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A descriptive chemical analysis of seaweeds, *Ulva* sp., *Saccharina latissima* and *Ascophyllum nodosum* harvested from Danish and Icelandic waters

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ABSTRACT

This study aimed to discuss the chemical composition of three seaweed species commonly found in Nordic countries and its potential use in feed rations for pigs and calves. Two brown seaweeds *Ascophyllum nodosum*, *Saccharina lastissima* and a green seaweed *Ulva* sp. harvested from Danish and Icelandic waters were analyzed for proximate, amino acids, minerals, fatty acids and non-starch polysaccharides composition. All studied seaweeds contained low protein concentrations (i.e. 11.4–15.9 g/100 g DM). The ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) was similar in all studied seaweeds (0.81−0.87). *Ulva* sp. had the highest ash concentration (48.2–54.4 g/100 g DM), followed by *S. latissima* (39.9 g/100 g DM) and *A. nodosum* (29.5 g/100 g DM). The most abundant macrominerals in the seaweeds were Ca, K and Na. Iodine was the most abundant micromineral in brown seaweeds (1.4–2.1 g/kg DM). Moreover, *Ulva* sp. had the highest Fe (5.1–8.0 g/kg DM), Mn (10.5 g/kg DM) and inorganic As (0.008 g/kg DM) concentrations. *Ascophyllum nodosum* had the highest crude fat concentration (3 g/100 g DM) and the highest concentration of polyunsaturated fatty acids (FAs) (37.9 g/100 g FA). Eicosapentaenoic acid (EPA) concentration was the highest in *A. nodosum* (7 g/100 g FA) followed by *S. latissima* (5 g/100 g FA) and *Ulva* sp. (2 g/100 g FA). Furthermore, concentration of α-linolenic acid, a precursor for EPA, was the highest in *Ulva* sp. (6.2–14.6 g/100 g FA). Total dietary fiber concentration was higher in the brown seaweeds (27.8–42.6 g/100 g DM) compared to the green seaweeds (17.9–21.5 g/100 g DM), where *S. latissima* had the highest soluble dietary fiber concentration. The high concentrations of ash and fiber may limit inclusion levels of the analyzed whole seaweeds in feed rations, mainly due to dilution of other nutrients in the feed, reduced digestibility of the feed and possible toxicities (i.e. high inorganic As). On the other hand, high concentrations of essential and valuable microminerals including I, Cu, Fe, Mn, Se were also detected in the studied seaweeds. High soluble dietary fiber concentration in *S. latissima* can be of

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interest as a fermentable substrate for probiotic bacteria. The easily digestible nutrients including, crude protein and fat were low in the seaweeds. However, the protein, fat, ash and dietary fiber fractions of the studied seaweeds were characterized by high concentrations of EAA, EFA, essential microminerals and interesting monomers of functional polysaccharides, respectively; which indicate possibilities for future use of seaweed-extracts in feed rations.

1. Introduction

Marine macroalgae, commonly known as seaweeds, can be used as a food and feed ingredient as well as for other industrial applications (i.e. bioenergy, biopolymers, etc.). Recently, the use of whole seaweeds and seaweed extracts as feed ingredients has gained increased interest in Europe.

Seaweeds are particularly interesting, as they have zero requirement of land-based resources (Ortiz et al., 2006; Øverland et al., 2019). Based on their pigmentation, among other characteristics, seaweeds are categorized into three groups; green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) (Makkar et al., 2016). The brown seaweeds *Ascophyllum nodosum* (Linnaeus; Le Jolis) and *Saccharina latissima* (Linnaeus; C.E.Lane, C.Mayes, Druehl & G.W.Saunders), and the green seaweed *Ulva* sp., used in this study are commonly found in Northern European waters (Moy and Christie, 2012; Mac Monagail et al., 2017). Furthermore, some of these seaweeds have traditionally been used as a feed for ruminants in some countries (Makkar et al., 2016).

