion of dairy cows. *Livestock Science, 125*(2), 223-231. doi:https://doi.org/10.1016/j.livsci.2009.04.012

Franklin, S. T., Martin, K. R., Baer, R. J., Schingoethe, D. J., & Hippen, A. R. (1999). Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. *J Nutr, 129*(11), 2048-2054. doi:10.1093/jn/129.11.2048

Gagnon, M., Ouamba, A. J. K., LaPointe, G., Chouinard, P. Y., & Roy, D. (2020). Prevalence and abundance of lactic acid bacteria in raw milk associated with forage types in dairy cow feeding. *Journal of Dairy Science, 103*(7), 5931-5946. doi:https://doi.org/10.3168/jds.2019-17918

Ghadiryanfar, M., Rosentrater, K. A., Keyhani, A., & Omid, M. (2016). A review of macroalgae production, with potential applications in biofuels and bioenergy. *Renewable and Sustainable Energy Reviews, 54*, 473-481. doi:https://doi.org/10.1016/j.rser.2015.10.022

Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019a). Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. *Nutrients, 11*(8), 1771. doi:10.3390/nu11081771

Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019b). Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. *Nutrients, 11*, 1771. doi:10.3390/nu11081771

Greig, L. (2009). An Analysis of the Key Factors Influencing Farmer's Choice of Crop, Kibamba Ward, Tanzania. *Journal of Agricultural Economics, 60*, 699-715. doi:10.1111/j.1477-9552.2009.00215.x

Hamilton, S. L., Elliott, M. S., deVries, M. S., Adelaars, J., Rintoul, M. D., & Graham, M. H. (2022). Integrated multi-trophic aquaculture mitigates the effects of ocean acidification: Seaweeds raise system pH and improve growth of juvenile abalone. *Aquaculture, 560*, 738571. doi:https://doi.org/10.1016/j.aquaculture.2022.738571

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition – a review. *Lipids in Health and Disease, 6*(1), 25. doi:10.1186/1476-511X-6-25

Hemaiswarya, S., Raja, R., Ravikumar, R., & Carvalho, I. (2013). Microalgae taxonomy and breeding. *Biofuel Crops: Production, Physiology and Genetics*, 44-53.

Hoekstra, M. J., Westgate, S. J., & Mueller, S. (2017). Povidone-iodine ointment demonstrates in vitro efficacy against biofilm formation. *International Wound Journal, 14*(1), 172-179. doi:https://doi.org/10.1111/iwj.12578

Hong, Z. S., Kim, E. J., Jin, Y. C., Lee, J. S., Choi, Y. J., & Lee, H. G. (2015). Effects of Supplementing Brown Seaweed By-products in the Diet of Holstein Cows during Transition on Ruminal Fermentation, Growth Performance and Endocrine Responses. *Asian-Australasian journal of animal sciences, 28*(9), 1296-1302. doi:10.5713/ajas.15.0235

Hostens, M., Fievez, V., Vlaeminck, B., Buyse, J., Leroy, J., Piepers, S., De Vliegher, S., & Opsomer, G. (2011). The effect of marine algae in the ration of high-yielding dairy cows during transition on metabolic parameters in serum and follicular fluid around parturition. *J. Dairy Sci., 94*(9), 4603-4615. doi:10.3168/jds.2010-3899

Hove, P. R., Mobley, D., Magunda, F., & Call, D. R. (2020). Deploying Elemental Iodine in a Vapor Form to Disinfect Water and to Clear Biofilms. *International Journal of Environmental Research and Public Health, 17*(10). doi:10.3390/ijerph17103489

Hu, X., Li, S., Mu, R., Guo, J., Zhao, C., Cao, Y., Zhang, N., & Yunhe, F. (2022). The Rumen Microbiota Contributes to the Development of Mastitis in Dairy Cows. *Microbiology Spectrum, 10*. doi:10.1128/spectrum.02512-21

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., & Bohannan, B. J. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. *Applied and environmental microbiology, 67*(10), 4399-4406. doi:10.1128/AEM.67.10.4399-4406.2001

Huhtanen, P. (1993). The effects of concentrate energy source and protein content on milk production in cows given grass silage ad libitum. *Grass and Forage Science, 48*(4), 347-355. doi:https://doi.org/10.1111/j.1365-2494.1993.tb01868.x

IASRI. (2012). Lesson 1. Introduction and Significance of Dairy Microbiology. Retrieved from http://ecoursesonline.iasri.res.in/mod/resource/view.php?id=101481

IPCC. (2019). *IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse gas fluxes in Terrestrial Ecosystems*. Retrieved from

Isbell, F., Balvanera, P., Mori, A. S., He, J.-S., Bullock, J. M., Regmi, G. R., Seabloom, E. W., Ferrier, S., Sala, O. E., Guerrero-Ramírez, N. R., Tavella, J., Larkin, D. J., Schmid, B., Outhwaite, C. L., Pramual, P., Borer, E. T., Loreau, M., Omotoriogun, T. C., Obura, D. O., Anderson, M., Portales-Reyes, C., Kirkman, K., Vergara, P. M., Clark, A. T., Komatsu, K. J., Petchey, O. L., Weiskopf, S. R., Williams, L. J., Collins, S. L., Eisenhauer, N., Trisos, C. H., Renard, D., Wright, A. J., Tripathi, P., Cowles, J., Byrnes, J. E., Reich, P. B., Purvis, A., Sharip, Z., O'Connor, M. I., Kazanski, C. E., Haddad, N. M., Soto, E. H., Dee, L. E., Díaz, S., Zirbel, C. R., Avolio, M. L., Wang, S., Ma, Z., Liang, J., Farah, H. C., Johnson, J. A., Miller, B. W., Hautier, Y., Smith, M. D., Knops, J. M., Myers, B. J., Harmáčková, Z. V., Cortés, J., Harfoot, M. B., Gonzalez, A., Newbold, T., Oehri, J., Mazón, M., Dobbs, C., & Palmer, M. S. (2023). Expert perspectives on global biodiversity loss and its drivers and impacts on people. *Frontiers in Ecology and the Environment, 21*(2), 94-103. doi:https://doi.org/10.1002/fee.2536

Jadhav, P. V., Das, D. N., Suresh, K. P., & Shome, B. R. (2018). Threshold somatic cell count for delineation of subclinical mastitis cases. *Vet World, 11*(6), 789-793. doi:10.14202/vetworld.2018.789-793

Jenkins, T. C., & McGuire, M. A. (2006). Major advances in nutrition: impact on milk composition. *J Dairy Sci, 89*(4), 1302-1310. doi:10.3168/jds.S0022-0302(06)72198-1

Jing-Wei, Z., Yi-Yuan, S., Xin, L., Hua, Z., Hui, N., Luo-Yun, F., Ben-Hai, X., Jin-Jin, T., & Lin-Shu, J. (2021). Microbiome and Metabolic Changes of Milk in Response to Dietary Supplementation With Bamboo Leaf Extract in Dairy Cows. *Front Nutr, 8*, 723446. doi:10.3389/fnut.2021.723446

Johansson, M., Placha, O., Pickova, J., Andrén, A., Zamaratskaia, G., Spöndly, E., & Åkerstedt, M. (2013). Impact of crude protein content in silage and concentrate on protein and fatty acid profiles in bovine milk. *Czech Journal of Animal Science, 58*, 304-312. doi:10.17221/6860-CJAS

Karatzia et al. (2012). The influence of dietary Ascophyllum nodosum on haematologic parameters of dairy cows. *Italian Journal of Animal Science, 11*(2), e31. doi:10.4081/ijas.2012.e31

Karlsson, J. O., Parodi, A., van Zanten, H. H. E., Hansson, P.-A., & Röös, E. (2021). Halting European Union soybean feed imports favours ruminants over pigs and poultry. *Nature Food, 2*(1), 38-46. doi:10.1038/s43016-020-00203-7

Keenleyside, C., & Tucker, G. (2010). Farmland abandonment in the EU: an assessment of trends and prospects. *London: WWF and IEEP (November*.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial cell factories, 17*(1), 36-36. doi:10.1186/s12934-018-0879-x

Kim, B.-R., Jiwon, S., Guevarra, R., Lee, J., Kim, D., Seol, K.-H., Lee, J.-H., Kim, H. B., & Isaacson, R. (2017). Deciphering diversity indices for better understanding of the microbial communities. *Journal of microbiology and biotechnology, 27*. doi:10.4014/jmb.1709.09027

Kinley, R. D., Martinez-Fernandez, G., Matthews, M. K., de Nys, R., Magnusson, M., & Tomkins, N. W. (2020). Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *Journal of Cleaner Production, 259*, 120836. doi:https://doi.org/10.1016/j.jclepro.2020.120836

Kinnby, A., White, J. C. B., Toth, G. B., & Pavia, H. (2021). Ocean acidification decreases grazing pressure but alters morphological structure in a dominant coastal seaweed. *PLOS ONE, 16*(1), e0245017. doi:10.1371/journal.pone.0245017

Kotowski, M., Kotowska, D., Biró, M., Babai, D., Sharifian, A., Szentes, S., Łuczaj, Ł., & Molnár, Z. (2023). Change in European Forage and Fodder Plant Indicator Sets over the Past 250 Years. *Rangeland Ecology & Management, 88*, 159-173. doi:https://doi.org/10.1016/j.rama.2023.02.010

Kuehn, J. S., Gorden, P. J., Munro, D., Rong, R., Dong, Q., Plummer, P. J., Wang, C., & Phillips, G. J. (2013). Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. *PLOS ONE, 8*(4), e61959-e61959. doi:10.1371/journal.pone.0061959

Kumssa, D. B., Joy, E. J. M., & Broadley, M. R. (2021). Global Trends (1961–2017) in Human Dietary Potassium Supplies. *Nutrients, 13*(4), 1369. Retrieved from https://www.mdpi.com/2072-6643/13/4/1369

La Duc, M. T., Sumner, R., Pierson, D., Venkat, P., & Venkateswaran, K. (2004). Evidence of pathogenic microbes in the International Space Station drinking water: reason for concern? *Habitation (Elmsford), 10*(1), 39-48. doi:10.3727/154296604774808883

Lamminen, M., Halmemies-Beauchet-Filleau, A., Kokkonen, T., Jaakkola, S., & Vanhatalo, A. (2019). Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. *Animal Feed Science and Technology, 247*, 112-126. doi:10.1016/j.anifeedsci.2018.11.005

Lamminen, M., Halmemies-Beauchet-Filleau, A., Kokkonen, T., Simpura, I., Jaakkola, S., & Vanhatalo, A. (2017). Comparison of microalgae and rapeseed meal as supplementary protein in the grass silage based nutrition of dairy cows. *Animal Feed Science and Technology, 234*, 295. doi:10.1016/j.anifeedsci.2017.10.002

Leip, A., Weiss, F., Wassenaar, T., Domínguez, I., Fellmann, T., Loudjani, P., Tubiello, F., Grandgirard, D., Monni, S., & Biala, K. (2010). *Evaluation of the Livestock Sector's Contribution to the EU Greenhouse Gas Emissions (GGELS) – Final Report*.

Levitt, T. (2019). Nearly a fifth of the EU's budget goes on livestock farming, says Greenpeace. *The Guardian*.

Li, J., Cao, D., Huang, Y., Chen, B., Chen, Z., Wang, R., Dong, Q., Wei, Q., & Liu, L. (2022). Zinc Intakes and Health Outcomes: An Umbrella Review. *Frontiers in Nutrition, 9*. doi:10.3389/fnut.2022.798078

Li, N., Wang, Y., You, C., Ren, J., Chen, W., Zheng, H., & Liu, Z. (2018). Variation in Raw Milk Microbiota Throughout 12 Months and the Impact of Weather Conditions. *Scientific reports, 8*(1), 2371. doi:10.1038/s41598-018-20862-8

Lim, D. H., Mayakrishnan, V., Lee, H. J., Ki, K. S., Kim, T. I., & Kim, Y. (2020). A comparative study on milk composition of Jersey and Holstein dairy cows during the early lactation. *J Anim Sci Technol, 62*(4), 565-576. doi:10.5187/jast.2020.62.4.565

Liu, A. G., Ford, N. A., Hu, F. B., Zelman, K. M., Mozaffarian, D., & Kris-Etherton, P. M. (2017). A healthy approach to dietary fats: understanding the science and taking action to reduce consumer confusion. *Nutr J, 16*(1), 53. doi:10.1186/s12937-017-0271-4

Liu, G., Yu, X., Li, S., Shao, W., & Zhang, N. (2020). Effects of dietary microalgae (Schizochytrium spp.) supplement on milk performance, blood parameters, and milk fatty acid composition in dairy cows. *Czech Journal of Animal Science, 65*, 162-171. doi:10.17221/19/2020-CJAS

Loza, C., Verma, S., Wolffram, S., Susenbeth, A., Blank, R., Taube, F., Loges, R., Hasler, M., Kluß, C., & Malisch, C. S. (2021). Assessing the Potential of Diverse Forage Mixtures to Reduce Enteric Methane Emissions In Vitro. *Animals : an open access journal from MDPI, 11*(4). doi:10.3390/ani11041126

Lucey, J. A. (2015). Raw Milk Consumption: Risks and Benefits. *Nutrition today, 50*(4), 189-193. doi:10.1097/NT.0000000000000108

Madeira, M. S., Cardoso, C., Lopes, P. A., Coelho, D., Afonso, C., Bandarra, N. M., & Prates, J. A. M. (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. *Livestock Science, 205*, 111-121. doi:https://doi.org/10.1016/j.livsci.2017.09.020

Makkar, H., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2015). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology, 212*. doi:10.1016/j.anifeedsci.2015.09.018

Manuelian, C. L., Penasa, M., Visentin, G., Zidi, A., Cassandro, M., & De Marchi, M. (2018). Mineral composition of cow milk from multibreed herds. *Anim Sci J, 89*(11), 1622-1627. doi:10.1111/asj.13095

Manuelian, C. L., Vigolo, V., Burbi, S., Righi, F., Simoni, M., & De Marchi, M. (2022). Detailed comparison between organic and conventional milk from Holstein-Friesian dairy herds in Italy. *Journal of Dairy Science, 105*(7), 5561-5572. doi:https://doi.org/10.3168/jds.2021-21465

Manzocchi, Guggenbühl, B., Kreuzer, M., & Giller, K. (2020). Effects of the substitution of soybean meal by spirulina in a hay-based diet for dairy cows on milk composition and sensory perception. *J Dairy Sci, 103*(12), 11349-11362. doi:10.3168/jds.2020-18602

Manzocchi, E., Hengartner, W., Kreuzer, M., & Giller, K. (2020). Effect of feeding hay vs. silages of various types to dairy cows on feed intake, milk composition and coagulation properties. *Journal of Dairy Research*, *87*(3), 334-340. doi:10.1017/S0022029920000801

Marangoni, F., Pellegrino, L., Verduci, E., Ghiselli, A., Bernabei, R., Calvani, R., Cetin, I., Giampietro, M., Perticone, F., Piretta, L., Giacco, R., Vecchia, C., Brandi, M., Ballardini, D., Banderali, G., Bellentani, S., Canzone, G., Cricelli, C., Faggiano, P., & Poli, A. (2018). Cow's Milk Consumption and Health: A Health Professional's Guide. *Journal of the American College of Nutrition, 38*, 1-12. doi:10.1080/07315724.2018.1491016

Matsushima, J. K. (1979). Classification of Feeds. In J. K. Matsushima (Ed.), *Feeding Beef Cattle* (pp. 19-42). Berlin, Heidelberg: Springer Berlin Heidelberg.

McGill, C. R., Fulgoni, V. L., 3rd, DiRienzo, D., Huth, P. J., Kurilich, A. C., & Miller, G. D. (2008). Contribution of dairy products to dietary potassium intake in the United States population. *J Am Coll Nutr*, *27*(1), 44-50. doi:10.1080/07315724.2008.10719673

McGregor, R. A., & Poppitt, S. D. (2013). Milk protein for improved metabolic health: a review of the evidence. *Nutr Metab (Lond), 10*(1), 46. doi:10.1186/1743-7075-10-46

McKay, Z. C., Lynch, M. B., Mulligan, F. J., Rajauria, G., Miller, C., & Pierce, K. M. (2019). The effect of concentrate supplementation type on milk production, dry matter intake, rumen fermentation, and nitrogen excretion in late-lactation, spring-calving grazing dairy cows. *J Dairy Sci, 102*(6), 5042-5053. doi:10.3168/jds.2018-15796

Mertens, D. R. (2009). *Maximizing forage use by dairy cows*.

Mohit, L. (2021). *Microalgae-based Products Market*. Retrieved from https://www.persistencemarketresearch.com/market-research/microalgae-market.asp

Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L., & Bahcevandziev, K. (2020). Seaweed Potential in the Animal Feed: A Review. *Journal of Marine Science and Engineering, 8*(8). doi:10.3390/jmse8080559

Moran, C. A., Morlacchini, M., & Fusconi, G. (2017). Enhancing the DHA content in milk from dairy cows by feeding ALL-G-RICH™. *Journal of Applied Animal Nutrition, 5*, e11. doi:10.1017/jan.2017.9

Moran, C. A., Morlacchini, M., Keegan, J. D., & Fusconi, G. (2018). The effect of dietary supplementation with Aurantiochytrium limacinum on lactating dairy cows in terms of animal health, productivity and milk composition. *J Anim Physiol Anim Nutr (Berl), 102*(2), 576-590. doi:10.1111/jpn.12827

Mungai, E., Behravesh, C. B., & Gould, L. H. (2015). Increased Outbreaks Associated with Nonpasteurized Milk, United States, 2007–2012. *Emerging Infectious Disease journal, 21*(1), 119. doi:10.3201/eid2101.140447

Nantapo, C. T. W., & Muchenje, V. (2013). Winter and spring variation in daily milk yield and mineral composition of Jersey, Friesian cows and their crosses under a pasture-based dairy system. *South African*

Journal of Animal Science, 43, s17-s21. Retrieved from http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0375-15892013000600004&nrm=iso

National Research Council. (1988). *Designing Foods: Animal Product Options in the Marketplace*. Washington, DC: The National Academies Press.

NDNS. (2020). *National Diet and Nutrition Survey (NDNS). Results from Years 9-11 (combined) of the Rolling Programme (2016/17 to 2018/19)*. Retrieved from

Newton, E. E. (2019). *A critical review of aflatoxin contamination reduction measures for effective climate change catastrophe resiliency and dairy food safety within Thailand.* (Msc Global Health). University of London, St. George's,

Newton, E. E., Lamminen, M., Ray, P., Mackenzie, A. M., Reynolds, C. K., Lee, M. R. F., Halmemies-Beauchet-Filleau, A., Vanhatalo, A., & Stergiadis, S. (2022). Macromineral and trace element concentrations in milk from Finnish Ayrshire cows fed microalgae (Spirulina platensis) and rapeseed (Brassica napus). *J Dairy Sci*, *105*(11), 8866-8878. doi:10.3168/jds.2022-22050

Newton, E. E., Pétursdóttir, Á. H., Beauclercq, S., Clarke, J., Desnica, N., & Stergiadis, S. (2023). Variation in macrominerals and trace elements in cows' retail milk and implications for consumers nutrition. *Food Chemistry*, *418*, 135809. doi:https://doi.org/10.1016/j.foodchem.2023.135809

Newton, E. E., Pétursdóttir, Á. H., Ríkharðsson, G., Beaumal, C., Desnica, N., Giannakopoulou, K., Juniper, D., Ray, P., & Stergiadis, S. (2021). Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations. *Foods*, *10*(7), 1526. Retrieved from https://www.mdpi.com/2304-8158/10/7/1526

Newton., Theodoridou, K., Terré, M., Huws, S., Ray, P., Reynolds, C. K., Prat, N., Sabrià, D., & Stergiadis, S. (2023). Effect of dietary seaweed Ascophyllum nodosum supplementation on milk mineral concentrations, transfer efficiency, and hematological parameters in lactating Holstein cows. *J Dairy Sci*, *106*(10), 6880-6893. doi:10.3168/jds.2022-23074

Noor, H. (2018). Potential of Carrageenans in Foods and Medical Applications. *GHMJ (Global Health Management Journal)*, *2*, 32-36. doi:10.35898/ghmj-22188

NRC. (2001). *Nutrient Requirements of Dairy Cattle* (Seventh Revised Edition ed.). Washington, D.C.: National Academy Press.

Nyström, H. F., Brantsæter, A. L., Erlund, I., Gunnarsdottir, I., Hulthén, L., Laurberg, P., Mattisson, I., Rasmussen, L. B., Virtanen, S., & Meltzer, H. M. (2016). Iodine status in the Nordic countries - past and present. *Food & Nutrition Research*, *60*, 31969-31969. doi:10.3402/fnr.v60.31969

Ohnishi, M., Sawada, T., Marumo, K., Harada, K., Hirose, K., Shimizu, A., Hayashimoto, M., Sato, R., Uchida, N., & Kato, H. (2012). Antimicrobial susceptibility and genetic relatedness of bovine Stenotrophomonas maltophilia isolates from a mastitis outbreak. *Lett Appl Microbiol*, *54*(6), 572-576. doi:10.1111/j.1472-765X.2012.03246.x

Oldenbroek, J. K. (1988). *Feed intake and energy utilization in dairy cows of different breeds.* Wageningen University & Research, Wageningen. Retrieved from https://edepot.wur.nl/206154

Paul, B., Muhimuzi, F., Bacigale, S., Wimba, B., Chiuri, W., Amzati, G., & Maass, B. (2016). Towards an assessment of on-farm niches for improved forages in Sud-Kivu, DR Congo. *Journal of Agriculture and Rural Development in the Tropics and Subtropics, 117*, 243-254.

Payling, L. M., Juniper, D. T., Drake, C., Rymer, C., & Givens, D. I. (2015). Effect of milk type and processing on iodine concentration of organic and conventional winter milk at retail: Implications for nutrition. *Food Chemistry, 178*, 327-330. doi:https://doi.org/10.1016/j.foodchem.2015.01.091

Petzer, I. M., Karzis, J., Donkin, E. F., Webb, E. C., & Etter, E. M. C. (2017). Validity of somatic cell count as indicator of pathogen-specific intramammary infections. *J S Afr Vet Assoc, 88*(0), e1-e10. doi:10.4102/jsava.v88i0.1465

Pincay-Figueroa, P., López González, F., Velarde-Guillén, J., Heredia Nava, D., Martínez-Castañeda, F., Vicente Mainar, F., Martínez-Fernández, A., & Arriaga-Jordán, C. (2016). Cut and carry vs. grazing of cultivated pastures in small- scale dairy systems in the central highlands of Mexico. *Journal of Agriculture and Environment for International Development, 110*, 349-363. doi:10.12895/jaeid.20162.496

Piste, P., Sayaji, D., & Avinash, M. (2012). Calcium and its Role in Human Body. *Int J Res Pharm Biomed Sci, 4*, 2229-3701.

Qin, N., Faludi, G., Beauclercq, S., Pitt, J., Desnica, N., Pétursdóttir, Á., Newton, E. E., Angelidis, A., Givens, I., Juniper, D., Humphries, D., Gunnlaugsdóttir, H., & Stergiadis, S. (2021). Macromineral and trace element concentrations and their seasonal variation in milk from organic and conventional dairy herds. *Food Chem, 359*, 129865. doi:10.1016/j.foodchem.2021.129865

Qin, N., Pétursdóttir, Á. H., Humphries, D. J., Desnica, N., Newton, E. E., Vanhatalo, A., Halmemies-Beauchet-Filleau, A., Bell, L., Givens, D. I., Juniper, D. T., Gunnlaugsdóttir, H., & Stergiadis, S. (2023). Mineral concentrations in milk from cows fed seaweed (Saccharina latissima) under different basal protein supplementation. *Food Chem., 403*, 134315. doi:10.1016/j.foodchem.2022.134315

Ramin, M., & Huhtanen, P. (2013). Development of equations for predicting methane emissions from ruminants. *Journal of Dairy Science, 96*(4), 2476-2493. doi:https://doi.org/10.3168/jds.2012-6095

Rauw, W. M., Gómez Izquierdo, E., Torres, O., García Gil, M., de Miguel Beascoechea, E., Rey Benayas, J. M., & Gomez-Raya, L. (2023). Future farming: protein production for livestock feed in the EU. *Sustainable Earth Reviews, 6*(1), 3. doi:10.1186/s42055-023-00052-9

Rey-Crespo, F., López-Alonso, M., & Miranda, M. (2014). The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. *Animal, 8*(4), 580-586. doi:10.1017/S1751731113002474

Ritota, M., Di Costanzo, M. G., Mattera, M., & Manzi, P. (2017). New Trends for the Evaluation of Heat Treatments of Milk. *J Anal Methods Chem, 2017*, 1864832. doi:10.1155/2017/1864832

Robertson, G. L. (2011). Heat Treatment of Milk | Ultra-High Temperature Treatment (UHT): Aseptic Packaging. In (pp. 708-713).

Robinson, T. P., Wint, G. R., Conchedda, G., Van Boeckel, T. P., Ercoli, V., Palamara, E., Cinardi, G., D'Aietti, L., Hay, S. I., & Gilbert, M. (2014). Mapping the global distribution of livestock. *PLOS ONE, 9*(5), e96084. doi:10.1371/journal.pone.0096084

Roland, L., Drillich, M., & Iwersen, M. (2014). Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation, 26*(5), 592-598. doi:10.1177/1040638714546490

Romero-Velarde, E., Delgado-Franco, D., García-Gutiérrez, M., Gurrola-Díaz, C., Larrosa-Haro, A., Montijo-Barrios, E., Muskiet, F. A. J., Vargas-Guerrero, B., & Geurts, J. (2019). The Importance of Lactose in the Human Diet: Outcomes of a Mexican Consensus Meeting. *Nutrients, 11*(11). doi:10.3390/nu11112737

Roohani, N., Hurrell, R., Kelishadi, R., & Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *J Res Med Sci, 18*(2), 144-157.

Rooke, J. (2001). Forage Evaluation in Ruminant Nutrition. *The Journal of Agricultural Science, 136*(4), 471-475. doi:10.1017/S0021859601228937

Roque, B. M., Salwen, J. K., Kinley, R., & Kebreab, E. (2019). Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *Journal of Cleaner Production, 234*, 132-138. doi:https://doi.org/10.1016/j.jclepro.2019.06.193

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLOS ONE, 16*(3), e0247820. doi:10.1371/journal.pone.0247820

Roy, D., Ye, A., Moughan, P. J., & Singh, H. (2020). Composition, Structure, and Digestive Dynamics of Milk From Different Species—A Review. *Frontiers in Nutrition, 7*. doi:10.3389/fnut.2020.577759

Ryan, M. P., & Pembroke, J. T. (2018). Brevundimonas spp: Emerging global opportunistic pathogens. *Virulence, 9*(1), 480-493. doi:10.1080/21505594.2017.1419116

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., van der Lelie, D., & Dow, J. M. (2009). The versatility and adaptation of bacteria from the genus Stenotrophomonas. *Nature Reviews Microbiology, 7*(7), 514-525. doi:10.1038/nrmicro2163

Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S., & Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology, 12*(1), 76. doi:10.1186/s40104-021-00593-z

Sadhasivam, S., Seedevi, P., Ramasamy, P., Subhapradha, N., Vairamani, S., & Shanmugam, A. (2012). Heavy metal accumulation in seaweeds and sea grasses along southeast coast of India. *Journal of Chemical and Pharmaceutical Research, 2012*, 4240-4244.

Saker, K., Allen, V., Fontenot, J., Bagley, C., Ivy, R., Evans, R., & Wester, D. (2001). Tasco-Forage: II. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract. *Journal of animal science, 79*, 1022-1031. doi:10.2527/2001.7941022x

Sandström, V., Chrysafi, A., Lamminen, M., Troell, M., Jalava, M., Piipponen, J., Siebert, S., van Hal, O., Virkki, V., & Kummu, M. (2022). Food system by-products upcycled in livestock and aquaculture feeds can increase global food supply. *Nature Food, 3*(9), 729-740. doi:10.1038/s43016-022-00589-6

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging infectious diseases, 17*(1), 7-15. doi:10.3201/eid1701.p11101

Schwalfenberg, G. K., & Genuis, S. J. (2017). The Importance of Magnesium in Clinical Healthcare. *Scientifica (Cairo), 2017*, 4179326. doi:10.1155/2017/4179326

Selvaggi, G., Monstrey, S., Van Landuyt, K., Hamdi, M., & Blondeel, P. (2003). The Role of Iodine in Antisepsis and Wound Management: A Reappraisal. *Acta chirurgica Belgica, 103*, 241-247. doi:10.1080/00015458.2003.11679417

Senbeta, E. K. (2018). Effect of Cattle Breed on Milk Composition in the same Management Conditions.

Silva, I., & Sant'Ana, M. (2016). Sodium, Potassium and Other Essential Mineral Contents on Dairy Milk Available in the Markets of a Northeastern Capital in Brazil: A Sodium Intake Estimation for Children. *Food and Public Health, 6*, 165. doi:10.5923/j.fph.20160606.03

Sinedino, L., Honda, P., Souza, L., Lock, A., Boland, M., Staples, C., Thatcher, W., & Santos, J. (2017). Effects of Supplementation with Docosahexaenoic Acid on Reproduction of Dairy Cows. *Reproduction, 153*, REP-16. doi:10.1530/REP-16-0642

Singh, B., Chopra, R., Rai, S., Verma, M., & Mohanta, R. (2014). Effect of Feeding Seaweed as Mineral Source on Mineral Metabolism, Blood and Milk Mineral Profile in Cows. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 86*. doi:10.1007/s40011-014-0413-9

Singh, B., Chopra, R., Rai, S., Verma, M., & Mohanta, R. (2015). Nutritional Evaluation of Seaweed on Nutrient Digestibility, Nitrogen Balance, Milk Production and Composition in Sahiwal Cows. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci., 87*. doi:10.1007/s40011-015-0616-8

Singh, B. K., Chopra, R. C., Rai, S. N., Verma, M. P., & Mohanta, R. K. (2017). Nutritional Evaluation of Seaweed on Nutrient Digestibility, Nitrogen Balance, Milk Production and Composition in Sahiwal Cows. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 87*(2), 437-443. doi:10.1007/s40011-015-0616-8

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. *Journal of Dairy Science, 99*(10), 8472-8476. doi:https://doi.org/10.3168/jds.2015-10810

Stehfest, E., van Zeist, W.-J., Valin, H., Havlik, P., Popp, A., Kyle, P., Tabeau, A., Mason-D'Croz, D., Hasegawa, T., Bodirsky, B. L., Calvin, K., Doelman, J. C., Fujimori, S., Humpenöder, F., Lotze-Campen, H., van Meijl, H., & Wiebe, K. (2019). Key determinants of global land-use projections. *Nature Communications, 10*(1), 2166. doi:10.1038/s41467-019-09945-w

Stergiadis, S., Berlitz, C. B., Hunt, B., Garg, S., Ian Givens, D., & Kliem, K. E. (2019). An update to the fatty acid profiles of bovine retail milk in the United Kingdom: Implications for nutrition in different age and gender groups. *Food Chemistry, 276*, 218-230. doi:https://doi.org/10.1016/j.foodchem.2018.09.165

Stergiadis, S., Qin, N., Faludi, G., Beauclercq, S., Pitt, J., Desnica, N., Pétursdóttir Á, H., Newton, E. E., Angelidis, A. E., Givens, I., Humphries, D. J., Gunnlaugsdóttir, H., & Juniper, D. T. (2021). Mineral Concentrations in Bovine Milk from Farms with Contrasting Grazing Management. *Foods, 10*(11). doi:10.3390/foods10112733

Stone, M. S., Martyn, L., & Weaver, C. M. (2016). Potassium Intake, Bioavailability, Hypertension, and Glucose Control. *Nutrients, 8*(7). doi:10.3390/nu8070444

Suttle, N. (2010). Mineral nutrition of livestock: Fourth edition. *Mineral Nutrition of Livestock: Fourth Edition*, 1-547.

Sutton, J. D. (1989). Altering Milk Composition by Feeding. *Journal of Dairy Science, 72*(10), 2801-2814. doi:https://doi.org/10.3168/jds.S0022-0302(89)79426-1

Talukder, M., & Ahmed, H. M. M. (2017). Effect of somatic cell count on dairy products: a review. *Asian Journal of Medical and Biological Research, 2017*, 1-9. doi:10.3329/ajmbr.v3i1.32030

Taylor, G. R., & Butler, M. (1982). A comparison of the virucidal properties of chlorine, chlorine dioxide, bromine chloride and iodine. *Journal of Hygiene, 89*(2), 321-328. doi:10.1017/S0022172400070856

Thomas, M. K., Vriezen, R., Farber, J. M., Currie, A., Schlech, W., & Fazil, A. (2015). Economic Cost of a Listeria monocytogenes Outbreak in Canada, 2008. *Foodborne Pathogens and Disease, 12*(12), 966-971. doi:10.1089/fpd.2015.1965

Thorning, T. K., Raben, A., Tholstrup, T., Soedamah-Muthu, S. S., Givens, I., & Astrup, A. (2016). Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. *Food & Nutrition Research, 60*, 32527-32527. doi:10.3402/fnr.v60.32527

Till, B. E., Huntington, J. A., Kliem, K. E., Taylor-Pickard, J., & Sinclair, L. A. (2020). Long term dietary supplementation with microalgae increases plasma docosahexaenoic acid in milk and plasma but does not affect plasma 13,14-dihydro-15-keto PGF(2α) concentration in dairy cows. *J Dairy Res, 87*(1), 14-22. doi:10.1017/s002202991900102x

Till, B. E., Huntington, J. A., Posri, W., Early, R., Taylor-Pickard, J., & Sinclair, L. A. (2019). Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows. *J. Dairy Sci., 102*(12), 10934-10946. doi:10.3168/jds.2019-16391

Trøan, G., Dahl, L., Meltzer, H. M., Abel, M. H., Indahl, U. G., Haug, A., & Prestløkken, E. (2015). A model to secure a stable iodine concentration in milk. *Food Nutr Res, 59*, 29829. doi:10.3402/fnr.v59.29829

Tseng, C. K. (1944). Agar: A Valuable Seaweed Product. *The Scientific Monthly, 58*(1), 24-32. Retrieved from http://www.jstor.org/stable/18137

The Dairy Products (Hygiene) Regulations 1995, (1995).

UN. (2022). *2022 Revision of World Population Prospects*. Retrieved from https://population.un.org/wpp/

van der Reijden, Galetti, V., Herter-Aeberli, I., Zimmermann, M. B., Zeder, C., Krzystek, A., Haldimann, M., Barmaz, A., Kreuzer, M., Berard, J., & Schlegel, P. (2019). Effects of feed iodine concentrations and milk processing on iodine concentrations of cows' milk and dairy products, and potential impact on iodine intake in Swiss adults. *British Journal of Nutrition, 122*(2), 172-185. doi:10.1017/S0007114519001041

van der Reijden, O. L., Zimmermann, M. B., & Galetti, V. (2017). Iodine in dairy milk: Sources, concentrations and importance to human health. *Best Pract Res Clin Endocrinol Metab, 31*(4), 385-395. doi:10.1016/j.beem.2017.10.004

van Hal, O., de Boer, I. J. M., Muller, A., de Vries, S., Erb, K. H., Schader, C., Gerrits, W. J. J., & van Zanten, H. H. E. (2019). Upcycling food leftovers and grass resources through livestock: Impact of livestock system and productivity. *Journal of Cleaner Production, 219*, 485-496. doi:https://doi.org/10.1016/j.jclepro.2019.01.329

Vanbergue, E., Peyraud, j.-l., & Hurtaud, C. (2018). Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows. *J. Dairy Sci., 85*, 1-8. doi:10.1017/S0022029918000390

Vanbergue, E., Peyraud, J. L., Ferlay, A., Miranda, G., Martin, P., & Hurtaud, C. (2018). Effects of feeding level, type of forage and milking time on milk lipolytic system in dairy cows. *Livestock Science, 217*, 116-126. doi:https://doi.org/10.1016/j.livsci.2018.09.019

Vaskova, H., & Buckova, M. (2016). Measuring the lactose content in milk. *MATEC Web of Conferences, 76*, 05011. doi:10.1051/matecconf/20167605011

Vasudevan, P., & Tandon, M. (2010). Antimicrobial properties of iodine based products. *Journal of Scientific and Industrial Research, 69*, 376-383.

Velthuis, A. G. J., Meuwissen, M., & Huirne, R. B. M. (2009). Distribution of direct recall costs along the milk chain. *Agribusiness, 25*(4), 466-479. doi:https://doi.org/10.1002/agr.20220

Walsh, S., Buckley, F., Pierce, K., Byrne, N., Patton, J., & Dillon, P. (2008). Effects of breed and feeding system on milk production, body weight, body condition score, reproductive performance, and postpartum ovarian function. *J Dairy Sci, 91*(11), 4401-4413. doi:10.3168/jds.2007-0818

Wang, Z., Liang, Y., Lu, J., Wei, Z., Bao, Y., Yao, X., Fan, Y., Wang, F., Wang, D., & Zhang, Y. (2023). Dietary spirulina supplementation modifies rumen development, fermentation and bacteria composition in Hu sheep when consuming high-fat dietary. *Front Vet Sci, 10*, 1001621. doi:10.3389/fvets.2023.1001621

Weaver, C. M. (2013). Potassium and health. *Advances in nutrition (Bethesda, Md.), 4*(3), 368S-377S. doi:10.3945/an.112.003533

Whitehead, D. C., Goulden, K. M., & Hartley, R. D. (1985). The distribution of nutrient elements in cell wall and other fractions of the herbage of some grasses and legumes. *Journal of the Science of Food and Agriculture*, *36*(5), 311-318. doi:https://doi.org/10.1002/jsfa.2740360502

Williams, J. E., Spiers, D. E., Thompson-Golden, L. N., Hackman, T. J., Ellersieck, M. R., Wax, L., Colling, D. P., Corners, J. B., & Lancaster, P. A. (2009). Effects of Tasco in Alleviation of Heat Stress in Beef Cattle. *The Professional Animal Scientist*, *25*(2), 109-117. doi:https://doi.org/10.15232/S1080-7446(15)30693-8

Willis, C., Jørgensen, F., Aird, H., Elviss, N., Fox, A., Jenkins, C., Fenelon, D., Sadler-Reeves, L., & McLauchlin, J. (2018). An assessment of the microbiological quality and safety of raw drinking milk on retail sale in England. *Journal of Applied Microbiology*, *124*(2), 535-546. doi:https://doi.org/10.1111/jam.13660

Wilson, R. L., Jensen, K. S., Etter, S. J., & Ahola, J. K. (2011). Variation in nutritive quality and mineral content of grazed and clipped forage from native range in southwest Idaho1. *The Professional Animal Scientist*, *27*(5), 435-448. doi:https://doi.org/10.15232/S1080-7446(15)30516-7

Winders, T. M., Boyd, B. M., Hilscher, F. H., Stowell, R. R., Fernando, S. C., & Erickson, G. E. (2020). Evaluation of methane production manipulated by level of intake in growing cattle and corn oil in finishing cattle. *Transl Anim Sci*, *4*(4), txaa186. doi:10.1093/tas/txaa186

Woodside, J. V., & Mullan, K. R. (2021). Iodine status in UK–An accidental public health triumph gone sour. *Clinical endocrinology*, *94*(4), 692-699. doi:https://doi.org/10.1111/cen.14368

Xue, F., Sun, F., Jiang, L., Hua, D., Wang, Y., Nan, X., Zhao, Y., & Xiong, B. (2019). Effects of Partial Replacment of Dietary Forage Using Kelp Powder (Thallus laminariae) on Ruminal Fermentation and Lactation Performances of Dairy Cows. *Animals : an open access journal from MDPI*, *9*(10). doi:10.3390/ani9100852

Yang, Guo, X., Liu, W., Tian, Y., Gao, P., Ren, Y., Zhang, W., Jiang, Y., & Man, C. (2020). The complex community structures and seasonal variations of psychrotrophic bacteria in raw milk in Heilongjiang Province, China. *LWT*, *134*, 110218. doi:https://doi.org/10.1016/j.lwt.2020.110218

Yang, L., Yang, Q., Yi, M., Pang, Z. H., & Xiong, B. H. (2013). Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in northern China. *Journal of Dairy Science*, *96*(11), 6863-6869. doi:https://doi.org/10.3168/jds.2013-6846

Zeinhom, M. M. A., Hassan, G. M., Salem, H. A. M., & Corke, H. (2021). Prevalence and Survival of Stenotrophomonas Species in Milk and Dairy Products in Egypt. *Foodborne Pathogens and Disease*, *18*(5), 337-345. doi:10.1089/fpd.2020.2893

Zhang, R., Huo, W., Zhu, W., & Mao, S. (2015). Characterization of bacterial community of raw milk from dairy cows during subacute ruminal acidosis challenge by high-throughput sequencing. *J Sci Food Agric*, *95*(5), 1072-1079. doi:10.1002/jsfa.6800

Zhang, Y., Wei, J., Qiu, Y., Niu, C., Song, Z., Yuan, Y., & Yue, T. (2019). Structure-Dependent Inhibition of Stenotrophomonas maltophilia by Polyphenol and Its Impact on Cell Membrane. *Frontiers in Microbiology, 10*. doi:10.3389/fmicb.2019.02646

Zhou, S., Li, W., & He, S. (2023). Microalgal diversity enhances water purification efficiency in experimental microcosms. *Frontiers in Ecology and Evolution, 11*. doi:10.3389/fevo.2023.1125743

Zurborg, C. E. (1978). Practical Experiences with Payment for Milk for Protein and Fat. *Journal of Dairy Science, 61*(6), 801-803. doi:https://doi.org/10.3168/jds.S0022-0302(78)83652-2

Chapter 2: "Effect of Macroalgal Supplementation on Milk Quality"

Status: *In Press* as of the time of writing (Newton, E. E., K. Theodoridou, M. Terré, S. Huws, P. Ray, C.K. Reynolds, K. N. Prat, D. Sabrià, and S. Stergiadis. 2023. Effect of dietary seaweed (*Ascophyllum nodosum*) supplementation on milk mineral concentrations, transfer efficiency, and haematological parameters in lactating Holstein cows. J Dairy Sci *In Press*.)

Contribution: The animal trial was run in Spain with collaborators that worked with us through funding of SMARTCOW. Milk was separately measured for basic composition, and blood was measured for haematological parameters separately. Seaweed, feed, and milk were aliquoted and measured for a full mineral profile at UoR exclusively by myself. I performed all analysis by myself, modelled nutritional outcomes by myself, and wrote the research article as the primary author. The overall estimated percentage contribution made by myself for this paper would be 85%, factoring in the contribution of partners to the collection of feed intakes from the animal trial and analysis of milk basic composition, blood haematological values, and along with the text review by co-authors.

Objectives: the present study aimed to (i) investigate the effect of feeding 330 g/d dried *A. nodosum* to Holstein dairy cows on DMI, milk yield and composition, including concentrations of milk macrominerals and trace elements, (ii) quantify the effect on animal haematological parameters, and (iii) estimate the impact that the consumption of milk from seaweed-fed cows may have on consumer mineral intakes.

Hypothesis: For this experiment, I hypothesized that macroalgal supplementation of dairy cows would increase iodine concentrations in milk and lead to a favourable animal haematological parameter panel but lead to reduced milk yield along with reduced fat and protein concentrations.

Overview: Seaweed (*Ascophyllum nodosum*) inclusion in dairy cow diets did not affect productivity and feed efficiency, or measured haematological parameters, but increased the milk concentrations of Mg, P, and I. These effects may be explained by the higher intakes when corn meal was substituted with seaweed in dairy cows' diets, as transfer efficiencies from feed to milk were similar (in case of I) or lower (in case of Mg and P). Milk concentrations of Ca, K, Mn, Zn and Mo were not affected by feeding seaweed in the present study. Based on reported Spanish population milk intakes, the contribution of milk towards I supply would be increased substantially when seaweed is fed to dairy cows.

2.1 Abstract

This study investigated the effect of feeding seaweed (*Ascophyllum nodosum*) to dairy cows on milk mineral concentrations, feed-to-milk mineral transfer efficiencies and haematological parameters. Lactating Holstein cows (n=46) were allocated to one of two diets (n=23 each): (i) control (CON; without seaweed), and (ii) seaweed (SWD; replacing 330 g/d of dried corn meal in CON with 330 g/d dried *A. nodosum*). All cows were fed the CON diet for 4 weeks before the experiment (adaptation period); and animals were then fed the experimental diets for 9 weeks. Samples included sequential 3-week composite feed samples, a composite milk sample on the last day of each week, and a blood sample at the end of the study. Data were statistically analysed using a linear mixed effects model with diet, week, and their interaction as fixed factors; cow (nested within diet) as a random factor and data collected on the last day of the adaptation period as covariates. Feeding SWD increased milk concentrations of Mg (+6.6 mg/kg), P (+56 mg/kg), and I (+1720 μg/kg). It also reduced transfer efficiency of Ca, Mg, P, K, Mn, and Zn, and increased transfer efficiency of Mo. Feeding SWD marginally reduced milk protein concentrations while there was no effect of SWD feeding on cows' haematological parameters. Feeding *A. nodosum* increased milk I concentrations, which can be beneficial when feed I concentration is limited or in demographics or populations with increased risk of I deficiency (e.g., female adolescents, pregnant women, nursing mothers). However, care should also be taken when feeding SWD to dairy cows because, in the present study, milk I concentrations were particularly high and could result in I intakes that pose a health risk for children consuming milk.