Due to the low lipid and digestible carbohydrate content, seaweeds are not considered a good source of digestible energy for monogastric animals (Holdt and Kraan, 2011). Protein content in seaweeds is highly variable depending on the species, but is often low in brown seaweeds (Holdt and Kraan, 2011). Furthermore, digestibility of the protein fraction can vary depending on its interactions with cell-wall polysaccharides and phenolic compounds (Holdt and Kraan, 2011). Nevertheless, seaweeds contain high concentrations of minerals, vitamins and non-starch polysaccharides (NSP) (Okolie et al., 2017; Øverland et al., 2019). Due to the high NSP content, seaweeds may be used as a source of dietary fiber (Ortiz et al., 2006), which could be a fermentable substrate for ruminal and intestinal microbiota. The composition of cell-wall polysaccharides differs between green and brown seaweeds. While sulfated galactan and xylan are the main NSP in green seaweeds; laminarin, alginate and fucoidan are the main NSP in brown seaweeds (Stiger-Pouvreau et al., 2016; Okolie et al., 2017; Øverland et al., 2019). Laminarin, alginate and fucoidan are reported to have immunomodulatory and prebiotic properties (Okolie et al., 2017). As an example, laminarin and fucoidan promoted the growth of beneficial bacterial species in the intestinal microbiome of pigs, including different species of *Lactobacillus* and *Bifidobacteria* while supressing the growth of pathogenic *Escherichia coli* (O'Doherty et al., 2010; Walsh et al., 2013). Although the lipid content is low, seaweeds can be a natural source of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) (Holdt and Kraan, 2011) and antioxidants such as tocopherols and carotenoids (Lynch et al., 2010; Okolie et al., 2017; Øverland et al., 2019).

The increased interest in seaweeds as a functional feed ingredient for swine and ruminant nutrition ranges from using them to reduce diarrhea incidences in weanling piglets and pre-weaned calves to reducing enteric methane emissions from cows. Many of these desirable functional properties are obtained when using specific extracts (i.e. up-concentrated compounds) from these seaweeds (O'Doherty et al., 2010; Sweeney et al., 2012; Walsh et al., 2013; Sweeney et al., 2017). However, the use of whole seaweeds over seaweed extracts could be practically desirable as it does not require additional processing aside from the already laborious and costly drying. Furthermore, the use of whole seaweeds will not require registration as a feed additive. In-depth knowledge on the biochemical composition of candidate seaweeds is needed to increase understanding of the potential of using whole seaweeds as functional and/or nutritional feed ingredients. As a result, this study aimed to provide a detailed description of the chemical composition of *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva* sp. harvested from Danish and Icelandic waters with the perspective of using them as an alternative feed ingredient for piglets and calves.

2. Material and methods

2.1. Seaweeds

Three different species of seaweeds were used in this study. *Ascophyllum nodosum* was purchased from a commercial producer, whereas *Saccharina latissima* and *Ulva* sp. were harvested in large volumes of 400−600 kg. The brown seaweed *A. nodosum* (Thorverk HF®, Reykhólar, Iceland) was harvested at the Breiðafjörður bay in November 2017 (Iceland; N: 65.47166°, W: 22.39222°) and July 2018 (Iceland; N: 65.17694°, W: 22.61333°). Thereafter, *A. nodosum* batches were air dried for 90 min at 80−85 °C and ground to a particle size of 0.2 mm. The representative subsamples for laboratory analysis were obtained from an 80 kg batch of dried *A. nodosum*. The brown seaweed *Saccharina latissima* was cultivated at a site near Hjarnø Hage (Denmark; N: 55.813822°, E: 10.112930°), and harvested in May 2018. The green seaweed *Ulva* sp. was harvested in Mariager Fjord (Denmark; N: 56.41413°, E: 10.11560°), and in Skive Fjord (Denmark; N: 56.560288°, E: 9.055511°) in June and August 2018, respectively. Species diversity of *Ulva* has recently been proven to be considerably larger than expected, and species determination is not possible based on morphology (Steinhagen et al., 2019). Hence, *Ulva* sp. is used throughout this paper.

Saccharina latissima and *Ulva* sp. were oven dried in trays at low temperature (40−50 °C) for two days and ground to a particle size of 0.8 mm. Representative subsamples for laboratory analyses were taken after drying and homogenizing the biomass, and thus represent the bulk biomass composition.

2.2. Chemical analyses

2.2.1. Dry matter

Dry matter content of dried and ground seaweeds was analysed by oven drying the samples for 20 h at 103 °C. Dry matter content of representative seaweed samples was analysed in duplicates and mean ± standard deviation (SD) were calculated.