Keywords: bovine milk, minerals, seaweed, iodine, *Ascophyllum nodosum*

2.2 Introduction

Dairy products are significant sources of dietary essential minerals such as Ca, Se, Mg, Zn, and I across the world for multiple demographics, and the extent of their contribution is influenced by country-specific agricultural and dietary circumstances (Haug et al., 2007, Hettinga & van Valenberg, 2017, Smith et al., 2021). Macrominerals Ca and Mg are necessary for the development and maintenance of healthy bones (Thorning et al., 2016). Microminerals Se and Zn play an important role in numerous biochemical pathways and cellular functions in human bodies (Chasapis et al., 2020, Kieliszek et al., 2022). Finally, sufficiency of I is important not only for the avoidance of goitre, but also for foetal and infant neurological development, as even mild maternal deficiency has been found to negatively affect intelligence quotients of their offspring (Bath et al., 2013).

Mineral concentrations in bovine milk can be modulated by a host of factors such as breed, feed composition, stage of lactation, climate, and processing; of which one of the most influential is seasonal differences (Nada et al., 2010, Stergiadis et al., 2021). Given that climate change continues to pose a substantial barrier to the attainment of food security at the farm level, production systems must adapt quickly to maintain production and product quality while adopting potentially sweeping changes (Gornall et al., 2010, Thornton et al., 2018, Ahmed et al., 2022). In turn, changes to feeding regimes with differing mineral concentrations may affect their concentrations in milk and dairy products (Newton et al., 2021, Stergiadis et al., 2021). The term "mineral transfer efficiency" has been used to describe the proportions of elements consumed that are secreted in milk (Kronqvist, 2011, Newton et al., 2021). Transfer efficiencies are a function of dietary supply and can be affected by the mineral content of the feed and the interaction that certain feed components have on minerals' bioavailability and absorption, such as glucosinolates that are found in commonly-fed cruciferous vegetables and are known to reduce I transfer from feed-to-milk (Papas et al., 1979, Bischoff, 2016). Therefore, any systemic changes to feeding practice on the farm, specifically regarding diet composition, should be contextually assessed to gauge the transfer efficiency of minerals to milk and the resulting impacts on nutritional security of dairy consumers.

The inclusion of phycological products into dairy cattle feed, specifically that of seaweed, or seaweed-based products (such as certain species of red seaweed within the *Asparagopsis* genus, or certain species of brown seaweed within the *Ascophyllum* genus) has recently gained interest as a way of reducing enteric methane emission and promoting smallholder resiliency (Duarte et al., 2017). Recent research has shown marked reduction of methane emissions in ruminants, when *Asparagopsis armata* and *Asparagopsis taxiformis* were offered in the diets of dairy cows (-43%) and beef steers (-80%), respectively (Roque et al., 2019, Roque et al., 2021). Additionally, brown seaweed *Ascophyllum nodosum* feeding has been shown to increase I content of milk by +1192 μg/kg when fed at 170g/cow/d, in comparison with a control diet (Antaya et al., 2015, Antaya et al., 2019). Similar results have also been observed when a 91:9 mixture of brown seaweeds *A. nodosum:Laminaria digitata* was fed at 158 g/cow/d, leading to an increase of +1649 μg/kg milk when compared with the control diet (Newton et al., 2021). Seaweeds can be a rich source of minerals due to its large capacity for mineral bioaccumulation, but their concentrations can vary across species, seasons, and processing methods among other factors (Nitschke and Stengel, 2015, Nitschke and Stengel, 2016). Alongside the potential accumulation of beneficial minerals, there are concerns that some harvested seaweeds (either wild or cultivated), may contain heavy metals that are potentially harmful to animals physiologically and/or subsequently, the consumer of animal-based products (Roleda et al., 2018). The potentially harmful nature of bioaccumulated heavy metals introduces consideration for the measurement of health parameters for the animal, therefore this study additionally implemented haematological monitoring to gauge potentially harmful outcomes from feeding seaweed.

The brown seaweed *A. nodosum* represents a significant portion of current research interest, specifically in Europe, wherein it is by far the species with the largest wild collection for Ireland and Iceland, as well as placing 4th in highest tonnage collected in the world (FAO, 2021). Therefore, *A. nodosum* is a rather plentiful and accessible seaweed for feeding to livestock in Europe. However, if seaweed is to be used as a widespread feed ingredient for dairy cows, effects on milk composition must be considered. Therefore, the present study aimed to (i) investigate the effect of feeding 330 g/d dried *A. nodosum* to Holstein dairy cows on DMI, milk yield and composition, including concentrations of milk macrominerals and trace elements, (ii) quantify the effect on animal haematological parameters, and (iii) estimate the impact that the consumption of milk from seaweed-fed cows may have on consumer mineral intakes.

2.3 Materials and Methods

2.3.1 Experimental conditions

The study was conducted at Institute of Agrifood Research and Technology (IRTA) dairy research farm in Monells (Girona, Spain) from June to August 2021. In total 23 primiparous and 23 multiparous Holstein dairy cows, were selected based on initial body weight (BW; 697 ± 65.6 kg), average milk yield (33.9 ± 5.27 kg/d), and days in milk (DIM; 168 ± 59.8). Cows were blocked by parity (primiparous and multiparous), days in milk (DIM), and milk yield, and randomly assigned to two dietary treatments equally distributed in four different pens; within a randomized complete block design in which they remained in the experimental diets and groups across the 9-week experiment. All cows (from both groups) were fed the CON diets continuously for four weeks prior to the introduction to the experimental diets (adaptation period). Diets were fed as a total mixed ration (TMR) at 48:52 forage:concentrate ratio and represented a control diet (without seaweed; CON) and a diet replacing 330 g/d of dried corn meal with 330 g/d of dried seaweed (*A. nodosum*) (SWD) (SeaLac Ltd, Kiltimagh, Ireland). Animals were fed twice daily at 0800h and 1900h for a period of 64d, and all received a total of 300 g/d dried pelleted soybean in the milking parlour (150 g of dried soya per milking). Pens were equipped with 20 cubicles bedded with a mixture of compost and sawdust, 4 electronic water troughs, and 15 electronic feed bins (MooFeeder, MooSystems, Cortes, Spain) that allowed the registration of individual daily feed intake by identifying the animal when it entered into the feed bin and by the difference between the weight of feed before and after feeding. Diet formulations are presented in Table 2.1. Mineral concentrations of TMR fed to cows of the experimental diets are presented in Table 2.2.

Table 2.1
Measured ingredients and basic composition of total mixed ration (TMR) of cows fed the experimental diets (Control, no seaweed, CON; 330 g/d dried *Ascophyllum nodosum* supplement, SWD)

	CON	SWD
Diet Formulation, g/kg DM		
Corn meal	198	187
Alfalfa silage	159	159
Ryegrass silage	132	132
Soybean hulls	102	102
Corn silage	93	94
Oat hay	90	90
Wheat meal	88	88
Soybean meal	82	82
Wheat middlings	38	38
Barley straw	8	8
Calcium carbonate	4	3
Magnesium oxide	2	2
Premix[1,2]	2	2
Sodium chloride	2	1
Ascophyllum nodosum	0	12
TMR basic composition; g/kg		
Neutral detergent fibre (NDF)	426	419
Acid detergent fibre (ADF)	278	261
Crude protein (CP)	168	163
Ash	92	90
Fat	32	31

Abbreviations: CON = Control Diet; SWD = Seaweed-supplemented Diet
[1] Premix contained: Vitamin A: 2,250,000 IU/kg; Vitamin D3 665,000 IU/kg; Vitamin E: 8,800 mg/kg; Manganese (Manganese oxide): 30,000 mg/kg; Cupper (Cupper sulphate): 5,000 mg/kg; Zinc (Zinc oxide): 30,000 mg/kg; Iodine (Potassium iodide: 250 mg/kg; Cobalt (Cobalt acetate): 40 mg/kg; Selenium (sodium selenite): 150 mg/kg; Iron (iron carbonate) 20,000 mg/kg, Butylhydroxytoluene 1,500 mg/kg, sepiolite 279,949 mg/kg
[2] Iodine was removed from the premix in SWD ration to ensure that I supply in the diet does not exceed EFSA's regulations of 5 mg I/kg DM (EFSA, 2013)

Table 2.2
Mineral concentrations of total mixed ration (TMR) fed to cows of the experimental diets (Control, no seaweed, CON; 330 g/d dried *Ascophyllum nodosum* supplement, SWD) and cows' total mineral intakes per day.

	CON n=9			SWD n=9		
	AVG	SD	RANGE	AVG	SD	RANGE
Macromineral concentrations of experimental diets (g/kg DM)[1]						
Calcium	6.84	0.311	6.54 – 7.34	7.04	0.212	6.78 – 7.36
Magnesium	2.58	0.047	2.52 – 2.65	2.84	0.154	2.62 – 3.01
Phosphorus	3.96	0.121	3.75 – 4.06	4.15	0.067	4.08 – 4.24
Potassium	19.38	1.070	18.21 – 21.03	19.49	0.829	18.62 –
Sodium	1.44	0.019	1.41 – 1.46	1.17	0.055	1.10 – 1.25
Trace element concentrations of experimental diets (mg/kg DM unless indicated by [2])[1]						
Copper	17.15	1.407	15.11 – 18.91	13.00	1.962	10.91 –
Iron	211	42.9	140 - 251	634	45.3	605 - 712
Iodine[2]	509	77.7	424 - 635	6087	1534.8	4217 - 8470
Manganese	35.2	3.71	30.2 – 40.1	86.8	8.58	75.2 – 97.7
Molybdenum	1.39	0.030	1.35 – 1.43	1.14	0.021	1.11 – 1.16
Zinc	33.4	4.23	26.4 – 36.7	70.6	5.98	62.6 – 77.4
Heavy metal concentrations of experimental diets (mg/kg DM unless indicated by [2])[1]						
Arsenic[2]	195	10.1	181 - 205	452	38.1	396 - 496
Cobalt	389	121.8	307 - 598	329	120.3	225 - 533
Macromineral intakes from experimental diets (g/d)[3]						
Calcium	165	21.5	120 - 220	179	22.7	137 - 226
Magnesium	62.1	6.65	46.6 – 77.2	71.9	9.33	53.6 – 92.3
Phosphorus	95.3	11.91	67.8 – 121.7	105.3	13.07	79.0 – 130.0
Potassium	467	63.6	338 - 630	495	63.9	374 - 621
Sodium	34.5	3.92	25.8 – 43.8	29.7	3.83	22.8 – 38.0
Trace element intakes from experimental diets (mg/d unless indicated by [4])[3]						
Copper	414	66.9	273 - 566	331	71.8	219 - 497
Iron[4]	5.08	1.181	2.92 – 7.53	16.10	2.446	11.74 –
Iodine	12	2.30	8.87 – 18.5	153	37.9	88 - 238
Manganese	843	109.7	631 - 1105	2203	351.1	1456 - 2997
Molybdenum	33.4	3.48	25.8 – 41.9	28.9	3.62	22.2 – 35.5
Zinc	801	127.1	552 - 1098	1790	263.8	1214 - 2376
Heavy metal intakes from experimental diets (mg/d)[3]						
Arsenic	4.68	0.551	3.67 – 6.15	11.44	1.541	8.30 – 14.9
Cobalt	9.38	3.918	6.06 – 17.41	8.40	3.372	4.52 – 16.26

Abbreviations: CON = Control Diet; SWD = Seaweed Diet; AVG = Average Value; SD = Standard Deviation; RANGE = Minimum and Maximum values

[1] Concentrations of minerals in experimental diets accounts for all weeks 2-9 of the 9-week experiment

[2] Expressed in μg/kg DM

[3] Concentrations of mineral intakes in experimental diets account for weeks 2,4,6,8 of the 9-week experiment

[4] Expressed in g/d DM

2.3.2 Experimental sampling

Samples of TMR were obtained weekly to determine DM and mineral concentrations. Samples of feed were frozen at -20ºC and composited every 3 weeks and analysed. Fortnightly, individual milk samples were collected for morning and afternoon milking and composited on the basis of milk yield to produce a daily sample for each cow. Two EDTA treated tubes (Vacutainer, Becton Dickinson, Madrid Spain) for blood samples (5 mL) were obtained from the coccygeal vein at day 64 of the study. One tube was refrigerated for further haematological analysis, and the other was centrifuged at 1,500 x g for 10 min and resulting plasma frozen at -20°C.

2.3.3 Experimental analysis

Feed was analysed for DM (method 934.01; oven drying in 100 °C until constant weight), N (method 984.13; copper catalyst Kjeldalh method), EE (method 920.39; ether extraction), and ash (method 942.05; heat at 600oC for 2h) following AOAC (1990) and NDF according to Van Soest et al. (1991) using sodium sulphite and heat stable amylase and expressed inclusive of residual ash. Non-fibre carbohydrates were calculated as 100 minus the summary of CP, NDF, EE, and ash. Milk was analysed for fat, protein, lactose, and urea concentrations using infrared spectroscopy (MilkoScan™ 7; Foss Iberia S.A., Barcelona, Spain) and somatic cell counts were analysed by Fossomatic 7 (Foss Iberia S.A., Barcelona, Spain).

Mineral concentrations of feed and milk were determined by utilizing a protocol based on US-EPA method 3051A (microwave assisted acid digestion of sediment, sludges, soils, and oils;(EPA, 2007), using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7000, Agilent, Singapore). Modifications included the amount of milk and acid that was used to digest the milk, as well as the concentrations of diluted solution that was then analysed, described below. All samples analysed for mineral concentrations were assessed in the present study in duplicates and were validated using ERM®- BD150 certified reference material (CRM) skimmed milk powder for milk, and IPE 993 black poplar hybrids leaf (*Populus x euramericana*) from Lienden, Netherlands, for TMR. Seaweed I concentrations were validated in reference to a chemistry analysis report generated by JHG Analytical Services Ltd in place of a specific *A. nodosum* reference material. Digestion and subsequent extraction was accomplished using Ethos Easy Microwave Digestion System with the heating of a 7.5 mL HNO3 + 2.5 mL HCl solution, and either 1 mL of milk or 0.5 g of feed to form a solution. The subsequent acid and sample solution was then subjected to a 15 min heating phase to reach 180°C, maintained at 180°C for 10 min, and then allowed to cool until it reached ambient temperature. The digested solution from microwave vessels was filtered through Cytiva Whatman™ 540 hardened ashless 110 mm diameter filter paper into Corning™ Falcon™ 50 mL polypropylene conical centrifuge tubes. The resulting solution was then diluted to a total weight of 50 g with ultrapure H2O, and then again at factors of 1:4

and 1:10 with ultrapure H2O into Corning™ Falcon™ 15 mL polypropylene conical centrifuge tubes for analysis.

Standards were created and later adjusted to encompass expected sample values based on preliminary testing within the same acid concentration for each final diluted sample. Trace element (Mn, Fe, Co, Cu, Zn, As, Mo) standards except for I were created with SPEX CertiPrep™ multi-element standard. Standards for I were created with ROMIL PrimAg® Mono-Component Reference Solutions. Macromineral (Ca, Mg, P, K, Na) standards were created with the element specific Fisher Chemical 1000ppm standard.

Mineral concentrations (mg/kg milk) were calculated as follows:

$$\frac{\left(\boldsymbol{Analytical\ reading}\ \left(\frac{\boldsymbol{\mu g}}{\boldsymbol{L}}\right)-\boldsymbol{blank}\ \left(\frac{\boldsymbol{\mu g}}{\boldsymbol{L}}\right)\right)\times \boldsymbol{Dilution\ Factor}}{\boldsymbol{1000}\ \times\left(\frac{\boldsymbol{measured\ sample\ mass\ (g)}}{\boldsymbol{diluted\ solution\ mass\ (g)}}\right)}$$

where (i) dilution factor was the fractional dilution performed to maintain read values within standard brackets (4 and 10 for micro- and macrominerals respectively), (ii) diluted solution mass was the total mass of the solution when brought up to 50 g with ultrapure H2O, and (iii) measured sample mass was the weight of the sample delivered to the microwave digestion vessel.

Transfer efficiencies from feed to milk (g into milk per 100 g ingested) were calculated as follows:

$$\boldsymbol{100}\ \times\left(\frac{\left(\boldsymbol{milk\ mineral\ concentration}\ \left(\frac{\boldsymbol{\mu g}}{\boldsymbol{kg\ milk}}\right)\times \boldsymbol{milk\ output}\ \left(\frac{\boldsymbol{kg}}{\boldsymbol{day}}\right)\right)}{\left(\boldsymbol{diet\ mineral\ concentration}\ \left(\frac{\boldsymbol{\mu g}}{\boldsymbol{kg\ DM}}\right)\times \boldsymbol{feed\ intake}\ \left(\frac{\boldsymbol{kg\ DM}}{\boldsymbol{day}}\right)\right)}\right)$$

Plasma haptoglobin was measured using the commercial kit Tridelta PHASE haptoglobin assay (Tridelta Development Ltd., Maynooth, Ireland). Whole blood in EDTA tubes was refrigerated for analysis in the following 12 hours for haematological parameters (White Blood Cell Count, Neutrophils, Lymphocytes, Monocytes, Eosinophils) using Element HT5 analyser (Heska, Colorado, US).

2.3.4 Statistical analysis

Data for milk production and composition, efficiency parameters, and mineral concentrations and transfer efficiencies were analysed using a linear mixed effects model in Minitab 20 (Minitab LLC, Pennsylvania, US). Diet, week, and their interaction were used as fixed factors, and cow (nested within treatment) as a random factor. Data collected on the last day of the adaptation period (before introducing experimental diets) were used as covariate for all measured variables, except for mineral transfer efficiencies. Normality of residuals were evaluated visually, and no data showed deviation from normality except for SCC which was log-transformed prior to performing the linear mixed model. Where necessary, Tukey's least significant difference test ($P < 0.05$) was used for pairwise comparison for the means, where the mixed effects model showed a significant effect of week or the diet x week interaction. Haematological data were analysed by general linear models in Minitab 20, using diet as fixed factor.

2.4 Results

2.4.1 Milk basic composition and efficiency parameters

There was a significant effect of diet on the concentrations of protein (P = 0.016) in milk (Table 2.3), but the numerical differences were small with SWD milk containing 0.06 g less protein per 100 g milk, when compared with CON milk. There was a significant effect (P < 0.001) of the diet × week interaction for DMI, feed, and ECM efficiency (Table 2.3). In Week 2, SWD fed cows has higher DMI but lower efficiency measurements than CON cows; but these differences were not significant in the following weeks (Figure 2.1).

Table 2.3
Means, Standard error (SE), and ANOVA P-values for the effect of dietary treatment (Control, no seaweed, CON; 330g/d *Ascophyllum nodosum* supplement, SWD) on animal diet data, milk production and basic composition, and efficiency parameters

	Dietary Treatment			ANOVA P-values[1]		
Parameters	CON n=92	SWD n=92	SE	Diet	Week	Diet × Week
Dry Matter Intake (kg/d)	24.7	24.7	0.34	0.972	<0.001	<0.001
Milk Yield (kg/d)	32.1	32.2	0.29	0.806	0.480	0.398
Milk Fat (g/100g milk)	3.63	3.65	0.058	0.841	<0.001	0.563
Milk Protein (g/100g milk)	3.38	3.32	0.016	0.016	<0.001	0.460
Milk Lactose (g/100g milk)	4.91	4.89	0.014	0.293	<0.001	0.124
Milk Urea (mg/L)	225	212	7.2	0.120	<0.001	0.470
Milk SSC (x1000/mL) [2]	143	85	36.8	0.677	0.025	0.973
Milk Fat:Protein	1.07	1.10	0.015	0.128	0.013	0.557
ECM [3]	31.7	31.6	0.34	0.918	0.001	0.328
Feed Efficiency (kg milk/kg of DMI)	1.31	1.31	0.01	0.865	<0.001	<0.001
ECM Efficiency (ECM/kg of DMI)	1.30	1.27	0.011	0.072	<0.001	<0.001

Abbreviations: CON = Control Diet; SWD = Seaweed Diet; n = number of records; AVG = Average Value; SE = Standard Error; ANOVA = Analysis of Variance; SCC = Somatic Cell Count; ECM = Energy Corrected Milk yield; DMI = Dry Matter Intake

[1] Significances were declared at $P < 0.05$.

[2] P-values were generated from the common logarithm of somatic cell count (SCC) values.

[3] Energy Corrected Milk Yield = milk yield (kg) × (0.01 + 0.0122 milk fat (g/kg) + 0.0077 milk protein (g/kg) + 0.053 milk lactose (g/kg))

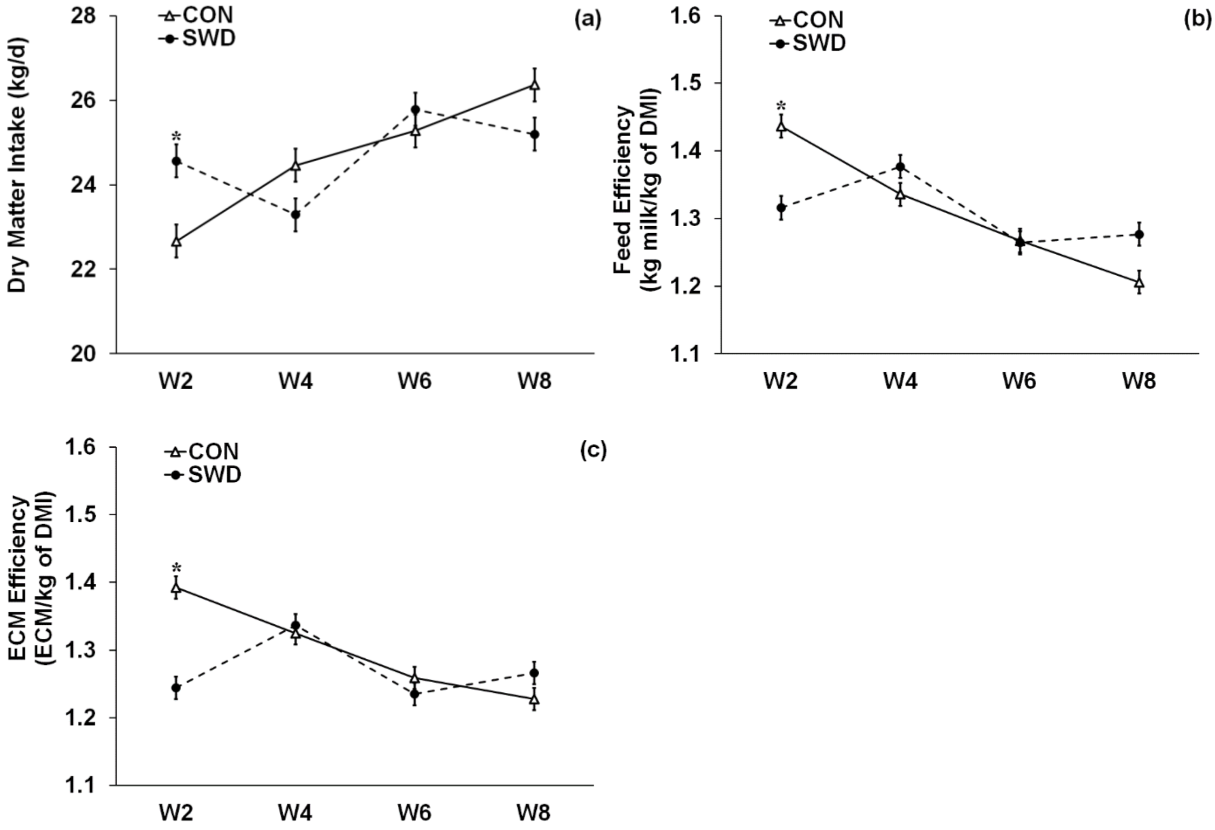

Figure 2.1. Interaction means ± SE (error bars) for the effects of dietary treatment (Control, no seaweed, CON; 330 g/d dried *Ascophyllum nodosum* supplement, SWD) and week (W2, W4, W6, W8) on animal DMI (a; kg/d), milk feed efficiency (b; kg milk / kg of DMI; $P < 0.001$), and ECM efficiency (c; ECM / kg of DMI; $P < 0.001$). Means for diet treatments within a week denoted with * are significantly different ($P < 0.05$).

2.4.2 Milk mineral composition and transfer efficiencies

There was a significant effect of diet on the concentrations of Mg (P = 0.007), P (P = 0.030), and I (P < 0.001) in milk (Table 2.4). The SWD diet, compared to CON diet, increased Mg by 6.8% (+6.6 mg/kg milk), P by 5.3% (+56 mg/kg milk) and I by 1036% (+1720 μg/kg milk). There was a significant effect of the diet × week interaction (P ≤ 0.001) for all measured mineral concentrations (Ca, Mg, P, K, Na, I, Mn, Mo, Zn) (Figure 2.2). The SWD milk contained more Ca, P, and Mo than CON milk in Week 4, but not in Week 8. The SWD milk contained more Mg in Weeks 4 and 8, Zn in Week 6, and Na in Week 8. The SWD milk contained less K in Week 6, Mo in Week 6, Zn in Week 4, Ca in Week 6, as well as Mn in Week 4. Milk I concentrations were higher in SWD than CON milk throughout the experiment with the relative differences incrementally increasing from Week 2 to Week 8.

Table 2.4
Means, Standard error (SE), and ANOVA P-values for the effect of dietary treatment (Control, no seaweed, CON; 330g/d *Ascophyllum nodosum* supplement, SWD) on mineral concentrations of milk and feed-to-milk transfer efficiency.

	Dietary Treatment			ANOVA P-values[1]		
Minerals	CON n=92	SWD n=92	SE	Diet	Week	Diet × week
Macromineral concentrations (mg/kg)						
Calcium	1243	1279	18.0	0.183	0.003	<0.001
Magnesium	96.8	103.4	1.63	0.007	<0.001	<0.001
Phosphorus	1064	1120	17.5	0.030	0.004	<0.001
Potassium	1707	1658	21.0	0.088	<0.001	<0.001
Sodium	305	314	5.7	0.262	0.010	<0.001
Trace element concentrations (μg/kg)						
Iodine	166	1886	70.1	<0.001	<0.001	<0.001
Manganese	32.7	32.8	1.45	0.970	<0.001	<0.001
Molybdenum	46.2	47.3	1.60	0.657	<0.001	<0.001
Zinc	4369	4335	162.5	0.888	<0.001	<0.001
Macromineral transfer efficiency (%; g in milk per 100g ingested)[2]						
Calcium	24.6	22.9	0.50	0.021	<0.001	<0.001
Magnesium	5.0	4.6	0.09	0.005	<0.001	0.001
Phosphorus	36.1	34.1	1.45	0.049	<0.001	<0.001
Potassium	12.0	10.9	0.39	0.045	<0.001	<0.001
Sodium	30.2	32.2	1.06	0.191	<0.001	<0.001
Trace element transfer efficiency (%; g in milk per 100g ingested)[2]						
Iodine	44.6	41.8	2.44	0.434	<0.001	<0.001
Manganese	0.13	0.05	0.004	<0.001	<0.001	<0.001
Molybdenum	4.46	5.25	0.223	0.016	<0.001	<0.001
Zinc	17.0	7.8	0.55	<0.001	<0.001	<0.001

Abbreviations: CON = Control Diet; SWD = Seaweed Diet; n = number of records; AVG = Average Value; SE = Standard Error; ANOVA = Analysis of Variance
[1] Significances were declared at $P < 0.05$.
[2] The covariate was not used as week 0 TMR mineral content was not measured

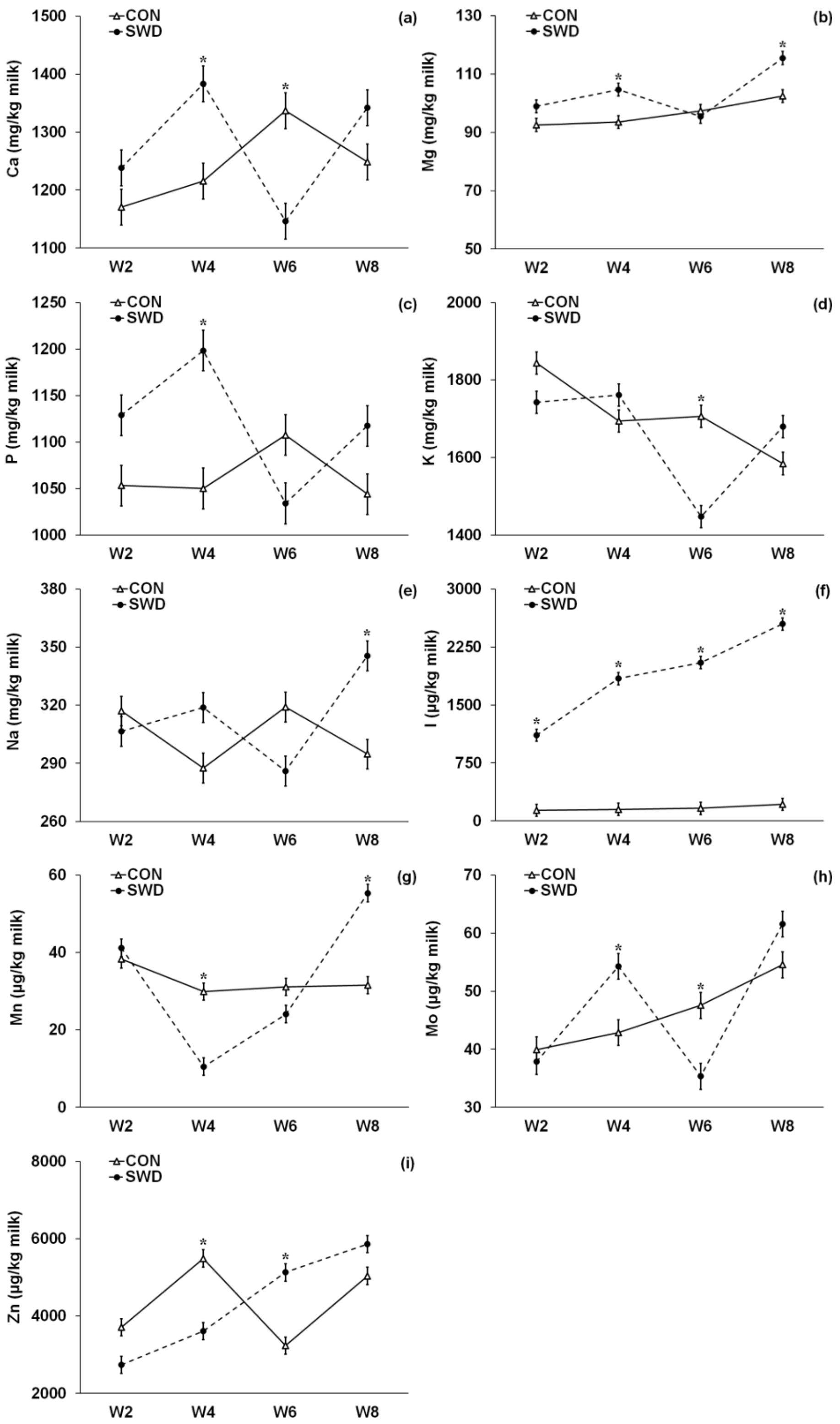

Figure 2.2. Interaction means ± SE (error bars) for the effects of dietary treatment (Control, no seaweed, CON; 330 g/d dried *Ascophyllum nodosum* supplement, SWD) and week (W2, W4, W6, W8) on the concentration of Ca (a; mg/kg; $P < 0.001$), Mg (b; mg/kg; $P = 0.001$), P (c; mg/kg; $P < 0.001$), K (d; mg/kg; $P < 0.001$), Na (e; mg/kg; $P < 0.001$), I (f; µg/kg; $P < 0.001$), Mn (g; µg/kg; $P < 0.001$), Mo (h; µg/kg; $P < 0.001$), and Zn (i; µg/kg; $P < 0.001$) in milk. Means for diet treatments within a week denoted with * are significantly different ($P < 0.05$).

There was a significant effect of diet on the feed-to-milk transfer efficiency of Ca ($P = 0.021$), Mg ($P = 0.005$), P ($P = 0.049$), K ($P = 0.045$), Mn ($P < 0.001$), Mo ($P = 0.016$), and Zn ($P < 0.001$) (Table 2.4). For every 100 g of individual mineral intake SWD diet transferred 1.7 g less Ca, 0.4 g less Mg, 2.0 g less P, 1.1 g less K, 0.08 g less Mn, and 9.2 g less Zn, but 0.79 g more Mo, compared with the CON diet. There was a significant effect ($P \leq 0.001$) for the diet × week interaction for all measured mineral transfer efficiencies (Figure 2.3). Transfer efficiencies for Ca, Mg, P, and K were lower in SWD cows than in CON cows in Weeks 2 and 6 but the differences in Weeks 4 and 8 were not significant. Transfer efficiencies for Na and Mo were higher in SWD cows than in CON cows in Weeks 4 and 8 but the differences in Weeks 2 and 6 were not significant. Transfer efficiencies for Zn in CON milk was higher throughout the experiment, but maximum relative difference has been observed in Week 4. I transfer efficiencies from feed to milk were higher in CON cows, than in SWD cows in Weeks 2 and 4, but there was no difference between milk from the two experimental groups in Weeks 6 and 8. Mn transfer efficiencies were lower in SWD cows than in CON cows in Weeks 2, 4, and 6.

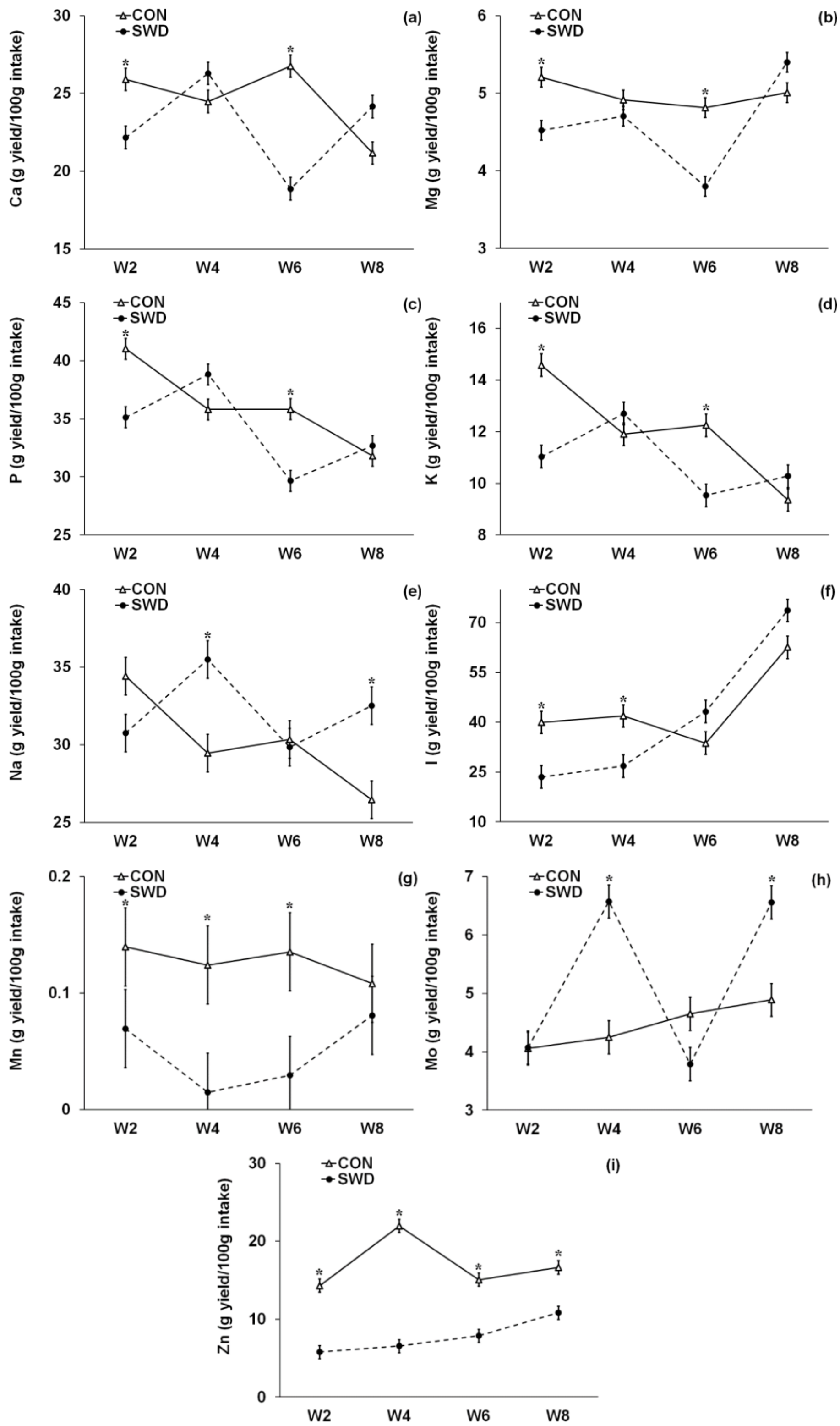

Figure 2.3 Interaction means ± SE (error bars) for the effects of dietary treatment (Control, no seaweed, CON; 330 g/d dried *Ascophyllum nodosum* supplement, SWD) and week (W2, W4, W6, W8) on the transfer efficiencies of Ca (a; g yield/100g intake), Mg (b; g yield/100g intake; $P = 0.001$), P (c; g yield/100g intake; $P < 0.001$), K (d; g yield/100g intake; $P < 0.001$), Na (e; g yield/100g intake; $P < 0.001$), I (f; g yield/100g intake; $P < 0.001$), Mn (g; g yield/100g intake; $P < 0.001$), Mo (h; g yield/100g intake; $P < 0.001$), and Zn (i; g yield/100g intake; $P < 0.001$) from feed to milk. Means for diet treatments within a week denoted with * are significantly different ($P < 0.05$).

2.4.3 Cow haematological parameters

Diet did not influence the concentrations of the assessed blood plasma parameters (Table 2.5).

Table 2.5
Means, Standard error (SE), and ANOVA P-values for the effect of dietary treatment (Control, no seaweed, CON; 330g/d *Ascophyllum nodosum* supplement, SWD) on cows' hematological parameters at the end of the experiment.

	Dietary Treatment			ANOVA P-values[1]
Parameters	CON n=23	SWD n=23	SE	Diet
White Blood Count (10^9/L)	7.38	7.01	0.258	0.320
Neutrophils (10^9/L)	2.58	2.18	0.172	0.107
Lymphocytes (10^9/L)	4.45	4.51	0.225	0.843
Monocytes (10^9/L)	0.117	0.116	0.0120	0.959
Eosinophils (10^9/L)	0.240	0.210	0.0380	0.579
Neutrophils (%)	34.8	31.1	2.13	0.232
Lymphocytes (%)	60.6	64.3	2.44	0.294
Monocytes (%)	1.57	1.66	0.146	0.691
Eosinophil (%)	3.03	2.94	0.403	0.879
RBC (10^12/L)	5.91	5.83	0.124	0.625
Haemoglobin (g/dL)	10.5	10.5	0.13	0.697
Haematocrit (%)	29.1	29.1	0.41	0.958
MCV (fL)	49.6	50.2	0.81	0.583
MCH (pg)	17.9	18.2	0.30	0.441
MCHC (g/dL)	36.0	36.2	0.22	0.488
RDW-CV (%)	21.5	20.8	0.44	0.255
PLT (10^9/L)	231	240	26.0	0.807
MPV (fL)	6.59	6.62	0.113	0.850

Abbreviations: CON = Control Diet; SWD = Seaweed Diet; n = number of records; SE = Standard Error; ANOVA = Analysis of Variance; MSV = Mean cell volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; RDW-CV = Red blood cells distribution width; PLT = Platelet count; PMV = Mean platelet volume; RBC = Red blood cells
[1] Significances were declared at $P < 0.05$.

2.5 Discussion

2.5.1 Effect of seaweed supplementation on milk production, milk composition, and efficiency parameters

In the present study, diet seaweed supplementation reduced milk protein concentration and there was a significant diet × week interaction for DMI (kg/d), protein and fat concentrations, and feed efficiency. The 0.06 g/100g milk average drop in milk protein concentration when cows fed SWD was similar to that in Newton et al. (2021) when 50 g/d of a brown seaweed mix (91% *A. nodosum*:9% *L. digitata*) was fed to dairy cows, resulting in a 0.13 g/100g reduction in milk protein concentration. This small change may be due to the replacement of corn meal in the control diet with *A. nodosum* in the

SWD diets and resulting effects on microbial protein synthesis. Qin et al. (2023) saw no change in protein % when cows were fed 35.7 g/cow/d on DM basis of *Saccharina latissima.*

There was a significant week effect seen in the current study for basic milk components, but this was expected as stage of lactation also affects milk composition (Laben, 1963, Heck et al., 2009, Forsbäck et al., 2010). Therefore, the observed differences over time could have also been an effect of the lactation stage. Cows within the trial had an average DIM of 161 days, ranging from 34 – 315 DIM at the start of the experiment, and milk protein concentration typically increases as lactation progresses and milk yield declines Waite et al. (1956).

2.5.2 Effect of seaweed supplementation on milk mineral concentrations and associated transfer efficiencies

2.5.2.1 Macromineral concentrations (Ca, Mg, P, K, Na)

In the present study, diet seaweed supplementation increased milk Mg and P concentrations. This reflects the numerically higher intakes of these two minerals from the diet, as SWD cows were fed +9.8 g/d and +10.0 g/d more Mg and P, respectively, than CON cows. Indeed, transfer efficiencies of Mg and P from feed to milk were lower in SWD cows, reflecting the greater intakes of these minerals. Previous work has shown that higher Mg and P intakes can increase their concentrations in milk (Withers et al., 1999, Gustafson et al., 2007). Withers et al. (1999) showed that as farming practices/systems reduced nutrient loss and increased delivery of P in cows' diets, milk P concentrations increased, while other studies indicated that an increase in diet Mg intake leads to increased concentrations within raw milk (Zwierzchowski and Ametaj, 2019).

Additionally, seaweed supplementation had a significant negative effect on the transfer efficiencies of macrominerals Ca and K. However, these decreases in transfer efficiencies did not reflect in lower concentrations of Ca or K in milk, again due to the higher intakes of Ca and K from SWD diets. In the case of K, this would have been further explained by the fact that most ingested K is excreted in urine, rather than milk; a relationship which is not true for Ca (Williams et al., 1990, Martín-Tereso and Verstegen, 2011). By nature of the physiological process of excretion from ingested minerals into milk, the proportion of diversion towards urine/faeces over milk increases as intake increases (López-Alonso, 2012). Therefore, a slight increase in the macrominerals intake for the SWD group may have led to reduced transfer efficiency, with SWD cows experiencing a drop of 1.7%, 0.4%, 2.0%, and 1.1% for Ca, Mg, P, and K respectively; similar to Qin et al. (2023) which saw significantly reduced transfer efficiencies by 1.1% and 1.2% for Ca and Na respectively when comparing a control diet with a diet containing 35.7 g/cow/d on DM basis of *S. latissima*.

An effect of diet × week interaction was found to be significant for all measured macrominerals. However, there were no consistent patterns and the relative differences between the experimental groups were 6-20% variation of all measured weeks within each group. The between-week variation tended to be higher in SWD milk than in CON milk, reflecting the higher intakes, while there were contradictory relationships between CON and SWD milk macromineral concentrations (e.g., Ca being higher in CON in Week 4 but lower in Week 6 when compared with SWD milk).

2.5.2.2 Trace element concentrations (I, Mn, Mo, Zn)

In the present study, seaweed supplementation had a significant effect on milk I concentrations. Antaya et al. (2019) also reported a rise in milk I by roughly four times that of control when cows were fed 113 g/d of *A. nodosum*. In a separate study (Antaya et al., 2015) milk I concentrations reached 1,370 μg/L when cows were fed 170 g/d of *A. nodosum* meal. Qin et al. (2023) reported increases of from 208 μg/kg to 695 μg/kg milk I when cows were fed *S. latissima*. Other work has reported even higher I concentrations (2,471 ug/kg) in milk of cows fed 50 g/d of *A. nodosum* and *L. digitata*. Given the lack of goitrogenic compounds within the diet, such as thiocyanate, glucosinolates, or goitrin mainly found in rapeseed or cruciferous vegetables among others, milk I concentrations are positively correlated to diet I concentrations (Flachowsky et al., 2013, Bertinato, 2021). In the present study, analysis of TMR I concentrations in weeks 2-9 showed 5,625 μg/kg DM for the SWD group and 472 μg/kg DM for the CON group, with I intakes averaging 153 mg/d for the SWD group and 12 mg/d for the CON group. Interestingly, the difference between milk I concentrations between SWD and CON milk was incrementally increasing throughout the period of SWD feeding in the present experiment, indicating that these differences may have increased further if SWD feeding had continued for longer; although the effect beyond Week 9 was not investigated in the present study. Previous work found milk I concentration was maximized within 6 weeks when larger amounts of I were fed (12.3 mg/kg DM compared to ~5.6 mg/kg DM in the present work), which was assumed to be due to the Wolff-Chaikoff effect in which over time excessive I triggers reduced absorption and thus reduced excretion through milk (Newton et al., 2021). The pattern of increasing milk I concentrations within the SWD group without an abrupt reduction in transfer efficiency may indicate that the Wolff-Chaikoff effect has not been triggered physiologically during the sampling period.

Seaweed supplementation reduced transfer efficiency of Mn and Zn, and this can be explained by the fact that larger intakes per day would lead to lower transfer efficiencies, as previously shown by Gustafson et al. (2007). For Mn and Zn, SWD added +1360 mg/d and +989 mg/d respectively compared to the CON diet. In the present study differences in calculated transfer efficiency were not associated with changes in milk concentrations of Mn, Zn and Mo. Interestingly, I transfer efficiencies were increased across the experiment for the SWD group. This is a phenomenon also experienced to some degree in Newton et al. (2021), wherein the introduction of a diet containing high amounts of I (via a *A. nodosum* and *L. digitata*) initially reduced transfer efficiency of I, but then stabilized potentially because the amounts were not high enough to trigger the Wolf-Chaikoff effect that would divert I from the mammary gland to the kidneys.

2.5.3 Effect of seaweed supplementation on cow health indicators

The effect of seaweed supplementation did not affect any of the assessed hematological parameters. The parameters assessed in the present study are related to clinical anaemia, renal insufficiency, myeloproliferative disorders, and hyperthyroidism among other conditions (Roland et al., 2014). The present work provides evidence that *A. nodosum* can be offered up to 330 g/d in dairy cows without a negative impact of these parameters. The SWD diet was specifically designed to feed I below the EFSA's upper limit of 5 mg I/kg complete feed (EFSA, 2013), but SWD cows consumed on average ~6.1 mg I/kg DM as a result of (i) discrepancies between book values (used to develop the experimental diets) and the actual I concentrations in the feed ingredients, and (ii) the fact that the predicted forage intake was lower than the actual forage intake, thus increasing the relative contribution (g/kg DM) of SWD-containing concentrate in the total diet. Although the diet I concentration in the present study was lower than in previous work (Newton et al., 2021, Qin et al., 2023), these results emphasize the need to measure feedstuff I content when SWD is fed, in order to ensure that over-supplementation of I does not occur.

2.5.4 Nutritional implications of milk from seaweed-fed cows for consumers

The ANIBES report (anthropometric data, macronutrients and micronutrients intake, practice of physical activity, socioeconomic data and lifestyles in Spain) was used in calculations requiring the average milk consumption rates of males and females in Spain by age group (Partearroyo et al., 2019); The EFSA's DRV Finder: Dietary Reference Values for the EU presents an adequate intake (AI) of I as 105, 125, 150, and 200 μg/d for individuals aged 9-12, 13-17, 18+, and pregnant or nursing women respectively (EFSA (2019). Therefore, based on the recorded liquid milk intakes from the ANIBES report and the milk I concentrations in the present study, CON milk would contribute (expressed as % AI) (i) 40, 32, 18% and 20% in men of age groups 9-12, 13-17, 18-64 and 65-75, respectively, (ii) 32, 23, 19% and 21% in women of age groups 9-12, 13-17, 18-64, and 65-75, respectively, and (iii) 14% in pregnant or nursing women. On the other hand, average consumption of milk from the SWD group would substantially exceed the AI of I in all cases (expressed as % AI) (i) 453, 364, 201% and 222% in males of age groups 9-12, 13-17, 18-64 and 65-75, respectively, (ii) 367, 258, 215% and 241% in females of age groups 9-12, 13-17, 18-64 and 65-75, respectively, and (iii) 161% in pregnant or nursing women. Previous work in Spain (Donnay and Vila, 2012) reported that milk and dairy products are a major contributor to the reduction of the previously observed I deficiency; and the population of Spain generally maintains optimum I nutrition. However, previous reports have indicated low urinary I levels in certain demographics in Spain (37% of children surveyed had below 100 ug/L) (Ansótegui and Knörr, 2012). SWD milk, produced in the present study, would provide a substantial amount of I, and if such levels also appear within retail products this could provide a strategy to enhance public I sufficiency with special considerations for demographics reported as deficient (children; (Ansótegui and Knörr, 2012)) or having higher requirement for daily I intakes such as pregnant or nursing women (de Escobar et al., 2007, EFSA, 2019). I status is crucial for pregnant women but also for women who may become pregnant, as it influences foetal development from conception; thus making it important to keep an adequate I status throughout as pregnant women may not be aware of their pregnancy status, and make essential dietary changes, for weeks after conception (Branum and Ahrens, 2017). Milk with increased I concentrations could be a gateway towards increased I supply, particularly within these vulnerable populations. However, several studies have identified the potential risk when feeding SWD to dairy cows, of producing milk with such high I concentrations that certain consumer demographics (especially young children) would reach their upper tolerable limit (UL) under typical daily intakes, which may also be the case for adults with higher than typical dairy consumption (Newton et al., 2021, Newton et al., 2022, Qin et al., 2023).

The UL for I set by EFSA is 200, 250, 300, 450, 500, and 600 μg/d for the age groups of 1-3, 4-6, 7-10, 11-14, 15-17, and 18+ (including pregnant/lactating women), respectively with there being no difference of sex. In the present study, based on the I concentrations of SWD milk and the current milk intakes of the Spanish population, the contribution towards UL if all milk consumed was from SWD-fed cows would be 117%, 86%, 52% and 58% for the age groups 9-12, 13-17, 18-64 and 65-75, respectively. For the same age groups, it would require consumption of 207 mL/d (already met with average consumption), 261 mL/d (+20.6% average consumption), 331 mL/d (+99.6% average consumption), and 331 mL/d (+79.3% average consumption), to reach their UL. This highlights the risk of I overconsumption, especially in children, adolescents, or individuals with high dairy consumption if the I concentrations of the SWD milk from the present study was seen at retail level. The amounts required to reach the UL are not only realistic, but already higher (for children) or very similar (for adolescents) to those already consumed by these demographics. The risk would be even higher for toddlers (age 1-3 years), which (at a UL of 200 μg/d; (EFSA, 2019)) they would reach their UL by drinking only 110 mL/d; an amount that most toddlers exceed, or would be recommended to exceed, as part of their daily diet.

Excess I intake causes the Wolff-Chaikoff effect mentioned above, a regulatory process that reduces thyroidal hormone synthesis (generally lasting for 24 hours) in vulnerable individuals (e.g. those with autoimmune disease, subacute thyroiditis, or a hemithyroidectomy), and failure of adaptation to this regulatory event can lead to transient or even permanent thyroidal dysfunction (Pramyothin et al., 2011, Leung and Braverman, 2014). Additionally, the consequences of I overconsumption during pregnancy are not well understood, and while targeted I delivery for pregnant women is crucial for preventing foetal neurodevelopmental problems (Zimmermann, 2012) the limited ability of the foetus to cope with excess I may also cause issues such as neonatal airway obstruction due to goitre size or congenital hypothyroidism (Farebrother et al., 2019). However, even at I concentrations at the SWD milk in this study, it would require 331 mL/d for a pregnant woman to reach their UL, and the risk of I overconsumption from milk from SWD-fed cows would be much lower than that for toddlers or adolescents. On the contrary, CON milk would only contribute 10%, 8%, 5% and 5% of the UL for the age groups 9-12, 13-17, 18-64 and 65-75, respectively; and would require consumption 2260 mL/d, 2976 mL/d, 3759 mL/d and 3759 mL/d, in order to reach their UL. These amounts appear to be very high, and it appears that CON milk can be a good source of I in Spanish diets, without posing any risks around increased I intake.

Milk I concentrations were highly variable between diets and between weeks within the same diet. Milk from the CON group averaged 166 μg/kg milk, which is slightly lower for previous studies in Spain that showed concentrations typically higher than 205 μg/kg (Donnay and Vila (2012). Therefore, when considering the implications of the contribution of milk to I intakes in Spanish population in the current study (which uses CON milk for the control milk I concentration), these may be slightly lower than that typically found. Other studies have also reported that there might be a large between-country variation (34-550 μg/kg in data from 20 industrialized countries; van der Reijden et al. (2017)) while within-country geographical and seasonal variation has also been high in previous work in the UK (<0.01-1604 μg/kg; Coneyworth et al. (2020)) and the US (~129-687 μg/kg; Roseland et al. (2020)); which indicates the need for country-specific, or even region-specific, research on the contribution of milk and dairy products on I supply to the population. In addition, it should be noted that estimates in the present study have assumed that all milk consumed came from cows fed SWD. In practice, this is unlikely to happen and milk from farms using SWD would likely have their milk bulked at the dairy plant with milk from other farms not feeding SWD, which would reduce I concentration of the raw milk that is processed.

Milk Mg concentrations in the current study did not differ when SWD was fed to an extent that would have a meaningful impact on human nutrition or health. Based on consumer milk intakes, and the most extreme possible difference in Mg concentrations in CON vs SWD milk (Week 2, 91 mg/kg vs Week 8, 117 mg/kg, respectively), drinking CON or SWD milk would provide 6.1% or 7.8% of AI, respectively, averaged across demographics. Similarly, milk P concentrations did not differ to an extent that would have a meaningful impact on human nutrition or health. Based on consumer milk intakes, and the most extreme possible difference in P concentrations in CON vs SWD milk (Week 8, 1044 mg/kg vs Week 4, 1198 mg/kg, respectively), drinking CON or SWD milk would provide for 35.7% or 41.0% AI, respectively, across demographics.

2.6 Conclusions

Seaweed (*Ascophyllum nodosum*) inclusion in dairy cow diets did not affect productivity and feed efficiency, or measured hematological parameters, but increased the milk concentrations of Mg, P, and I. These effects may be explained by the higher intakes when corn meal was substituted with seaweed in dairy cows' diets, as transfer efficiencies from feed to milk were similar (in case of I) or lower (in case of Mg and P). Milk concentrations of Ca, K, Mn, Zn and Mo were not affected by feeding seaweed in the present study. Based on reported Spanish population milk intakes, the contribution of milk towards I supply would be increased substantially when seaweed is fed to dairy cows; which can be advantageous for consumers with higher I requirements or demographics known to be deficient (children, pregnant and nursing women, or women at childbearing age). However, care should be taken when feeding seaweed to dairy cows in order to avoid excessive I intakes, as this would result to milk I concentrations that could lead to I overconsumption by children.

2.7 Information

Author Contributions: Eric E. Newton: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization. Katerina Theodoridou: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition. Marta Terré: Project administration, Investigation, Resources, Writing - Review & Editing. Sharon Huws: Writing - Review and Editing. Partha Ray: Conceptualization, Methodology, Software, Supervision. Christopher K. Reynolds: Writing - Review & Editing, Supervision. N. Prat: Investigation, Resources. D. Sabrià: Investigation, Resources. Sokratis Stergiadis: Conceptualization, Methodology, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration. All authors reviewed and approved the manuscript.

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2.8 References

Ahmed, M., Hayat, R., Ahmad, M., Ul-Hassan, M., Kheir, A. M. S., Ul-Hassan, F., Ur-Rehman, M. H., Shaheen, F. A., Raza, M. A., & Ahmad, S. (2022). Impact of Climate Change on Dryland Agricultural Systems: A Review of Current Status, Potentials, and Further Work Need. Int. J. Plant Prod., 16(3), 341-363. doi:10.1007/s42106-022-00197-1

Ansótegui, J. A., & Knörr, J. I. (2012). Study of iodine intake in children from 6 months to three years-old in Guipúzcoa. An. Pediatr. (Barc), 76(2), 65-68. doi:10.1016/j.anpedi.2011.07.007

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. J. Dairy Sci., 102(9), 8040-8058. doi: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 diets. J. Dairy Sci., 98(3), 1991-2004. doi:10.3168/jds.2014-8851

AOAC. (1990). Official methods of analysis. Retrieved from Arlington, VA:

Bath, S. C., Steer, C. D., Golding, J., Emmett, P., & Rayman, M. P. (2013). Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet, 382(9889), 331-337. doi:10.1016/s0140-6736(13)60436-5

Bertinato, J. (2021). Iodine nutrition: Disorders, monitoring and policies. In Adv. Food Nutr. Res. (2021/06/12 ed., Vol. 96, pp. 365-415).

Bischoff, K. L. (2016). Chapter 40 - Glucosinolates. In R. C. Gupta (Ed.), Nutraceuticals (pp. 551-554). Boston: Academic Press.

Branum, A. M., & Ahrens, K. A. (2017). Trends in Timing of Pregnancy Awareness Among US Women. Matern. Child Health J., 21(4), 715-726. doi:10.1007/s10995-016-2155-1

Chasapis, C. T., Ntoupa, P.-S. A., Spiliopoulou, C. A., & Stefanidou, M. E. (2020). Recent aspects of the effects of zinc on human health. Arch. Toxicol., 94(5), 1443-1460. doi:10.1007/s00204-020-02702-9

Coneyworth, L., Coulthard, L., Bailey, E., Young, S., Stubberfield, J., Parsons, L., Saunders, N., Homeer, E., & Welham, S. (2020). Geographical and seasonal variation in Iodine content of cow's milk in the UK and consequences for the consumer´s supply. J. Trace Elem. Med. Biol., 59, 126453. doi:10.1016/j.jtemb.2020.126453

de Escobar, G. M., Obregón, M. J., & del Rey, F. E. (2007). Iodine deficiency and brain development in the first half of pregnancy. Public Health Nutr., 10(12a), 1554-1570. doi:10.1017/s1368980007360928

Donnay, S., & Vila, L. (2012). Eradication of iodine deficiency in Spain. Close, but not there yet. Endocrinol. Nutr., 59(8), 471-473. doi:10.1016/j.endoen.2012.05.003

Duarte, C. M., Wu, J., Xiao, X., Bruhn, A., & Krause-Jensen, D. (2017). Can Seaweed Farming Play a Role in Climate Change Mitigation and Adaptation? Front. Mar. Sci., 4(100). doi:10.3389/fmars.2017.00100

EFSA. (2013). EFSA Panel on Additives Products or Substances used in Animal Feed: Scientific Opinion on the safety and efficacy of iodine compounds (E2) as feed additives for all species: calcium iodate anhydrous and potassium iodide, based on a dossier submitted by HELM AG. EFSA J., 11(2), 3101. doi:10.2903/j.efsa.2013.3101

EFSA. (2019). DRV Finder. Retrieved from https://multimedia.efsa.europa.eu/drvs/index.htm

EPA, U. S. (2007). Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils. In. Washington, DC.

FAO. (2021). FAO Global Fishery and Aquaculture Production Statistics (FishStatJ). Retrieved from: https://www.fao.org/fishery/en/global-search?q=statistics%20software%20fishstatj%20en&lang=en

Farebrother, J., Zimmermann, M. B., & Andersson, M. (2019). Excess iodine intake: sources, assessment, and effects on thyroid function. Ann. N. Y. Acad. Sci., 1446(1), 44-65. doi:10.1111/nyas.14041

Flachowsky, G., Franke, K., Meyer, U., Leiterer, M., & Schöne, F. (2013). Influencing factors on iodine content of cow milk. Eur. J. Nutr., 53. doi:10.1007/s00394-013-0597-4

Forsbäck, L., Lindmark-Månsson, H., Andrén, A., Åkerstedt, M., Andrée, L., & Svennersten-Sjaunja, K. (2010). Day-to-day variation in milk yield and milk composition at the udder-quarter level. J. Dairy Sci., 93(8), 3569-3577. doi:10.3168/jds.2009-3015

Gornall, J., Betts, R., Burke, E., Clark, R., Camp, J., Willett, K., & Wiltshire, A. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 365(1554), 2973-2989. doi:10.1098/rstb.2010.0158

Gustafson, G. M., Salomon, E., & Jonsson, S. (2007). Barn balance calculations of Ca, Cu, K, Mg, Mn, N, P, S and Zn in a conventional and organic dairy farm in Sweden. Agric. Ecosyst. Environ., 119(1), 160-170. doi:10.1016/j.agee.2006.07.003

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition--a review. Lipids Health Dis., 6, 25. doi:10.1186/1476-511x-6-25

Heck, J. M. L., van Valenberg, H. J. F., Dijkstra, J., & van Hooijdonk, A. C. M. (2009). Seasonal variation in the Dutch bovine raw milk composition. J. Dairy Sci., 92(10), 4745-4755. doi:10.3168/jds.2009-2146

Hettinga, K., & van Valenberg, H. (2017). Chapter 20 - Contribution of Dairy to Nutrient Intake in the Western Diet. In R. R. Watson, R. J. Collier, & V. R. Preedy (Eds.), Nutrients in Dairy and their Implications on Health and Disease (pp. 251-258): Academic Press.

Kieliszek, M., Bano, I., & Zare, H. (2022). A Comprehensive Review on Selenium and Its Effects on Human Health and Distribution in Middle Eastern Countries. Biol. Trace Elem. Res., 200(3), 971-987. doi:10.1007/s12011-021-02716-z

Kronqvist, C. (2011). Minerals to dairy cows with focus on calcium and magnesium balance. Retrieved from https://pub.epsilon.slu.se/8302/1/kronqvist_c_110831.pdf

Laben, R. C. (1963). Factors Responsible for Variation in Milk Composition. J. Dairy Sci., 46(11), 1293-1301. doi:10.3168/jds.S0022-0302(63)89264-4

Leung, A. M., & Braverman, L. E. (2014). Consequences of excess iodine. Nat. Rev. Endocrinol., 10(3), 136-142. doi:10.1038/nrendo.2013.251

López-Alonso, M. (2012). Trace minerals and livestock: not too much not too little. ISRN Vet. Sci., 2012, 704825. doi:10.5402/2012/704825

Martín-Tereso, J., & Verstegen, M. (2011). A novel model to explain dietary factors affecting hypocalcaemia in dairy cattle. Nutr. Res. Rev., 24, 228-243. doi:10.1017/S0954422411000126

Nada, V., Hruskar, M., Marković, K., Banović, M., & Baric, I. (2010). Essential minerals in milk and their daily intake through milk consumption. Mljekarstvo, 60.

Newton, E. E., Lamminen, M., Ray, P., Mackenzie, A. M., Reynolds, C. K., Lee, M. R. F., Halmemies-Beauchet-Filleau, A., Vanhatalo, A., & Stergiadis, S. (2022). Macromineral and trace element concentrations in milk from Finnish Ayrshire cows fed microalgae (*Spirulina platensis*) and rapeseed (*Brassica napus*). J. Dairy Sci., 105(11), 8866-8878. doi:10.3168/jds.2022-22050

Newton, E. E., Petursdottir, A. H., Rikharethsson, G., Beaumal, C., Desnica, N., Giannakopoulou, K., Juniper, D., Ray, P., & Stergiadis, S. (2021). Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations. Foods, 10(7), 1526. doi:10.3390/foods10071526

Nitschke, U., & Stengel, D. (2016). Quantification of iodine loss in edible Irish seaweeds during processing. J. Appl. Phycol., 28. doi:10.1007/s10811-016-0868-6

Nitschke, U., & Stengel, D. B. (2015). A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products. Food Chem., 172, 326-334. doi:10.1016/j.foodchem.2014.09.030

Papas, A., Ingalls, J. R., & Campbell, L. D. (1979). Studies on the effects of rapeseed meal on thyroid status of cattle, glucosinolate and iodine content of milk and other parameters. J. Nutr., 109(7), 1129-1139. doi:10.1093/jn/109.7.1129

Partearroyo, T., Samaniego-Vaesken, M. L., Ruiz, E., Aranceta-Bartrina, J., Gil, Á., González-Gross, M., Ortega, R. M., Serra-Majem, L., & Varela-Moreiras, G. (2019). Current Food Consumption amongst the Spanish ANIBES Study Population. Nutrients, 11(11). doi:10.3390/nu11112663

Pramyothin, P., Leung, A. M., Pearce, E. N., Malabanan, A. O., & Braverman, L. E. (2011). Clinical problem-solving. A hidden solution. N. Engl. J. Med., 365(22), 2123-2127. doi:10.1056/NEJMcps1008908

Qin, N., Pétursdóttir, Á. H., Humphries, D. J., Desnica, N., Newton, E. E., Vanhatalo, A., Halmemies-Beauchet-Filleau, A., Bell, L., Givens, D. I., Juniper, T.D., Gunnlaugsdóttir, H., & Stergiadis, S. (2023). Mineral concentrations in milk from cows fed seaweed (Saccharina latissima) under different basal protein supplementation. Food Chemistry, 403, 134315. doi:https://doi.org/10.1016/j.foodchem.2022.134315

Roland, L., Drillich, M., & Iwersen, M. (2014). Hematology as a diagnostic tool in bovine medicine. J. Vet. Diagn. Invest., 26(5), 592-598. doi:10.1177/1040638714546490

Roleda, M., Marfaing, H., Desnica, N., Jónsdóttir, R., Skjermo, J., Rebours, C., & Nitschke, U. (2018). Variations in polyphenol and heavy metal contents of wild-harvested and cultivated seaweed bulk biomass: Health risk assessment and implication for food applications. Food Control, 95. doi:10.1016/j.foodcont.2018.07.031

Roque, B. M., Salwen, J. K., Kinley, R., & Kebreab, E. (2019). Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. J. Clean. Prod., 234, 132-138. doi:10.1016/j.jclepro.2019.06.193

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. PLOS ONE, 16(3), e0247820. doi:10.1371/journal.pone.0247820

Roseland, J. M., Phillips, K. M., Patterson, K. Y., Pehrsson, P. R., Bahadur, R., Ershow, A. G., & Somanchi, M. (2020). Large Variability of Iodine Content in Retail Cow's Milk in the U.S. Nutrients, 12(5). doi:10.3390/nu12051246

Smith, N. W., Fletcher, A. J., Hill, J. P., & McNabb, W. C. (2021). Modeling the Contribution of Milk to Global Nutrition. Front. Nutr., 8, 716100. doi:10.3389/fnut.2021.716100

Stergiadis, S., Qin, N., Faludi, G., Beauclercq, S., Pitt, J., Desnica, N., Pétursdóttir Á, H., Newton, E. E., Angelidis, A. E., Givens, I., Humphries, D. J., Gunnlaugsdóttir, H., & Juniper, D. T. (2021). Mineral Concentrations in Bovine Milk from Farms with Contrasting Grazing Management. Foods, 10(11). doi:10.3390/foods10112733

Thorning, T. K., Raben, A., Tholstrup, T., Soedamah-Muthu, S. S., Givens, I., & Astrup, A. (2016). Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. Food Nutr. Res., 60, 32527. doi:10.3402/fnr.v60.32527

Thornton, P., Dinesh, D., Cramer, L., Loboguerrero, A. M., & Campbell, B. (2018). Agriculture in a changing climate: Keeping our cool in the face of the hothouse. Outlook Agric., 47(4), 283-290. doi:10.1177/0030727018815332

van der Reijden, O. L., Zimmermann, M. B., & Galetti, V. (2017). Iodine in dairy milk: Sources, concentrations and importance to human health. Best Pract. Res. Clin. Endocrinol. Metab., 31(4), 385-395. doi:10.1016/j.beem.2017.10.004

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74(10), 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Waite, R., White, J. C. D., & Robertson, A. (1956). 613. Variations in the chemical composition of milk with particular reference to the solids-not-fat: I. The effect of stage of lactation, season of year and age of cow. J. Dairy Res., 23(1), 65-81. doi:10.1017/S0022029900008049

Williams, P. H., Gregg, P. E. H., & Hedley, M. J. (1990). Fate of potassium in dairy cow urine applied to intact soil cores. New Zealand J. Agric. Res., 33(1), 151-158. doi:10.1080/00288233.1990.10430672

Withers, P. J. A., Peel, S., Mansbridge, R. M., Chalmers, A. C., & Lane, S. J. (1999). Transfers of phosphorus within three dairy farming systems receiving varying inputs in feeds and fertilizers. Nutr. Cycling Agroecosyst., 55(1), 63-75. doi:10.1023/A:1009860915462

Zimmermann, M. B. (2012). The effects of iodine deficiency in pregnancy and infancy. Paediatr. Perinat. Epidemiol., 26 Suppl 1, 108-117. doi:10.1111/j.1365-3016.2012.01275.x

Zwierzchowski, G., & Ametaj, B. N. (2019). Mineral Elements in the Raw Milk of Several Dairy Farms in the Province of Alberta. Foods, 8(8). doi:10.3390/foods8080345

Chapter 3: "Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations"

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Contribution: The animal trial was run in Iceland with collaborators that worked with us through the Agricultural Fund of Iceland (Framleiðnisjóður landbúnaðarins, Grant Numbers 18-039 and 20-025) for funding the research work. Milk was separately measured for basic composition and all minerals except iodine. Milk and feed was aliquoted and measured for iodine at UoR exclusively by myself. I performed all analysis by myself, modelled nutritional outcomes by myself, and wrote the article as the primary author. The overall estimated percentage contribution made by myself would be 85%, factoring in the contribution of partners to the collection of feed intakes from the animal trial and analysis of milk basic composition, non-iodine minerals, and along with the text review by co-authors.

Objectives: This study aimed to (i) investigate the effect of feeding a mixture of seaweed (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) to dairy cows at different dietary inclusion rates (0.75% and 1.5% of the concentrate dry matter (DM)) on milk yield and basic composition, along with the concentrations of macro-minerals, trace elements and heavy metals, and (ii) estimate the impact that the consumption of milk from seaweed-fed cows may have on consumer mineral intakes.

Hypothesis: For this experiment, I hypothesized that macroalgal supplementation of dairy cows would increase iodine and arsenic concentrations in milk but lead to reduced milk yield along with reduced fat and protein concentrations.

Overview: Overall, the study found that seaweed supplementation did not affect cow productivity or milk basic composition except for a small reduction in protein and casein content. Mineral contents were reduced when seaweed supplementation occurred with Cu and Se, and increased in I and As. The increase in I and As were linked to a higher innate dietary supply of the seaweed, while the lower concentrations of Cu and Se were most likely due to the reduced transfer efficiency caused my physiological phenomena. The fluctuations in seaweed supplemented cows' milk were then projected in terms of population nutrition and health. Because this study took place in Iceland, this was most applicable to this specific country, and the theoretical mineral intakes were calculated with reported recent milk consumption amounts. It was found that the highest-supplemented cows' milk was entirely unsuitable for any demographic, while control milk did not entirely fulfil the RNI for any Icelandic demographic. The middle-supplemented cows' milk was found to both deliver more than enough I in terms of RNI, but not so much so to surpass the UL.

3.1 Abstract

This study investigated the effect of seaweed supplementation in dairy cow diets on milk yield, basic composition, and mineral concentrations. 37 Icelandic cows were split into three diet treatments: control (CON, no seaweed), low seaweed (LSW, 0.75% concentrate dry matter (DM), 13–40 g/cow/day), and high seaweed (HSW, 1.5% concentrate DM, 26–158 g/cow/day). Cows were fed the same basal diet of grass silage and concentrate for a week, and then were introduced to the assigned experimental diets for 6 weeks. The seaweed mix of 91% *Ascophyllum nodosum*: 9% *Laminaria digitata* (DM basis), feed, and milk samples were collected weekly. Data were analysed using a linear mixed effects model, with diet, week, and their interaction as fixed factors, cow ID as random factor, and the pre-treatment week data as a covariate. When compared with CON milk, LSW and HSW milk had, respectively, less Se (−1.4 and −3.1 μg/kg milk) and more I (+744 and +1649 μg/kg milk), while HSW milk also had less Cu (−11.6 μg/kg milk) and more As (+0.17 μg/kg milk) than CON milk. The minimal changes or concentrations in milk for Se, Cu, and As cannot be associated with any effects on consumer nutrition, but care should be taken when I-rich seaweed is fed to cows to avoid excessive animal I supply and milk I concentrations.

Keywords: milk; iodine; minerals; seaweed; *Laminaria digitata*; *Ascophyllum nodosum*; Icelandic cow

3.2 Introduction

Seaweed is an underexploited potential animal feed source that has recently gained increased attention due to its high concentration of specific minerals, macronutrients, and bioactive compounds, spearheaded by indications that certain seaweed species have been shown to markedly reduce enteric methane emissions (Cherry et al., 2019; Kinleyet al., 2020; Makkar et al., 2016; Roque et al., 2019; Roque, Salwen, Kinley, & Kebreab, 2019). Seaweed farming and wild harvesting have a number of benefits, including faster growth rates from traditional crops farmed on land and less of a vulnerability to the meteorological effects of climate change, therefore reducing the increasing competition between food and feed production from traditional land-based agricultural production (Gegg & Wells, 2017). Global seaweed production has increased by almost 27% between 2011 and 2015, resulting in a total output of 30 million tons at the end of this period, and continues to grow, with the vast majority of seaweed being from farmed aquaculture (Ferdouse et al., 2018). Given the current state of harvesting seaweed, and its potential benefits on animal nutrition and health, there is an increasing interest towards alternative applications for the growing industry, such as the sustainable seaweed supplementation of animal diets which may yield potential benefits for ruminant health and nutrition along with resulting benefits to human health(Antaya et al., 2019; Braden et al., 2004; Saker, Fike, Veit, & Ward, 2004).

Of the several seaweed species that have been previously explored, *Ascophyllum nodosum* and *Laminaria digitata* have been identified as potential candidates for experimental animal feeding

(Belanche, Jones, Parveen, & Newbold, 2016; Karatzia et al., 2012; Moneda et al., 2019). *A. nodosum* is a brown coldwater alga which is found in much of the Northern Atlantic Ocean, including Norway, the United Kingdom (UK), Iceland, and the eastern seaboard of the United States and Canada (Buschmann et al., 2017; Ugarte & Sharp, 2012). *A. nodosum* is either gathered by hand (e.g., Scotland and Ireland) or by mechanical harvesting (e.g., Norway and Iceland) and is one of the main species harvested in Europe; its use as a biostimulant for agricultural opportunities have been recently researched and it is currently used in much of phycological industrial applications, such as fertilizer and alginate production, along with a function as an animal feed supplement (Borges, Araujo, Azevedo, & Pinto, 2020; Capuzzo, 2016; Shukla et al., 2019). *L. digitata*, is a less harvested but still common seaweed found within the Northern Atlantic Ocean, with an estimated total harvested amount annually (<150 tons in the wild) being lower than *A. nodosum* in Ireland (Edwards & Watson, 2015). *L. digitata* is one of the most exploited types of seaweed off the coast of France, where it has been harvested for alginates at around 50,000 tons a year as of 2011 (Davoult et al., 2011). These two species (i) have been shown to illicit a positive effect on rumen function, animal health, energy utilization, and milk quality and safety (Antaya et al., 2019; Belanche et al., 2016; Brantsæter et al., 2018; Cherry et al., 2019; Karatzia et al., 2012; Rayman & Bath, 2015; Rey-Crespo, López-Alonso, & Miranda, 2014; Zhou et al., 2018); (ii) are good sources of minerals, such as iodine (I), calcium (Ca), phosphorus (P), selenium (Se), magnesium (Mg), and zinc (Zn) representing excellent candidates for feed mineral supplementation (Circuncisão, Catarino, Cardoso, & Silva, 2018; Lorenzo et al., 2017; Pereira, 2011); (iii) are excellent sources of essential amino acids for the animal, such as theanine, valine, methionine, isoleucine, leucine, phenylalanine, lysin, histidine, and arginine—of which, for many, dairy serves as a source of (Gaillard et al., 2018; Lorenzo et al., 2017; D. G. Smith & Young, 1955); and (iv) are readily available in Europe, Scandinavia, and the eastern seaboard of North America (Buschmann et al., 2017; Ugarte et al., 2012).

While previous findings regarding the effect of seaweed supplementation in ruminant diets on animal health and rumen function are promising, the impact on milk quality should also be considered. Milk and dairy products are rich in minerals and are large suppliers of I, Ca, P, Se, Mg and Zn in human diets (Górska-Warsewicz, Rejman, Laskowski, & Czeczotko, 2019; Haug, Høstmark, & Harstad, 2007). These minerals can exert positive effects on human health as they are associated with reduced risk of cardiometabolic diseases and other noncommunicable diseases, therefore providing a source of nutrition and the potential to reduce healthcare expenses (Thorning et al., 2016). A common characteristic of most seaweeds is the high mineral content, and therefore, supplementation of dairy cow diets with this phycological product may influence mineral concentrations in the milk (Pereira, 2011). Previous work has found that dietary supplementation of cow diets with *A. nodosum* has increased milk I concentrations by approximately 309%, to 481 μg/L average across three periods, when offered at 113 g per cow per day, and by approximately 671% to 1370 μg/L when cows were offered 170 g per head per day (Antaya et al., 2019; Antaya et al., 2015). In another study, supplementation of dairy cow diets with a blend of seaweeds, including *Ulva rigida*, *Laminaria ochroleuca*, *Saccharina latissima*, *Saccorhiza polyschides*, *Mastocarpus stellatus*, and *Sargassum muticum* resulted in higher milk I content, indicating that dietary supplementation of seaweed could be used as a potential strategy to increase milk I content (Antaya et al., 2019; Rey-Crespo et al., 2014). However, seaweed may contain heavy metals, including cadmium (Cd), lead (Pb), mercury (Hg), copper (Cu), molybdenum (Mo), and arsenic (As) (Besada, Andrade, Schultze, & Gonzalez, 2009; Pomin, 2011; J. L. Smith, Summers, & Wong, 2010). Some of these heavy metals are considered contaminants in the food chain and there is a requirement to maintain their concentrations in foods below certain thresholds, although there are currently no published maximum statutory limits for As, Cd, or Hg in milk in Europe; while Pb is limited to 20 μg/kg milk (Commission Regulation (EC), 2006; EFSA, 2014; Rey-Crespo et al., 2014). Brown macroalgae in particular may contain high concentrations of total As but usually with low levels of the toxic inorganic As (e.g., *A. nodosum*), however, *L. digitata* is a notable exception to this as it can contain high concentrations of both (Pétursdóttir & Gunnlaugsdóttir, 2019; Ronan et al., 2017). Supplementation of dairy cow diets with a mixture of U. rigida (green seaweed), S. muticum (brown seaweed) and S. polyschides (brown seaweed), increased As content in milk while Cd and Pb concentration was unaffected (Rey-Crespo et al., 2014).

While there is increasing interest of several seaweed species as animal feed, high seasonal and between-species variation in mineral and chemical composition of seaweed species (El-Said & El-Sikaily, 2012; Moneda et al., 2019; Mwalugha, Wakibia, Kenji, & Mwasaru, 2015) suggests a need for vigilant screening of seaweeds, as well as the development of corresponding animal feeding strategies. This will ensure that seaweed supplementation to dairy cow diets improves or at least maintains milk yield, quality, and safety characteristics. Therefore, the present study aimed to (i) investigate the effect of feeding a mixture of seaweed (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) to dairy cows at different dietary inclusion rates (0.75% and 1.5% of the concentrate dry matter (DM)) on milk yield and basic composition, along with the concentrations of macrominerals, trace elements and heavy metals, and (ii) estimate the impact that the consumption of milk from seaweed-fed cows may have on consumer mineral intakes.

3.3 Materials and Methods

3.3.1 Experimental design

The current study was conducted during the winter indoor period at Stóra-Ármót farm, Selfoss, Iceland. Animal procedures were reviewed by The Icelandic Food and Veterinary Authority and confirmed that the experiment did not require a license according to the regulation no. 460/2017. 37 lactating dairy cows of the Icelandic breed were blocked into three groups of 11 to 13 cows each, balanced for parity, lactation stage, milk yield and milk contents of fat, protein, and somatic cell count (SCC). Before the experiment began, all cows received a basal diet made up of 4.8–11.4 kg DM concentrate feed (ingredients list presented in Supplementary Material, Table S3.1) according to milk yield, topped up with ad libitum supply of grass silage. Each group was assigned to one of three experimental diets (i) without seaweed supplementation (control, CON), (ii) with seaweed supplementation at 0.75% seaweed in concentrate, DM basis (low seaweed, LSW; 13–40 g seaweed/cow/day), and (iii) 1.5% seaweed in concentrate, DM basis (high seaweed, HSW; 26–158 g seaweed/cow/day). The seaweed mix comprised of 91% *Ascophyllum nodosum* and 9% *Laminaria digitata*, on DM basis. These seaweeds were selected because they represent species with high commercial potential as they are abundant and easy to access; the dietary inclusion rate was based on not exceeding maximum levels of heavy metals according to the European Commission. In addition, the specific inclusion rates were developed to be representative of local production – as *L. digitata* production is lower than *A. nodosum*. Commission Regulation for maximum levels for As in animal feed, where 2 mg/kg diet DM of inorganic As in the seaweed mixture was the limiting factor (European Commission, 2015). The chemical composition of silage and concentrate are shown in Table 3.1 and mineral composition of silage, concentrate, and concentrate with seaweed are shown in Table 3.2. The average chemical composition and mineral composition of the three experimental diets are presented in Tables S3.2 and S3.3, respectively, in the Supplementary Material. Animal data (estimated bodyweight, lactation stage, parity) are presented in Table 3.3. Feed intake was calculated as described by Butler et al. (2008), using estimated bodyweight and milk yield.

Table 3.1. Means, standard deviation (SD), minimum and maximum values for the chemical composition of silage and concentrate used in the animal trial.

	Silage				Concentrate [1]			
Chemical Composition (g/kg Dry Matter)	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Dry Matter (g/kg fresh)	301	7.0	290	309	895	1.2	894	897
Ash	70	2.4	67	74	89	2.7	83	93
NCDG [2]	768	12.2	750	780	NM [3]	NM [3]	NM [3]	NM [3]
Crude Protein	166	11.6	143	178	213	3.3	203	220
Neutral Detergent Fiber	501	15.5	483	525	118	6.6	103	129
Acid Detergent Fiber	301	13.6	285	328	564	46.0	501	647
Single Cell Protein	111	5.8	101	118	NM [3]	NM [3]	NM [3]	NM [3]
Indigestible Neutral Detergent Fiber	87	12.1	78	113	NM [3]	NM [3]	NM [3]	NM [3]
Sugar	47	9.9	30	62	NM [3]	NM [3]	NM [3]	NM [3]
Fat	59	4.7	54	65	26	1.2	24	28
Ammonia	0.7	0.14	0.5	0.9	NM [3]	NM [3]	NM [3]	NM [3]
Starch	NM [3]	NM [3]	NM [3]	NM [3]	276	9.4	252	286
Water-soluble Carbohydrates	NM [3]	NM [3]	NM [3]	NM [3]	136	12.2	115	160

[1] Compound feed in the form of pellet comprising of soybean, wheat, corn, barley, sugar beet flour, molasses, shell lime, hard fat, mono-calcium phosphate, magnesium phosphate, salt, and mineral/vitamin supplement in ratios as presented in Table S3.1 in the Supplementary Material. [2] Neutral Detergent Cellulase Digestible Organic Matter. [3] not measured.

Table 3.2. Means, standard deviation (SD), minimum and maximum values for the mineral composition of silage, concentrate and seaweed used in the animal trial.

	Silage				**Concentrate** [1]				**Concentrate with Seaweed** [2]			
Minerals (mg/kg Dry Matter)	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Aluminium (Al)	552	698.8	164	2107	510	166.1	408	701.6	419	50.3	383	454
Arsenic (As)	0.07	0.045	0.04	0.17	0.77	0.526	0.41	1.373	1.06	0.106	0.98	1.13
Cadmium (Cd)	0.02	0.005	0.01	0.02	0.08	0.022	0.05	0.097	0.11	0.004	0.11	0.12
Calcium (Ca)	4373	484.8	3859	5220	14,945	342.8	14,632	15,311	14,979	446.1	14,663	15,294
Chromium (Cr)	37	40.7	12	128	26.1	8.0	19.2	34.78	22	3.0	20	24
Cobalt (Co)	0.66	0.461	0.37	1.68	3.60	0.785	2.82	4.393	2.67	0.184	2.54	2.80
Copper (Cu)	11	1.4	9	13	71	10.2	60	80.88	57	3.8	55	60
Iodine (I)	0.22	0.138	0.14	0.53	4.2	0.33	3.6	4.499	18	6.2	8	26
Iron (Fe)	1468	1317.2	685	4392	917	260.9	723	1212.0	760	50.1	725	795
Lead (Pb)	0.09	0.081	0.04	0.27	0.37	0.084	0.29	0.460	0.20	0.000	0.20	0.20
Magnesium (Mg)	1939	182.1	1757	2260	4936	250.8	4783	5225	4670	166.4	4552	4788
Manganese (Mn)	73	11.6	54	89	181	36.8	145	218.0	193	28.1	173	213
Mercury (Hg)	0.01	0.000	0.01	0.01	0.00	0.00	0.00	0.00	0.003	0.0035	0.000	0.005
Molybdenum (Mo)	0.67	0.366	0.37	1.46	2.2	0.39	1.8	2.517	1.94	0.209	1.80	2.09
Nickel (Ni)	13	9.6	6	34	11.9	2.31	9.3	13.72	11.3	1.06	10.5	12.0
Phosphorus (P)	3091	632.2	2635	4429	7208	257.1	5912	7372	7005	416.3	6710	7299
Potassium (K)	20,399	2102.5	17,581	24,024	11,991	739.6	11,254	12,733	12,814	935.2	12,152	13,475
Selenium (Se)	0.17	0.055	0.10	0.28	0.94	0.052	0.89	0.993	0.98	0.039	0.96	0.01
Sodium (Na)	1067	220.6	730	1331	3279	157.6	3111	3424	3082	184.4	2951	3212
Tin (Sn)	0.09	0.038	0.06	0.17	0.12	0.017	0.10	0.130	0.13	0.004	0.13	0.14
Zinc (Zn)	43	5.1	36	51	153	29.9	126	185.2	130	13.7	120	139

[1] Compound feed in the form of pellet comprising of soybean, wheat, corn, barley, sugar beet flour, molasses, shell lime, hard fat, mono-calcium phosphate, magnesium phosphate, salt, and mineral/vitamin supplement (their proportional contribution is presented in Table S3.1 in the Supplementary Material. [2] Containing 15 g/kg DM seaweed mixture on DM basis. Seaweed mixture was made of 91% *Ascophyllum nodosum* + 9% *Laminaria digitata*.

Table 3.3. Means, standard error (SE) and ANOVA p-values for the effect of the dietary treatment (Control, no seaweed, CON; Low Seaweed, 0.75% concentrate DM, LS; High Seaweed, 1.5% concentrate DM, HS) on animal data, milk production and basic composition and efficiency parameters.

	Diet				ANOVA *p*-Values [1]		
Parameters	**CON** ***n* = 66**	**LSW** ***n* = 78**	**HSW** ***n* = 78**	**SE**	**Diet**	**Week**	**Diet × Week**
Animal Data							
Parity	2.0	2.1	2.3	0.15			
Lactation weeks	20.0	24.0	21.9	1.79			
Bodyweight (kg)	445	446	450	5.0			
Animal Diet							
Dry Matter Intake [2] (kg/d)	14.3	14.3	14.5	0.08	0.075	0.041	0.943
Forage:concentrate	44.7	45.4	44.8	1.80	0.942	<0.001	0.793
Silage Intake (kg DM/d)	6.35	5.43	6.47	0.254	0.946	<0.001	0.776
Concentrate Intake (kg DM/d)	8.01	7.85	8.02	0.251	0.872	<0.001	0.844
Seaweed Intake (g DM/d)	0.00 [c]	12.8 [b]	50.2 [a]	0.004	<0.001	<0.001	<0.001
Milk Production							
Yield (kg/d)	25.3	24.9	26.5	0.60	0.097	0.041	0.943
ECMY [3] (kg/d)	27.0	25.9	27.1	0.70	0.399	0.133	0.775
Milk Composition							
Fat (g/100 g)	4.56	4.46	4.35	0.082	0.157	0.843	0.878
Protein (g/100 g)	3.33 [a]	3.27 [a]	3.20 [b]	0.027	0.004	<0.001	0.632
Casein (g/100 g)	2.43 [a]	2.39 [a]	2.33 [b]	0.234	0.006	<0.001	0.694
Lactose (g/100 g)	4.58	4.62	4.63	0.030	0.517	<0.001	0.767
Whey Protein (g/100 g)	0.90	0.88	0.87	0.008	0.111	<0.001	0.627
Urea (mmol/L)	6.37	6.13	3.08	0.139	0.283	<0.001	0.109
Free Fatty Acids (mmol/L)	0.80	0.87	0.91	0.038	0.144	<0.001	0.929
Fat:Protein	1.37	1.37	1.36	0.028	0.931	0.198	0.579
Somatic Cell Count (x10^3/ml)	181	206	193	65.1	0.965	0.255	0.699
Efficiency (g/kg DMI)							
Feed Efficiency	1753	1730	1807	29.6	0.134	0.022	0.899
Fat Efficiency	80.4	76.8	77.8	2.15	0.461	0.335	0.637
Protein Efficiency	58.3	56.2	57.3	0.86	0.191	0.244	0.894

[1] Significances were declared at $p < 0.05$. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test ($p < 0.05$). [2] calculated as described by Butler et al. (2008): DMI (kg/day) = 0.025 live weight (LW) (kg) + 0.125 milk yield (kg/day). 3 Energy Correct Milk Yield = milk yield (kg) × [0.01 + 0.0122 milk fat (g/kg) + 0.0077 milk protein (g/kg) + 0.053 milk lactose (g/kg)] (Sjaunja, 1991).