2.2.2. Crude protein and amino acids profile

Nitrogen content was determined by the Dumas method using a Vario MAX CN analyzer (Elementar Analyse systems GmbH, Hanau, Germany) (Hansen, 1989). A factor of 6.25 was used for the conversion of N into crude protein (CP). Duplicate analyses of nitrogen were performed on representative seaweed samples and mean ± standard deviation (SD) were calculated. The samples were hydrolysed for 23 h at 110 °C (European Commission, 2009) for determination of the amino acid (AA) composition. Methionine and cysteine were oxidised with performic acid before hydrolysis. All other amino acids, except for tryptophan, were analysed without previous performic acid oxidation. Thereafter, ion exchange chromatography was used to separate hydrolysed AA. Amino acids were then measured by photometric detection after ninhydrin reaction. A correction factor of 1.06 was used for serine, valine and isoleucine because these are prone to oxidation (Rudemo et al., 1980). Tryptophan was hydrolysed under alkaline conditions for 20 h at 110 °C and measured with HPLC fluorescent detection (European Commission, 2009).

2.2.3. Crude ash, minerals and heavy metals content

Crude ash content was determined by weighing following combustion of the samples for 6 h at 525 °C. The macrominerals, i.e. Ca, Mg, P, K and Na, were analyzed using an X-series II inductively coupled plasma mass spectrophotometer (Thermo Electron Coorperation, Bremen, Germany) equipped with a Meinhard nebulizer and a Peltier cooled quartz impact bead spray chamber at 3 °C. Each representative sample was analysed in duplicates and average and SD were calculated. Concentrations of micromineral and heavy metals (Cu, Fe, Mn, Zn, As, Cd, Pb, Se, Cr, Co, Ni, Sr, Ba) were determined using inductively coupled plasma mass spectrometry (iCAPq ICP-MS, Thermo Fischer, Bremen, Germany). Briefly, 0.2 *g* dry sub-samples were digested in closed quartz vessels in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) using 5 ml of concentrated nitric acid (SPS science, Courtabeuf, France). The digests were subsequently diluted with Milli-Q water and the content of the micromineral and heavy metals were quantified by ICP-MS (Thermo Fischer, Bremen, Germany) using external calibration with internal standardization (^{97}Rh). For determination of iodine (I) concentration, the principles of the standardized method (EN17050:2017, 2017EN0:, 2017EN17050:2017, 2017) were followed. Briefly, 0.15−0.20 *g* of dry sample was weighed into tubes (Sarstedt, Nümbrecht, Germany). Subsequently, 5 ml Milli-Q® water and 1 ml 25 % tetra-methyl-ammonium-hydroxide (TMAH, Merck, Darmstadt, Germany) was added. The tubes were then sealed and placed in a preheated oven for 3 h at 90 °C followed by cooling and diluting to a final volume of 20 ml with Milli-Q water. To remove coarse particles, the samples were centrifuged at 10.000 × *g* for 20 min. Prior to analysis, the supernatants were filtered through 0.45 µm syringe filters and the samples were then diluted with Milli-Q water prior to analysis. The iodine quantification was performed by ICP-MS (Thermo Fischer, Bremen, Germany) using external calibration with internal standardization (^{125}Te). For determination of inorganic arsenic (iAs) the principles of the standardised method (EN17374:2020, 2020EN4:, 2020EN17374:2020, 2020) were followed. Briefly, 0.2−0.3 *g* of dry sample material was weighed into 15 ml polypropylene tubes (Sarstedt, Nümbrecht, Germany). Subsequently, 10 ml of 0.1 M nitric acid (Merck, Darmstadt, Germany) in 3% (V/V) hydrogen peroxide solution was added. The tubes were then sealed and placed in a preheated water bath for 60 min at 90 °C. The extracts were centrifuged at 10,000 × *g* for 10 min and the supernatants were used for the analysis of iAs. The content of iAs was determined using anion-exchange chromatography for selective separation of iAs from organoarsenic compounds coupled to ICP-MS (8900 ICP-QQQ, Agilent Technologies, Santa Clara, USA) as an arsenic-selective detector. External calibration curve was used for the quantification of iAs. For all minerals and heavy metals reported in the present study, certified stock solutions were used for preparation of the calibration standard and internal standard (SPS science, Courtabeuf, France). The standard deviation was calculated based on intra-coefficient of variance (intra-CV). The intra-CV for Cu, Fe, Mn, Zn, Se, Cr, Co, Sr and Ba was 6%, and for As, Cd, Pb, Ni and inorganic As was 8%.