The experiment was carried out over a 7-week period between December 2018 and January 2019. All animals were fed the CON diet for two weeks before the commencement of the 7-week period. The starting week was used as a covariate, where all cows were fed the basal diet, and this was followed by 6-week measurement period where animals were offered experimental diets. Seaweed was gradually introduced to diets. In week 1 of the measurement period, seaweed was provided at approximately 0.25% (13 g/cow/day) and 0.50% (26 g/cow/day) of concentrate DM for LSW and HSW groups, respectively. In weeks 2 to 5, seaweed was provided at 0.75% (19–40 g/cow/day) and 1.5% (79–158 g/cow/day) of concentrate DM for LSW and HSW groups, respectively. In week 6, dietary inclusion rate of seaweed returned to approximately 0.25% (13 g/cow/day) and 0.50% (26 g/cow/day) of concentrate DM for LSW and HSW groups, respectively. Cows were milked twice daily. Milk samples were collected from each cow at the end of each experimental week during the morning and evening milkings, and composite milk samples were stored frozen (at −18 °C) in a 50 ml polypropylene tube. Samples of grass silage were collected once a week (n = 7) during the experimental period and immediately frozen at −18 °C. Samples of concentrate without seaweed were collected in experimental weeks 1, 3, and 5 (n = 3), while samples of concentrate with seaweed were collected in weeks 3 and 5 (n = 2). All feed samples were stored at −18 °C until further analysis.

3.3.2 Analysis of milk and feed for chemical composition

The basic composition (fat, protein, casein, lactose, urea, free fatty acids (FFA)) and somatic cell count (SCC) of milk was analysed using Fourier Transform Infrared Spectroscopy (Combifoss 6000, FOSS, Hilleroed, Denmark) in the laboratories of Auðhumla (Selfoss, Iceland). Samples of silages and concentrates were analysed for chemical composition (crude protein, CP; fat; sugar; starch; sugar, neutral detergent fiber, NDF; acid detergent fiber, ADF; water soluble carbohydrates, WSC; single cell protein, SCP; indigestible NDF, iNDF; neutral detergent cellulase digestible organic matter, NCDG) at the laboratories of Efnagreining (Hvanneyri, Iceland).

3.3.3 Quantification of mineral concentrations in milk and feed

Concentrations of macrominerals, trace elements (except for I) and heavy metals in milk, silage and concentrate feed were quantified according to Nordic-Baltic Committee on Food Analysis (NMKL) method 186, using an Ultra wave Acid Digestion System (Milestone Inc., Sorisole, Italy) for the digestion of samples. An Agilent 7900 quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, Singapore) was used. It was combined with an ultra-high matrix introduction (UHMI) system with a quartz cyclonic spray chamber and MicroMist nebulizer (Glass Expansion, Weilburg, Germany). Concentrations of I in milk and feed samples were quantified according to previously published methods by Payling et al (2015) and British Standards Institution Publication (BS EN 17050:2017), respectively, using ICP-MS (Agilent 7000, Agilent, Singapore). For Sn, Cd, Cr, Ni, Pb and Hg, the majority of the individual measurements (88% for Sn, 96% for Cd, 59% for Cr, 53% for Ni, 82% for Pb and 92% for Hg) were below the limits of quantification (LOQ; Sn, 0.266 μg/kg milk; Cd, 0.099 μg/kg milk; Cr, 0.696 μg/kg milk; Ni, 1.457 μg/kg milk; Pb, 0.335 μg/kg milk; Hg, 0.243 μg/kg milk); and the results of these elements were thus not included in statistical analysis. The scatter plots of all measurements of mineral concentrations in the three experimental treatments, and in relation to LOQ, are presented in supplementary Figure S3.1 (macro-minerals), Figure S3.2 (trace elements) and Figure S3.3 (heavy metals). Transfer efficiencies from feed to milk were calculated as follows: 100 × [milk mineral concentration (ug/kg milk) × milk output (kg/d) / diet mineral concentration (ug/kg dry matter) × feed intake (kg dry matter/day)].

3.3.4 Statistical analysis

Data were analysed using a mixed effects model in Minitab 18. In the model, diet, experimental week, and their interaction were used as fixed factors, while cow was set as the random factor (Minitab, 2019). Measurements from the week before the 6-week measurement period, when all cows were fed the same basal diet, were used as a covariate in the model. Normality of residuals were evaluated visually and, while most data showed no deviation from normality, SCC, milk I content, and I intake were log10 transformed prior to analysis so that their residuals were normalized. Fischer's least significance difference test ($p < 0.05$) was used for pairwise comparison of the means, where the mixed effect model showed a significant effect of diet, experimental week, or their interaction.

3.4 Results

3.4.1 Animal and diet parameters

The experimental groups were balanced for parity, lactation stage and bodyweight (Table 3.3). Parity ranged 1–4, 1–5, and 1–5, in CON, LSW and HSW groups, respectively. Lactation stage in weeks ranged 1–42, 1–68, and 1–47 in CON, LSW and HSW groups, respectively. The dietary treatment influenced seaweed intake which increased from CON to LSW, and LSW to HSW cows, in line with the experimental design (Table 3.3). Seaweed intake significantly differed between experimental groups, averaging 0 g, 12.8 g, and 50.2 g for CON, LSW, and HSW groups, respectively (Table 3.3). The DMI, forage:concentrate ratio, silage intake and concentrate intake varied by experimental week (Table 3.3).

3.4.2 Milk yield, basic Composition, and efficiency

Milk from HSW group cows had 4.1% and 2.2% less protein (g/100 g) and 4.3% and 2.6% less casein (g/100 g), when compared with CON and LSW milk, respectively (Table 3.3). There was a significant effect of dietary treatment on milk protein and casein concentration. However, milk production, and other compositional and efficiency parameters were not influenced by dietary supplementation of seaweed (Table 3.3). Milk yield, milk composition (e.g., contents of protein, casein, lactose, whey protein, and urea), and feed efficiency varied with experimental week (Table 3.3). There was no significant diet × sampling week interaction on milk production, milk basic composition, or efficiency parameters (Table 3.3).

3.4.3 Milk mineral concentrations

Dietary supplementation of seaweed influenced milk concentrations of Cu, I, Se, and As, with CON milk having a 32.5% higher Cu concentration compared to HSW milk (Table 3.4). When compared with CON milk, I concentrations were greater in LSW (+90.5%) and HSW milk (+200.8%); while HSW milk had higher (+57.8%) concentrations of I than LSW milk (Table 3.4). However, the trend was the opposite for Se concentration in milk. When compared with CON milk, concentrations of Se were lower in LSW milk (−6.0%) and HSW milk (−13.4%); while HSW contained less Se (−8.5%) than LSW milk (Table 3.4). The concentration of As in HSW milk was higher compared with LSW and CON milk (+28.8% and +36.7%, respectively) (Table 3.4).

Table 3.4. Means, standard error (SE) and ANOVA p-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on milk mineral concentrations.

	Diet				**ANOVA *p*-Values** [1]		
Minerals	**CON** ***n* = 66**	**LSW** ***n* = 78**	**HSW** ***n* = 78**	**SE**	**Diet**	**Week**	**Diet × Week**
Macrominerals (mg/kg)							
Calcium (Ca)	1129	1076	1053	29.7	0.192	<0.001	0.797
Magnesium (Mg)	110.4	103.0	99.2	4.30	0.179	0.021	0.481
Phosphorus (P)	881.8	866.8	851.0	26.72	0.708	<0.001	0.892
Potassium (K)	1471	1433	1423	40.2	0.661	<0.001	0.711
Sodium (Na)	432.9	435.2	403.0	20.31	0.422	0.033	0.525
Essential Trace Elements (μg/kg)							
Copper (Cu)	47.3 [a]	40.9 [ab]	35.7 [b]	3.05	0.034	<0.001	0.364
Iron (Fe)	223.9	224.1	223.9	9.72	1.000	0.020	0.337
Iodine (I)	821.5 [c]	1565.3 [b]	2470.8 [a]	60.98	<0.001	<0.001	<0.001
Manganese (Mn)	27.5	28.4	27.4	1.06	0.717	0.009	0.173
Molybdenum (Mo)	52.5	51.9	49.4	1.62	0.346	<0.001	0.296
Nickel (Ni)	2.49	1.60	1.40	0.440	0.182	<0.001	0.105
Selenium (Se)	23.2 [a]	21.8 [b]	20.1 [c]	0.50	<0.001	<0.001	0.987
Zinc (Zn)	4720	4683	4406	125.5	0.137	<0.001	0.842
Non-Essential Trace Elements (μg/kg)							
Aluminium (Al)	63.7	57.3	60.1	4.53	0.577	<0.001	0.202
Cobalt (Co)	0.52	0.48	0.43	0.029	0.088	<0.001	0.140
Heavy Metals (μg/kg)							
Arsenic (As)	0.455 [b]	0.483 [b]	0.622 [a]	0.0416	0.013	<0.001	0.102

[1] Significances were declared at $p < 0.05$. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test ($p < 0.05$).

The effect of sampling week was significant for all macrominerals, trace elements, and heavy metals assessed (Table S3.4). Individual significant differences between weeks are presented in detail in the Supplementary Material (Table S3.4). The I concentration in milk was influenced by the dietary treatment × sampling week interaction (Figure 3.1A). Milk I concentration was highest in HSW milk, intermediate in LSW and lowest in CON milk throughout seaweed supplementation period. Their relative difference in milk I concentrations between all experimental groups was higher during weeks 2 and 3 compared with the rest of weeks. HSW contained significantly more I across the experiment than LSW, except for Week 6 where there was no difference between the experimental groups.

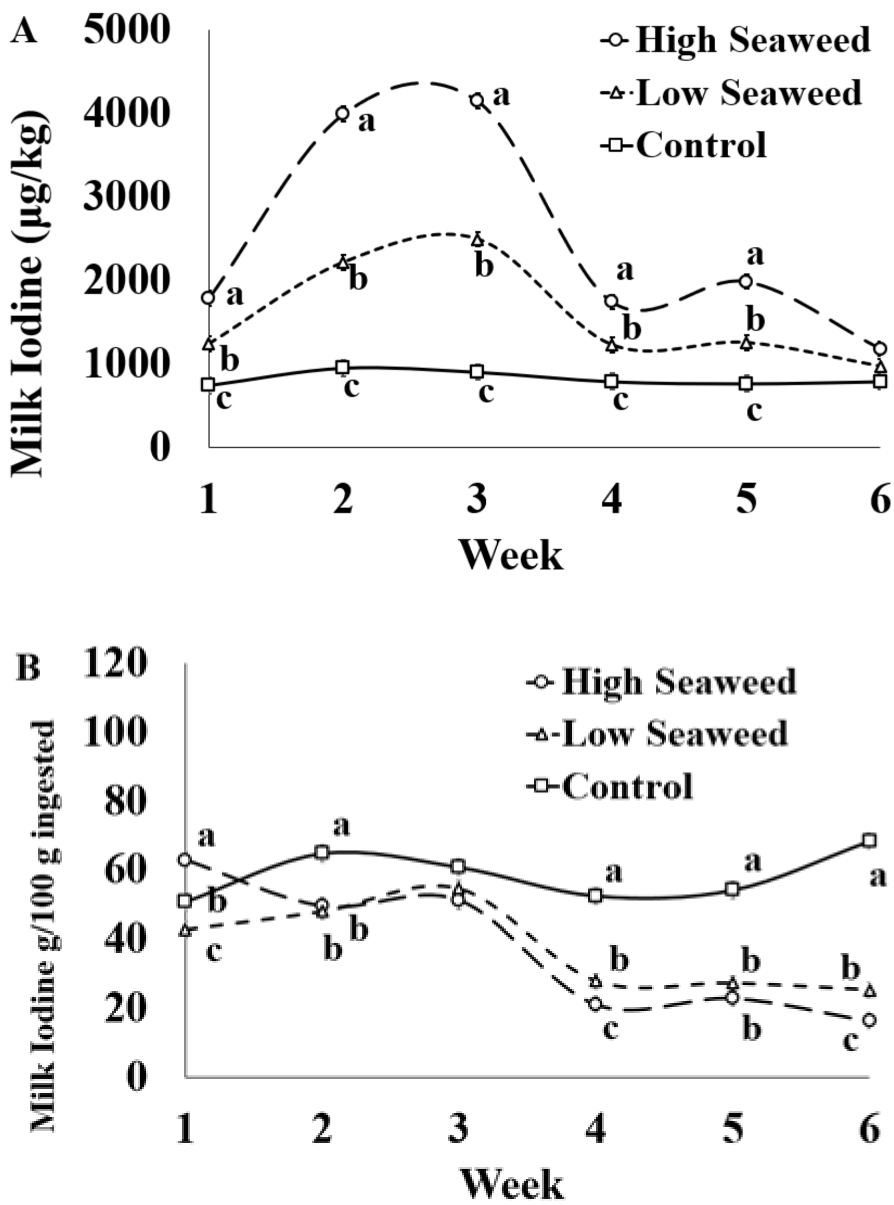


Figure 3.1. Interaction means ± SE (error bars) for the effects of dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) and week on the concentration of iodine in milk (µg/kg; panel (A); $p < 0.001$) and transfer efficiency (g milk/100 g ingested; panel (B); $p < 0.001$). Means for diet treatments within a week with different letters are significantly different according to Fisher's Least Significant Difference test ($p < 0.05$).

3.4.4 Estimated mineral transfer efficiencies from feed to milk

There was a significant effect of dietary treatment on the estimated transfer efficiency of Cu, I, Se, and Co. The transfer efficiency of Cu was higher (+0.5 µg/kg intake) in CON milk than HSW milk (Table 3.5). Transfer efficiency of I was higher (+21 µg/kg intake) in CON milk when compared to LSW and HSW milk (Table 3.5). Similarly, transfer efficiency of Se was higher (+0.7 µg/kg intake and

+1.0 µg/kg intake) in CON milk than in LSW and HSW milk, respectively (Table 3.5). The transfer efficiency of Co was higher (+0.005 µg/kg intake and +0.009 µg/kg intake) in CON milk than LSW and HSW milk, respectively (Table 3.5).

Table 3.5. Means, standard error (SE) and ANOVA p-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on estimated transfer efficiency of minerals from feed into milk.

	Diet				**ANOVA *p*-Values** [1]		
Minerals (g in Milk/100 g Ingested)	**CON *n* = 66**	**LSW *n* = 78**	**HSW *n* = 78**	**SE**	**Diet**	**Week**	**Diet × Week**
Macrominerals							
Calcium (Ca)	19.7	18.4	18.0	0.67	0.170	<0.001	0.830
Magnesium (Mg)	8.7	7.9	7.6	0.51	0.294	<0.001	0.846
Phosphorus (P)	29.2	28.1	27.8	0.99	0.590	<0.001	0.944
Potassium (K)	16.2	16.1	15.8	0.72	0.912	<0.001	0.834
Sodium (Na)	33.3	32.4	29.9	1.74	0.341	0.031	0.488
Essential Trace Elements							
Copper (Cu)	0.20 [a]	0.17 [a,b]	0.15 [b]	0.145	0.042	<0.001	0.308
Iron (Fe)	0.04	0.04	0.04	0.002	0.638	<0.001	0.364
Iodine (I)	58.7 [a]	37.7 [b]	37.5 [b]	1.70	<0.001	<0.001	<0.001
Manganese (Mn)	0.04	0.04	0.04	0.002	0.950	<0.001	0.279
Molybdenum (Mo)	6.8	6.6	6.3	0.23	0.378	<0.001	0.436
Nickel (Ni)	0.05	0.03	0.02	0.011	0.215	<0.001	0.087
Selenium (Se)	7.2 [a]	6.5 [b]	6.2 [b]	0.22	0.007	<0.001	0.961
Zinc (Zn)	8.5	8.4	7.9	0.23	0.128	<0.001	0.690
Non-Essential Trace Elements							
Aluminium (Al)	0.03	0.02	0.03	0.002	0.563	<0.001	0.252
Cobalt (Co)	0.044 [a]	0.039 [a,b]	0.035 [b]	0.0025	0.037	<0.001	0.128
Heavy metals							
Arsenic (As)	0.22	0.20	0.19	0.018	0.679	<0.001	0.252

[1] Significances were declared at $p < 0.05$. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test ($p < 0.05$).

The effect of sampling week was significant on the transfer efficiency of all assessed macrominerals, trace elements, and heavy metals and individual significant differences between weeks are presented in detail in the Supplementary Material (Table S3.5).

The only significant effects of the dietary treatment × sampling week interaction mineral transfer efficiency from feed to milk was for I (Table 3.5). In Week 1, I transfer efficiencies were highest in HSW milk, intermediate in CON milk and lowest in LSW milk. Between Weeks 2 and 6, I transfer efficiencies were higher in CON milk than in LSW and HSW milk (except for Week 3), while LSW also resulted on higher I transfer efficiencies than HSW milk in Weeks 4 and 6 (Figure 3.1B).

3.5 Discussion

3.5.1 Effect of seaweed supplementation on milk yield, basic composition and efficiency parameters

In the present study, seaweed supplementation of dairy cow diets did not affect productivity, efficiency, and the basic composition of milk, thus agreeing with previous studies feeding *Ascophyllum nodosum* and *Undaria pinnatifida* (Antaya et al., 2019; Chaves Lopez et al., 2016; Hong et al., 2015). Given that main drivers for productivity, production efficiency and milk composition are animal breed, intakes, and types of forages and concentrates (Stergiadis et al., 2015; Stergiadis et al., 2012), it is likely that the relatively small amount of seaweed supplementation to dairy cow diets (0 to 158 g/cow/day) in the present study was not adequate to cause any impact on these parameters. In contrast, Singh, Chopra, Rai, Verma, and Mohanta (2015) reported that *S. wightii* supplementation at 20% to concentrate DM showed increased milk production in dairy cows. This discrepancy could be attributed to the much larger degree of supplementation as they administered approximately 955 g of seaweed per cow per day and indicated that there might be unidentified bioactive substances within the seaweed that may have positively affected milk yield at such high supplementation rates. The only milk composition parameters affected by seaweed supplementation in the present study were milk protein and casein contents, which were both reduced in case of HSW diets. This is different than the studies of Hong et al (2015) and Lopez et al. (2016), which saw no change in milk protein concentrations with increasing brown seaweed by-products or *A. nodosum* supplementation, respectively. However, the differences in the present study were numerically marginal as HSW contained only 1.3 g/kg less protein and 1.0 g/kg less casein than CON milk.

3.5.2 Effect of seaweed on milk mineral concentrations and estimated mineral transfer efficiencies from feed to milk

3.5.2.1 Trace elements

The reduced Cu concentration in milk with increased seaweed supplementation in the present study is in contrast with other studies that showed seaweed supplementation did not impact milk Cu content (Rey-Crespo et al., 2014). In the current study, reduced Cu concentration was found in HSW milk despite the minimal difference in dietary intakes of Cu between experimental groups (614.5 mg/cow/day for CON, 413.1–934.1 mg/cow/day; 602.3 mg/cow/day for LS, 376.1–932.9 mg/cow/day; and 615.1 mg/cow/day for HSW, 403.6–926.9 mg/cow/day). This might indicate that the appearance of Cu into milk might be mediated by physiological or metabolic processes rather than simply Cu intake. Milk Cu concentrations are unaffected by high Cu intakes, but when Cu intakes are restricted below requirement there is a commensurate decrease in milk Cu concentrations (Suttle, 2010). Although Cu availability has not been assessed in this study, a possible explanation might be that Cu availability from the CON was higher than that in seaweed-supplemented diets (in line with the lower Cu transfer efficiency observed in the current study). However, it should be noted that Cu regulation is more complicated than a simple input/output relationship and involves several organ systems (Suttle, 2010). In general, differences between studies may also originate from the use of different species of seaweed, known to affect mineral concentrations (Pereira, 2011), which was a mix of *A. nodosum* and *L. digitata* in this study and a mix of *Ulva rigida*, *Sargassum muticum*, and *Saccorhiza polyschides* in the study of Rey-Crespo, López-Alonso and Miranda (2014), as well as the dietary supplementation level (158 g/cow/day maximum in the present study for the HSW group vs. 100 g/cow/day in study by Rey-Crespo, López-Alonso and Miranda (2014)).

In the current study, seaweed supplementation in dairy cow diets increased I concentrations in milk, which is in line with the findings from previous work that investigated the effect of feeding *A. nodosum* (Antaya et al., 2019; Chaves Lopez et al., 2016; Sorge et al., 2016) and kelp powder or *Thallus laminariae* to dairy cows on milk I concentration (Xue et al., 2019). Concentrations of I in raw milk are primarily influenced by diet I concentrations, but in-feed goitrogenic compounds, husbandry practices, and mammary gland hygiene management (teat-dipping) are also determinant factors (Antaya et al., 2019; Flachowsky et al., 2013; Schöne et al., 2009). Seaweed is a known rich source of I (Circuncisão et al., 2018; Pereira, 2011) and in the present study, I intake across the experimental period was 35.0 mg/cow/day (21.2–48.5 mg/cow/day), 107 mg/cow/day (60.4–163.6 mg/cow/day), and 178.7 mg/cow/day (60.4–281.1 mg/cow/d) for CON, LSW, and HSW cows, respectively. Therefore, LSW and HSW cows ingested 3.1 and 5.1 times more I, respectively, than CON cows, which could explain the higher concentration of I in the milk from LSW and HSW cows.

In the present study, the diet I concentration was 2.4 mg/kg DM for CON, 7.5 mg/kg DM for LSW and 12.3 mg/kg DM for HSW cows. Given the maximum permitted dietary I concentration is 5 mg/kg DM (EFSA Panel on Dietetic Products & Allergies, 2013), the I concentration in CON, LS and HS diets was 48%, 150%, and 246% of maximum permitted I concentration, respectively. Notably, at the peak of seaweed supplementation (weeks 2, 3, 5), dietary supply of I to LSW and HSW cows temporarily exceeded 2.2 and 4.0 times of the maximum permitted supply. This indicates that care should be taken when seaweed is supplemented in dairy cow diets for long periods because small amounts of I-rich seaweed may supply far higher amounts of I in dairy cow diets than the maximum permitted intakes. The upper tolerable limit dietary I for cattle is reported to be 50 mg/kg of diet DM (NRC, 2005). At an average DMI of 14.4 kg/day, as calculated in the present study, the maximum tolerable limit for I intake would be 720 mg/cow/day. Therefore, although LSW and HSW diets exceeded permitted dietary supplementation of I for cattle, I intake by LSW and HSW cows in the current study was, respectively, on average 15% and 25% of the upper tolerable limit for cattle, and never exceeded the 40% of upper tolerable limit. Although the dietary I supply in the current study was much lower than the upper tolerable limit, after one week adaptation in seaweed diets and two weeks after peak seaweed supplementation, the I transfer from feed to milk dropped from 55% and 51% to 28% and 21% in LSW and HSW cows, respectively. In mammals, excessive I intake triggers the Wolff-Chaikoff effect reducing I absorption from the gut to blood (Arriagada et al., 2015). A similar mechanism may not be excluded in dairy cows and therefore, the rapid increase in I supply may have triggered a reduced absorption of I and subsequent supply in the mammary gland and/or a down regulation of the Na^+/I^- symporter system in the mammary gland; both of which would reflect in reduced I concentrations in milk despite the high intakes. After the end of the experiment, I was monitored for 3 more weeks in the cows that consumed LSW and HSW diets and the transfer efficiencies of I returned to the pre-supplementation levels (52% and 57% for LSW and HSW cows, respectively), only a week after removal of seaweed from the diet, which may indicate that this impact is reversible, at least after the exposure duration to LSW and HSW diets investigated in the present study, when I supply returns to recommended levels; possibly because the Na+/I- symporter system returns to pre-high dose levels.

The reduced Se concentration in milk with increased seaweed supplementation in the present study is in contrast with the findings of a previous study that reported that seaweed supplementation did not impact milk Se concentration (Rey-Crespo et al., 2014). Even though Se intake was not different between experimental groups in the current study (8.4 mg/cow/day for CON (5.7–11.5 mg/cow/day), 8.3 mg/cow/day for LSW (5.3–11.5 mg/cow/day), and 8.5 mg/cow/day for HSW (5.7–11.4 mg/cow/day)), there was still a decrease in milk Se concentration in the LSW and HSW groups. This indicates that the resulting concentrations might be influenced by physiological or metabolic processes rather than being a direct effect of Se intake. Milk Se concentrations are influenced by cow supplementation and feed types (varying widely between different areas (Ammerman & Miller, 1975; Grace, Lee, Mills, & Death, 1997), and has been shown to be increased (albeit short-lived) with dietary Se increases (Suttle, 2010). Another explanation could be in that an increase in sulphur supplied from seaweed, as sulphate is a typical component of marine algal polysaccharides, may antagonize selenium absorption, or that the form of Se found within the treatment feed may affect uptake (Galbraith et al., 2016; Netto et al., 2014; Rey-Crespo et al., 2014). Reduced transfer efficiency might be a consequence of an interaction between Se and Se antagonists thus reducing the uptake and transfer of selenium from feed into milk. The differences between Rey-Crespo, López-Alonso and Miranda (2014) and this study can also be explained via the differing species and amount fed to the cows, as described above for Cu.

The decreased Co transfer efficiency with increased seaweed supplementation in the present study was not reflected in the Co content between the experimental groups. This is likely due to differences between the groups and the total Co transfer efficiency results numerically extremely small, as the difference between the highest and lowest transfer efficiency is 0.009%.

3.5.2.2 Heavy metals

Increasing seaweed supplementation in cow diets increased As concentrations, thus being in line with Rey-Crespo, López-Alonso and Miranda (2014). This is expected, as the most prominent heavy metal in algae is As, hence the EU there is relevant regulation regarding the maximum amount in algae in feed (Pétursdóttir et al., 2019). As intake across the experimental period was 6.0 mg/cow/day for CON (2.8–14.81 mg/cow/day), 6.7 mg/cow/day for LSW (3.1–15.2 mg/cow/day), and 9.3 mg/cow/day for HSW (4.5–15.6 mg/cow/day). The higher dietary intake of As when seaweed was fed is the most possible reason for the increased As content in milk, as As intake leads to increased milk As content (Saeed Akhtar, 2019). Any amount of inorganic As (which is more toxic than organic As (Cubadda, et al., 2017)) in feed or product is recommended to be avoided, and US NRC reports that the maximum tolerable dosage for cattle is 50 mg/kg diet DM (Bampidis, Nistor, & Nitas, 2013). At an average DMI of 14.4 kg/day in the present study, the maximum tolerable limit for inorganic As intake would be 720 mg/cow/day, which is 46 times higher than the maximum As intake in the present study (15.6 mg/cow/day). In the present study, the analysis did not differentiate between organic or inorganic As and diets were designed to supply less than the maximum limits of As in dairy cow diets (2 mg/kg inorganic As and 40 mg/kg total As in the seaweed mixture (European Commission, 2015)).

3.5.3. Nutritional implications of milk from seaweed-fed cows for consumers (I, Cu, Se, As)

Milk is a good source of several macrominerals and trace elements and this has particular importance for different demographics which may have higher requirements or rely more on milk for the supply of minerals across infancy, adolescence, and adulthood. In the present study, the concentrations of Cu, I, Se, and As were affected by seaweed supplementation in dairy cow diets and this would have an effect on consumer intakes of these minerals when consuming milk from seaweed-fed cows. To assess the impact of seaweed supplementation of dairy diets on consumers' mineral intakes, the mineral intakes from the milk of experimental groups was calculated by multiplying the recorded average milk intakes in Iceland (kg of liquid milk per person per day) with the concentrations of I, Cu, Se, and As (ug, or mg, per kg milk). Following that, the calculated mineral intakes were compared against the nutritional recommendations (reference nutrient intakes (RNI) and upper limits (UL)) by the Icelandic Directorate of Health (Iceland, 2013) to assess the % contribution that milk would provide to the RNI, but also investigate whether consumption of any minerals exceeds UL, when milk from different experimental groups would be consumed.

The average consumption of milk in Iceland is 285 g/day, according the most recent available milk sale records (2020) from Icelandic Dairies Association (Samtök afurðastöðva í mjólkuriðnaði); based on this, CON, LSW, and HSW milks would cover 2.7–4.5%, 2.3–3.9%, and 2.0–3.4% of the RNI for Cu in children <10 years of age, respectively; 1.5–1.9 %, 1.3–1.7%, and 1.1–1.5% in adolescents and adults ≥10 years of age, respectively; and 1.0–1.4%, 0.9–1.2%, and 0.8–1.0% in nursing mothers and pregnant women, respectively. Given that this amount does not represent a considerable proportion of RNI for Cu for all age groups at Icelandic levels of consumption, it is unlikely that these differences will have a relevance to consumers' nutrition and health.

Based on the above referenced average consumption of milk in Iceland, the CON, LSW and HSW milks would cover 196–470%, 373–895% and 589–1413% of the RNI for I in children <10 years of age, respectively; 157%, 299% and 471% of the RNI for I in adolescents and adults ≥10 years of age, respectively; and 117–134%, 224–256% and 353–404% in nursing mothers and pregnant women, respectively. Even consumption of CON milk from the present study, and under the stated milk intakes in Iceland, would provide more than the required I to the population to meet their RNI for I. This is of particular importance because I deficiency prevails globally, occurring in 435.5 million (56.9% of the population) and almost 2 billion (35.2% of the population) people in Europe and globally, respectively (WHO, 2007). Although in Iceland this was not a public health issue for years, more recent studies have highlighted that specific demographics (including pregnant women) had suboptimal I intake and have associated this with the reduction in milk, dairy and fish consumption (Adalsteinsdottir et al., 2020). The results for the CON milk in the present study reinforce the important role that milk can play in providing the required amounts of I in human diets. Interestingly, the milk I content of the CON milk (822 μg/kg) was substantially higher than that in countries neighbouring Iceland (e.g., 331 μg/kg milk in conventional UK milk (Qin et al., 2021) 232 μg/L in winter low-fat Norwegian milk (Dahl, Opsahl, Meltzer, & Julshamn, 2003); and 670 μg/kg in Irish milk that involved pre- and post-milking teat dipping in I-containing solution (O'Brien, Gleeson, & Jordan, 2013)). These higher concentrations may be due to the experimental farm being on the banks of the Ölfusá River, a body of water that carries glacial water (commonly rich in I) and at close proximity to the sea (~20 km) (Hansen, 2019; Nyström et al., 2016). Coastal areas have more I in the soil and subsequently produce forage that may also have higher I concentrations (Jensen et al., 2019). The potential effect of Icelandic cow genetics may not be excluded as it is known that breed can also be a driver for milk I concentrations (Franke, Meyer, Wagner, & Flachowsky, 2009; Qin et al., 2021). The combination of even standard milk being rich in I, and the relatively high average consumption of milk in Iceland (26th in the world and 23rd in Europe, (FAOSTAT, 2013)), contributed to a high calculated contribution of milk towards the RNI for I.

However, supplementation of dairy diets with seaweed would exacerbate an excessive I intake. The upper limit for I in adults is 600 μg/day, and high consumption of I may induce hypothyroidism, in which susceptible individuals fail to adapt to the acute Wolff-Chaikoff effect, or hyperthyroidism in which vulnerable individuals increase thyroid hormone production due to the rich I substrate, inducing thyrotoxicosis (Leung & Braverman, 2014). When comparing these intakes with the recommended UL for adults (Iceland, 2013), consumption of CON, LSW and HSW milk would provide 39%, 75% and 118% of the upper limit. This highlights that, although high in I, CON and LSW milk would not provide an amount that would be considered a risk (at a consumption rate of 285 g/day) but drinking milk from the HSW group at the average Icelandic intake levels would exceed the UL for I. From a different perspective, the UL for I intake in adults would be reached by drinking 730 g of CON milk, 383 g of LSW milk, or 243 g of HSW milk. Although Icelandic guidelines were not available for UL in children and adolescents, EFSA (2006) recommends that UL for children <10 years of age to be 200–300 μg/day and UL for adolescents (10–17 years of age) to be 450–500 μg/day. Based on this, the UL can be reached by children drinking 243–365 g of the CON milk, 128–192 g of the LSW milk and 81–122 g of the HSW milk. For adolescents, the UL can be reached by children drinking 548–608 g of the CON milk, 287–319 g of the LSW milk and 182–203 g of the HSW milk. It is important however to note that in the present study, I concentrations in LSW and HSW averaged 7.5 and 12.3 mg/kg DM, respectively, while CON diet contained 2.4 mg/kg DM. Such high diet I concentrations as in LSW and HSW groups are unlikely to be provided in commercial herds because I supplementation in dairy diets ought to be less than 5 mg/kg DM (NRC, 2001). Although these diets do not represent potential commercial examples, and therefore it is unlikely that milk with such high I content would reach the Icelandic market, the findings highlight that extreme care should be taken when seaweed is supplemented to dairy cow diets because even small amounts of I-rich seaweed can not only exceed I allowances in dairy cow diets, but also drastically increase milk I concentrations and potentially pose a nutritional risk to the consumers.

Based on the above-referenced average consumption of milk in Iceland, CON, LSW, and HSW milks would cover 22–44%, 21–42%, and 19–38% of the RNI for Se in children <10 years of age, respectively; 12–17%, 11–16%, and 11–14% in adolescents and adults ≥10 years of age, respectively; and 11%, 11%, and 10% for nursing mothers and pregnant women, respectively. Although milk appears to be among the main suppliers of Se in the Icelandic diets, and seaweed supplementation in dairy diets influences milk Se concentrations, the numerical differences are rather small. As a result, the consumption of CON, LSW or HSW milk would marginally differentiate the proportionate contribution of milk to RNI for Se and it is unlikely that consuming milk from different groups would impact consumer nutrition and health.

As is a toxic heavy metal and should generally be avoided in foodstuffs, as previous nutritional research council reports have not found a biochemical process in which As is required, and that the concept of As essentiality is still to be researched (Hughes, Beck, Chen, Lewis, & Thomas, 2011). The WHO provisional guideline recommendation is that As intake should not exceed 10 μg/L in drinking water (Ravenscroft, Brammer, & Richards, 2009). The milks in the present study contained 0.46 μg/kg (CON), 0.48 μg/kg (LSW) and 0.62 μg/kg (HSW), thus all having extremely low As concentrations, being only 4.6%, 4.8% and 6.2% of the maximum recommended concentrations in water. Notably, this recommendation for milk As content is paired with the Tropical Agriculture Association's (TAA) published requirements for humans living in temperate conditions to drink 3 L of water per day (Grandjean, 2009); which would provide a recommended maximum As supply of 30 μg/day. In the present study, considering above-referenced average consumption of milk in Iceland, CON, LSW, and HSW milks would account for 0.13, 0.14, and 0.18 μg/day, respectively, which represents 0.4–0.6% of the maximum recommended As intake. Therefore, milk cannot be considered a source of As and the consumption of milk of any experimental group is not associated with any potential As-related risks in human nutrition and health; a finding which also aligns with previous studies using other seaweeds (*Ulva rigida, Sargassum muticum, Saccorhiza polyschides,* fed at 80.0:17.5:2.5 ratio at 100 g per animal per day) (Rey-Crespo et al., 2014). In addition, it should be noted that the present study has not differentiated between organic and inorganic As, a parameter that also influences toxicity with inorganic As posing a higher toxicity (Cubadda et al., 2017). Therefore, the intakes of inorganic As could be smaller given that a fraction of As in milk, might be present as organic As, however, since the total As concentration is so low a distinction between inorganic and organic As is not relevant from a toxicological point of view (Jackson, Taylor, Punshon, & Cottingham, 2012). In general, milk is not a source of heavy metals in human diets as only traces were detected, mostly below an already extremely low LOQ, which are far below the maximum recommended levels for milk, and this is not expected to be associated with effects on human health.

3.6 Conclusions

Seaweed supplementation (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) did not affect cow productivity or milk basic composition, except for a small reduction in milk protein and casein content. However, seaweed supplementation reduced contents of Cu and Se in milk and increased contents of I and As in milk. The increases in milk I and As contents are likely due to the higher dietary supply of I and As, although the lower concentrations of Cu and Se seem to be more associated with a reduction in their transfer efficiencies from diet to milk, when seaweed was included in cows' diets. Despite the lower milk Cu and Se contents when seaweed was supplemented in dairy diets, the subsequent calculated contribution of the different milks on Cu and Se reference nutrient intakes (based in Icelandic population milk intakes and nutritional guidelines) were marginally different and unlikely to be related with any effect on consumers nutrition or health. This study further emphasizes the main role that milk plays in I supply as even consumption of the control milk would provide more than the required I to the population to meet their RNI for I. However, the findings also showed that extreme care should be taken when seaweed is supplemented to dairy diets because even small amounts of I-rich seaweed can exceed the cow dietary I allowances but also drastically increase milk I concentrations and potentially pose a nutritional risk for consumers. Seaweed supplementation of dairy diets increased As concentrations in milk but milk from all experimental groups contained only traces of As and consumption cannot not be associated with any potential As-related risks in human nutrition and health.

3.7 Supplemental Material

The following are available online at www.mdpi.com/xxx/s1, Table S3.1: Ingredient composition (g/100 g dry matter) of the concentrate feed fed during the animal trial, Table S3.2: Means, standard deviation (SD), minimum and maximum values for the average chemical composition of the three experimental diets used in the animal trial, Table S3.3: Means, standard deviation (SD), minimum and maximum values for the average mineral concentrations of the three experimental diets used in the animal trial, Figure S1: title, Video S1: title, Table S3.4: Means, standard error (SE) and ANOVA p-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on mineral composition within the resulting milk, Table S3.5: Means, standard error (SE) and ANOVA p-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on transfer efficiency of minerals from feed to milk per week, Figure S3.1: Scatter plots of all measurements of macromineral concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM), Figure S3.2: Scatter plots of all measurements of trace element concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM). The horizontal dotted lines represent limits of quantification for each element, Figure S3.3: Scatter plots of all measurements of heavy metal concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM). The horizontal dotted lines represent limits of quantification for each element.

Table S3.1

Ingredient composition (g/kg dry matter) of the concentrate feed fed during the animal trial.

Soybean	310
Wheat	210
Corn	151
Barley	104.5
Sugar Beet Flour	80
Molasses	60
Shell Lime	25
Hard Fat	20
Mono-calcium Phosphate	15
Magnesium Phosphate	15
Salt	7
Minerals/Vitamins	2.5

Table S3.2

Measured means, standard deviation (SD), minimum and maximum values for the average chemical composition (g/kg dry matter (DM)) of the three experimental diets (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) used in the animal trial.

	Control				**Low-Seaweed**				**High-Seaweed**			
Chemical composition (g/kg DM)	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Dry Matter (g/kg fresh)	629	4	624	634	618	4	613	623	634	4	629	639
Ash	80	3	76	84	79	3	75	83	81	3	76	85
Crude Protein	192	07	176	201	189	7	173	198	193	7	177	202
Neutral Detergent Fiber	289	11	273	306	287	10	270	303	288	11	272	305
Acid Detergent Fiber	446	31	404	504	439	31	398	496	449	32	407	508
Fat	41	3	37	45	40	3	37	44	41	3	37	44

Table S3.3

Estimated means, standard deviation (SD), minimum and maximum values for the average total fed mineral concentrations (mg/kg dry matter (DM)) for all three experimental diets (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) used in the animal trial

	Control				**Low-Seaweed**				**High-Seaweed**			
Minerals (mg/kg DM)	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Aluminium (Al)	517	320.1	258	1365	521	324.2	245	1501	527	317.0	252	1477
Arsenic (As)	0.41	0.202	0.22	0.92	0.47	0.200	0.23	0.97	0.64	0.169	0.32	1.00
Cadmium (Cd)	0.05	0.010	0.03	0.07	0.05	0.011	0.03	0.07	0.05	0.010	0.03	0.07
Calcium (Ca)	10206	820.5	8714	11755	10182	900.5	7787	11951	10371	896.1	8066	12565
Chromium (Cr)	31	19.4	16	82	31	19.6	15	91	30	19.2	15	89
Cobalt (Co)	2.20	0.446	1.54	3.08	2.18	0.467	1.30	3.12	2.21	0.457	1.34	3.13
Copper (Cu)	42	6.4	31	58	42	6.9	28	58	42	6.8	28	59
Iodine (I)	2.4	0.37	1.6	3.0	7.5	1.57	4.5	10.9	12.3	3.91	4.4	20.2
Iron (Fe)	1147	618.7	709	2829	1153	625.8	707	3113	1152	613.1	711	3054
Lead (Pb)	0.23	0.059	0.15	0.33	0.23	0.061	0.13	0.33	0.23	0.059	0.13	0.34
Magnesium (Mg)	3152	1080.8	606	4147	3155	1067.3	570	4292	3199	1077.6	572	4367
Manganese (Mn)	128	18.8	96	172	128	19.6	89	173	128	19.4	90	173
Mercury (Hg)	0.005	0.0008	0.003	0.006	0.005	0.0008	0.003	0.006	0.005	0.0008	0.002	0.006
Molybdenum (Mo)	1.44	0.274	1.07	1.93	1.43	0.283	0.94	1.97	1.44	0.277	0.97	1.99
Nickel (Ni)	12	4.8	8	25	12	4.9	8	26	12	4.8	8	26
Phosphorus (P)	5402	444.9	4572	6337	5373	455.6	4302	6510	5397	452.2	4265	6623
Potassium (K)	15842	1106.2	13803	18517	16011	1187.8	13793	19788	16292	1200.0	13836	19897
Selenium (Se)	0.58	0.071	0.44	0.71	0.58	0.077	0.39	0.73	0.58	0.076	0.40	0.74
Sodium (Na)	2272	192.2	1833	2645	2308	213.1	1653	2797	2463	234.9	1814	3038
Tin (Sn)	0.10	0.021	0.08	0.15	0.10	0.021	0.07	0.15	0.10	0.021	0.07	0.15
Zinc (Zn)	100	15.7	75	140	99	16.6	69	141	100	16.5	70	141

Table S3.4

Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on milk mineral concentrations

	Week						ANOVA P-values	
Minerals	**1 n=37**	**2 n=37**	**3 n=37**	**4 n=37**	**5 n=37**	**6 n=37**	**SE**	**Week**
Macrominerals (mg/kg)								
Calcium (Ca)	1127^{ab}	968^{d}	1150^{a}	1149^{a}	1073^{bc}	1049^{c}	26.5	<0.001
Magnesium (Mg)	116.9^{a}	92.2^{c}	107.7^{ab}	107.7^{ab}	101.2^{bc}	99.4^{bc}	5.26	0.021
Phosphorus (P)	864.2^{bc}	812.6^{c}	903.3^{ab}	922.2^{a}	835.2^{c}	861.4^{bc}	22.45	<0.001
Potassium (K)	1469^{ab}	1337^{d}	1512^{a}	1531^{a}	1418^{bc}	1387^{cd}	33.49	<0.001
Sodium (Na)	473.1^{a}	378.1^{c}	432.8^{ab}	411.3^{bc}	423.5^{abc}	423.6^{abc}	20.98	0.033
Essential trace elements (µg/kg)								
Copper (Cu)	47.7^{a}	43.1^{b}	48.7^{a}	40.0^{b}	34.6^{c}	33.8^{c}	2.16	<0.001
Iron (Fe)	237.4^{a}	231.6^{a}	237.4^{a}	217.6^{ab}	202.1^{b}	217.9^{ab}	9.37	0.02
Iodine (I)	1254.9^{c}	2383.6^{b}	2514.1^{a}	1251.3^{c}	1333.2^{c}	978.2^{d}	52.51	<0.001
Manganese (Mn)	26.1^{b}	27.3^{ab}	29.2^{a}	28.6^{a}	26.0^{b}	29.4^{a}	0.97	0.009
Molybdenum (Mo)	48.0^{c}	48.0^{c}	53.2^{b}	63.1^{a}	46.1^{c}	49.1^{c}	1.42	<0.001
Nickel (Ni)	1.06^{b}	1.12^{b}	1.32^{b}	4.60^{a}	2.05^{b}	0.81^{b}	0.546	<0.001
Selenium (Se)	23.0^{a}	22.7^{a}	23.6^{a}	22.1^{a}	19.3^{b}	19.6^{b}	0.58	<0.001
Zinc (Zn)	4794^{b}	4609^{bc}	5233^{a}	4482^{cd}	4323^{de}	4175^{e}	109.5	<0.001
Non-essential trace elements (µg/kg)								
Aluminium (Al)	59.0^{b}	43.5^{b}	85.1^{a}	56.7^{b}	58.6^{b}	59.4^{b}	6.11	<0.001
Cobalt (Co)	0.56^{a}	0.43^{bc}	0.57^{a}	0.48^{b}	0.44^{bc}	0.39^{c}	0.024	<0.001
Heavy metals (µg/kg)								
Arsenic (As)	0.63^{a}	0.53^{abc}	0.59^{ab}	0.52^{bc}	0.38^{d}	0.47^{cd}	0.041	<0.001

[1] Significances were declared at P < 0.05. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test (P < 0.05)

Table S3.5

Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no seaweed, CON; Low seaweed, 0.75% concentrate DM, LSW; High Seaweed, 1.5% concentrate DM, HSW) on transfer efficiency of minerals from feed to milk per week

	Week						ANOVA P-values	
Minerals	**1**	**2**	**3**	**4**	**5**	**6**		
(g in milk/100g ingested)	**n=37**	**n=37**	**n=37**	**n=37**	**n=37**	**n=37**	**SE**	**Week**
Macrominerals (mg/kg)								
Calcium (Ca)	19.2[bc]	16.8[e]	20.1[ab]	20.4[a]	18.2[cd]	17.6[de]	0.51	<0.001
Magnesium (Mg)	6.13[b]	4.57[c]	5.02[bc]	23.18[a]	4.86[bc]	4.72[bc]	0.546	<0.001
Phosphorus (P)	30.3[b]	27.8[c]	28.9[bc]	30.7[a]	24.3[d]	28.1[c]	0.79	<0.001
Potassium (K)	17.7[a]	14.7[e]	15.3[de]	16.7[b]	15.6[cd]	16.2[bc]	0.48	<0.001
Sodium (Na)	36.1[a]	28.1[c]	30.4[bc]	33.3[ab]	31.5[abc]	31.6[abc]	1.84	0.031
Essential trace elements (μg/kg)								
Copper (Cu)	0.17[b]	0.18[b]	0.23[a]	0.18[b]	0.14[c]	0.16[b]	0.010	<0.001
Iron (Fe)	0.04[c]	0.05[b]	0.05[b]	0.05[b]	0.01[d]	0.05[a]	0.002	<0.001
Iodine (I)	52.20[b]	54.19[ab]	55.65[a]	33.96[c]	34.91[c]	36.79[c]	1.34	<0.001
Manganese (Mn)	0.03[d]	0.04[c]	0.04[b]	0.04[b]	0.03[d]	0.05[a]	0.001	<0.001
Molybdenum (Mo)	5.06[e]	6.15[d]	7.92[b]	8.99[a]	4.31[f]	6.83[c]	0.207	<0.001
Nickel (Ni)	0.02[b]	0.02[b]	0.03[b]	0.10[a]	0.02[b]	0.01[b]	0.013	<0.001
Selenium (Se)	6.50[b]	6.63[b]	7.38[a]	6.88[b]	5.55[c]	6.83[b]	0.203	<0.001
Zinc (Zn)	6.8[e]	8.0[cd]	10.3[a]	8.5[bc]	7.5[d]	8.6[b]	0.21	<0.001
Non-essential trace elements (μg/kg)								
Aluminium (Al)	0.02[c]	0.02[c]	0.05[a]	0.03[b]	0.01[d]	0.04[b]	0.002	<0.001
Cobalt (Co)	0.04[c]	0.03[c]	0.06[a]	0.04[b]	0.03[d]	0.04[c]	0.002	<0.001
Heavy metals (μg/kg)								
Arsenic (As)	0.13[c]	0.15[c]	0.29[a]	0.25[b]	0.15[c]	0.24[b]	0.017	<0.001

[1] Significances were declared at P < 0.05. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test (P < 0.05)

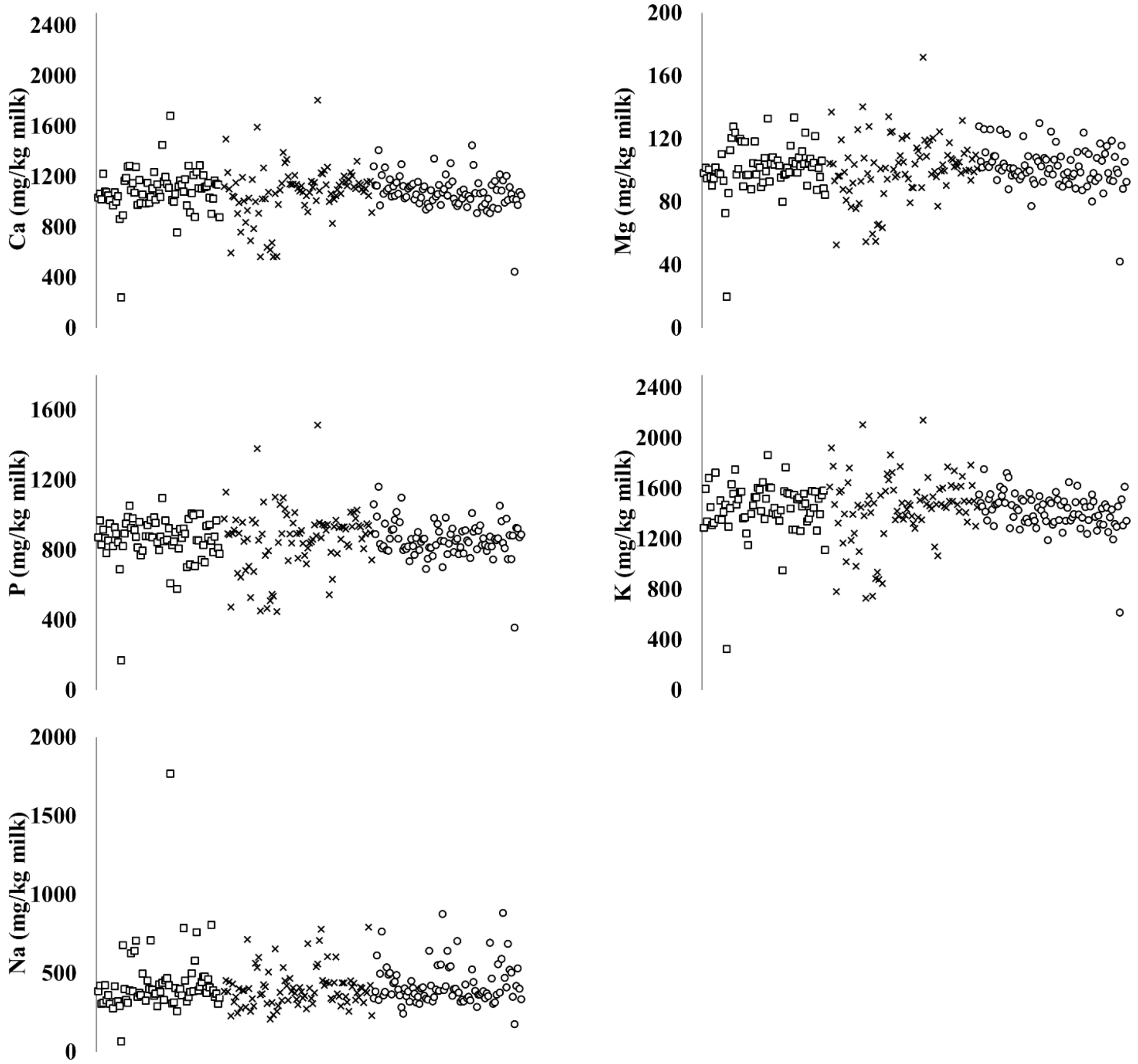
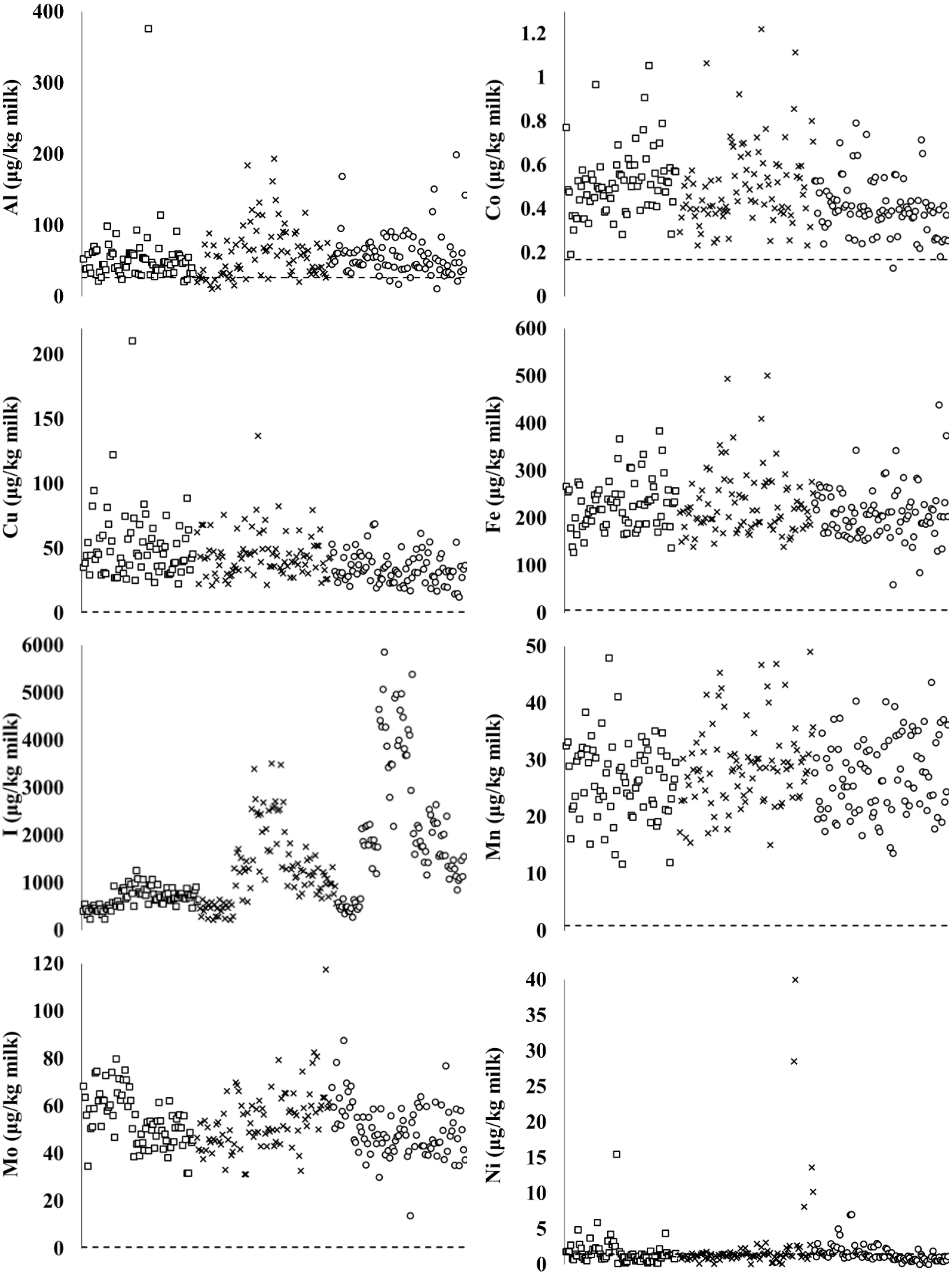


Figure S3.1

Scatter plots of all measurements of macromineral concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM).


Al (µg/kg milk)
0
100
200
300
400
Co (µg/kg milk)
0
0.2
0.4
0.6
0.8
1
1.2
Cu (µg/kg milk)
0
50
100
150
200
Fe (µg/kg milk)
0
100
200
300
400
500
600
I (µg/kg milk)
0
1000
2000
3000
4000
5000
6000
Mn (µg/kg milk)
0
10
20
30
40
50
Mo (µg/kg milk)
0
20
40
60
80
100
120
Ni (µg/kg milk)
0
5
10
15
20
25
30
35
40

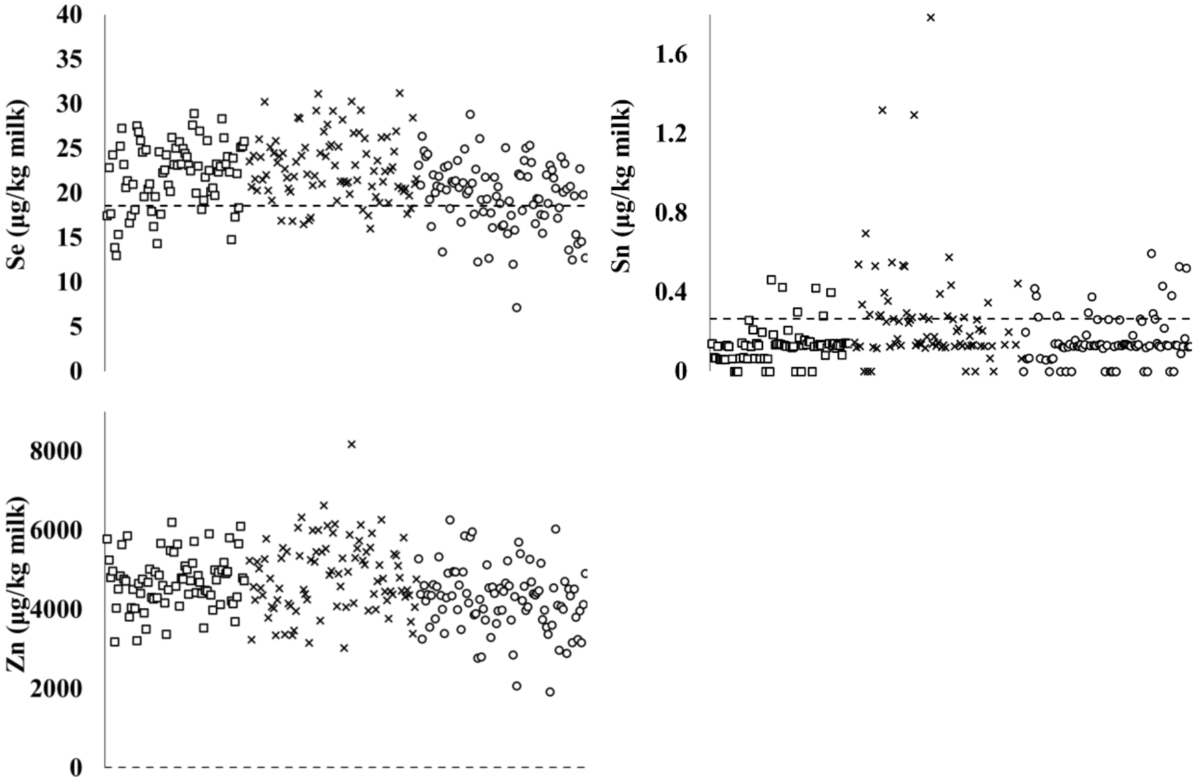


Figure S3.2

Scatter plots of all measurements of trace element concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM). The horizontal dotted lines represent limits of quantification for each element. The limits of quantification of trace elements are: Co, 0.169 µg/kg milk; Cu, 0.911 µg/kg milk; Fe, 6.417 µg/kg milk; Mn, 0.936 µg/kg milk; Mo, 0.625 µg/kg milk; Se, 18.599 µg/kg milk; Zn, 9.974 µg/kg milk; Al, 26.570 µg/kg milk; Ni, 1.457 µg/kg milk; Sn, 0.266 µg/kg milk. The proportions of individual measurements which were below LOQs were: Co, 1%; Cu, 0%; Fe, 0%; Mn, 0%; Mo, 0%; Se, 22%; Zn, 0%; Al, 9%; Ni, 53%; Sn, 88%.

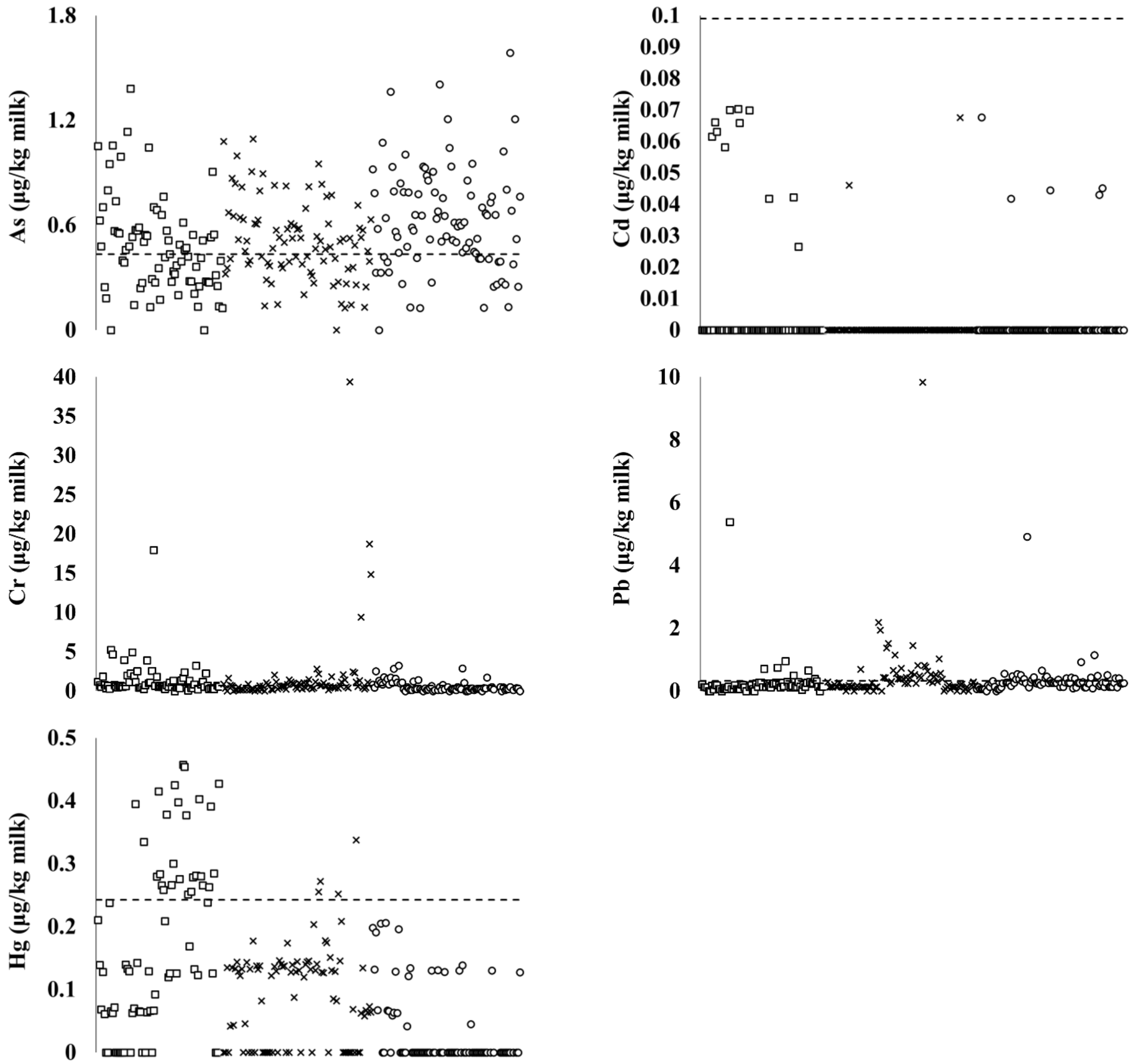


Figure S3.3

Scatter plots of all measurements of heavy metal concentrations in milk samples collected throughout the study from the three experimental groups (▢, control, no seaweed; ×, Low seaweed, 0.75% concentrate DM; ◯, High Seaweed, 1.5% concentrate DM). The horizontal dotted lines represent limits of quantification for each element. The limits of quantification of heavy metals are: As, 0.435 µg/kg milk; Cd, 0.099 µg/kg milk; Cr, 0.696 µg/kg milk; Hg, 0.243 µg/kg milk; Pb, 0.335 µg/kg milk. The proportions of individual measurements which were below LOQs were: As, 28%; Cd, 96%; Cr, 59%; Hg, 92%; Pb 82%.

3.8 Information

Author Contributions: Conceptualization, Á.H.P. and S.S.; data curation, E.E.N., K.G., S.S., C.B. and Á.H.P.; formal analysis, E.E.N., P.R. and S.S.; funding acquisition, Á.H.P. and S.S.; investigation, Á.H.P., K.G. and E.E.N.; methodology, Á.H.P., S.S., E.E.N., P.R. and D.J.; project administration, Á.H.P. and S.S.; resources, Á.H.P. and S.S.; software, Á.H.P., S.S. and P.R.; supervision, Á.H.P., S.S., P.R., N.D. and D.J.; validation, Á.H.P., E.E.N. and S.S.; visualization, E.E.N.; writing—Original draft, E.E.N. and S.S.; writing—Review and editing, E.E.N., Á.H.P., G.R., C.B., N.D., K.G., D.J., P.R., and S.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Animal procedures were reviewed by The Icelandic Food and Veterinary Authority and confirmed that the experiment did not require a license according to the regulation no. 460/2017.

Informed Consent Statement: Not applicable

Data Availability Statement: The raw data presented in this study are available on request from the corresponding author.

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3.9 References

Adalsteinsdottir, S., Tryggvadottir, E., Hrolfsdottir, L., Halldorsson, T., Birgisdottir, B., Hreidarsdottir, I., Hardardottir, H., Arohonka, P., Erlund, I., & Gunnarsdottir, I. (2020). Insufficient iodine status in pregnant women as consequence of dietary changes. Food & Nutrition Research, 64. doi:10.29219/fnr.v64.3653

Ammerman, C. B., & Miller, S. M. (1975). Selenium in Ruminant Nutrition: A Review1. Journal of Dairy Science, 58(10), 1561-1577. doi:https://doi.org/10.3168/jds.S0022-0302(75)84752-7

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. doi: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. doi:https://doi.org/10.3168/jds.2014-8851

Arriagada, A. A., Albornoz, E., Opazo, M. C., Becerra, A., Vidal, G., Fardella, C., Michea, L., Carrasco, N., Simon, F., Elorza, A. A., Bueno, M. S., Kalergis, M. A., & Riedel, C. A. (2015). Excess Iodide Induces an Acute Inhibition of the Sodium/Iodide Symporter in Thyroid Male Rat Cells by Increasing Reactive Oxygen Species. Endocrinology, 156(4), 1540-1551. doi:10.1210/en.2014-1371

Bampidis, V., Nistor, E., & Nitas, D. (2013). Arsenic, cadmium, lead and mercury as undesirable substances in animal feeds. 46, 17–22.

Belanche, A., Jones, E., Parveen, I., & Newbold, C. J. (2016). A Metagenomics Approach to Evaluate the Impact of Dietary Supplementation with *Ascophyllum nodosum* or *Laminaria digitata* on Rumen Function in Rusitec Fermenters. Frontiers in Microbiology, 7(299). doi:10.3389/fmicb.2016.00299

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

Borges, D., Araujo, R., Azevedo, I., & Pinto, I. S. (2020). Sustainable management of economically valuable seaweed stocks at the limits of their range of distribution: *Ascophyllum nodosum* (Phaeophyceae) and its southernmost population in Europe. Journal of Applied Phycology, 32(2), 1365-1375. doi:10.1007/s10811-019-02002-5

Braden, K. W., Blanton, J. R., Jr., Allen, V. G., Pond, K. R., & Miller, M. F. (2004). *Ascophyllum nodosum* supplementation: a preharvest intervention for reducing Escherichia coli O157:H7 and Salmonella spp. in feedlot steers. J Food Prot, 67(9), 1824-1828. doi:10.4315/0362-028x-67.9.1824

Brantsæter, A. L., Knutsen, H., Johansen, N., Nyheim, K., Erlund, I., Meltzer, H., & Henjum, S. (2018). Inadequate Iodine Intake in Population Groups Defined by Age, Life Stage and Vegetarian Dietary Practice in a Norwegian Convenience Sample. Nutrients, 10, 230. doi:10.3390/nu10020230

Buschmann, A., Camus, C., Infante Rosselot, J., Neori, A., Israel, A., Hernández-González, M., Pereda, S., Gomez Pinchetti, J. L., Golberg, A., Tadmor Shalev, N., & Critchley, A. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. European Journal of Phycology, 52, 391-406. doi:10.1080/09670262.2017.1365175

Butler, G., Nielsen, J. H., Slots, T., Seal, C., Eyre, M. D., Sanderson, R., & Leifert, C. (2008). Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. Journal of the Science of Food and Agriculture, 88(8), 1431-1441. doi:10.1002/jsfa.3235

Capuzzo, E. M., Torsten. (2016). Seaweed in the UK and abroad - status, products, limitations, gaps and Cefas role. Retrieved from

Chaves Lopez, C., Serio, A., Rossi, C., Mazzarrino, G., Marchetti, S., Castellani, F., Grotta, L., Fiorentino, F. P., Paparella, A., & Martino, G. (2016). Effect of diet supplementation with *Ascophyllum nodosum* on cow milk composition and microbiota. Journal of Dairy Science, 99(8), 6285-6297. doi:10.3168/jds.2015-10837

Cherry, P., Yadav, S., Strain, C. R., Allsopp, P. J., McSorley, E. M., Ross, R. P., & Stanton, C. (2019). Prebiotics from Seaweeds: An Ocean of Opportunity? Marine drugs, 17(6), 327. Retrieved from https://www.mdpi.com/1660-3397/17/6/327

Circuncisão, A. R., Catarino, M. D., Cardoso, S. M., & Silva, A. M. S. (2018). Minerals from Macroalgae Origin: Health Benefits and Risks for Consumers. Marine drugs, 16(11), 400. doi:10.3390/md16110400

Commission Regulation (EC). (2006). Consolidated text: Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. The Official Journal of the European Union L 187, 11-15.

Cubadda, F., Jackson, B. P., Cottingham, K. L., Van Horne, Y. O., & Kurzius-Spencer, M. (2017). Human exposure to dietary inorganic arsenic and other arsenic species: State of knowledge, gaps and uncertainties. The Science of the total environment, 579, 1228-1239. doi:10.1016/j.scitotenv.2016.11.108

Dahl, L., Opsahl, J., Meltzer, H., & Julshamn, K. (2003). Iodine concentration in Norwegian milk and dairy products. The British journal of nutrition, 90, 679-685. doi:10.1079/BJN2003921

Davoult, D., Engel, C., Arzel, P., Knoch, D., & Laurans, M. (2011). Environmental factors and commercial harvesting: exploring possible links behind the decline of the kelp *Laminaria digitata* in Brittany, France. Cahiers de Biologie Marine, 52, 429-435.

de la Moneda, A., Carro, M. D., Weisbjerg, M. R., Roleda, M. Y., Lind, V., Novoa-Garrido, M., & Molina-Alcaide, E. (2019). Variability and Potential of Seaweeds as Ingredients of Ruminant Diets: An In Vitro Study. Animals : an open access journal from MDPI, 9(10). doi:10.3390/ani9100851

Edwards, M., & Watson, L. (2015). Aquaculture Explained: Cultivating *Laminaria digitata*.

EFSA. (2006). Tolerable upper intake levels for vitamins and minerals Retrieved from

EFSA. (2013). Scientific Opinion on the safety and efficacy of iodine compounds (E2) as feed additives for all species: calcium iodate anhydrous and potassium iodide, based on a dossier submitted by HELM AG. EFSA Journal, 11. doi:10.2903/j.efsa.2013.3101

EFSA. (2014). Dietary exposure to inorganic arsenic in the European population. EFSA Journal, 12. doi:10.2903/j.efsa.2014.3597

El-Said, G., & El-Sikaily, A. (2012). Chemical composition of some seaweed from Mediterranean Sea coast, Egypt. Environmental Monitoring and Assessment, 185. doi:10.1007/s10661-012-3009-y

European Commission. (2015). Commission Regulation (EU) 2015/186 of 6 February 2015 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, fluorine, lead, mercury, endosulfan and Ambrosia seeds Text with EEA relevance. Retrieved from FAOSTAT. (2013). Food Supply - Livestock and Fish Primary Equivalent. Retrieved from: http://www.fao.org/faostat/en/#data/CL

Ferdouse, F. L. H., Susan; Smith, Rohan; Murúa, Pedro; Yang, Zhengyong. (2018). The Global Status of Seaweed Production, Trade and Utilization. Retrieved from Rome:

Flachowsky, G., Franke, K., Meyer, U., Leiterer, M., & Schöne, F. (2013). Influencing factors on iodine content of cow milk. European journal of nutrition, 53. doi:10.1007/s00394-013-0597-4

Franke, A. A., Bruhn, J. C., & Osland, R. B. (1983). Factors Affecting Iodine Concentration of Milk of Individual Cows. Journal of Dairy Science, 66(5), 997-1002. doi:https://doi.org/10.3168/jds.S0022-0302(83)81894-3

Gaillard, C., Bhatti, H. S., Novoa-Garrido, M., Lind, V., Roleda, M. Y., & Weisbjerg, M. R. (2018). Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. Animal Feed Science and Technology, 241, 210-222. doi:10.1016/j.anifeedsci.2018.05.003

Galbraith, M., Vorachek, W., Estill, C., Whanger, P., Bobe, G., Davis, T., & Hall, J. (2016). Rumen Microorganisms Decrease Bioavailability of Inorganic Selenium Supplements. Biological trace element research, 171. doi:10.1007/s12011-015-0560-8

Gegg, P., & Wells, V. (2017). UK Macro-Algae Biofuels: A Strategic Management Review and Future Research Agenda. Journal of Marine Science and Engineering, 5, 32. doi:10.3390/jmse5030032

Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019). Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. Nutrients, 11, 1771. doi:10.3390/nu11081771

Grace, N. D., Lee, J., Mills, R. A., & Death, A. F. (1997). Influence of Se status on milk Se concentrations in dairy cows. New Zealand Journal of Agricultural Research, 40(1), 75-78. doi:10.1080/00288233.1997.9513232

Grandjean, A. (2009). Water Requirements, Impinging Factors, and Recommended Intakes. Nutrients in Drinking Water.

Hansen, K. (2019). Iceland's Raging Rivers. Retrieved from https://earthobservatory.nasa.gov/images/145408/icelands-raging-rivers

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition--a review. Lipids in Health and Disease, 6, 25-25. doi:10.1186/1476-511X-6-25

Hong, Z. S., Kim, E. J., Jin, Y. C., Lee, J. S., Choi, Y. J., & Lee, H. G. (2015). Effects of Supplementing Brown Seaweed By-products in the Diet of Holstein Cows during Transition on Ruminal Fermentation, Growth Performance and Endocrine Responses. Asian-Australasian journal of animal sciences, 28(9), 1296-1302. doi:10.5713/ajas.15.0235

Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S., & Thomas, D. J. (2011). Arsenic exposure and toxicology: a historical perspective. Toxicological sciences : an official journal of the Society of Toxicology, 123(2), 305-332. doi:10.1093/toxsci/kfr184

Iceland, D. o. H. o. (2013). Ráðlagðir dagskammtar (RDS) af ýmsum vítamínum 2013.

Jackson, B. P., Taylor, V. F., Punshon, T., & Cottingham, K. L. (2012). Arsenic concentration and speciation in infant formulas and first foods. Pure and applied chemistry. Chimie pure et appliquee, 84(2), 215-223. doi:10.1351/PAC-CON-11-09-17

Jensen, H., Orth, B., Reiser, R., Bürge, D., Lehto, N., Almond, P., Gaw, S., Thomson, B., Lilburne, L., & Robinson, B. (2019). Environmental Parameters Affecting the Concentration of Iodine in New Zealand Pasture. Journal of Environment Quality, 48. doi:10.2134/jeq2019.03.0128

Karatzia, M., Christaki, E., Bonos, E., Karatzias, C., & Florou-Paneri, P. (2012a). The influence of dietary *Ascophyllum nodosum* on haematologic parameters of dairy cows. Italian Journal of Animal Science, 11. doi:10.4081/ijas.2012.e31

Karatzia, M., Christaki, E., Bonos, E., Karatzias, C., & Florou-Paneri, P. (2012b). The influence of dietary *Ascophyllum nodosum* on haematologic parameters of dairy cows. Italian Journal of Animal Science, 11(2), e31. doi:10.4081/ijas.2012.e31

Kinley, R. D., Martinez-Fernandez, G., Matthews, M. K., de Nys, R., Magnusson, M., & Tomkins, N. W. (2020). Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. Journal of Cleaner Production, 259, 120836. doi:https://doi.org/10.1016/j.jclepro.2020.120836

Leung, A. M., & Braverman, L. E. (2014). Consequences of excess iodine. Nature reviews. Endocrinology, 10(3), 136-142. doi:10.1038/nrendo.2013.251

Lorenzo, J. M., Agregán, R., Munekata, P. E. S., Franco, D., Carballo, J., Şahin, S., Lacomba, R.. & Barba, F. J. (2017). Proximate composition and nutritional value of three macroalgae: *Ascophyllum*

nodosum, *Fucus vesiculosus* and *Bifurcaria bifurcata*. Marine drugs, 15(11). doi:10.3390/md15110360
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. doi:https://doi.org/10.1016/j.anifeedsci.2015.09.018
Moneda, A., Carro, M., Weisbjerg, M., Roleda, M., Lind, V., Novoa-Garrido, M., & Molina-Alcaide, E. (2019). Variability and Potential of Seaweeds as Ingredients of Ruminant Diets: An In Vitro Study. Animals, 9. doi:10.3390/ani9100851
Mwalugha, H., Wakibia, J., Kenji, G., & Mwasaru, M. (2015). Chemical Composition of Common Seaweeds from the Kenya Coast. Journal of Food Research, 4, 28. doi:10.5539/jfr.v4n6p28
Netto, A. S., Zanetti, M. A., Correa, L. B., Del Claro, G. R., Salles, M. S. V., & Vilela, F. G. (2014). Effects of dietary selenium, sulphur and copper levels on selenium concentration in the serum and liver of lamb. Asian-Australasian journal of animal sciences, 27(8), 1082-1087. doi:10.5713/ajas.2013.13818
NordVal International. (2007). Trace elements - As, Cd, Hg, Pb and other elements. Determination by ICP-MS after pressure digestion. (NMKL 186, 2007) In.
NRC. (2001). Nutrient Requirements of Dairy Cattle (Seventh Revised Edition ed.). Washington, D.C.: National Academy Press.
NRC. (2005). Mineral Tolerance of Animals. Washington D.C.: The National Academies Press.
Nyström, H. F., Brantsæter, A. L., Erlund, I., Gunnarsdottir, I., Hulthén, L., Laurberg, P., Mattisson, I., Rasmussen, L. B., Virtanen, S., & Meltzer, H. M. (2016). Iodine status in the Nordic countries - past and present. Food Nutr Res, 60, 31969. doi:10.3402/fnr.v60.31969
O'Brien, B., Gleeson, D. E., & Jordan, K. (2013). Iodine concentrations in milk. Retrieved from http://hdl.handle.net/11019/524
Payling, L. M., Juniper, D. T., Drake, C., Rymer, C., & Givens, D. I. (2015). Effect of milk type and processing on iodine concentration of organic and conventional winter milk at retail: Implications for nutrition. Food Chemistry, 178, 327-330. doi:https://doi.org/10.1016/j.foodchem.2015.01.091
Pereira, L. (2011). A review of the nutrient composition of selected edible seaweeds. In (pp. 15-47).
Pétursdóttir, Á. H., & Gunnlaugsdóttir, H. (2019). Selective and fast screening method for inorganic arsenic in seaweed using hydride generation inductively coupled plasma mass spectrometry (HG-ICPMS). Microchemical Journal, 144, 45-50. doi:https://doi.org/10.1016/j.microc.2018.08.055
Pinheiro, J. B., Douglas. (2000). Mixed-Effects Models in S and S-PLUS: Springer.
Pomin, V. H. (2011). Seaweed: Ecology, Nutrient Composition, and Medicinal Uses. New York: Nova Science Publishers, Inc.
Qin, N., G. Faludi, S. Beauclercq, J. Pitt, N. Desnica, Á. Pétursdóttir, E. E. Newton, A. Angelidis, I. Givens, D. Juniper, D. Humphries, H. Gunnlaugsdóttir, and S. Stergiadis. 2021. Macromineral and

trace element concentrations and their seasonal variation in milk from organic and conventional dairy herds. Food Chemistry 359:129865.
Ravenscroft, P., Brammer, H., & Richards, K. (2009). Arsenic Pollution: A Global Synthesis. RGS-IBG Book Series, 1. doi:10.1002/9781444308785
Rayman, M., & Bath, S. (2015). The new emergence of iodine deficiency in the UK: consequences for child neurodevelopment. Annals of Clinical Biochemistry, 52. doi:10.1177/0004563215597249
Rey-Crespo, F., López-Alonso, M., & Miranda, M. (2014). The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. Animal, 8(4), 580-586. doi:10.1017/S1751731113002474
Ronan, J. M., Stengel, D. B., Raab, A., Feldmann, J., O'Hea, L., Bralatei, E., & McGovern, E. (2017). High proportions of inorganic arsenic in *Laminaria digitata* but not in *Ascophyllum nodosum* samples from Ireland. Chemosphere, 186, 17-23. doi:https://doi.org/10.1016/j.chemosphere.2017.07.076
Roque, B. M., Brooke, C. G., Ladau, J., Polley, T., Marsh, L. J., Najafi, N., Pandey, P., Singh, L., Kinley, R., Salwen, K. J., Eloe-Fadrosh, E., Kebreab, E., & Hess, M. (2019). Effect of the macroalgae *Asparagopsis taxiformis* on methane production and rumen microbiome assemblage. Animal Microbiome, 1(1), 3. doi:10.1186/s42523-019-0004-4
Roque, B. M., Salwen, J. K., Kinley, R., & Kebreab, E. (2019). Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. Journal of Cleaner Production, 234, 132-138. doi:10.1016/j.jclepro.2019.06.193
Saeed Akhtar, A. A., Iqra Naeem Mahnoor Tariq, Aneela Hameed,. (2019). Comparative Assessment of Arsenic Contamination
in Raw Milk, Infant Formulas and Breast Milk. Journal of Dairy & Veterinary Sciences, 13(1).
Saker, K. E., Fike, J. H., Veit, H., & Ward, D. L. (2004). Brown seaweed- (TascoTM) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. Journal of Animal Physiology and Animal Nutrition, 88(3-4), 122-130. doi:https://doi.org/10.1111/j.1439-0396.2003.00468.x
Schöne, F., Leiterer, M., Lebzien, P., Bemmann, D., Spolders, M., & Flachowsky, G. (2009). Iodine concentration of milk in a dose–response study with dairy cows and implications for consumer iodine intake. Journal of Trace Elements in Medicine and Biology, 23(2), 84-92. doi:https://doi.org/10.1016/j.jtemb.2009.02.004
Shukla, P. S., Mantin, E. G., Adil, M., Bajpai, S., Critchley, A. T., & Prithiviraj, B. (2019). *Ascophyllum nodosum*-Based Biostimulants: Sustainable Applications in Agriculture for the Stimulation of Plant Growth, Stress Tolerance, and Disease Management. Frontiers in Plant Science, 10(655). doi:10.3389/fpls.2019.00655
Singh, B., Chopra, R., Rai, S., Verma, M., & Mohanta, R. (2015). Nutritional Evaluation of Seaweed on Nutrient Digestibility, Nitrogen Balance, Milk Production and Composition in Sahiwal Cows.

Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences, 87. doi:10.1007/s40011-015-0616-8

Sjaunja, L. O. B., L; Junkkarinen, L; Pedersen, Janne; Setala, J. (1991). A Nordic proposal for an energy corrected milk (ECM) formula. state of the art 1990: 1156-1157, 1192. Retrieved from https://eurekamag.com/research/002/007/002007239.php

Smith, D. G., & Young, E. G. (1955). The combined amino acids in several species of marine algae. Journal of Biological Chemistry, 217, 845-853.

Smith, J. L., Summers, G., & Wong, R. (2010). Nutrient and heavy metal content of edible seaweeds in New Zealand. New Zealand Journal of Crop and Horticultural Science, 38, 19-28. doi:10.1080/01140671003619290

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. Journal of Dairy Science, 99(10), 8472-8476. doi:https://doi.org/10.3168/jds.2015-10810

Stergiadis, S., Bieber, A., Franceschin, E., Isensee, A., Eyre, M. D., Maurer, V., Chatzidimitriou, E., Cozzi, G., Bapst, B., Stewart, G., Gordon, A., & Butler, G. (2015). Impact of US Brown Swiss genetics on milk quality from low-input herds in Switzerland: Interactions with grazing intake and pasture type. Food Chemistry, 175, 609-618. doi:https://doi.org/10.1016/j.foodchem.2014.11.079

Stergiadis, S., Leifert, C., Seal, C. J., Eyre, M. D., Nielsen, J. H., Larsen, M. K., Slots, T., Steinshamn, H., & Butler, G. (2012). Effect of Feeding Intensity and Milking System on Nutritionally Relevant Milk Components in Dairy Farming Systems in the North East of England. Journal of Agricultural and Food Chemistry, 60(29), 7270-7281. doi:10.1021/jf301053b

Suttle, N. (2010). Mineral nutrition of livestock: Fourth edition. Mineral Nutrition of Livestock: Fourth Edition, 1-547.

Thorning, T., Raben, A., Tholstrup, T., Soedamah-Muthu, S., Givens, I., & Astrup, A. (2016). Milk and dairy products: Good or bad for human health? An assessment of the totality of scientific evidence. Food & Nutrition Research, 60. doi:10.3402/fnr.v60.32527

Ugarte, R., & Sharp, G. (2012). Management and production of the brown algae *Ascophyllum nodosum* in the Canadian maritimes. Journal of Applied Phycology, 24(3), 409-416. doi:10.1007/s10811-011-9753-5

WHO. (2007). Iodine deficiency in Europe : a continuing public health problem. In. Geneva: World Health Organization.

Xue, F., Sun, F., Jiang, L., Hua, D., Wang, Y., Nan, X., Zhao, Y., & Xiong, B. (2019). Effects of Partial Replacement of Dietary Forage Using Kelp Powder (*Thallus laminariae*) on Ruminal Fermentation and Lactation Performances of Dairy Cows. Animals : an open access journal from MDPI, 9(10), 852. doi:10.3390/ani9100852

Zhou, M., Hünerberg, M., Chen, Y., Reuter, T., McAllister, T. A., Evans, F., Critchley, A. T., & Guan, L. L. (2018). Air-dried brown seaweed, *Ascophyllum nodosum*, alters the rumen microbiome in a manner that changes rumen fermentation profiles and lowers the prevalence of foodborne pathogens. mSphere, 3(1). doi:10.1128/mSphere.00017-18

Chapter 4: "Effect of Dietary Seaweed Supplementation on Icelandic Cow Milk Microbiota Composition"

Status: In-review with co-authors

Contribution: The animal trial was run in Iceland with collaborators that worked with us through the Agricultural Fund of Iceland (Framleiðnisjóður landbúnaðarins, Grant Numbers 18-039 and 20-025) for funding the research work. Following iodine analysis of the milk, milk was then subjected to DNA extraction and 16S rRNA gene sequencing. Milk aliquoted analysed for bacterial populations at UoR exclusively by myself. I performed all analysis by myself and wrote the article as the primary author. The overall estimated percentage contribution made by myself would be 95%, factoring in the contribution and experimental planning assistance by co-authors.

Objectives: This study aimed investigate the effect of feeding a mixture of seaweed (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) to dairy cows on (i) milk bacterial relative abundances, (ii) correlations between iodine and milk bacterial relative abundances, and (iii) correlations between SCC and milk bacterial relative abundances.

Hypothesis: For this experiment, I hypothesized that macroalgal supplementation of dairy cows would decrease the relative abundances of large contributors to the overall milk bacterial profile. Additionally, I hypothesize there to be a negative correlation between iodine and milk bacterial relative abundances and a positive correlation between SCC and milk bacterial relative abundances.

Overview: Overall seaweed supplementation (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) had a significant effect ($P < 0.1$) on specifically the specific order of *Caulobacterales*, and the genera of *Stenotrophomonas* and *Brevundimonas*. While the species data presented the existence of largely non-harmful bacteria, these two groups have corresponding species of the same genera that have been shown to be damaging to consumers and the dairy industry as a whole. Additionally, alpha diversity measures of Chao1 and ACE showed a significant difference ($P < 0.1$) between the two experimental groups, with SWD indicating a higher score, along with having a significantly higher observed species count. Finally, there was found to be a negative correlational effect of *Brevundimonas* and *Stenotrophomonas* relative abundance with milk SSC content and a negative correlational effect of *Brevundimonas* relative abundance with milk iodine content. This gives valuable insight as to the effect that phycological supplementation has on the milk microbiota and also points toward a possible boost to product microbial resiliency that a shift in feeding paradigm could lead to.

4.1 Abstract

Following an animal trial that showed remarkably increased iodine concentrations within the resulting raw milk when Icelandic cattle were fed a seaweed mix (9:91) of *Laminaria digitata* and *Aschophyllum nodosum* at a maximum amount of 160g/cow/day, milk was analysed for possible bacterial population differences. The current study aimed to investigate the effect of seaweed supplementation in dairy cow diets on the milk microbiota. The milk from twenty individual Icelandic cows was divided into 2 groups: (i) control (CON, n=10, no seaweed), and (ii) seaweed (SWD, n=10, 1.5% seaweed in concentrate, 160 g/cow/day). Groups were balanced for parity, lactation stage, milk yield, milk fat and protein concentration, and somatic cell count. The analysed weeks were chosen according to the weeks wherein peak iodine content was found in milk within the supplemented group (weeks 5, 6, and 7 of the 12-week study). DNA extraction was performed using a QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) with slight modifications. Data was subjected to ANOVA using linear mixed effects model in Minitab 18. Within the model, diet, experimental week, and their interactions were used as fixed factors, while cow was set as the random factor. Seaweed supplementation in dairy cow diets did not influence the relative abundance of milk bacteria at the phylum level. However, relative abundance of the genera of *Brevundimonas* ($P < 0.001$) (and the corresponding order *Caulobacterales* ($P = 0.005$) and *Stenotrophomonas* ($P = 0.048$) was lower in the milk from seaweed-fed cows compared to control cows. Seaweed supplementation in dairy cow diets had a tendency to increase the alpha diversity metrics of Chao1 ($P = 0.059$), and ACE ($P = 0.088$), as well as observed species ($P = 0.091$). Additionally, *Stenotrophomonas* ($P = 0.032$) and *Brevundimonas* ($P = 0.006$) were negatively correlated to milk SCC. Finally, *Brevundimonas* ($P = 0.004$) was negatively correlated with iodine concentration. Significant reductions of certain taxonomic populations within the milk when seaweed mix is added to dairy cow diets can be linked to specific genus groups that are home to pathogenic bacteria that can affect the safety and bacterial resilience of the product within the supply chain. This would be particularly useful in preserving food security for those that consume raw milk, or products made from such, while also reducing product losses and increasing animal health and welfare due to a reduction in occurrences of mastitis due to a small dietary addition.