2.2.4. Crude fat and fatty acid composition

The crude fat content was determined by Soxhlet extraction with petroleum ether (Soxtec 2050, Foss Analytical, Hillerød, Denmark) after hydrolysis with hydrochloric acid (Stoldt, 1952). A modified Bligh and Dyer method was used to extract lipids using methanol and chloroform after acidification of the seaweed samples with 3 M HCl for 1 h (Bligh and Dyer, 1959; Jensen, 2008) and C17:0 (Heptadecanoic acid, Sigma-Aldrich, St. Louis, MO) was used as an internal standard. Extracted lipids, quantified as fatty acid (FA) methyl esters, were analyzed using gas chromatography (Hewlett-Packard 6890 series, Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was equipped with an automatic column injector (Hewlet Packard 7673), a capillary column of 30 cm ×0.32 mm (inner diameter), 0.25 µm film thickness (Omegawax 320; Supelco, Sigma-Aldrich), and a flame ionization detector. Retention times obtained by the gas chromatograph were compared with the retention times of the external standards (GLC-68C, Nu-Prep-Check, Elysian, MN, USA) in order to identify specific fatty acids (FA) in the samples (Bligh and Dyer, 1959; Jensen, 2008). Fatty acid analysis in representative seaweed samples was performed in duplicates and average and SD were calculated.

2.2.5. Content of total dietary fiber, non-starch polysaccharides and lignin-like substances

The analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was performed sequentially using the Ankom Technology method (ANKOM, 2016) as described by Mertens (2002). Heat stable amylase was used for determination of NDF. Values were corrected for ash using ash residue obtained after ADL determination.

The composition of total dietary fiber and NSP was analysed after enzymatic extraction of starch as described by Knudsen (1997). Total NSP and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by colorimetry for uronic acids using a modification of the Theander et al. (1994) and Englyst et al. (1992) procedures as described by Bach Knudsen (1997).

This method divide total NSP into cellulose and soluble and insoluble non-cellulosic polysaccharides (NCP) based on the analysis of monomeric constituents. Cellulose was determined as the difference of glucose content of NSP when the swelling step with 12 M H_2SO_4 was included ($NSP_{Glucose\ (12\ M\ H2SO4)}$) or omitted ($NSP_{Glucose\ (2\ M\ H2SO4)}$).

Total NCP = glucose + galactose + xylose + arabinose + mannose + rhamnose + fucose + uronic acids

Klason lignin was measured as the insoluble residue after hydrolysis with 12 M H_2SO_4 and the content of total dietary fibre (DF) was calculated.

DF = Total NSP + Klason lignin

The fraction that was analysed as lignin in the present study represents the fraction in seaweeds that was insoluble in sulphuric acid, and consequently indigestible and not fermentable. However, it is uncertain if this is actually lignin or if it represents other acid insoluble components in seaweeds. Therefore, henceforth, this fraction will be referred to as lignin-like substances.

3. Results

3.1. Crude protein and amino acid profile

The selected seaweeds contained between 11–16 % CP (DM basis) (Table 1). In addition, the AA profile was similar among the studied seaweeds (Table 1). The ratio between essential AA (EAA) and non-essential AA (NEAA) was similar for the brown seaweeds and *Ulva* sp. harvested in May (approx. 0.86; Table 1). *Ulva* sp. harvested in August had a lower EAA:NEAA ratio of 0.81. The most abundant EAA in the three studied seaweed species were leucine (5.5–6.0 g/100 g CP) and lysine (3.4–4.9 g/100 g CP), whereas tryptophan and methionine were the least abundant EAA (Table 1). On average, the lysine concentration was 30 % higher in the brown seaweeds compared to the green seaweeds. Moreover, lysine concentration was the highest in *A. nodosum*. The concentration of cysteine in *Ulva* sp. from the June harvest was 32 % lower compared to the brown seaweeds and 25 % lower compared to *Ulva* sp. from the August harvest. Methionine concentration was also the lowest in *Ulva* sp. from the June harvest. Methionine concentration was the

Table 1
Dry matter, crude protein concentrations (mean ± SD[1]) and amino acids (AA) composition (mean) in dried and ground *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva* sp.