4.2 Introduction

Recent interest has arisen regarding the advantages that a paradigm shift in production animal diets via supplementation of seaweed can have not only for the animals, but also for the environment, and health of consumers (Costa et al., 2021; Makkar et al., 2016; Morais et al., 2020). Dietary inclusion of macroalga *Ascophyllum nodosum* has been shown to increase energy utilization and elicit a protective effect on the dairy cow liver (Karatzia et al., 2012), and Wang et al. (2009) reported that phlorotannins in *A. nodosum* exhibit bacteriostatic and bactericidal effects on various *Escherichia coli* strains, a bacterial pathogen that can cause clinical health problems in cattle (Burvenich et al., 2003). In addition, feeding macroalga *Asparagopsis taxiformis* to beef cattle reduced methane production by up to 80% at a supplementation of 0.50% organic matter intake, mainly due to the anti-methanogenic capability of bromoform, which can be found in various species across the phycological spectrum (Abbott et al., 2020; Machado et al., 2014; Roque et al., 2021). Nutritionally, consumption of milk from cows fed seaweed-supplemented diets could increase critical nutrient intake – thereby being of benefit to chronically deficient populations, or for demographics requiring a larger amount of minerals. Like others, I have observed a significant increase in the concentration of iodine in milk from cows *fed A. nodosum* (Antaya et al., 2014; Chaves Lopez et al., 2016; Newton et al., 2021). Certain seaweeds such as *A. nodosum* contain bioactive phenolic compounds such as alginic acid, fucoidans, mannitol, and laminarin (Shukla et al., 2019). Some seaweeds and macroalgal oils can also contain monoterpenes (Cikoš et al., 2019; Naylor et al., 1983). Monoterpenes have been found to exhibit antimicrobial activity across a wide range of bacteria (Ahmed et al., 2020; Nogueira et al., 2021; Trombetta et al., 2005). Additionally, terpenes have also been found to transfer from feed to cow milk (Lejonklev et al., 2013; Viallon et al., 2000).

While milk from a healthy cow has been considered in the past to be sterile in a vacuum, the process of industrial milking and animal agriculture has rendered this idea largely not as applicable given the open-system aspect of the mammary gland after parturition (Taponen et al., 2019). Milk microbiota can be an indicator for animal health and more specifically dysbiosis which can manifest in clinical mastitis (Rainard, 2017). The teat canal, serving as the primary defence of the cistern of the udder in cows can become compromised and lead to internal colonization of bacteria which can lead to mastitis, and this can be evidenced in milk bacterial analysis (Derakhshani et al., 2018). Analysis of infected glands within the anatomical udder has shown a consistency between bacterial populations identified in infected glands and the associated produced milk (Andrews et al., 2019). Therefore, a transference of antimicrobial properties from feed to milk might influence the clinical state of the udder, not only in anatomical areas in which there is direct milk to epithelial cell contact, but in all parts of the teat with contact being made during regular milking activity. Furthermore, raw milk can be quickly colonized by bacteria, whether this be from milking equipment, bedding, soil, or from cows afflicted by udder disease, typically bovine mastitis, which can harbour biofilm-creating pathogens that are eventually shed (Gomes et al., 2016; Quigley et al., 2013). Seen from the transfer of minerals from feed to milk in previously-cited work, the natural elemental bioaccumulation of seaweed described in Cherry et al. (2019), could lead to an increased mineral content within the milk that could theoretically have an effect on the milk microbiota. Iodine, much like what has been found to be increased in seaweed-supplemented cow milk, has been used clinically for its antibacterial properties throughout modern medicine and as a disinfectant within solution and vapor (Durani and Leaper, 2008; Eggers, 2019; Hove et al., 2020). Therefore, the feeding of seaweed to cows which would increase the concentration of iodine within the raw milk, could influence the milk microbiota and thus alter the quality of the dairy product.

A host of pathogenic bacteria are commonly identified as being the cause of disease outbreaks in raw milk such as *Salmonella*, *Campylobacter*, *E. coli*, *Y. enterocolitica*, and *L. monocytogenes*; and this can either be through direct passage from the blood into the milk via a systemic infection from the udder specifically as a result of mastitis, or environmental contamination via faeces or other sources (Claeys et al., 2013). Overall, pathogenic bacteria as a whole constitute around 90% of all dairy-related diseases (Berhe et al., 2020). Even when milk is pasteurized, thermoduric bacteria can still survive, which includes Bacillus, Clostridium, and Enterococci (Hileman, 1940; Mane and Gandhi, 2010; Thomas, 2014). Contamination can also occur during processing and packaging of milk and dairy products even after heat treatment has occurred (Fischer et al., 2011). This risk is relatively high in developing countries that lack the ability to, or will not, enforce safety standards for their dairy industry (Dhanashekar et al., 2012). All of this culminates in a possible risk to consumers, not unlike all foodstuffs, in that there is always a chance for dairy products to contain pathogenic bacteria that can be detrimental to human health by way of raw milk, dairy products made from raw milk, and generally domestically produced milk in developing countries. This risk also exists, although to a lesser extent, in supply chains with broad and encompassing safety standards, given the occurrence of outbreaks after the milk processing stage during packaging, on the shelf at the supermarket, or within the refrigerator at home. An inherent resilience by means of antimicrobial elemental fortification or food additive addition - such as increased levels of iodine or other bioactive compounds because of a change in supplementation - could be a useful tool in a reduction of potential illness from consumption of dairy products. The addition of a small supplement to cow feed can lead to milk that contains minerals that have been proven to be antimicrobial leading to microbiological safety improvements for humans or the animal itself. This study aimed to gain insight into the improvement or degradation of these qualitative aspects as they relate to the milk microbiota and the differences between experimental groups therein associated with a seaweed-supplemented diet for dairy cows.

4.3 Materials and Methods

4.3.1 Experimental design

The study was conducted during the winter period at the Stóra-Ármót farm, Selfoss, Iceland and was approved by The Icelandic Food and Veterinary Authority and confirmed that the experiment did not require a license according to the regulation no. 460/2017. In the current study, 20 experimental Icelandic breed cows were assigned to one of two diets (i) without seaweed supplementation (control, CON), and (ii) with 1.5% seaweed in concentrate, dry matter (DM) basis (seaweed, SWD; 26 to 158 g/cow/day) such that groups (CON and SWD) were balanced for parity, lactation stage, milk yield, and milk fat and protein concentration, and somatic cell count (SCC). The animal trial continued for 12 weeks with a diet adaptation period of one week. Samples from weeks 5, 6 and 7 of the trial (referred to here as weeks 1, 2 and 3), where the mean iodine concentration was at its highest for the SWD treatment group, were included in this study. The seaweed mix comprised of 91% *Ascophyllum nodosum* and 9%

Laminaria digitata, on DM basis. For week 1 in the SWD group, a proportion of approximately 0.50% seaweed in concentrate was fed as a transitionary week, and then weeks 2 and 3, the full amount of 1.5% seaweed in concentrate was administered. Details concerning seaweed feeding regimen can be found in Newton et al. (2021). Cows in the SWD group were fed seaweed at an average of 89g/cow/day over the three-week period. The average milk iodine concentration as reported in Newton et al. (2021) for the weeks considered in this study was 718 and 1892 μg/kg for CON and SWD groups, respectively.

4.3.2 Milk sample collection and processing

Milk samples were collected from each cow on the last day of each experimental week during the morning and evening milking and composite milk samples (3 weeks × 1 sample/week × 20 cows = 60 samples) were stored at -18°C for further processing and analysis as described in Newton et al. (2021). Before collecting milk samples from each cow, their teats and udders were cleaned with washcloths that had been washed in soap and water in a washing machine. The cows were then equipped with a sterilized milk machine and the milk was extracted. After the group was milked, the milking system was washed again (twice a day after morning and evening milking). Milk was stripped before milking and the milk was collected via a specifically designed sampling device attached to the machine into cleaned and sterile sampling cups and poured into sterile test tubes for analysis. Cows that showed signs of mastitis during the experiment was not used for sampling and other data collection.

4.3.3 DNA extraction and 16S rRNA gene sequencing

DNA was extracted from all 60 milk samples (following fat removal) using a QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following a protocol previously used by Chambers et al. (2015). 0.1 mL of each milk sample was centrifuged at 16,000 × g for 1 min at -9°C to remove fat to improve DNA extraction efficiency. Extract samples were then sent to Novogene (Beijing, China) for microbial amplicon-based 16S rRNA library preparation and sequencing.

Amplicon was sequenced using Illumina paired-end platform generating 250 bp paired-end raw reads, merged, and pre-treated to obtain clean tags. Chimeric sequences were removed to generate effective tags used in analysis via -Usearch64 -cluster_otus. Alpha diversity statistical indices were comprised of 26,087 reads chosen for normalization. Reads were classified both into diet groups (CON and SWD), along with specification within taxonomic groups Operational Taxonomic Units (OTUs) were obtained by clustering with 97% identity on the effective tags of all samples, and then identified. Taxonomic assignment of OTU was done via silva_138.1. Shannon diversity was generated by using the software qiime1.9alpha_diversity.py.

Region 16SV34 was amplified using Primer 341F-CCTAYGGGRBGCASCAG (806R-GGACTACNNGGGTATCTAAT). Sequencing library (Illumina Novaseq 6000) was created using internal Novogene methods, and a NEBNext ® Ultra IIDNA Library Prep Kit (Cat No. E7645) was used.

4.3.4 Statistical analysis

Taxonomic data were subjected to ANOVA using linear mixed effects model in Minitab 18. The model had diet, experimental week, and their interactions as fixed factors and cow as the random factor (Pinheiro, 2000). Normality of residuals was evaluated with Minitab 18, and non-normal data was log-transformed prior to analysis so that their residuals were normalized. Comparisons of means were done using Fisher method. Statistical significance was declared at $P < 0.05$ and $P < 0.1$ was considered a tendency. Spearman correlational analysis was performed using Minitab18. The correlational results between relative abundance and other variables milk iodine concentration and SCC was determined using the correlational function method in Minitab.

4.4 Results

4.4.1 The relative abundance of bacteria in the milk microbiota at different taxonomic levels

Seaweed supplementation did not influence the relative abundance of bacterial phyla, but the relative abundance of phylum *Bacteroidetes* was different between weeks ($P = 0.030$; Figure 4.1) with greater abundance in week 2 and 3 compared to week 1 (1.9 vs 1.4%). The order *Caulobacterales* had lower abundance in the milk from cows fed seaweed compared to CON cows ($P = 0.005$; Figure 4.1). Regardless of the diet, the relative abundance of order *Caulobacterales* gradually increased over time with greater abundance in week 3 compared to week 1 (1.3 vs 0.5%). The cows in the CON group had greater abundance of genera *Stenotrophomonas* ($P = 0.048$; Figure 4.1) and *Brevundimonas* ($P <0.001$; Figure 4.1) in their milk compared to cows fed seaweed.

Figure 4.1

The effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD), week, and their interaction on the relative abundance (%) of bacterial populations in the milk.

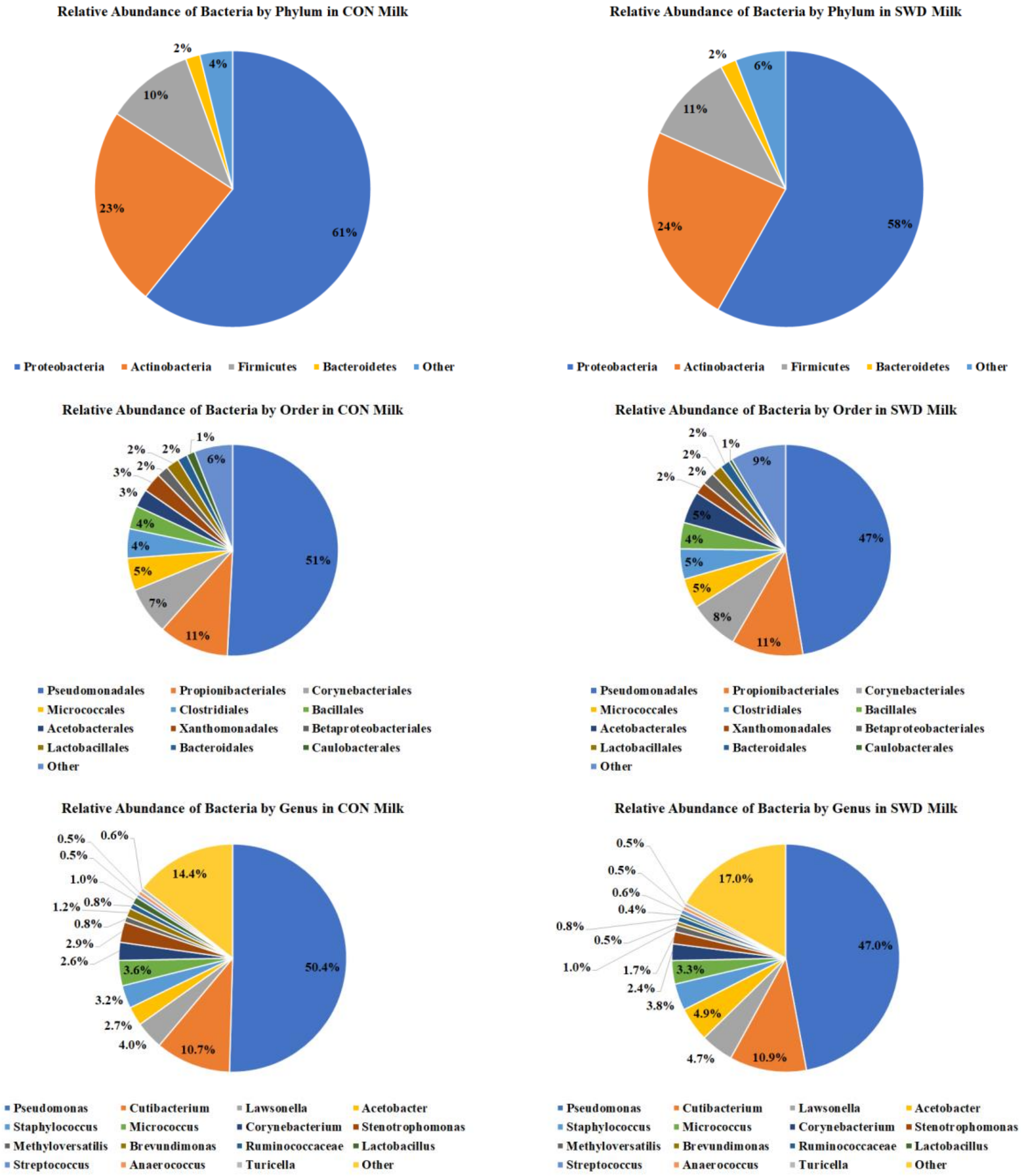

Table 4.1

Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD) on alpha diversity metrics within the resulting milk

	Diet			ANOVA P-values[2]		
Alpha diversity	**CON n=30**	**SWD n=30**	**SE[1]**	**Diet**	**Week**	**Diet x Week**
Observed species	870	963	34.2	0.091	0.612	0.688
Shannon	3.86	3.99	0.149	0.520	0.045	0.924
Simpson	0.74	0.75	0.023	0.675	0.105	0.821
Chao1	959	1075	38.5	0.059	0.700	0.682
ACE	990	1100	40.2	0.088	0.563	0.695

[1] Standard error

[2] Significances were declared at $P < 0.1$.

Table 4.2

The correlational effect of SCC and relative abundance (%) of bacterial genera and corresponding orders in the milk across all experimental samples.

Genus	Spearman ρ	P-value[1]	Order	Spearman ρ	P-value[1]
Pseudomonas	-0.125	0.342	*Pseudomonadales*	-0.126	0.336
Cutibacterium	0.043	0.744	*Propionibacteriales*	0.040	0.761
Lawsonella	-0.040	0.763	*Corynebacteriales*	0.067	0.611
Acetobacter	0.001	0.996	*Micrococcales*	0.053	0.687
Staphylococcus	-0.052	0.693	*Clostridiales*	0.075	0.571
Micrococcus	0.054	0.682	*Bacillales*	-0.093	0.482
Corynebacterium	0.228	0.080	*Acetobacterales*	-0.012	0.927
Stenotrophomonas	-0.277	0.032	*Xanthomonadales*	-0.286	0.027
Methyloversatilis	-0.001	0.991	*Betaproteobacteriales*	-0.029	0.828
Brevundimonas	-0.353	0.006	*Lactobacillales*	0.075	0.571
Ruminococcaceae	0.014	0.914	*Bacteroidales*	-0.043	0.746
Lactobacillus	-0.057	0.667	*Caulobacterales*	-0.356	0.005
Streptococcus	0.084	0.522	Other[2]	0.556	<0.000
Anaerococcus	0.142	0.278			
Turicella	0.133	0.313			
Other[2]	0.422	0.001			

[1]Statistical significances were declared at $P < 0.05$.

[2]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

Table 4.3

The correlational effect of iodine concentration and relative abundance (%) of bacterial genera and corresponding orders in the milk across all experimental samples

Genus	Spearman ρ	P-value[1]	Order	Spearman ρ	P-value[1]
Pseudomonas	-0.155	0.238	*Pseudomonadales*	-0.154	0.239
Cutibacterium	0.015	0.908	*Propionibacteriales*	0.016	0.905
Lawsonella	0.114	0.387	*Corynebacteriales*	0.095	0.472
Acetobacter	0.073	0.580	*Micrococcales*	0.009	0.946
Staphylococcus	0.176	0.179	*Clostridiales*	0.156	0.233
Micrococcus	0.034	0.795	*Bacillales*	0.147	0.262
Corynebacterium	0.058	0.661	*Acetobacterales*	0.072	0.586
Stenotrophomonas	-0.207	0.113	*Xanthomonadales*	-0.215	0.100
Methyloversatilis	0.108	0.410	*Betaproteobacteriales*	0.154	0.241
Brevundimonas	-0.369	0.004	*Lactobacillales*	0.097	0.459
Ruminococcaceae	-0.049	0.708	*Bacteroidales*	0.146	0.267
Lactobacillus	-0.065	0.621	*Caulobacterales*	-0.361	0.005
Streptococcus	0.150	0.252	Other[2]	0.439	<0.000
Anaerococcus	-0.016	0.906			
Turicella	0.018	0.890			
Other[2]	0.383	0.002			

[1]Statistical significances were declared at $P < 0.05$.

[2]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

4.4.2 Bacteria diversity in the milk microbiota

The alpha diversity of the microbiota in milk from SWD group had a tendency to be higher compared to CON group with greater values for three alpha diversity metrics namely observed species, Chao1, and ACE (963 vs 870, P = 0.09; 1075 vs 959, P = 0.06; and 1100 vs 990, P = 0.09, respectively) Measuring the difference between the unique evolutionary history of two groups by examining the fraction of phylogenetic branch length while accounting for differences in the relative abundances produces the weighted unifrac score (C. Lozupone et al., 2011). This was then subjected to a t-test accounting for a statistically significant value of 0.031.

4.4.3 Association of bacterial abundance with somatic cell count and iodine concentrations in milk

The relative abundance of genera *Stenotrophomonas* (and corresponding order *Xanthomonadales*) and *Brevundimonas* (and corresponding order *Caulobacterales*) was negatively correlated to milk somatic cell count (SCC) (ρ = -0.277 and –0.286, P = 0.032 and 0.027; ρ = -0.353 and -0.356, P = 0.006 and 0.005, Table 4.2). Each significant genus spearman rho value was within proximity to the corresponding order, with *Stenotrophomonas* within 0.009 to the associated order (*Xanthomonadales*), and *Brevundimonas* within 0.003 to the associated order (*Caulobacterales*). The taxonomic relative abundance of "other" was found to have a positive rho value when correlating relative abundance and SCC and be significant with the difference in spearman values between genus and order being that of 0.134.

The relative abundance of genera *Brevundimonas* (and corresponding order *Caulobacterales*) was negatively correlated to iodine concentration (ρ = -0.369 and -0.361, P = 0.004 and 0.005, Table 4.6). The relative abundance of genus labelled "other" (and order labelled as "other") was positively correlated to iodine concentration (ρ = 0.383 and 0.439, P = 0.002 and <0.001, Table 4.3). Each significant genus spearman rho was within proximity to the corresponding order, with *Brevundimonas* within 0.008 to the associated order (*Caulobacterales*), and the genera labelled "other" was within 0.056 of the corresponding order of "other".

4.5 Discussion

In the current study, lower abundance of the genus *Stenotrophomonas* in the milk from cows fed seaweed was likely due to the direct effect of seaweed-borne bioactive compounds such as polyphenols on *Stenotrophomonas* in the milk. Polyphenols have been shown to be found in cows' milk transferred from feed (Besle et al., 2010). Additionally, polyphenols have been shown to have an inhibitory effect on *Stenotrophomonas maltophilia* via damage to cell membrane integrity and alteration of cell morphology (Zhang et al., 2019). *Stenotrophomonas rhizophila* and *S. maltophilia* are two major *Stenotrophomonas spp.* that have been found in the raw milk from dairy cows (Boubendir et al., 2016). While *S. rhizophila* has not been reported for its pathogenic relevance in animals or humans, *S. maltophilia* caused mastitis in a mouse model and has been linked to the occurrence of mastitis in dairy cows (Hu et al., 2022; Kuehn et al., 2013; Ohnishi et al., 2012). In addition to a link to reduced milk production, *S. maltophilia* has been detected in various dairy products with the ability to survive for prolonged periods, contributing to poor dairy product quality as well as creating opportunities for potential human infection from dairy products (Zeinhom et al., 2021). Furthermore, *S. maltophilia* is an opportunistic pathogen and can cause various nosocomial and community-acquired infections in humans (Brooke, 2021). Another major concern is that *S. maltophilia* has intrinsic resistance to many antibiotics that are considered high priority-critically important to treat human infections (Ohnishi et al., 2012; Ryan et al., 2009).

In the current study, like *Stenotrophomonas*, relatively lower abundance of *Brevundimonas* in the milk from seaweed-fed cows was likely due to a direct antimicrobial effect of the residue of seaweed-borne bioactive compounds on *Brevundimonas*. *Brevundimonas* is a psychotropic bacterial genus found in raw milk samples (Yang et al., 2020). *Brevundimonas* can be commonly found in tap water and thus its presence in the milk collected in the current study could be linked to the contamination of raw milk or milking equipment or udder surface (Li et al., 2018). *Brevundimonas* has also been linked to mastitis (Zhang et al., 2015), along with higher prevalence within mastitic milk samples (Kuehn et al., 2013). Furthermore, *Brevundimonas* spp. are emerging as global pathogens with *Brevundimonas diminuta* and *Brevundimonas vesicularis* being responsible food-borne outbreaks (Ryan & Pembroke, 2018). Even though the resolution of taxonomic composition in the current study was to the species level, the study findings discussed above suggests that feeding seaweed to dairy cows could be a potential dietary strategy to reduce the abundance of opportunistic pathogens like *S. maltophilia* and *B. diminuta or B. vesicularis* in the milk and hence reduced threat to animal and human health.

A greater number of observed species in SWD milk compared to CON milk found in this study is likely due to the effect of iodine, seaweed-related bioactive compounds, and/or flavonoids (Fiordalisi et al., 2016; Jing-Wei et al., 2021) on the dominant genus of *Pseudomonas* that while not statistically different in this study between animal groups in terms of relative abundance, but had a numerical difference. *Pseudomonas* has been shown a significant swing in comparative population in similar seaweed diet milk (Chaves Lopez et al., 2016).While the difference in relative abundance of *Pseudomonas* between SWD and CON milk was not statistically significant in the current study, a small alteration in the population makeup might be enough to make room for a niche to fill for additional bacterial groups, leading to a higher number of observed species within SWD milk. Treatment using iodine-based ointment, generally at 1% inclusion, has been shown to be extremely effective against members of the *Pseudomonas* genus, thus removing a small portion of the population, allowing the entry of possibly more iodine-resistant bacteria (Hoekstra, Westgate, & Mueller, 2017). This explanation above as a plausible reason for relatively higher number of observed species in SWD milk is reinforced by the tendency effect ($P < 0.1$) of diet on both Chao1 and ACE indexes, indicating a larger species richness (Hughes et al., 2001; Kim et al., 2017). While in the context of liquid milk, a reduced number of *Pseudomonas* is relatively beneficial, technological advantages elicited by SWD milk may not be as helpful, as increased richness may lead to negative effects when producing dairy products such as cheese, yogurt, or butter.

The negative correlation between milk SCC and the relative abundance of genera *Stenotrophomonas* and *Brevundimonas* observed in the current study could be likely due to a selective decline in the abundance of non-pathogenic species of genera *Stenotrophomonas* and *Brevundimonas*. A decline in non-pathogenic species might have caused relative increase in the abundance of pathogenic species in genera *Stenotrophomonas* and *Brevundimonas*, leading to increased SCC in the milk. Since milk SCC has always been considered an indicator for detecting mammary gland infection in dairy cows and SWD milk had lower relative abundance of genera *Stenotrophomonas* and *Brevundimonas* compared to CON milk in the current study, it could be argued that seaweed feeding might cause the mammary gland to be more susceptible to infection (Petzer et al., 2017). However, it is important to consider that SCC must be higher than a threshold value for it to be an indicator of the likelihood of mammary gland infection (Jadhav et al., 2018). In addition, lack of species level resolution in the microbiota composition determined in the current study makes it impossible to put forward any conclusion about the association between seaweed feeding and susceptibility of mammary gland infection in dairy cows.

The negative correlation between milk iodine content and the relative abundance of genus *Brevundimonas* observed in this study could be likely due to the inhibition of this group of bacteria by relatively high iodine concentrations in SWD milk as explained by lower relative abundance of *Brevundimonas* in SWD milk compared to control. Susceptibility of this bacterial group to iodine is due to the fact that iodine can bind to tyrosine residues and will oxidize sulfhydryl groups leading to inhibition (La Duc et al., 2004).

4.6 Conclusions

Seaweed supplementation (9% *Laminaria digitata* + 91% *Ascophyllum nodosum*) had a significant effect ($P < 0.1$) on specifically the specific order of *Caulobacterales*, and the genera of *Stenotrophomonas* and *Brevundimonas*. While the species data presented the existence of largely non-harmful bacteria, these two groups have corresponding species of the same genera that have been shown to be damaging to consumers and the dairy industry as a whole. Additionally, alpha diversity measures of Chao1 and ACE showed a significant difference ($P < 0.1$) between the two experimental groups, with SWD indicating a higher score, along with having a significantly higher observed species count. Finally, there was found to be a negative correlational effect of *Brevundimonas* and *Stenotrophomonas* relative abundance with milk SSC content and a negative correlational effect of *Brevundimonas* relative abundance with milk iodine content. This gives valuable insight as to the effect that phycological supplementation has on the milk microbiota and also points toward a possible boost to product microbial resiliency that a shift in feeding paradigm could lead to.

4.7 Supplemental Material

Table S4.1

The effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD), week, and their interaction on the relative abundance (%) of bacterial phyla in the milk.

	Diet			ANOVA P-values[2]		
Phylum	CON (%) n=30	SWD (%) n=30	SE[1]	Diet	Week	Diet × Week
Proteobacteria	60.83	58.15	2.767	0.515	0.180	0.427
Actinobacteria	23.35	23.54	1.900	0.968	0.080	0.248
Firmicutes	10.31	10.55	0.737	0.774	0.734	0.585
Bacteroidetes	1.68	1.85	0.211	0.779	0.030	0.551
Other[3]	3.83	5.91	0.770	0.071	0.512	0.402

[1]Standard Error

[2]Statistical significances were declared at $P < 0.05$.

[3]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

Table S4.2

The effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD), week, and their interaction on the relative abundance (%) of bacterial orders in the milk.

	Diet			ANOVA P-values[2]		
	CON (%)	SWD (%)				
Order	**n=30**	**n=30**	**SE[1]**	**Diet**	**Week**	**Diet x Week**
Pseudomonadales	50.82	47.36	2.508	0.247	0.644	0.969
Propionibacteriales	10.75	10.97	1.053	0.902	0.374	0.299
Corynebacteriales	7.26	7.67	0.653	0.600	0.316	0.133
Micrococcales	4.98	4.57	0.563	0.631	0.270	0.399
Clostridiales	4.49	4.63	0.290	0.869	0.465	0.358
Bacillales	3.57	4.04	0.369	0.592	0.321	0.402
Acetobacterales	2.73	4.87	1.389	0.218	0.703	0.618
Xanthomonadales	2.98	1.80	0.526	0.053	0.186	0.663
Betaproteobacteriales	1.75	1.91	0.161	0.593	0.106	0.965
Lactobacillales	2.01	1.64	0.461	0.930	0.998	0.750
Bacteroidales	1.52	1.50	0.134	0.993	0.073	0.773
Caulobacterales	1.25	0.51	0.220	0.005	0.022	0.151
Other[3]	5.90	8.51	0.883	0.051	0.321	0.738

[1]Standard error

[2]Statistical significances were declared at $P < 0.05$.

[3]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

Table S4.3

The effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD), week, and their interaction on the relative abundance (%) of bacterial genera in the milk.

	Diet			ANOVA P-values[2]		
	CON (%)	SWD (%)				
Genus	**n=30**	**n=30**	**SE[1]**	**Diet**	**Week**	**Diet x Week**
Pseudomonas	50.42	46.97	2.511	0.222	0.783	0.054
Cutibacterium	10.69	10.92	1.048	0.893	0.553	0.890
Lawsonella	3.98	4.66	0.560	0.315	0.634	0.257
Acetobacter	2.71	4.85	1.389	0.271	0.752	0.152
Staphylococcus	3.24	3.78	0.362	0.494	0.327	0.254
Micrococcus	3.59	3.32	0.468	0.714	0.995	0.831
Corynebacterium	2.56	2.36	0.262	0.820	0.652	0.548
Stenotrophomonas	2.89	1.72	0.527	0.048	0.134	0.671
Methyloversatilis	0.81	0.95	0.183	0.615	0.317	0.670
Brevundimonas	1.24	0.50	0.220	<0.000	0.109	0.284
Ruminococcaceae	0.79	0.77	0.050	0.742	0.936	0.973
Lactobacillus	1.00	0.41	0.403	0.303	0.511	0.741
Streptococcus	0.49	0.62	0.089	0.289	0.520	0.507
Anaerococcus	0.54	0.52	0.063	0.814	0.914	0.616
Turicella	0.55	0.48	0.103	0.791	0.993	0.786
Other[3]	14.44	16.96	1.101	0.085	0.850	0.053

[1]Standard error

[2]Statistical significances were declared at $P < 0.05$.

[3]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

Table S4.4

Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD) on bacterial relative abundance (%) within the resulting milk

	Diet			ANOVA P-values[2]		
	CON (%)	SWD (%)				
Class	n=30	n=30	SE[1]	Diet	Week	Week x Diet
Gammaproteobacteria	55.91	51.61	2.468	0.181	0.741	0.940
Alphaproteobacteria	4.81	6.38	1.428	0.640	0.939	0.849
Actinobacteria	23.23	23.44	1.891	0.973	0.083	0.236
Bacilli	5.57	5.68	0.591	0.689	0.632	0.516
Clostridia	4.49	4.63	0.266	0.869	0.465	0.357
Bacteroidia	1.68	1.85	0.211	0.778	0.030	0.552

[1]Standard error

[2]Statistical significances were declared at $P < 0.05$.

[3]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

Table S4.5

Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no seaweed, CON; Seaweed, 1.5% concentrate DM, SWD) on bacterial relative abundance (%) within the resulting milk

	Diet			ANOVA P-values[2]		
	CON (%)	SWD (%)				
Family	n=30	n=30	SE[1]	Diet	Week	Week x Diet
Pseudomonadaceae	50.42	46.97	2.511	0.245	0.636	0.971
Acetobacteraceae	2.73	4.87	1.389	0.218	0.703	0.618
Propionibacteriaceae	10.73	10.96	1.052	0.904	0.374	0.301
Corynebacteriaceae	7.12	7.52	0.644	0.597	0.302	0.140
Lactobacillaceae	1.00	0.41	0.403	0.334	0.664	0.511
Xanthomonadaceae	2.98	1.80	0.526	0.052	0.189	0.659
Micrococcaceae	3.83	3.60	0.493	0.712	0.318	0.362
Staphylococcaceae	3.26	3.80	0.363	0.519	0.348	0.471
Caulobacteraceae	1.25	0.51	0.220	0.004	0.023	0.151
Sphingobacteriaceae	0.09	0.25	0.137	0.516	0.565	0.045
Ruminococcaceae	2.17	2.14	0.127	0.887	0.568	0.541
Rhodocyclaceae	0.81	0.96	0.149	0.655	0.734	0.125

[1]Standard error

[2]Statistical significances were declared at $P < 0.05$.

[3]Includes taxonomic groups that had abundance of <0.50% in both experimental groups, plus unidentified operational taxonomic units.

4.8 Information

Author Contributions: Conceptualization, P.R., E.E.N., S.S.; data curation, E.E.N., P.R.; formal analysis, E.E.N., P.R. and S.S.; funding acquisition, Á.H.P. and S.S.; investigation, P.R. and E.E.N.; methodology, Á.H.P., S.S., E.E.N., P.R.; project administration, P.R. and S.S.; resources, Á.H.P., P.R. and S.S.; supervision, S.S., P.R., and C.K.R; validation, Á.H.P., E.E.N., P.R., C.K.R., and S.S.; visualization, E.E.N.; writing—Original draft, E.E.N. and P.R.; writing—Review and editing, E.E.N., Á.H.P., G.R., C.K.R., P.R., and S.S. All authors have read and agreed to the published version of the manuscript.

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4.9 References

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

Ahmed, A., Diab, H., Alkahtani, M., Alshehri, M., Saber, H., Mahmoud, H., Dandrawy, M., Elmansi, A., Shati, A., & Ezzat, A. (2020). Molecular epidemiology of virulent E. coli among rural small scale dairy herds and shops: Efficacy of selected marine algal extracts and disinfectants. International Journal of Environmental Health Research, 1-23. https://doi.org/10.1080/09603123.2020.1727422.

Andrews, T., Neher, D. A., Weicht, T. R., & Barlow, J. W. (2019). Mammary microbiome of lactating organic dairy cows varies by time, tissue site, and infection status. PLOS ONE, 14(11), e0225001. https://doi.org/10.1371/journal.pone.0225001.

Antaya, N. T., Soder, K. J., Kraft, J., Whitehouse, N., Guindon, N. E., Erickson, P., Conroy, A. B., & Brito, A. (2014). 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. J. Dairy Sci., 98. https://doi.org/10.3168/jds.2014-8851.

Berhe, G., Wasihun, A. G., Kassaye, E., & Gebreselasie, K. (2020). Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health, 20(1), 894. https://doi.org/10.1186/s12889-020-09016-6.

Besle, J. M., Viala, D., Martin, B., Pradel, P., Meunier, B., Berdagué, J. L., Fraisse, D., Lamaison, J. L., & Coulon, J. B. (2010). Ultraviolet-absorbing compounds in milk are related to forage polyphenols. J. Dairy Sci., 93(7), 2846-2856. https://doi.org/https://doi.org/10.3168/jds.2009-2939.

Boubendir, A., Serrazanetti, D. I., Hamidechi, M. A., Vannini, L., & Guerzoni, M. E. (2016). Changes in bacterial populations in refrigerated raw milk collected from a semi-arid area of Algeria. Annals of Microbiology, 66(2), 777-783. https://doi.org/10.1007/s13213-015-1163-5.

Brooke, J. (2021). Advances in the Microbiology of Stenotrophomonas maltophilia. Clinical microbiology reviews, 34, e0003019. https://doi.org/10.1128/CMR.00030-19.

Burvenich, C., Van Merris, V., Mehrzad, J., Diez-Fraile, A., & Duchateau, L. (2003). Severity of E. coli mastitis is mainly determined by cow factors. Vet Res, 34(5), 521-564. https://doi.org/10.1051/vetres:2003023.

Chambers, L., Yang, Y., Littier, H., Ray, P., Zhang, T., Pruden, A., Strickland, M., & Knowlton, K. (2015). Metagenomic Analysis of Antibiotic Resistance Genes in Dairy Cow Feces following Therapeutic Administration of Third Generation Cephalosporin. PLOS ONE, 10(8), e0133764. https://doi.org/10.1371/journal.pone.0133764.

Chaves Lopez, C., Serio, A., Rossi, C., Mazzarrino, G., Marchetti, S., Castellani, F., Grotta, L., Fiorentino, P. F., Paparella, A., & Martino, G. (2016). Effect of diet supplementation with *Ascophyllum nodosum* on cow milk composition and microbiota. Journal of Dairy Science, 99(8), 6285-6297. https://doi.org/https://doi.org/10.3168/jds.2015-10837.

Cherry, P., O'Hara, C., Magee, P. J., McSorley, E. M., & Allsopp, P. J. (2019). Risks and benefits of consuming edible seaweeds. Nutrition reviews, 77(5), 307-329. https://doi.org/10.1093/nutrit/nuy066.

Cikoš, A. M., Jurin, M., Čož-Rakovac, R., Jokić, S., & Jerković, I. (2019). Update on Monoterpenes from Red Macroalgae: Isolation, Analysis, and Bioactivity. Marine drugs, 17(9). https://doi.org/10.3390/md17090537.

Claeys, W. L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., De Zutter, L., Huyghebaert, A., Imberechts, H., Thiange, P., Vandenplas, Y., & Herman, L. (2013). Raw or heated cow milk consumption: Review of risks and benefits. Food Control, 31(1), 251-262. https://doi.org/https://doi.org/10.1016/j.foodcont.2012.09.035.

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, n/a(n/a). https://doi.org/https://doi.org/10.1111/jpn.13509.

Derakhshani, H., Fehr, K. B., Sepehri, S., Francoz, D., De Buck, J., Barkema, H. W., Plaizier, C. J., & Khafipour, E. (2018). Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. J. Dairy Sci., 101(12), 10605-10625. https://doi.org/https://doi.org/10.3168/jds.2018-14860.

Dhanashekar, R., Akkinepalli, S., & Nellutla, A. (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. Germs, 2(3), 101-109. https://doi.org/10.11599/germs.2012.1020.

Durani, P., & Leaper, D. (2008). Povidone–iodine: use in hand disinfection, skin preparation and antiseptic irrigation. International Wound Journal, 5(3), 376-387. https://doi.org/https://doi.org/10.1111/j.1742-481X.2007.00405.x.

Eggers, M. (2019). Infectious Disease Management and Control with Povidone Iodine. Infectious diseases and therapy, 8(4), 581-593. https://doi.org/10.1007/s40121-019-00260-x.

Fiordalisi, S. A. L., Honorato, L. A., Loiko, M. R., Avancini, C. A. M., Veleirinho, M. B. R., Filho, L., & Kuhnen, S. (2016). The effects of Brazilian propolis on etiological agents of mastitis and the viability of bovine mammary gland explants. J Dairy Sci, 99(3), 2308-2318. https://doi.org/10.3168/jds.2015-9777.

Fischer, W. J., Schilter, B., Tritscher, A., & Stadler, R. (2011). Contaminants of Milk and Dairy Products: Contamination Resulting from Farm and Dairy Practices. Encyclopedia of Dairy Sciences, 887-897. https://doi.org/10.1016/B978-0-12-374407-4.00104-7.

Gomes, F., Saavedra, M. J., & Henriques, M. (2016). Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. Pathogens and Disease, 74(3). https://doi.org/10.1093/femspd/ftw006.

Hileman, J. L. (1940). Thermoduric Bacteria in Pasteurized Milk. A Review of Literature. J. Dairy Sci., 23(11), 1143-1160. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(40)95602-8.

Hoekstra, M. J., Westgate, S. J., & Mueller, S. (2017). Povidone-iodine ointment demonstrates in vitro efficacy against biofilm formation. International Wound Journal, 14(1), 172-179. https://doi.org/https://doi.org/10.1111/iwj.12578.

Hove, P. R., Mobley, D., Magunda, F., & Call, D. R. (2020). Deploying Elemental Iodine in a Vapor Form to Disinfect Water and to Clear Biofilms. International Journal of Environmental Research and Public Health, 17(10). https://doi.org/10.3390/ijerph17103489.

Hu, X., Li, S., Mu, R., Guo, J., Zhao, C., Cao, Y., Zhang, N., & Yunhe, F. (2022). The Rumen Microbiota Contributes to the Development of Mastitis in Dairy Cows. Microbiology Spectrum, 10. https://doi.org/10.1128/spectrum.02512-21.

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., & Bohannan, B. J. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. Applied and environmental microbiology, 67(10), 4399-4406. https://doi.org/10.1128/AEM.67.10.4399-4406.2001.

Jadhav, P. V., Das, D. N., Suresh, K. P., & Shome, B. R. (2018). Threshold somatic cell count for delineation of subclinical mastitis cases. Vet World, 11(6), 789-793. https://doi.org/10.14202/vetworld.2018.789-793.

Jing-Wei, Z., Yi-Yuan, S., Xin, L., Hua, Z., Hui, N., Luo-Yun, F., Ben-Hai, X., Jin-Jin, T., & Lin-Shu, J. (2021). Microbiome and Metabolic Changes of Milk in Response to Dietary Supplementation With Bamboo Leaf Extract in Dairy Cows. Front Nutr, 8, 723446. https://doi.org/10.3389/fnut.2021.723446.

Karatzia, M., Christaki, E., Bonos, E., Karatzias, C., & Florou-Paneri, P. (2012). The influence of dietary *Ascophyllum nodosum* on haematologic parameters of dairy cows. Italian Journal of Animal Science, 11(2), e31. https://doi.org/10.4081/ijas.2012.e31.

Kim, B.-R., Jiwon, S., Guevarra, R., Lee, J., Kim, D., Seol, K.-H., Lee, J.-H., Kim, H.-B., & Isaacson, R. (2017). Deciphering diversity indices for better understanding of the microbial communities. Journal of microbiology and biotechnology, 27. https://doi.org/10.4014/jmb.1709.09027.

Kuehn, J. S., Gorden, P. J., Munro, D., Rong, R., Dong, Q., Plummer, P. J., Wang, C., & Phillips, G. J. (2013). Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. PLOS ONE, 8(4), e61959-e61959. https://doi.org/10.1371/journal.pone.0061959.

La Duc, M. T., Sumner, R., Pierson, D., Venkat, P., & Venkateswaran, K. (2004). Evidence of pathogenic microbes in the International Space Station drinking water: reason for concern? Habitation (Elmsford), 10(1), 39-48. https://doi.org/10.3727/154296604774808883.

Lejonklev, J., Løkke, M. M., Larsen, M. K., Mortensen, G., Petersen, M. A., & Weisbjerg, M. R. (2013). Transfer of terpenes from essential oils into cow milk. J. Dairy Sci., 96(7), 4235-4241. https://doi.org/10.3168/jds.2012-6502.

Li, N., Wang, Y., You, C., Ren, J., Chen, W., Zheng, H., & Liu, Z. (2018). Variation in Raw Milk Microbiota Throughout 12 Months and the Impact of Weather Conditions. Scientific reports, 8(1), 2371. https://doi.org/10.1038/s41598-018-20862-8.

Machado, L., Magnusson, M., Paul, N. A., de Nys, R., & Tomkins, N. (2014). Effects of Marine and Freshwater Macroalgae on In Vitro Total Gas and Methane Production. PLOS ONE, 9(1), e85289. https://doi.org/10.1371/journal.pone.0085289.

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.

Mane, N. V., & Gandhi, M. B. (2010). Studies on proteolytic thermoduric psychrotrophic bacteria in milk and fermented milk products. Journal of Environmental Research and Development, 5, 384-392.

Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L., & Bahcevandziev, K. (2020). Seaweed Potential in the Animal Feed: A Review. Journal of Marine Science and Engineering, 8(8). https://doi.org/10.3390/jmse8080559.

Naylor, S., Hanke, F. J., Manes, L. V., & Crews, P. (1983). Chemical and Biological Aspects of Marine Monoterpenes. In Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products (pp. 189-241). Vienna: Springer Vienna.

Newton, E. E., Pétursdóttir, Á. H., Ríkharðsson, G., Beaumal, C., Desnica, N., Giannakopoulou, K., Juniper, D., Ray, P., & Stergiadis, S. (2021). Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations. Foods, 10(7). https://doi.org/10.3390/foods10071526.

Nogueira, J. O. e., Campolina, G. A., Batista, L. R., Alves, E., Caetano, A. R. S., Brandão, R. M., Nelson, D. L., & Cardoso, M. d. G. (2021). Mechanism of action of various terpenes and phenylpropanoids against Escherichia coli and Staphylococcus aureus. FEMS Microbiology Letters, 368(9). https://doi.org/10.1093/femsle/fnab052.

Ohnishi, M., Sawada, T., Marumo, K., Harada, K., Hirose, K., Shimizu, A., Hayashimoto, M., Sato, R., Uchida, N., & Kato, H. (2012). Antimicrobial susceptibility and genetic relatedness of bovine Stenotrophomonas maltophilia isolates from a mastitis outbreak. Lett Appl Microbiol, 54(6), 572-576. https://doi.org/10.1111/j.1472-765X.2012.03246.x.

Petzer, I. M., Karzis, J., Donkin, E. F., Webb, E. C., & Etter, E. M. C. (2017). Validity of somatic cell count as indicator of pathogen-specific intramammary infections. J S Afr Vet Assoc, 88(0), e1-e10. https://doi.org/10.4102/jsava.v88i0.1465.

Pinheiro, J. B., Douglas. (2000). Mixed-Effects Models in S and S-PLUS: Springer.

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2013). The complex microbiota of raw milk. FEMS Microbiology Reviews, 37(5), 664-698. https://doi.org/10.1111/1574-6976.12030.

Rainard, P. (2017). Mammary microbiota of dairy ruminants: fact or fiction? Vet Res, 48(1), 25. https://doi.org/10.1186/s13567-017-0429-2.

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. PLOS ONE, 16(3), e0247820. https://doi.org/10.1371/journal.pone.0247820.

Ryan, M. P., & Pembroke, J. T. (2018). Brevundimonas spp: Emerging global opportunistic pathogens. Virulence, 9(1), 480-493. https://doi.org/10.1080/21505594.2017.1419116.

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., van der Lelie, D., & Dow, J. M. (2009). The versatility and adaptation of bacteria from the genus Stenotrophomonas. Nature Reviews Microbiology, 7(7), 514-525. https://doi.org/10.1038/nrmicro2163.

Shukla, P. S., Mantin, E. G., Adil, M., Bajpai, S., Critchley, A. T., & Prithiviraj, B. (2019). *Ascophyllum nodosum*-Based Biostimulants: Sustainable Applications in Agriculture for the Stimulation of Plant Growth, Stress Tolerance, and Disease Management. Frontiers in Plant Science, 10(655). https://doi.org/10.3389/fpls.2019.00655.

Taponen, S., McGuinness, D., Hiitiö, H., Simojoki, H., Zadoks, R., & Pyörälä, S. (2019). Bovine milk microbiome: a more complex issue than expected. Vet Res, 50(1), 44. https://doi.org/10.1186/s13567-019-0662-y.

Thomas, A. (2014). Thermoduric Bacteria in Milk- A Review.

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., & Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrobial agents and chemotherapy, 49(6), 2474-2478. https://doi.org/10.1128/AAC.49.6.2474-2478.2005.

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J. P., Coulon, J. B., & Berdagué, J.-l. (2000). Transfer of monoterpenes and sesquiterpenes from forages into milk fat. Lait, 80, 635-641.

Wang, Y., Xu, Z., Bach, S., & McAllister, T. (2009). Sensitivity of Escherichia coli to Seaweed (*Ascophyllum nodosum*) Phlorotannins and Terrestrial Tannins. Asian-Australasian journal of animal sciences, 22. https://doi.org/10.5713/ajas.2009.80213.

Yang, X., Guo, X., Liu, W., Tian, Y., Gao, P., Ren, Y., Zhang, W., Jiang, Y., & Man, C. (2020). The complex community structures and seasonal variations of psychrotrophic bacteria in raw milk in Heilongjiang Province, China. LWT, 134, 110218. https://doi.org/https://doi.org/10.1016/j.lwt.2020.110218.

Zeinhom, M. M. A., Hassan, G. M., Salem, H. A. M., & Corke, H. (2021). Prevalence and Survival of Stenotrophomonas Species in Milk and Dairy Products in Egypt. Foodborne Pathogens and Disease, 18(5), 337-345. https://doi.org/10.1089/fpd.2020.2893.

Zhang, R., Huo, W., Zhu, W., & Mao, S. (2015). Characterization of bacterial community of raw milk from dairy cows during subacute ruminal acidosis challenge by high-throughput sequencing. J Sci Food Agric, 95(5), 1072-1079. https://doi.org/10.1002/jsfa.6800.

Zhang, Y., Wei, J., Qiu, Y., Niu, C., Song, Z., Yuan, Y., & Yue, T. (2019). Structure-Dependent Inhibition of Stenotrophomonas maltophilia by Polyphenol and Its Impact on Cell Membrane. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.02646.

Chapter 5: “Effect of Dietary Microalgae Supplementation on Finnish Ayrshire Cow Mineral Use Efficiency and Milk Nutritional Profile ”

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Contribution: The animal trial was run in Finland with collaborators that worked with us through the European Regional Development Fund and Raisioagro Ltd. (Raisio, Finland) and conducted in cooperation with Cursor Ltd. (Kotka, Finland). Milk, feed, faeces, and serum was aliquoted and measured for iodine at UoR exclusively by myself. Non-iodine minerals was analysed by partners at Harper Adams University. I performed all analysis by myself, modelled nutritional outcomes by myself, and wrote the article as the primary author. The overall estimated percentage contribution made by myself would be 85%, factoring in the contribution of partners to the collection of feed intakes from the animal trial, and milk basic composition and the non-iodine minerals, along with the text review by co-authors.

Objectives: The study aimed to: (i) investigate the comparative effects of including *S. platensis* and rapeseed meal in dairy cow diets on milk, faeces, and plasma concentrations of macro- and microminerals, and (ii) estimate the effect that the consumption of the produced milk may have on consumers’ mineral intakes, based on milk intakes and dietary guidelines from the Finnish Institute for Health and Welfare National FinDiet 2017 Survey and the 2012 Nordic Nutritional Recommendations.

Hypothesis: For this experiment, I hypothesized that microalgal supplementation of dairy cows would not change iodine concentrations of milk, faeces, or serum – but diets with higher rapeseed inclusion would limit iodine concentrations within milk and serum. Given that rapeseed is a commonly fed within Finland, I hypothesized that I would show reduced iodine intake as a result of decreased iodine concentrations within milk.

Overview: Overall, Microalgal (*Spirulina platensis*) inclusion in dairy cow diets did not affect milk and plasma concentrations of macrominerals and trace elements. Rapeseed inclusion in cow diets did not affect blood plasma mineral concentrations but reduced milk I concentrations, an effect which may be associated with glucosinolates, which are known to reduce dietary I availability to the mammary gland. Based on Finnish population milk intakes and nutritional guidelines, the contribution of milk for I supply of the different population demographics could be reduced by approximately 25 to 50% when rapeseed partially or wholly substitutes other protein feeds. This may increase the need for higher I supply from either consuming more milk and dairy, or increasing the intake of other diet sources (fish, shellfish, I-fortified foods), especially in consumers with higher I requirements (pregnant and nursing women). This study showed that feeding microalgae to dairy cows maintained mineral concentrations in cows’ blood and the milk produced. However, supplemental dietary I is recommended when rapeseed is fed to dairy cows, to prevent subsequent production of milk with lower I concentrations.

5.1 Abstract

Given the lack of research regarding the effect of microalgal supplementation in dairy cows on milk mineral concentrations, this study investigated the effect of feeding different protein supplements in dairy cow diets on milk, faeces, and blood plasma mineral concentrations, associated milk and blood plasma transfer efficiencies, and apparent digestibility. Lactating Finnish Ayrshire cows (n = 8) were allocated at the start of the trial to 4 diets used in a replicated 4 × 4 Latin square design experiment: (1) control diet (CON), (2) a pelleted rapeseed supplement (RSS; 2,550 g/d), (3) a mixture of rapeseed and *Spirulina platensis* (RSAL; 1,280 g of RSS + 570 g of *S. platensis* per day), and (4) *S. platensis* (ALG; 1,130 g of *S. platensis* per day). In each of the 4 experimental periods, a 2-wk adaptation to the experimental diets was followed by a 7-d sampling and measurement period. Feed samples were composited per measurement period, milk, and feed samples (4 consecutive days; d 17–20), and blood plasma samples (d 21) were composited for each cow period (n = 32). Data were statistically analysed using a linear mixed effects model with diet, period within square, square and their interaction as fixed factors, and cow within square as a random factor. Cows fed ALG were not significantly different in their milk or blood plasma mineral concentrations compared with CON, although feeding ALG increased faecal concentrations of macrominerals (Ca and Mg) and trace elements (Co, Cu, Fe, I, Mn, and Zn), and reduced their apparent digestibility, compared with CON. When compared with CON and ALG, milk from cows fed RSAL and RSS had lower milk I concentrations (−69.6 and −102.7 µg/kg of milk, respectively), but total plasma I concentrations were not affected significantly. Feeding *S. platensis* to dairy cows did not affect mineral concentrations in cows' blood or milk, but care should be taken when rapeseed is fed to avoid reducing milk I concentrations which may in turn reduce consumers' I intake from milk and dairy products.

Keywords: bovine, milk, minerals, microalgae, rapeseed

5.2 Introduction

Microalgae is often considered to be a possible partial solution to food security-related problems stemming from land scarcity and climate change within the agricultural sector (Ullmann and Grimm, 2021). The benefits from growing microalgae are numerous, as algal aquacultural systems can be located in nonarable land and utilise wastewater, reducing the cost of production and providing a more sustainable and eco-friendly solution to deliver biomass for animal feed (Dębowski et al., 2020). In 2019, an estimated 56,456 tons of microalgae were cultivated, the vast majority of which, 56,208 tons, were of *Spirulina* (Cai et al., 2021). Given the applicability of microalgal farming across several disciplines, and the potential benefits, there has been increasing interest in feeding microalgae as a protein source to ruminants and the effect this might have on the quality of the milk from microalgal-fed cows (Halmemies-Beauchet-Filleau et al., 2018; Lamminen et al., 2019). Previous work has shown that microalgae has potential to be included with rapeseed in dairy diets as a source of protein (Lamminen et al., 2019). Some microalgae species, although CP-rich, have palatability issues that have been seen to reduce feed intake in ruminants when substituted for conventional feeds such as bromegrass (Bromus madritensis) hay, soybean meal (Glycine max), or corn (Zea mays), but this could be potentially overcome if microalgae was used as a pelleted supplement or deodorization with ethanol (Van Emon et al., 2015; Cuellar-Bermúdez et al., 2017; Halmemies-Beauchet-Filleau et al., 2018; Lamminen et al., 2019). Of the approximately 25,000 microalgal species currently identified, *Spirulina platensis* (also known as *Arthrospira platensis*) has been considered as a potential for animal feed (Vale et al., 2020). *Spirulina platensis* is the most cultivated photosynthetic prokaryote, given its array of use in food and animal feed, and estimates for production are ~56,000 tons, with the majority grown in China and the Asia-Pacific region (Cai et al., 2021). This phycological species contains large concentrations of minerals such as calcium (Ca; 6,000 mg/100 g), magnesium (Mg; 100 mg/100 g), phosphorus (P; 10,088 mg/100 g), potassium (K; 2,502 mg/100 g), and sodium (Na; 14,004 mg/100 g; Seghiri et al., 2019), and its production is projected to continue to increase in availability given the wide uses for the product (Lum et al., 2013). Milk and dairy products are a rich source of I, Ca, P, Se, Mg, and Zn (Haug et al., 2007), which can have beneficial effects on human health such as increased bone health, a protective effect against certain cancers, and reduction in childhood obesity that can lead to the development of type 2 diabetes (Thorning et al., 2016). Published reports in Finland have shown that dairy products, including milk, provide 18% of K, 36% of P, 67% of Ca, 23% of Se, 27% of Zn, and 32% of I as a portion of total daily intake for women (Kaartinen et al., 2020). Milk products are estimated to provide between 13 and 64% of the recommended daily intake for I depending on the country (van der Reijden et al., 2017). This is even more important with the consideration that 1.9 billion individuals on the planet would be described as having inadequate I intake, with Europe making up the largest portion (59.9%; de Benoist et al., 2003). In 2017, it was estimated that 2 billion people still suffer from I deficiency, and this is realized in 50 million cases of clinical symptoms globally requiring intervention (Biban and Lichiardopol, 2017). Previous studies have shown that a cows' diet is a major factor influencing milk mineral concentrations (Qin et al., 2021; Stergiadis et al., 2021). Notably, inclusion of macroalgae in a cows' diet has been found to increase milk concentration of I and decrease milk concentration of Cu (Newton et al., 2021). Certain protein sources (e.g., rapeseed, cassava, sorghum, soy, cruciferous vegetables), in particular those rich in goitrogens (e.g., thiocyanate, glucosinolates, flavonoids, goitrin), can also play an important role in milk I concentrations (Flachowsky et al., 2014; Bertinato, 2021). Goitrogens can be found in several animal feeds and some can inhibit the activity of the thyroid peroxidase enzyme (Babiker et al., 2020). Depending on the type of goitrogen, this results in a suppression of thyroid gland function, reduction of production of thyroid hormones, inability of the thyroid to properly uptake and process I, leading to lower I excretion into the milk, or all of these. (Petroski and Minich, 2020; Bertinato, 2021). Given that dairy products are major suppliers of minerals in human diets, and the fact that changes in a cow's diet may affect resulting mineral concentrations, supplementing microalgae to dairy diets may well affect milk mineral concentrations and mineral supply to consumers. To the best of our knowledge, there are no published studies concerning the effect of feeding *S. platensis* on milk mineral concentrations. In the present study, I hypothesize that the inclusion of rapeseed or microalgae as a protein source in cows' diets will affect milk mineral concentrations. Therefore, the present study aimed to: (1) investigate the comparative effects of including *S. platensis* and rapeseed meal in dairy cow diets on milk, faeces, and plasma concentrations of macro- and microminerals, and (2) estimate the effect that the consumption of the produced milk may have on consumers' mineral intakes, based on milk intakes and dietary guidelines from the Finnish Institute for Health and Welfare National FinDiet 2017 Survey (Kaartinen et al., 2020) and the 2012 Nordic Nutritional Recommendations (NNR, 2014).

5.3 Materials and Methods

5.3.1 Experimental design

The current study was conducted from May to July, 2014, at the University of Helsinki research farm, Helsinki, Finland. All animal procedures were approved by the National Animal Experiment Board in Finland according to the guidelines of the European Union Directive 2010/63/EU and the current Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific or Educational Purposes 497/2013). Lactating Finnish Ayrshire multiparous cows (n = 8) were selected based on similar lactation stage and milk yield; specifically, the means at the start of the experimental work are as follows: milk yield = 35.8 ± 3.08 kg/d; BW = 718 ± 54.4 kg; BCS = 2.89 ± 0.330. Cows were randomly allocated in a replicated balanced 4 × 4 Latin square study with 4 dietary treatments and four 21-d periods, which consisted of 2 wk of diet adaptation, followed by a 7-d sampling and measurement period. The dietary treatments were (1) control diet (CON), (2) pelleted rapeseed supplementation (RSS; 2,550 g/d), (3) supplementation of a mixture of RSS and *S. platensis* (RSAL; 1,280 g of RSS + 570 g of *S. platensis* per day), and (4) *S. platensis* supplementation (ALG; 1,130 g/d of *S. platensis*). Rapeseed supplement consisted of 695 g/kg of rapeseed meal, 138 g/kg of turnip rape (*Brassica rapa*) cake, 117 g/kg of molassed sugar beet (*Beta vulgaris*) pulp, and 50 g/kg of molasses. The CON diet consisted of grass silage, cereal-sugar beet pulp, and mineral-vitamin supplement (53:47 forage to concentrate). All treatments that received protein supplementation (RSS, RSAL, ALG) were nearly iso-nitrogenous (120–127 g of N per day between rapeseed and *S. platensis*, 277–305 g of N per day for all concentrates). Crude protein coming from the concentrate feeds was based on cereals (CON), rapeseed (RSS), rapeseed and microalgae (RSAL), and microalgae (ALG). Diet ingredient profiles of the 4 treatments can be found in previously published work (Lamminen et al., 2017). In short, all 4 experimental groups were fed cereal-sugar beet pulp (7.87–10.50 kg of DM per day), and a mineral-vitamin supplement (0.30 kg of DM per day). Algae-containing groups (RSAL and ALG) were fed molassed sugar beet pulp (0.09 and 0.18 kg of DM per day, respectively) and molasses (0.03 and 0.06 kg of DM per day, respectively), along with *S. platensis* at 0.57 kg of DM per day and 1.13 kg of DM per day, respectively. Rapeseed-containing treatments (RSS and RSAL) were a rapeseed supplement (2.55 and 1.28 kg of DM per day, respectively) and were selected due to the prevalence of rapeseed in common feed ingredient on Finnish dairy farms. *Spirulina platensis* was purchased from Duplaco B.V. The microalgae was produced in open raceway ponds, centrifuged after harvesting, and subsequently dried. The same grass silage was used across all treatments as the basal forage, which was preserved from the secondary growth of a mixed sward of timothy (Phleum pratense) and meadow fescue (Festuca pratensis). Pre-wilted grass silage was ensiled with formic acid-based additive, applied at a rate of 6 L/1,000 kg of fresh matter. Mineral compositions of individual feeds and mineral-vitamin supplement are shown in Table 5.1. Chemical composition of the feed ingredients (silage, cereal-sugar beet pulp, molassed sugar beet pulp, molasses, mineral-vitamin supplement, rapeseed supplement, and *S. platensis*) can be found in Table 5.2. The concentrate feeds were provided at 12 kg/d on fresh matter basis (4 times daily, at 0600, 1100, 1700, and 1930 h), and grass silage was offered ad libitum. Animals had ad libitum access to water. A mineral-vitamin supplement was provided (Pihatto-Melli Plus, Raisioagro Ltd.) by inclusion in a paste made of microalgae, molasses, and water, which was then mixed with the other concentrate components. Mineral composition of the 4 experimental diets is shown in Table 5.3. Mineral intakes from the 4 experimental diets are also presented in Table 5.3. The experimental diets were number coded, and the codes and group allocation were under the responsibility of the study coordinator. Research personnel knew the contents of the diets (as they were responsible to prepare them), but the interpretation of the codes was not revealed to other animal caretakers.

5.3.2 Sample collection

Detailed sampling information can be found in Lamminen et al. (2017). In brief, feed samples (from every feed ingredient) were collected daily and composited per sample of feed per period, except for the mineral-vitamin supplement, which were composited to provide 1 sample for the entire experiment, and the molasses products, which were composited by combining periods 1 and 2, and then 3 and 4, resulting in 2 representative samples. Samples for each composite diet ingredient were stored at −20°C until further analysis. Milk samples were obtained daily for 4 consecutive days. Cows were milked twice daily (a.m. and p.m.), and samples taken from each milking were combined into 1 representative daily sample based on milk yield. Milk samples from 4 consecutive days were then composited based on milk yield to represent 1 sample per cow per period, and stored at −20°C. Faeces were collected as rectal grab samples twice daily from d 17 to 20 of each period, and composited, which resulted in 1 faecal sample per cow per period. Representative composite samples were collected and frozen at −20°C for future analysis. Blood was taken and processed akin to Puhakka et al. (2016) by drawing from the coccygeal vein 3 times (0530, 0830, and 1130 h) on the 21st day of the period, and plasma was later composited to give 1 sample per cow per period. Samples were collected into 10-mL test tubes (Vacutainer; BD Medical) containing an anticoagulant agent (lithium heparin). After collection, samples were placed immediately on ice until centrifuged (2,220 × g for 10 min at room temperature, 21°C) to separate plasma. Plasma was stored in polypropylene tubes at −20°C. No samples were missing or excluded from the experiment.

5.3.3 Quantification of mineral concentrations in feed, milk, faeces, and blood plasma

Concentrations of I in feed, milk, and faecal samples were quantified according to British Standards Institution Publication (BS EN 17050:2017), using ICP-MS (Agilent 7000, Agilent) with slight modifications, as published in Newton et al. (2021). In brief, feed and faeces were extracted using a tetramethylammonium hydroxide solution and heated, syringe filtered, and then diluted for analysis. Milk I was extracted with a 2% tetramethylammonium hydroxide solution, syringe filtered, and then diluted for analysis (Newton et al., 2021). Dried feed, faeces, and milk concentrations of all other minerals were analysed according to previous publications (Cope et al., 2009; McCaughern et al., 2020). In brief, approximately 0.5 g of previously dried and milled sample or liquid was digested with 1 mL of concentrated trace element-grade HCl (Fisher Scientific) and 6 mL of concentrated trace element-grade nitric acid (HNO3; Fisher Scientific) in DigiPREP tubes, and placed in a DigiPREP heating block (QMX Laboratories Ltd.). The temperature of the heating block was increased in a stepwise fashion to 100°C and maintained at this temperature for 45 min. Digested samples were then diluted to 50 mL using type-1 water. Once cooled, digested samples were then diluted 1:20 using an acidic diluent consisting of 0.50% trace element-grade HNO3 (Fisher Scientific), 2.00% HPLC-grade methanol (Sigma-Aldrich), and 0.05% Triton X-100 (Fisher Scientific) in type-1 water. Concentrations of I in heparinized plasma samples were quantified according to previously published methods of human serum analysis by Yu et al. (2018), which, in brief, involved digestion of the composite samples with aqueous ammonia, isopropanol, and ultrapure water, and utilizing a rhenium internal standard for accuracy. Concentrations of all other plasma sample minerals analysed used methods by McCaughern et al. (2020). Briefly, plasma samples were diluted 1:50 in an acidic diluent, which contained 0.50% concentrated HNO3 (Fisher Scientific), 2.00% HPLC-grade methanol (Sigma-Aldrich), and 0.05% Triton X-100 (Fisher Scientific) in type-1 water.

Table 5.1

Mineral concentrations of feed components fed to experimental treatments. Average, standard deviation, range mineral values per feed component fed to experimental treatments (Control, no microalgae, CON; Rapeseed supplement, RSS; 570g/day Rapeseed + microalgae supplement, RSAL; 1130g/day microalgae supplement, ALG) each day.

	Grass Silage			Cereal-Sugar Beet Pulp			Rapeseed[1]			Algae[2]			Molassed Sugar Beet Pulp[2]			Molasses[2]			Mineral-Vitamin Supplement[3]
	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG
Macrominerals (g/kg DM)																			
Calcium	5.5	*0.36*	5.1 - 6.0	1.3	*0.13*	1.2 - 1.5	8.3	*0.48*	7.8 - 9.0	2.9	*0.18*	2.7 - 3.2	10.6	*0.38*	10.0 - 11.0	5.7	*1.00*	4.6 - 7.2	273
Magnesium	1.8	*0.09*	1.7 - 1.9	0.02	*0.036*	0.02 - 0.02	4.7	*0.21*	4.4 - 4.9	3.1	*0.06*	3.0 - 3.2	2.0	*0.09*	1.9 - 2.1	0.42	*0.016*	0.40 - 0.44	69
Phosphorus	2.1	*0.10*	2.0 - 2.2	3.1	*0.06*	3.0 - 3.2	10.0	*0.51*	9.2 - 10.4	10.7	*0.20*	10.4 - 10.9	1.0	*0.06*	0.9 - 1.1	1.4	*0.33*	1.0 - 1.9	0.3
Potassium	30	*1.3*	27 - 31	6.0	*0.04*	5.7 - 5.8	14	*0.9*	13 - 15	14	*0.5*	14 - 15	8.3	*0.39*	7.8 - 8.9	46	*0.5*	45 - 46	0.5
Sodium	0.09	*0.007*	0.08 - 0.10	0.54	*0.027*	0.51 - 0.58	0.80	*0.029*	0.76 - 0.83	7.3	*0.57*	6.7 - 8.2	6.4	*0.17*	6.2 - 6.6	16	*0.4*	16 - 17	80
Sulphur	3.1	*0.29*	2.7- 6.5	1.9	*0.15*	1.7 - 2.1	8.0	*0.37*	7.3 - 8.3	7.9	*0.90*	6.9 - 9.2	7.0	*0.24*	6.6 - 7.2	4.7	*0.61*	4.1 - 5.7	12
Trace elements (μg/kg DM unless indicated by [4])																			
Cobalt	31	*19.1*	0 - 48	15	*9.7*	5 - 28	108	*11.3*	92 - 123	291	*2.6*	288 - 295	408	*182.2*	267 - 716	503	*30.0*	480 - 553	0.02
Copper[4]	5.5	*0.73*	4.4 - 6.4	6.1	*1.93*	4.8 - 9.3	5.4	*0.21*	5.1 - 5.7	1.5	*0.99*	0.2 - 2.9	7.3	*1.13*	5.4 - 8.1	3.4	*1.06*	2.4 - 4.9	0.4
Iron[4]	334	*12.4*	323 - 355	114	*5.5*	106 - 120	181	*7.1*	170 - 187	902	*30.0*	875 - 949	394	*25.5*	360 - 421	252	*25.4*	228 - 290	7
Iodine	136	*2.5*	134 - 141	95	*0.0*	95 - 95	126	*61.8*	83 - 230	1719	*149.7*	1518 -1931	1342	*66.6*	1265 - 1411	335	*71.3*	234 - 431	0.07
Manganese[4]	28	*1.9*	25 - 30	23	*0.7*	22 - 23	66	*2.9*	62 - 70	37	*1.2*	36 - 39	145	*166.8*	46 - 429	17	*2.9*	14 - 21	0.92
Zinc[4]	32	*1.6*	30 - 34	31	*1.7*	29 - 33	58	*1.6*	56 - 59	15	*1.3*	14 - 17	46	*4.7*	40 - 51	55	*48.7*	27 - 138	1.5

[1] Only fed to RSS and RSAL treatments

[2] Only fed to RSAL and ALG treatments

[3] Expressed in g/kg DM, the same MVS was used across the experiment, therefore the values are constant

[4] Expressed in mg/kg DM

Table 5.2

Chemical composition (g/kg DM) of experimental feed[1]

	Silage	Cereal-sugar beet pulp	Molassed sugar beet pulp	Molasses	Mineral-vitamin supplement	Rapeseed supplement	*S. platensis*
Dry matter (g/kg)	288	899	878	710	992	866	946
Ash	81.7	31.6	67.8	103	918	66.1	71.7
Crude protein	133	119	113	106		311	697
Crude fat		48.4	2.93			41.5	51.3
NDF[2]	480	363	338			272	0
Starch							66.2

[1] Blank entries were not analysed

[2] Results of silage analysed without heat stable amylase and expressed inclusive of residual ash (NDF), results of concentrate components analysed with heat stable amylase and expressed inclusive of residual ash (aNDF).

5.3.4 Statistical analysis

ANOVA was performed by linear mixed effects model in Minitab 18 (Minitab, 2019). Diet, period within square, square, and their interaction were used as fixed factors, and cow (experimental unit) within square as a random factor. Normality of residuals were evaluated visually, and no data showed deviation from normality. Tukey's least significance difference test ($P < 0.05$) was used for pairwise comparison of the means, where the mixed effects model showed a significant effect of diet or period. Mineral transfer efficiencies from feed to milk were calculated as follows: [100 × (milk mineral concentration, µg/kg) × milk output (kg/d)]/[(diet mineral concentration, µg/kg of DM) × DMI (kg/d)]. Apparent digestibility from feed to faeces were calculated as follows: 100 − [(100 × (faeces mineral concentration, µg/ kg) × faeces output (kg/d)]/[(diet mineral concentration, µg/kg of DM) × (DMI, kg/d)]. Acid insoluble ash was used as a marker to calculate faecal output.

5.4 Results

5.4.1 Milk mineral concentrations and transfer efficiencies

I found a significant effect of dietary treatment on the concentrations of I in milk ($P < 0.001$; Table 5.4), with average CON and ALG milk both containing 273% more than RSS milk and 98% more than RSAL milk. Transfer efficiency of Ca ($P = 0.020$), Mg ($P = 0.003$), P ($P < 0.001$), Na ($P = 0.018$), and I ($P < 0.001$) from feed to milk were influenced by dietary treatment (Table 5.4). Transfer efficiency of Ca, Mg, and P was highest in CON, lowest in RSS, and had intermediate values in ALG and RSAL, although not all differences were statistically significant. The Na transfer efficiency was higher for CON than in ALG. Transfer efficiency of I was higher in CON and ALG than in RSS and RSAL.

5.4.2 Faecal mineral concentrations and apparent digestibility

Dietary treatments influenced faecal concentrations of Ca ($P < 0.001$), Mg ($P < 0.001$), P ($P < 0.001$), S ($P = 0.027$), Co ($P < 0.001$), Cu ($P < 0.001$), Fe ($P < 0.001$), I ($P < 0.001$), Mn ($P < 0.001$), Mo ($P = 0.008$), and Zn ($P < 0.001$; Table 5.5). Faecal concentrations of Ca, Mg, S, Mn, and Zn were highest in RSAL cows, lowest in CON, and intermediate in faeces from RSS and ALG cows; in some cases, differences were not statistically significant. The group fed RSAL had faeces that contained 36, 18, 21, 24, and 17% more Ca, Mg, S, Mn, and Zn, respectively, than CON. Faecal P concentrations were higher for RSS and RSAL than for CON and ALG, with average RSS and RSAL faeces containing 24% more P than average CON and ALG. Cows fed RSAL and ALG excreted faeces with more Co, Cu, and I than CON and RSS cows. The RSAL and ALG groups had faeces that contained 50, 21, and 47% more Co, Cu, and I, respectively, than average CON and RSS faeces. Faecal concentrations of Mo were highest in RSS and lowest in CON, with intermediate values in RSAL and ALG. Cows fed ALG had faeces that contained 6, 24, and 33% more Fe than RSAL, RSS, and CON, respectively, and I detected an increasing Fe content as CON < RSS < RSAL < ALG, with all differences significant. I found a significant effect of dietary treatment on the apparent digestibility of Ca ($P < 0.001$), Mg ($P < 0.001$), Co ($P < 0.001$), Cu ($P = 0.001$), Fe ($P < 0.001$), I ($P = 0.001$), Mn ($P = 0.010$), and Zn ($P = 0.001$) in faeces (Table 5.5). Apparent digestibility of all these minerals (except Mg) was higher in treatments fed microalgae (RSAL, ALG) compared with those that were not (CON, RSS). Magnesium apparent digestibility was highest for RSAL, lowest for CON, and had intermediate values for RSS and ALG; however, the difference between ALG with RSS and RSAL was not statistically significant

Table 5.3

Mineral concentrations of total experimental diets via analysis of individual ingredients. Average, standard deviation, range (i) mineral concentrations of feed and (ii) mineral intakes, per experimental treatments (Control, no microalgae, CON; 2550 g/day rapeseed supplement, RSS; 1280 g/day rapeseed supplement + 570g/day *S. platensis* supplement, RSAL; 1130g/day *S. platensis* supplement, ALG).

	CON			RSS			RSAL			ALG		
	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE	AVG	*SD*	RANGE
Macrominerals concentrations of experimental diets (g/kg DM)												
Calcium	7.1	*0.16*	6.9 - 7.3	7.8	*0.20*	7.6 - 8.2	7.6	*0.21*	7.2 - 7.9	7.3	*0.20*	6.9 - 7.5
Magnesium	2.4	*0.05*	2.3 - 2.5	2.8	*0.06*	2.7 - 2.9	2.7	*0.08*	2.6 - 2.8	2.5	*0.06*	2.4 - 2.6
Phosphorus	2.5	*0.04*	2.5 - 2.6	3.3	*0.05*	3.2 - 3.3	3.1	*0.10*	3.0 - 3.3	2.9	*0.06*	2.8 - 3.0
Potassium	18.2	*0.81*	17.2 - 19.2	19.5	*0.96*	18.4 - 21.0	19.0	*1.28*	16.8 - 20.7	18.7	*1.32*	16.5 - 20.2
Sodium	1.3	*0.06*	1.3 - 1.5	1.3	*0.06*	1.3 - 1.4	1.6	*0.14*	1.4 - 1.9	1.8	*0.10*	1.6 - 1.9
Sulphur	2.7	*0.17*	2.5 - 3.0	3.3	*0.14*	3.2 - 3.5	3.2	*0.18*	3.0 - 3.5	3.0	*0.22*	2.8 - 3.4
Trace elements concentrations of experimental diets (mg/kg DM unless indicated by [1])												
Cobalt[1]	318	*15.5*	295 - 349	322	*14.2*	304 - 351	332	*26.9*	308 - 388	337	*20.7*	312 - 378
Copper	10.3	*0.65*	9.6 - 11.4	10.1	*0.54*	9.5 - 11.0	10.2	*0.92*	9.3 - 12.2	10.1	*0.85*	9.3 - 11.4
Iron	318	*8.7*	308 - 332	327	*9.6*	316 - 341	343	*8.9*	332 - 355	360	*8.3*	348 - 370
Iodine	1.06	*0.045*	1.01 – 1.15	1.04	*0.047*	0.97 – 1.11	1.10	*0.094*	1.01 – 1.31	1.15	*0.076*	1.06 – 1.28
Manganese	37.4	*1.06*	35.5 - 38.9	41.9	*0.91*	40.4 - 42.9	40.7	*1.45*	38.4 - 42.4	39.1	*2.18*	37.5 - 43.4
Zinc	51.3	*1.50*	49.6 - 54.4	53.8	*0.96*	52.1 - 54.9	52.5	*2.33*	50.1 - 57.1	50.7	*1.43*	48.2 - 52.4
Macrominerals intakes from experimental diets (g/day)												
Calcium	162	*8.0*	147 - 174	183	*7.7*	172 - 194	175	*12.9*	149 - 189	166	*10.1*	153 - 184
Magnesium	54.8	*2.28*	50.4 - 57.0	64.9	*2.98*	60.9 - 68.3	61.0	*4.10*	52.4 - 64.5	57.1	*2.97*	53.4 - 61.2

Phosphorus	57.7	*2.56*	52.7 - 61.3	76.3	*3.03*	71.6 - 81.0	71.0	*4.29*	62.2 - 75.7	66.0	*3.67*	60.0 - 71.6
Potassium	416	*33.3*	363 - 458	456	*42.8*	406 - 530	440	*63.2*	315 - 520	430	*58.0*	336 - 502
Sodium	30.6	*0.24*	30.3 - 31.0	31.3	*0.21*	31.1 - 31.7	35.9	*0.43*	35.3 - 36.5	40.3	*0.75*	39.5 - 41.5
Sulphur	60.7	*4.09*	55.7 - 69.8	77.5	*6.63*	69.6 - 89.1	73.1	*7.99*	57.5 - 86.6	68.9	*8.64*	59.9 - 82.8
Trace elements intakes from experimental diets (mg/day)												
Cobalt	7.2	*0.15*	7.0 - 7.4	7.5	*0.22*	7.2 - 7.7	7.6	*0.21*	7.3 - 7.8	7.7	*0.18*	7.4 - 7.9
Copper	235	*16.5*	211 - 261	237	*9.6*	218 - 246	234	*10.5*	215 - 250.	230	*10.0*	217 - 245
Iron	7252	*331.1*	6793 - 7803	7643	*551.3*	6788 - 8596	7897	*679.5*	6500 - 8914	8215	*590.4*	7318 - 9103
Iodine	24.1	*0.15*	23.7 - 24.2	24.2	*0.27*	23.9 - 24.6	25.2	*0.36*	24.5 - 25.6	26.1	*0.21*	25.9 - 26.5
Manganese	852	*34.2*	802 - 886	981	*54.7*	906 - 1057	935	*73.6*	784 - 1005	892	*57.5*	805 - 966
Molybdenum	9.9	*1.43*	7.4 - 11.4	11.1	*1.25*	9.4 - 12.7	10.4	*1.97*	6.7 - 12.3	9.7	*1.39*	8.1 - 12.1
Zinc	1169	*31.4*	1123 - 1220	1258	*53.5*	1182 - 1349	1205	*64.3*	1074 - 1301	1157	*57.0*	1064 - 1241

[1]Expressed in µg/kg DM

Table 5.4

Mineral composition of milk. Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no microalgae, CON; 2550 g/day rapeseed supplement, RSS; 1280 g/day rapeseed supplement + 570g/day *S. platensis* supplement, RSAL; 1130g/day *S. platensis* supplement, ALG).

	Diet					ANOVA P-values[1]	
	CON	RSS	RSAL	ALG			
Minerals	n=8	n=8	n=8	n=8	SE	Diet	Period
Macrominerals (mg/kg)							
Calcium	1229	1139	1178	1173	43.9	0.287	0.001
Magnesium	123.7	115.3	118.3	119.7	4.68	0.342	<0.001
Phosphorus	957.5	919.7	936.1	935.3	28.85	0.713	<0.001
Potassium	1510	1468	1471	1508	36.2	0.758	<0.001
Sodium	597.1	531.4	601.3	576.3	37.46	0.383	0.009
Sulphur	667.3	650.1	669.2	667.6	48.87	0.991	0.136
Trace elements (µg/kg)							
Copper	119.0	261.4	186.8	209.8	98.02	0.780	0.526
Iron	852.3	942.3	756.7	1213.7	163.53	0.257	0.303
Iodine	140.3 [a]	37.6 [c]	70.7 [b]	140.2 [a]	22.06	<0.001	0.101
Manganese	70.3	51.4	50.4	58.0	9.19	0.413	0.067
Zinc	4275	3976	4197	4286	219.4	0.452	0.002
Macrominerals transfer efficiency (g in milk/100 g ingested)							
Calcium	20.3 [a]	17.5 [b]	18.7 [a,b]	19.4 [a,b]	1.04	0.020	<0.001
Magnesium	6.0 [a]	5.0 [c]	5.3 [b,c]	5.7 [a,b]	0.20	0.003	<0.001
Phosphorus	44.4 [a]	33.9 [c]	36.3 [b,c]	38.7 [b]	1.62	<0.001	<0.001
Potassium	9.8	9.0	9.1	9.7	0.38	0.137	<0.001
Sodium	51.7 [a]	47.1 [a,b]	45.1 [a,b]	38.4 [b]	2.70	0.018	0.002
Sulphur	29.6	23.3	24.7	26.4	2.01	0.143	0.071
Trace elements transfer efficiency (g in milk/100 g ingested)							
Copper	1.3	3.2	2.2	2.5	1.16	0.700	0.466
Iron	0.32	0.34	0.27	0.40	0.055	0.414	0.153
Iodine	15.5 [a]	4.3 [b]	7.2 [b]	14.2 [a]	2.17	<0.001	0.032
Manganese	0.22	0.15	0.15	0.18	0.027	0.194	0.019
Zinc	9.8	8.9	9.6	10.1	0.51	0.227	<0.001

[1] Significances were declared at $P < 0.05$. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test ($P < 0.05$)

5.4.3 Plasma mineral concentrations

The effect of dietary treatment did not influence the concentrations of any of the assessed minerals in blood plasma (Table 5.6).

5.5 Discussion

5.5.1 Effect of protein supplementation on milk mineral concentrations and mineral transfer efficiencies from feed to milk

Milk I concentrations were significantly lowered when fed rapeseed (RSS, RSAL) as opposed to diets that did not contain rapeseed (CON, ALG).

Table 5.5

Mineral composition of faeces. Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no microalgae, CON; 2550 g/day rapeseed supplement, RSS; 1280 g/day rapeseed supplement + 570g/day *S. platensis* supplement, RSAL; 1130g/day *S. platensis* supplement, ALG).

	Diet					ANOVA P-values[1]	
	CON	RSS	RSAL	ALG			
Minerals	n=8	n=8	n=8	n=8	SE	Diet	Period
Macrominerals (g/kg DM)							
Calcium	9.91 [c]	11.64 [b]	13.51 [a]	13.10	0.539	<0.001	0.006
Magnesium	3.73 [c]	4.95 [a,b]	5.23 [a]	4.73 [b]	0.080	<0.001	0.005
Phosphorus	4.61 [b]	6.42 [a]	5.81 [a]	5.24 [b]	0.221	<0.001	0.499
Potassium	9.47	9.61	8.04	8.12	0.792	0.275	0.837
Sodium	1.91	1.55	2.18	2.16	0.377	0.342	0.412
Sulphur	3.36 [b]	3.86 [a,b]	4.05 [a]	3.89 [a,b]	0.136	0.027	0.020
Trace elements (µg/kg DM unless indicated by [2])							
Cobalt	470.4 [b]	489.6 [b]	738.9 [a]	709.0 [a]	39.72	<0.001	0.010
Copper[2]	22.3 [b]	22.5 [b]	27.1 [a]	27.2 [a]	0.94	<0.001	0.039
Iron[2]	665.0 [d]	710.4 [c]	831.1 [b]	882.5 [a]	15.65	<0.001	0.033
Iodine	413.2 [b]	402.4 [b]	592.5 [a]	612.6 [a]	29.21	<0.001	0.045
Manganese[2]	81.8 [c]	96.1 [b]	101.8 [a]	96.7 [b]	2.50	<0.001	0.130
Molybdenum	1312 [b]	1566 [a]	1470 [a,b]	1487 [a,b]	63.6	0.008	<0.001
Zinc[2]	97.2 [c]	105.5 [b]	115.0 [a]	108.7 [b]	2.61	<0.001	<0.001
Macrominerals apparent digestibility (100 - (g in faeces/100 g ingested))							
Calcium	49.4 [a]	48.2 [a]	37.2 [b]	37.0 [b]	1.97	<0.001	0.007
Magnesium	43.7 [a]	37.9 [a,b]	30.3 [c]	34.0 [b,c]	1.93	<0.001	0.012
Phosphorus	33.7	32.1	34.0	37.7	2.64	0.335	0.827
Potassium	80.9	82.9	85.1	84.9	1.42	0.167	0.904
Sodium	48.2	60.9	50.4	58.0	8.94	0.437	0.422
Sulphur	53.9	59.2	54.6	54.7	1.99	0.247	0.001
Trace elements apparent digestibility (100 - (g in faeces/100 g ingested))							
Cobalt	46.5 [a]	46.8 [a]	21.4 [b]	26.1 [b]	3.85	<0.001	0.006
Copper	21.3 [a]	22.5 [a]	5.6 [b]	5.2 [b]	3.27	0.001	0.002
Iron	23.9 [a]	24.0 [a]	14.3 [b]	14.3 [b]	1.46	<0.001	<0.001
Iodine	85.8 [a]	86.4 [a]	80.8 [b]	81.2 [b]	1.02	0.001	0.018
Manganese	20.6 [a]	20.1 [a]	11.6 [b]	13.7 [a,b]	2.04	0.010	0.177
Zinc	31.1 [a]	31.6 [a]	22.6 [b]	25.1 [b]	1.51	0.001	<0.001

[1] Significances were declared at P < 0.05. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test (P < 0.05)

[2] Expressed in mg/kg DM

Given that there was found no difference between CON and ALG milks, it appears that the addition of microalgae did not affect I concentration. Rather, the main driver of I concentration in milk was the amount of rapeseed in the cows' diet. This appears to be a dose-dependent response, as was detected 37.6 μg/kg of I in RSS milk (from cows fed 2.6 kg of DM per day of rapeseed), and 70.7 μg/kg of I in RSAL milk (from cows fed 1.3 kg of DM per day of rapeseed). This decrease in milk I concentration with increasing rapeseed inclusion may be due to the glucosinolates found in rapeseed. Glucosinolates interfere with I metabolism in cows, causing I to be diverted to the kidneys rather than the mammary gland (Křížová et al., 2016). This is further reinforced by previous work (Franke et al., 2009) that reported a decrease in milk I concentration by half when rapeseed meal replaced distillers dried grains with solubles in cows' diets. The potential diversion of I toward the kidneys (rather than the mammary gland) is further supported by I transfer efficiency from feed to milk in the present study; for instance, 14.2 and 15.5% of dietary I appeared in milk for ALG and CON treatments, respectively, whereas there was detected a numerical dose-dependent response with only 4.3 and 7.2% of dietary I appearing in milk for RSS and RSAL treatments, respectively. Although milk I concentrations can vary widely based on dairy management practices, breed, teat dipping solutions, and diet composition (Flachowsky et al., 2014), the concentrations of 70.7 μg/kg, and furthermore 37.6 μg/kg (from cows fed rapeseed), are below the previously documented average I concentration of 150 μg/L in Finnish cow milk (Nyström et al., 2016). However, milk from the cows that did not consume rapeseed had similar values to those previously reported (Flachowsky et al., 2014; Nyström et al., 2016; Qin et al., 2021). Generally, it is recommended that when rapeseed is fed to dairy cows, additional I supplementation is to be provided, to reduce the risk of deteriorating milk nutritional quality by lowering I concentration (Flachowsky et al., 2014).

Table 5.6

Mineral composition of blood plasma. Means, standard error (SE) and ANOVA P-values for the effect of the dietary treatment (Control, no microalgae, CON; 2550 g/day rapeseed supplement, RSS; 1280 g/day rapeseed supplement + 570g/day *S. platensis* supplement, RSAL; 1130g/day *S. platensis* supplement, ALG).

	Diet					ANOVA P-values[1]	
	CON	RSS	RSAL	ALG			
Minerals	n=8	n=8	n=8	n=8	SE	Diet	Period
Macrominerals (mg/L)							
Calcium	114.1	122.5	117.6	117.2	3.84	0.486	0.300
Magnesium	25.5	27.4	25.9	25.6	0.90	0.270	0.264
Phosphorus	195.6	214.3	203.2	197.4	11.20	0.499	0.434
Potassium	215.4	240.5	218.5	219.2	8.34	0.127	0.421
Sodium	3940	4271	3999	3993	147.7	0.388	0.299
Sulphur	1390	1538	1435	1438	56.4	0.338	0.222
Trace elements (µg/L)							
Copper	903.4	961.6	920.9	962.5	70.54	0.711	0.630
Iron	2038	2010	1891	1949	163.6	0.883	0.459
Iodine	78.4	92.8	113.1	95.3	9.12	0.087	0.025
Manganese	2.74	2.30	3.40	2.75	0.441	0.387	0.117
Selenium	80.5	90.1	86.9	87.4	4.06	0.380	0.418
Zinc	834.0	871.7	876.9	857.0	49.41	0.812	0.634

[1] Significances were declared at P < 0.05. Means for diet treatment within a row with different letters are significantly different according to Fisher's Least Significant Difference test (P < 0.05)

In the present study, iodine was included in the mineral-vitamin supplement at 70 mg/kg (Table 5.1), and the whole diet of all experimental treatments contained approximately 1.0 to 1.1 mg of I per kg of DM (Table 5.3). Given that the European Food Safety Authority has maintained an upper level of I inclusion in dairy cows' diets of 5 mg of I per kilogram of feed DM (EFSA, 2013), the I supply in the present study was approximately 20% of the maximum recommendation, thus further contributing to the low I concentration of milk of RSS and RSAL in addition to the effect of rapeseed. Other than I, there was found no other statistically significant effects of diet on milk mineral concentrations, and, to the best of our knowledge, no other published studies have investigated the effect of feeding S. platensis to dairy cattle on milk mineral concentrations. In addition, this is the first study to present the effect of feeding rapeseed to dairy cattle on the wider milk mineral profile. Previous work has assessed the effect of feeding fava bean and rapeseed meal on the concentrations of Ca, P, and Mg in skim milk, and found these were not affected by their inclusion in the diet (Poulsen et al., 2021), which aligns with the effect of rapeseed and microalgae in the present study.

5.5.2 Effect of protein supplementation on faecal mineral concentrations and apparent digestibility

There was detected a significant effect of dietary microalgae on faecal I concentration, as RSAL and ALG treatments had increased faecal I compared with the CON and RSS treatments. This, however, did not appear to be a dose-dependent response, as there was only a 3.4% difference in faecal I concentration between the 2 treatments fed microalgae (RSAL and ALG), whereas the algae inclusion in the diet was 98% higher in the ALG than RSAL treatment. However, the extent of the difference on excretion, from 413.2 µg/kg of DM to 612.6 µg/kg of DM faecal I, may not be explained from the 2,000 µg/d difference in I intake between CON and ALG, and may also relate to a reduced apparent digestibility of I (81% in CON, 86% in ALG). A possible explanation that further adds to the reduced I apparent digestibility when algae is fed is that the I accumulation takes place in a form that is not as readily bioavailable in the gut and, therefore, a larger proportion of it ends up undigested in faeces (Iwamoto and Shiraiwa, 2012; Han et al., 2016). Faecal I output in cattle could be higher with high I intakes and when I is already sufficient in the diet (Hemken, 1970; Franke, 2009). Interestingly, the same pattern has been observed for several macrominerals (Ca, Mg) and trace elements (Co, Cu, Fe, Mn, Zn), where apparent digestibility was lower when cows were fed microalgae. Microalgal interactions with dietary minerals have been previously suggested for S. platensis in cows' diets, which resulted in reduced plasma Fe concentration due to a possible chelation with phycocyanin, a pigment-protein complex found in *Spirulina* (Bermejo et al., 2008; Suliburska et al., 2016). However, this reduction in plasma Fe concentration was not found, and increased excretion in macrominerals in the current trial did not influence the transfer into milk and subsequently milk nutritional quality.

5.5.3 Effect of protein supplementation on mineral concentrations of bovine plasma

The present study indicated that feed protein source (rapeseed, rapeseed and microalgae mix, and microalgae) had no significant effect on cow blood mineral concentrations. Despite the significant effect on milk I concentrations, there was found only a tendency ($P = 0.087$) for reduced plasma I concentrations (average 78.4 to 113.1 µg/L) when rapeseed was fed. Research on the effect of diet on total plasma I concentration is scarce, but it has been reported that total plasma I can be affected by several factors such as blood composition, BW, breed, analysis method, and timing of parturition (Tong et al., 1986; Dillon et al., 2003). Previous studies (Sorge et al., 2016) reported a range of 305 to 311 µg/L in cow serum I concentration when TMR was fed, values which are substantially higher than those observed in the present study. This may be due to their use of a TMR of various kinds of hay, corn, and cottonseed (*Gossypium hirsutum*), which resulted in a dietary I concentration of 2.17 g/kg of DM, which is double the average I concentration (1.09 g/kg of DM) of all experimental diets in the present study. In the same previous study (Sorge et al., 2016), the mean dietary I intake was 62.5 mg/d for the cows in the control group, whereas cows in the present study ingested, on average, 24.8 mg/d across all experimental treatments. Overall, it could be concluded that rapeseed, microalgae supplementation, or both in dairy cow diets does not affect blood plasma mineral concentrations, including that of I.

5.5.4 Nutritional implications of milk from microalgae-fed cows for consumers

According to the Finnish Institute for Health and Welfare National FinDiet 2017 Survey (Kaartinen et al., 2020) the intakes of all types of liquid milk by men in the Finnish population were 319, 233, and 193 g/d in the age groups of 18 to 44, 45 to 64, and 65 to 74 years, respectively; additionally, intake by women was 183, 165, and 145 g/d in the age groups of 18 to 44, 45 to 64, and 65 to 74 years, respectively. The 2012 Nordic Nutritional Recommendations present a recommended nutritional intake (RNI) for I as 150, 175, and 200 µg/d for adults (18+ years of age), pregnant women, and nursing women, respectively (NNR, 2014). Therefore, based on the recorded liquid milk intakes from the FinDiet 2017 Survey and the milk I concentrations in the present study, CON and ALG milk would contribute (expressed as % RNI) (1) 30, 22, and 18% in men of age groups 18 to 44, 45 to 64, and 65 to 74 years, respectively, (2) 17, 15, and 14% in women of age groups 18 to 44, 45 to 64, and 65 to 74 years, respectively, (3) 15% in pregnant women, and (4) 13% in nursing women. In addition, even for the demographic with the highest milk intake, contribution of the experimental milk to the I upper tolerable limit (600 µg/d; NNR, 2014) if consumed would be marginal; someone would need to ingest substantially large amounts of CON or ALG milk (approximately 4.17 L per day) to reach the upper tolerable limit, and hence there are no public health safety concerns around excessive I consumption via milk from cows consuming microalgae. Previous work in Finland (Nyström et al., 2016) reported that milk is a major source of I in the Finnish population; the adult population of Finland has also been classified as mildly I deficient. Previous work in Finland has suggested that goitre issues were frequent due to I deficiency 50 years ago, and iodized table salt and cattle feed (for increased milk I) has been used to attempt to remedy this issue (Elorinne et al., 2016). However, CON and ALG milk, produced in the present study, would provide less than 45 µg per day and contribute less than 30% of the recommended I intake (under the current milk intake levels). If such levels also appear within retail products, strategies to enhance milk I concentrations could be recommended, and the intake of I-rich foods could be increased (such as more milk and dairy, certain fish and seafood, or I-fortified foods; Bouga et al., 2018), with special considerations for pregnant and nursing women, as this could reduce the occurrence of I deficiency in the Finnish population. As previously discussed, the feeding of rapeseed to cows decreases milk concentrations of I, as there was found a significant difference between milk from cows fed rapeseed (RSAL, RSS) or diets containing no rapeseed (CON, ALG). By performing similar calculations (as above) regarding the contribution of RSAL and RSS milks to the RNI for I in the Finnish population, this would be (expressed as % RNI) (i) 9 to 15% along with 5 to 8% in men ages 18 to 44, 45 to 64, and 65 to 74, respectively, (ii) 7 to 9% along with 4 to 5% in women ages 18 to 44, 45 to 64, and 65 to 74, respectively, (iii) 7 and 4% in pregnant women, respectively, and (iv) 6 and 3% in nursing mothers, respectively. Therefore, given that these values are substantially lower compared with milk from cows not consuming rapeseed, and that milk I concentration is strongly correlated with dietary I intake (Flachowsky et al., 2014), supplementing additional I in cow diets that contain rapeseed can be recommended as a potential way to mitigate the negative effect of rapeseed on milk I concentrations and the undesirable implications for consumer I intake.

5.6 Conclusions

Microalgal (*Spirulina platensis*) inclusion in dairy cow diets did not affect milk and plasma concentrations of macrominerals and trace elements. Rapeseed inclusion in cow diets did not affect blood plasma mineral concentrations but reduced milk I concentrations, an effect which may be associated with glucosinolates, which are known to reduce dietary I availability to the mammary gland. Based on Finnish population milk intakes and nutritional guidelines, the contribution of milk for I supply of the different population demographics could be reduced by approximately 25 to 50% when rapeseed partially or wholly substitutes other protein feeds. This may increase the need for higher I supply from either consuming more milk and dairy, or increasing the intake of other diet sources (fish, shellfish, I-fortified foods), especially in consumers with higher I requirements (pregnant and nursing women). This study showed that feeding microalgae to dairy cows maintained mineral concentrations in cows' blood and the milk produced. However, supplemental dietary I is recommended when rapeseed is fed to dairy cows, to prevent subsequent production of milk with lower I concentrations.

5.7 Information

Author Contributions: Eric E. Newton: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization; Marjukka Lamminen: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—review and editing, project administration; Partha Ray: writing—review and editing, methodology, supervision; Alexander M. Mackenzie: methodology, validation, formal analysis, investigation, resources, data curation, writing—review and editing; Christopher K. Reynolds: writing—review and editing, supervision; Michael R. F. Lee: resources, writing—review and editing, project administration; Anni Halmemies-Beauchet-Filleau: resources, methodology, writing—review and editing, project administration; Aila Vanhatalo: conceptualization, methodology, resources, writing—original draft, writing—review and editing, supervision, project administration, funding acquisition; Sokratis Stergiadis: conceptualization, methodology, resources, data curation, writing—original draft, writing—review and editing, supervision, project administration. All authors reviewed and approved the manuscript.

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Conflicts of Interest: The authors declare that no conflict of interest.

5.8 References

Babiker, A., A. Alawi, M. Al Atawi, and I. Al Alwan. 2020. The role of micronutrients in thyroid dysfunction. Sudan J Paediatr 20(1):13-19.

Bermejo, P., E. Piñero, and Á. M. Villar. 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chemistry 110(2):436-445.

Bertinato, J. 2021. Iodine nutrition: Disorders, monitoring and policies. Advances in food and nutrition research 96:365-415.

Biban, B. G. and C. Lichiardopol. 2017. Iodine Deficiency, Still a Global Problem? Curr Health Sci J 43(2):103-111.

Bouga, M., M. E. J. Lean, and E. Combet. 2018. Contemporary challenges to iodine status and nutrition: the role of foods, dietary recommendations, fortification and supplementation. Proceedings of the Nutrition Society 77(3):302-313.

BSI. 2017. BS EN 17050:2017. Animal feeding stuffs: Methods of sampling and analysis. Determination of iodine in animal feed by ICP-MS. Page 18. The British Standards Institution, United Kingdom.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J Dairy Sci 92(5):2128-2135.

Cuellar-Bermúdez, S. P., B. Barba-Davila, S. O. Serna-Saldivar, R. Parra-Saldivar, J. Rodriguez-Rodriguez, S. Morales-Davila, K. Goiris, K. Muylaert, and C. Chuck-Hernández. 2017. Deodorization of Arthrospira platensis biomass for further scale-up food applications. Journal of the Science of Food and Agriculture 97(15):5123-5130.

de Benoist, B., M. Andersson, B. Takkouche, and I. Egli. 2003. Prevalence of iodine deficiency worldwide. The Lancet 362(9398):1859-1860.

Dębowski, M., M. Zieliński, J. Kazimierowicz, N. Kujawska, and S. Talbierz. 2020. Microalgae Cultivation Technologies as an Opportunity for Bioenergetic System Development—Advantages and Limitations. Sustainability 12(23).

Dillon, P., F. Buckley, P. O'Connor, D. Hegarty, and M. Rath. 2003. A comparison of different dairy cow breeds on a seasonal grass-based system of milk production: 1. Milk production, live weight, body condition score and DM intake. Livestock Production Science 83(1):21-33.

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.

Elorinne, A. L., G. Alfthan, I. Erlund, H. Kivimäki, A. Paju, I. Salminen, U. Turpeinen, S. Voutilainen, and J. Laakso. 2016. Food and Nutrient Intake and Nutritional Status of Finnish Vegans and Non-Vegetarians. PloS one 11(2):e0148235.

FAO. 2021. Seaweeds and Microalgae: An Overview for Unlocking Their Potential in Global Aquaculture Development.
Flachowsky, G., K. Franke, U. Meyer, M. Leiterer, and F. Schöne. 2014. Influencing factors on iodine content of cow milk. European journal of nutrition 53(2):351-365.
Franke, K. 2009. Effect of various iodine supplementations and species on the iodine transfer into milk and on serum, urinary and faecal iodine of dairy cows fed rations varying in the glucosinolate content.
Franke, K., U. Meyer, H. Wagner, and G. Flachowsky. 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.
Halmemies-Beauchet-Filleau, A., M. Rinne, M. Lamminen, C. Mapato, T. Ampapon, M. Wanapat, and A. Vanhatalo. 2018. Review: Alternative and novel feeds for ruminants: Nutritive value, product quality and environmental aspects. animal 12:1-15.
Han, W., W. Clarke, and S. Pratt. 2016. Cycling of iodine by microalgae: Iodine uptake and release by a microalgae biofilm in a groundwater holding pond. Ecological Engineering 94:286-294.
Haug, A., A. T. Høstmark, and O. M. Harstad. 2007. Bovine milk in human nutrition--a review. Lipids Health Dis 6:25-25.
Hemken, R. W. 1970. Iodine1. Journal of Dairy Science 53(8):1138-1143.
Iwamoto, K. and Y. Shiraiwa. 2012. Characterization of Intracellular Iodine Accumulation by Iodine-Tolerant Microalgae. Procedia Environmental Sciences 15:34–42.
Kaartinen, N., H. Tapanainen, H. Reinivuo, H. Pakkala, S. Aalto, S. Raulio, S. Männistö, T. Korhonen, S. Virtanen, K. Borodulin, S. Koskinen, and L. Valsta. 2020. The Finnish National Dietary Survey in Adults and Elderly (FinDiet 2017): Finnish Institute for Health and Welfare, Finland. EFSA Supporting Publications 17.
Křížová, Z., Trávníček, Jan, R. Konecný, J. Hladký, L. HaspHová, and R. Kala. 2016. The effect of feeding extracted rapeseed meal on the content of iodine in milk, urine and blood plasma in dairy cows.
Lamminen, M., A. Halmemies-Beauchet-Filleau, T. Kokkonen, S. Jaakkola, and A. Vanhatalo. 2019a. Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. Animal Feed Science and Technology 247:112-126.
Lamminen, M., A. Halmemies-Beauchet-Filleau, T. Kokkonen, I. Simpura, S. Jaakkola, and A. Vanhatalo. 2017. Comparison of microalgae and rapeseed meal as supplementary protein in the grass silage based nutrition of dairy cows. Animal Feed Science and Technology 234:295-311.
Lamminen, M., A. Halmemies-Beauchet-Filleau, T. Kokkonen, A. Vanhatalo, and S. Jaakkola. 2019b. The effect of partial substitution of rapeseed meal and faba beans by *Spirulina platensis* microalgae on milk production, nitrogen utilization, and amino acid metabolism of lactating dairy cows. Journal of dairy science 102.

Lum, K. K., J. Kim, and X. G. Lei. 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. Journal of Animal Science and Biotechnology 4(1):53.
McCaughern, J. H., A. M. Mackenzie, and L. A. Sinclair. 2020. Dietary starch concentration alters reticular pH, hepatic copper concentration, and performance in lactating Holstein-Friesian dairy cows receiving added dietary sulfur and molybdenum. Journal of Dairy Science 103(10):9024-9036.
Minitab. 2019. Minitab 18: Session Commands. Vol. 2022. Minitab Inc.
Newton, E. E., Á. H. Pétursdóttir, G. Ríkharðsson, C. Beaumal, N. Desnica, K. Giannakopoulou, D. Juniper, P. Ray, and S. Stergiadis. 2021. Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations. Foods 10(7).
NNR. 2012. Nordic Nutrition Recommendations 2012.
Nyström, H. F., A. L. Brantsæter, I. Erlund, I. Gunnarsdottir, L. Hulthén, P. Laurberg, I. Mattisson, L. B. Rasmussen, S. Virtanen, and H. M. Meltzer. 2016. Iodine status in the Nordic countries - past and present. Food & nutrition research 60:31969-31969.
Petroski, W. and D. M. Minich. 2020. Is There Such a Thing as "Anti-Nutrients"? A Narrative Review of Perceived Problematic Plant Compounds. Nutrients 12(10).
Poulsen, N. A., G. Giagnoni, M. Johansen, P. Lund, and L. B. Larsen. 2021. Effect of protein concentrate mixtures and dietary addition of exogenous phytase on major milk minerals and proteins, including casein phosphorylation. J Dairy Sci 104(9):9801-9812.
Puhakka, L., S. Jaakkola, I. Simpura, T. Kokkonen, and A. Vanhatalo. 2016. Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage–based diets. Journal of Dairy Science 99(10):7993-8006.
Qin, N., G. Faludi, S. Beauclercq, J. Pitt, N. Desnica, Á. Pétursdóttir, E. E. Newton, A. Angelidis, I. Givens, D. Juniper, D. Humphries, H. Gunnlaugsdóttir, and S. Stergiadis. 2021. Macromineral and trace element concentrations and their seasonal variation in milk from organic and conventional dairy herds. Food Chemistry 359:129865.
Seghiri, R., M. Kharbach, and A. Essamri. 2019. Functional Composition, Nutritional Properties, and Biological Activities of Moroccan *Spirulina* Microalga. Journal of Food Quality 2019:3707219.
Sorge, U. S., M. Henriksen, A. Bastan, N. Cremers, K. Olsen, and B. A. Crooker. 2016. Short communication: Iodine concentrations in serum, milk, and tears after feeding *Ascophyllum nodosum* to dairy cows—A pilot study. Journal of dairy science 99(10):8472-8476.
Stergiadis, S., N. Qin, G. Faludi, S. Beauclercq, J. Pitt, N. Desnica, Á. H. Pétursdóttir, E. E. Newton, A. E. Angelidis, I. Givens, D. J. Humphries, H. Gunnlaugsdóttir, and D. T. Juniper. 2021. Mineral concentrations in bovine milk from farms with con-trasting grazing management. Foods In press.
Suliburska, J., M. Szulińska, A. A. Tinkov, and P. Bogdański. 2016. Effect of *Spirulina maxima* Supplementation on Calcium, Magnesium, Iron, and Zinc Status in Obese Patients with Treated Hypertension. Biol Trace Elem Res 173(1):1-6.

Thorning, T., A. Raben, T. Tholstrup, S. Soedamah-Muthu, I. Givens, and A. Astrup. 2016. Milk and dairy products: Good or bad for human health? An assessment of the totality of scientific evidence. Food & Nutrition Research 60.

Tong, A., H. Doornenbal, and J. Newman. 1986. Blood composition of different beef breed types. Canadian Journal of Animal Science - CAN J ANIM SCI 66:915-924.

Ullmann, J. and D. Grimm. 2021. Algae and their potential for a future bioeconomy, landless food production, and the socio-economic impact of an algae industry. Organic Agriculture.

Vale, M. A., A. Ferreira, J. C. M. Pires, and A. L. Gonçalves. 2020. Chapter 17 - CO2 capture using microalgae. Pages 381-405 in Advances in Carbon Capture. M. R. Rahimpour, M. Farsi, and M. A. Makarem, ed. Woodhead Publishing.

van der Reijden, O., M. Zimmermann, and V. Galetti. 2017. Iodine in dairy milk: Sources, concentrations and importance to human health. Best Practice & Research Clinical Endocrinology & Metabolism 31.

Van Emon, M. L., D. D. Loy, and S. L. Hansen. 2015. Determining the preference, in vitro digestibility, in situ disappearance, and grower period performance of steers fed a novel algae meal derived from heterotrophic microalgae. J Anim Sci 93(6):3121-3129.

Yu, S., Y. Yin, Q. Cheng, J. Han, X. Cheng, Y. Guo, D. Sun, S. Xie, and L. Qiu. 2018. Validation of a simple inductively coupled plasma mass spectrometry method for detecting urine and serum iodine and evaluation of iodine status of pregnant women in Beijing. Scandinavian journal of clinical and laboratory investigation 78(6):501-507.

Chapter 6: General Discussion & Overall Conclusions

Below will be mentioned the various papers under their acronym that was used during my research. This is to provide a quick reference for each paper.

SPAN: "Effect of Macroalgal Supplementation on Milk Quality"

ICEL: "Effect of Dietary Seaweed Supplementation in Cows on Milk Macrominerals, Trace Elements and Heavy Metal Concentrations"

OMIC: "Effect of Dietary Seaweed Supplementation on Icelandic Cow Milk Microbiota Composition"

FINL: "Effect of Dietary Microalgae Supplementation on Finnish Ayrshire Cow Mineral Use Efficiency and Milk Nutritional Profile"

6.1 Logistical viability of phycological matter as a feed stuff for dairy cows

As was seen in FINL, a common forage, rapeseed, can be found to be implemented across Europe as a regular feed. Unfortunately, rapeseed has been found to be goitrogenic and reduces the transfer efficiency of iodine from feed to milk (Trøan et al., 2015). This results in a lower concentration of iodine in the milk, and thus not as nutritionally competitive product. Additionally, this effect has been observed to be consistent across different basal diets, typical of those used in European dairy systems (Franke et al., 2009; Trøan et al., 2015; van der Reijden et al., 2019). Therefore, within niche situations, seaweed, which often contains large amounts of iodine to counteract the goitrogenic effect while supporting the sustainable production of seaweed (Newton et al., 2021). In addition, a more sustainable option, microalgae has been shown to have no effect on milk mineral efficiency, and thus can be used as not just a supplement as is the case for seaweed, but for protein replacement (Newton et al., 2022). Sustainable being that the growth of algae can either be, in the case of seaweeds, brought in from shore and grown in areas that do not require land mass needed by terrestrial plants, or microalgae, commonly grown in circuit ponds, typically built upon non-arable land, therefore bringing aquaculture to non-utilisable land.

Based on my work thus far, the feeding of phycological matter to dairy cows seems to have strong viability in terms of improving nutritional concentrations within the resulting milk similar to many publications, Antaya et al. (2015) which saw increased iodine concentrations, and Rey-Crespo et al. (2014). In practice, this would mean that macroalgae could be used as an additive to a preexisting diet, and microalgae could be used to replace protein within a dairy cow's diet, such as soybean meal as previously discussed in Chapter 1. That being said, the logistical viability is tenuous at best. Issues concerning logistical viability are grounded in, generally, three different concepts - adoption, supply, and control.

While algae supplementation of dairy cow feed can improve nutritional or microbiological quality, the issue with adoption is a massive issue. Due to, understandably, large scale regulation and the existence of a disconnect between primary producers and research – phycological matter implementation into livestock feed most likely will be incredibly slow (Kinnby et al., 2021). This body of work aims to set the stage for increased investment within quality assessment of alternative foodstuff implementation and the resulting product, but each community, region, or country asks for different results to meet the needs of public nutrition and the health system or situation at the time (Bath et al., 2022). Not only this, but forage and concentrates differ wildly across these same areas, and this can have knock-on effects with the type and amount of algae that are delivered to the animal (Kotowski et al., 2023). Total replacement would be especially difficult, given the vast array of different types of feed, let alone overall agricultural practices. Therefore, in implementing phycological matter as an additive or protein replacement, nutritional studies validating each component would be required (Manzocchi et al., 2020). While it is understandable that progress may be slow, the adoption of algae supplementation into primary track production of milk and dairy products should be encouraged.

As for supply, as stated in the Chapter 1: Introduction, Europe represents 0.8% of world production of seaweed, and this being centralized in one country, Norway (FAO, 2021a). The viability of feeding just the seaweed that was examined within this thesis, or even what is currently produced is logistically nonviable. That being said, increased research towards understanding physiological changes and the resulting milk compositional variance when dairy cows' diets are replaced or supplemented with phycological matter enables scientists to pinpoint select species for cultivation and investment (Newton. et al., 2023). Therefore, while production within Europe may be small, this work serves to examine the knock-on effects of phycological supplementation of dairy cow diets to encourage greater interest, investment, and a future to the algae industry within Europe. This provides insight as to the limitations of my animal trials and studies – as a broad overview was provided resulting from multiple types of seaweed and a microalgal feed, different breeds of cows, different forages, different amounts, etc. While it is justifiably structured to provide a foundation for ongoing and future research, the exact physiological mechanisms for which responses were seen are not necessarily explainable at the molecular level.

Finally, concerning control, as seen in ICEL, SPAN, and FINL – livestock agriculture can be messy in terms of precision implementation of interventions (Newton. et al., 2023). While inputs can be/are measurable, output is a result of complex physiological and chemical reactions within a living animal. Due to the wealth of factors that may affect the point of consumption, providing a certain percentage of a diet as being replaced, or a certain amount to be supplemented and delivered to the dairy cow tends to be non-exact. This poses a problem when it comes to dairy quality, as milk composition and quality factors such as microbial or mineral concentration amounts can/are influenced by minute changes, resulting in radically different outcomes (Jenkins & McGuire, 2006). Additionally, aspects of palatability has been the subject of some study when it comes to phycological consumption (Lamminen et al., 2017). Generally, as seaweed was an added supplement, there were no palatability issues, and for microalgae, which was more of a protein replacement, the cows were provided with a full TMR and within this ration was mollassed ingredients. Therefore, from studies in this thesis and beyond, palatability seems to not necessarily be a problem at the levels at which cows' diets were supplemented. That being said, if commercial farms were to provide this product, either at higher levels, different breeds, different species, etc., – there should be assumed a potential palatability issue at certain points which require additional research. This would obviously prevent the delivery of exact amounts and would result in a differing product that what was seen within experimental animal trials. As seen from the work that forms the entire thesis, regulation of exact amounts of algae delivered to each cow was variable, and this precision cannot be expected from industrial dairy farms, large or small. Therefore, this work aims to provide a window at which algae can be implemented into the diet to where it is safe, but also flexible, while also providing what is needed and/or expected from dairy products.

6.2 Potential implications for the dairy supply chain

Key messages have arisen from my work across these 4 works that should be investigated further and taken into consideration by those who come after me, legislators, consumers, processors, producers, every part of the dairy food chain. Milk and dairy products continue to serve as a linchpin to European nutritional food security, and this serves as a very accessible way by which legislators can positively influence public health outcomes. With the reliance of milk and dairy products to provide for a large portion of individual's numerous mineral needs such as I, Ca, Zn, Mo, etc., increased investment in pre-farmgate food chain nutrition is crucial. Phycological markets (both macro- and microalgal) are rapidly increasing in size and profit potential, serving as a niche for the European workforce. This work has shown a maintenance or even increase in the nutritional content of milk from cows fed algae, and an investment by legislators to encourage this sector would lead to job creation, increased select markets to carve a niche among international competitors, increased nutritional security for those that consume the milk, and thus, increased health outcomes for the populace. Legislators need to incentivise processors or reward the nutritional content of milk, especially towards directions where a standard nutritional content would require preservation at critical management conditions (e.g. maintain an adequate milk iodine content during the grazing season). Within the current system, by-and-large, farmers are encouraged to only consider yield along with fat and protein concentrations within the dairy, as this is what modulates the price they are sold for. Subsidization of processors with the aim of increased nutrition within product would encourage increased research within food chain nutrition, and lead to increased niche markets to support smallholder farms. This investment in the future would support public nutrition and health, maintenance of grassroots farming, and could possibly decrease the contribution that agriculture has towards GHG emissions (in case that seaweeds with methane mitigation potential are used). However, at farm level extreme care should be taken when seaweed is supplemented to dairy cow diets because even small amounts of I-rich seaweed can not only exceed I allowances in dairy cow diets, but also drastically increase milk I concentrations and potentially pose a nutritional risk to the consumers.

6.2.1 Milk production, basic composition, and efficiencies

In the studies that examined DMI, ICEL, SPAN, and the preceding paper to FINL, there was no change in DMI when the experimental groups were supplemented with phycological matter. Therefore, there is no indication of palatability issues with the consumption of either as much as 158g/cow/day of 91:9 (DM basis) *A. nodosum:L. digitata*, 330g/cow/day of *A. nodosum*, and 1130 g/cow/day of *S. platensis* respectively. As for the effects of this phycological matter on basic milk composition there was found in SPAN feeding 300g/cow/day of *A. nodosum* to significantly reduce milk protein, and in ICEL a reduction in milk protein and casein. This change was however negligible, as the protein reduction in SPAN was from 3.38% to 3.32%, and ICEL was reduced from 3.33 to 3.20% and 2.43 - 2.33% for protein and casein respectively. That said, while the trial did experience tendencies ($0.05 < P < 0.10$) between control and experimental diets for SPAN (reduction of protein efficiency from 44.2 to 42.8g in milk/kg of DMI and ECMY efficiency from 1.30 to 1.27 ECMY/kg of DMI) and ICEL (increase in milk yield from 25.3 to 26.5kg/d), within these two animal trials that measured such factors, these were relatively small. This is in contrast to Singh et al. (2017) in that it is reported an increase in milk yield, but a number of differences exist between this body of work and this study, and to name a few the large difference in the degree of supplementation at 955g/cow/day, environment, and difference in seaweed species used. Therefore, from this body of work, there was not shown any significant contraindications to milk production and solids concentrations when feeding *A. nodosum* and a *A. nodosum* and *L. digitata* mix to cows, at rates of 330g/cow/day and 158g/cow/day, to Holstein dairy and Icelandic cows, respectively.

6.2.2 Animal mineral status

Given that an phycological feed supplement must be non-harmful to the animal before use, SPAN measured hematological parameters in cows at the end of the feeding trial. There was found no changes in the hematological factors (accounting for WBC, neutrophils, lymphocytes, monocytes, eosinophils, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC, RDW-CV, PLT, and MPV which can be indicators of infectious and inflammatory diseases, clinical anaemia, renal insufficiency, myeloproliferative disorders, hyperthyroidism, and mineral deficiencies to name a few (Roland, Drillich, & Iwersen, 2014)). Therefore, one can reason that there are no negative animal health implications by feeding 330g/d of *A. nodosum* to Holstein dairy cows, which are associated with these blood compositional indicators. It should be noted that even at feeding within a scientific experimental setting, under typical recommended commercial applications, the amount of I allowed in dairy cows' diet (5 mg/kg DM; (NRC, 2001; Woodside & Mullan, 2021)) was exceeded in both SPAN and ICEL.

While not exceeding the upper tolerable limit (50 mg/kg DM; Butler, Curran, and Gleeson (2017)), this highlights the requirement that for safe implementation of seaweed in dairy cows' diets, the I concentrations of the seaweed and the total diet must be taken into consideration in order to avoid feeding I beyond the legal allowances. Additionally, in FINL there was indicated what can be explained as the known potential goitrogenic effect of rapeseed, therefore it also should be noted that the supplementation of animals must not be one-dimensional in terms of input and expected transfer/eventual output of minerals, but account for other parameters (in this case recommend higher dietary I concentrations when rapeseed is part of the diet) because mineral recovery from feed to milk is complicated and affected by the basal diet, environment, animal physiology, elemental speciation, etc. and requires a holistic multidimensional approach. If one were to seek for I concentrations within milk to be maximized, then this would call for increased feeding of I relative to considerations of what the diet is composed of.

6.2.3 Milk mineral concentrations and efficiencies

Perhaps the largest contribution of this body of work involves the fate of minerals, especially I, from feed to milk. SPAN saw an increase in the milk mineral concentrations of Mg, P, and I much in line with Antaya et al. (2015) and Rey-Crespo et al. (2014). While the differences in Mg and P were relatively minimal, the increase in I was substantial, rising from 166 µg/kg to 1886µg/kg, when 330 g/d day of *A. nodosum* was fed. Additionally, there was a significant diet-related mineral transfer efficiency reduction in the group wherein seaweed was supplemented for minerals Ca (24.6 to 22.9%), Mg (5.0 to 4.6%), P (36.1 to 34.1%), K (12.0 to 10.9%), Na (30.2 to 32.2%), Mn (0.13 to 0.05%) and Zn (17.0 to 7.8%) with transfer efficiency increased in a group wherein seaweed was supplemented for Mo (4.46% to 5.25%). Despite these significant differences in mineral transfer efficiencies between diets, the numeral differences were not large and would not necessarily suggest a strong enough effect in that it noticeably increases milk concentrations, except for Zn which underwent a 9.2% change which would be considerable coming from high-yielding cows. This was seen again in ICEL when a macroalgal mix was introduced, wherein dose-dependently Cu and Se were reduced in the seaweed groups of increasing amounts, but increased in As and most importantly I, rising from 822µg/kg to 2471µg/kg. While SPAN fed more seaweed (330g/d *A. nodosum*) compared to ICEL (max 158g/d 91:9 (*A. nodosum:L. digitata*)), this was not reflected in the amount of I that was found within the resulting milk. The control group of ICEL exhibited quite high I concentrations, averaging 822µg/kg, therefore it may be concluded that the basal diet (with consideration for environmental mineral saturation of forage/water) and breed of cow, to name a few factors, could have resulted in this. These results reinforce the need to measure I concentrations from each phycological supplemental intervention, as well as the basal diet, in order to prevent over-supply of iodine and excessive amounts into milk. FINL fed *S. platensis*, which is a micro- rather than macro- algae, and interestingly saw no difference in milk I concentrations compared to the control group, but showed drastically reduced I concentrations within rapeseed supplemented cows – as previously has been observed in other studies due to the goitrogenic nature of rapeseed which can cause thyroidal disfunction leading to a diversion of I from the thyroid and towards the kidneys to be excreted via urine (Trøan et al., 2015). This body of work shows care should be taken when dairy cows are fed macroalgal supplementation because this can cause significantly increased concentrations of I within the milk, but this is not a requirement when microalgal is fed. Additionally, agrifood scientists should consider the entire feeding regime when it comes to delivering dairy product which serves as a basis for I consumption for a large proportion of the world, as to not reduce I too much when dairy cows are fed rapeseed or other goitrogen-containing feeds. Finally, standard mineral transfer efficiencies are shown in this body of work, allowing for the basis of any sort of research further on to build upon this foundation towards even more precise nutritional planning for increased populational nutrition from the consumption of dairy milk.

6.2.4 Milk bacteriological parameters

Given that there was shown a theme of I concentrations in milk being extremely important as a result of the animal trials of SPAN, ICEL, and FINL – a step further was taken to understand if the amount of minerals, specifically I, would have a significant effect on microbiological parameters within the milk. This is due to the fact that I is a known bacteriostatic/cidal element (dependent on concentrations) (Aftab et al., 2023). The specific parameters of relative abundance, alpha diversity, and the spearman correlational values of SCC and milk I concentrations and bacterial populations were analysed. There are, at present, limited resources regarding the effect that feeding phycological supplement to animals will have on the microbiological profile of the resulting milk. This would have large implications in terms of microbiological parameters for food safety, but also for technological advantages such as processes that rely upon relative abundances of particular bacteria such as cheese, yogurt, and other dairy products. There was found a theme of high I amounts within the milk matrix decreasing the relative abundance of the largest proportional groups of bacteria (Genus *Pseudomonas*), and increasing the abundance of smaller representatives classified as “other”. There was also found to be a corresponding decrease in certain taxonomic groups that are home to milk-relevant pathogens (Genera of *Stenotrophomonas* and *Brevundimonas*). Therefore, while preliminary, I would encourage and hope to aim towards building upon this foundation – spiking samples and understanding the value-addition that phycological supplementation has for milk bacteriological quality.

6.2.5 Populational mineral intakes

To capture the real-world applicatory implications that this research would have for milk consumers– in each of the animal trials and papers of SPAN, ICEL, and FINL – I nutritionally modelled the effect that drinking the experimental milk from each experimental group would have on the mineral intakes of the population, when compared with the corresponding control milk from each experiment. This was tailored specifically to the country that this trial was run in, therefore SPAN in Spain, ICEL in

Iceland, and FINL in Finland. In order to do this, nutritional surveys from the respective countries were used to capture the intakes and official governmental recommendations of nutritional intakes and upper limits for each nutrient.

I found that generally when consuming control experimental milk, and under the average intakes for the difference demographics in each country, milk fulfilled the RNI for I through milk alone in ICEL (<10 years of age, ≥ 10 years of age, and pregnant or lactating women (PLW)), yet in FINL it did not fulfil close to the recommendations for each demographic (~24, ~19, and ~16% for age groups of 18-44, 45-64, and 65-74 respectively) especially within pregnant (15%) and lactating (13%) women (PLW). This was mirrored in SPAN which showed that while the % of adequate intake of I was fulfilled slightly more than the FINL study (~36, ~27.5, ~18.5, and ~20.5% for age groups of 9-12, 13-17, 18-64, and 65-75 respectively) especially within PLW (14%), this was not anywhere near the full adequate intake that is hoped for. This was generally because of reduced intakes of milk depending on the country, and higher differences in consumption based on sex which is seen in FINL and SPAN studies with women tending to consume less milk than men (consistently throughout the lifetime in Finnish men vs women - 319 vs 183g/d, 233 vs 165g/d, and 193 vs 145g/d in the age groups of 18-44, 45-64, and 65-74 respectively; and in pre-teen years in Spanish men vs women - 252 vs 205g/d, 241 vs 171g/d, 160 vs 171g/d, and 177 vs 192g/d in the age groups of 9-12, 13-17, 18-64, and 65-75 respectively). I also found that feeding a phycological supplement generally did raise milk concentrations that would therefore be nutritional beneficial towards achievement of recommendations and enhance I intake for especially PLW (161% AI for PLW in SPAN, and 209% RNI for PLW in ICEL), but this then poses a problem for non-PLW demographics which may consume over their UL, especially children. Therefore, it is imperative that if phycological supplementation was to occur in dairy cows, producers, processors, and retailers should be aware of increased I content. This could also be used as a technological advantage as seaweed supplementation could occur in occasions were milk I content is known to be relatively low (e.g. during the grazing season in countries where grazing cows is common practice.)

6.3 Study limitations

6.3.1 SPAN limitations

A key point to consider within SPAN was the estimation of transfer efficiency of the animal from feed to excretion for the mineral panel. Unfortunately, this study did not consider urine analysis as the amount that was collected and able to be transferred to my study was limited. Thus, while I can assume that minerals that were not excreted through faeces ended up in urine, this was not measured. This analysis would have produced valuable validatory figures to further cement the analytical technique proposed in the paper. Additionally, while haematological composition analysis was performed, this was taken on the last day of the trial. Therefore there was no covariate for the haematological parameters, and thus no effect was able to be shown. Following, the parameters measured can be used as an animal health indicator, but can only really show if something is dramatically negatively impacted. Therefore more concise and valuable animal health indicating parameters could have been measured – as although this work was mainly designed to measure product quality, it is extremely important to measure the animal's health whilst producing that product. Therefore, going forward with future experimentation, when measuring transfer efficiency of feed to product – it is important to measure all excretion into all biofluids to hone in on the physiological cause of a change rather than a simple reporting of results. This will ensure that future research is more targeted, and manipulated more effectively to reach a desired outcome.

6.3.2 ICEL limitations

As ICEL was originally designed to limit and explore the concentrations of arsenic resulting from the feeding of seaweed to Icelandic dairy cattle, the study was designed in such that material was gathered in a less-suitable way for deriving the distinct fate of iodine. Firstly, as mentioned in the text, the location that the trial was performed sat on an iodine-rich environment, and therefore it becomes hard to discern the effect of the seaweed compared to the environment. Secondly, the sampling of feed from the trial resulted in the mixed TMR, meaning that the raw seaweed itself could not be analysed for iodine concentrations in the same instance and under the same circumstances as the milk was analysed. Thirdly, given that there was a physiological response by the dairy cow to the large ingestion of iodine, a more thorough paper could have been produced if the study was extended in time to allow for additional sample collection and insight as to the excretion response in order to provide better recommendations to farmers. Finally, while the study was meant to have direct draws to actual farming operations within Europe, ICEL utilised Icelandic cattle, a protected and isolated breed that may process and digest certain minerals at differing rates than other commonly used breeds in mainland Europe.

6.3.3 OMIC limitations

As an ad-hoc extension of ICEL, OMIC examined the abundance difference between milk from the high-seaweed dosage group and the control group at the timepoints wherein the iodine concentrations were highest in the high-seaweed dosage group. This allowed for sampling to occur at the peak and at the timepoints directly before or after the peak – at both sides of the bell curve of iodine concentrations. This allowed for possible implications to be derived, as potential pathogens could be identified as being lower in abundance compared to the control, or visa versa. Unfortunately, the link between a member of a taxonomic group of a pathogen behaving the same as the actual pathogen itself can be tenuous. Additionally, as the ICEL experiment was designed to measure product mineral concentrations, there is no way to discern possible contamination that could have occurred in the samples as a result of milking. All this being said, it was ensured that teats were cleaned before sampling – but given the fact that the samples would be analysed microbiologically, storage conditions could have been better. Limitations aside, this study does offer preliminary pilot data to then explore the effect that direct iodine has - excreted from ingestion of iodine sources – on a panel of pathogens. Going forward, when creating a biobank of samples from a study, analysis can be expanded with proper sterile technique and consistent recording of all factors within the trial, as increased value can be derived if not then, later – as was the case within this study.

6.3.4 FINL limitations

The FINL project was bolt-on work that expanded upon previous analysed material. This poses a limitation, as again, proper storage and recording can be unknown regarding certain portions, and thus more value could have been derived if increased record-keeping had occurred. Additionally, as was the case in SPAN, urine was not collected and could have been to deliver measured and accurate mineral transfer efficiency figures. Finally, cow numbers were limited, and thus a 4 x 4 Latin square design was implemented. While this is suitable for this work, an expanded amount of animal subjects would have been instrumental in additionally verifying trends between dietary groups. This is key as iodine persistence was seen in ICEL, and thus adaptation periods between square treatments could have been too short to completely rid the body, and thus excretion, of iodine. Finally, while blood iodine concentrations were measured at multiple points, overcoming issues found in SPAN, this only measures animal iodine status – which is only a small portion of what one could derive as animal health implications from the feeding of rapeseed and *Spirulina*.

6.4 Overall Conclusions

Overall, the feeding of phycological matter generally did not affect milk basic composition or yield significantly and therefore this indicates an animal foodstuff that lacks contraindications for the qualities that determine farmer profitability. Although, across all studies in this body of work involving macroalgae, there was an increase in the concentrations of certain minerals within the milk. This was especially pronounced in iodine, which when modelled, would exceed the upper limit tolerance of members of the population that consume large amounts for their size, and thus have a lower upper limit threshold, children. This interaction was shown to be a dose-dependent response, and therefore can be modulated pre-farmgate to control for iodine concentrations suitable for consumption by any demographic. The increased, but controllable, amount of iodine concentration within the milk from seaweed-fed cows could theoretically be used to fulfil the mineral requirements for all members of a population, increasing public nutrition, and thus public health outcomes. This is because even today within high-income countries, iodine deficiency persists and has pronounced consequences for the offspring of mothers who are iodine deficient during pregnancy. For microalgae, there seems to be no contraindications as well, as feeding *Spirulina* to dairy cows did not generally affect milk composition, and the animal trial presented more so conveyed the goitrogenic effect of rapeseed meal to cows rather than present anything that would indicate microalgal feeding as a problem. Thus, it was indicated that a holistic approach must be utilized when assessing dietary components of total rations for animals, as the entire diet must be considered and can be bolstered to deliver key nutrition to the animal and thus the animal products.

Going forward, an increased number of animal trials regarding phycological matter other than *A. nodosum*, *L. digitata*, and *Spirulina* are suggested, paired with the typical dietary components of farms on a per country or even region basis. This would allow farms across all of Europe to maintain the distinct feeding components for their practice, but allow farmers to adhere to bespoke suggestion to maintain or improve the nutritional quality of their products. This would in-turn provide larger opportunities for phycological matter to be utilised on farm, and thus increase the market potential of this good for all of Europe. Additionally, further research is required to understand the potential differences in transfer efficiencies and metabolism of minerals per breed of cow. This body of work used Finnish Ayrshires, Icelandic cattle, and Holstein cattle as is commonly used in the country that the animal trial was performed, but the nature of semi-isolated genetics, more so in the case of Iceland, poses an interesting question as to the role that foetal programming and/or genetics may have in the transference of minerals from feed to milk or even meat. Finally, regarding sustainability, more market-centred approach studies would be suggested to possibly encourage farming stakeholders to invest into phycological feeding within Europe. A recent continuing increase in consumer awareness of the negative consequences of animal production due to poor sustainable practices in some regions could mean that more profitability is associated with phycological feeding.