	Ascophyllum nodosum	Saccharina latissima	Ulva sp. (June)	Ulva sp. (August)	Soybean meal[2]
Dry matter, (g/100 g)	93.2 ± 0.00	94.0 ± 0.00	92.8 ± 0.00	94.5 ± 0.16	
Crude protein, (g/100 g DM)	11.4 ± 0.18	15.2 ± 0.00	15.9 ± 0.27	13.3 ± 0.18	53.1 ± 1.14
EAA[3], (g/100 g CP)					
Arginine	4.58	4.25	4.74	4.46	7.21 ± 0.07
Histidine	1.42	1.41	1.29	0.96	2.70 ± 0.12
Isoleucine	4.00	3.87	3.61	3.86	4.50 ± 0.14
Leucine	6.02	6.07	5.87	5.48	7.60 ± 0.13
Lysine	4.92	4.61	3.89	3.40	6.12 ± 0.21
Methionine	1.85	1.47	1.32	1.54	1.34 ± 0.07
Phenylalanine	3.73	3.73	4.70	4.08	5.10 ± 0.10
Threonine	4.25	4.01	4.21	4.75	3.82 ± 0.14
Tryptophan	1.12	1.05	1.17	0.92	1.35 ± 0.06
Valine	4.73	4.65	4.94	5.40	4.81 ± 0.11
NEAA[4] (g/100 g CP)					
Alanine	5.34	6.01	6.81	7.67	4.25 ± 0.15
Aspartic acid	10.1	9.26	9.76	10.03	9.37 ± 3.04
Cysteine	1.85	1.80	1.24	1.65	1.41 ± 0.08
Glutamine	12.1	10.8	9.49	10.82	17.4 ± 0.55
Glycine	6.09	5.35	5.23	4.82	4.14 ± 0.10
Ornithine	0.10	0.09	0.09	0.11	
Proline	3.53	3.45	3.69	3.21	4.76 ± 0.19
Serine	4.14	4.10	4.59	4.75	4.71 ± 0.2
Σ EAA, (g/100 g CP)	36.62	35.12	35.74	34.85	44.2 ± 1.00
Σ NEAA, (g/100 g CP)	43.18	40.85	40.90	43.06	47.9 ± 3.36
EAA: NEAA	0.85	0.86	0.87	0.81	0.93 ± 0.05
Σ Total AA, (g/100 g CP)	79.8	76.0	76.6	77.9	92.1 ± 4.23

[1] Standard deviation over duplicate analysis of a representative seaweed sample.
[2] Average and SD over several studies (Hulshof et al., 2016; Lagos and Stein, 2017; Cowieson et al., 2019; Oliveira et al., 2020).
[3] Essential amino acids.
[4] Non-essential amino acids.

highest in *A. nodosum,* and on average 29 % higher than that in the other seaweeds. The most abundant NEAA were glutamine and aspartic acid, which ranged from 9.5 to 12 and 9−10 g/100 g CP in all studied seaweeds, respectively.

3.2. Crude ash, minerals and heavy metals content

All seaweeds in this study contained high concentrations of crude ash (30−55 g/100 g DM; Table 2). The highest ash concentration was observed in *Ulva* sp. harvested in August, and the lowest in *A. nodosum*. Calcium, K and Na were the most abundant macrominerals in the three studied seaweeds. Moreover, *S. latissima* had three times higher K concentration than the other two seaweed species. The micromineral concentrations in *Ulva* sp. differed depending on the month of harvest. The concentrations of I, Cu, Fe, Mn, Zn, Cr, and Co were higher in *Ulva* sp. harvested in August compared to *Ulva* sp. harvested in June (Table 2). The two brown seaweeds, *A. nodosum* and *S. latissima* had a considerably higher I concentration compared to the green seaweed *Ulva* sp.. In contrast, *Ulva* sp. had a higher Fe concentration than both of the brown seaweeds. The Mn concentration was higher in *S. latissima* and *Ulva* sp. compared to *A. nodosum*. The highest Mn concentration was observed in *Ulva* sp. harvested in August and it was around 11 g/kg of DM. The concentration of Zn was the highest in *S. latissima*. Furthermore, Se concentration in *Ulva* sp. (i.e. in both harvest months) was approximately five times greater than that in *A. nodosum* and *S. latissima.*

Regarding potentially toxic heavy metals, Sr concentrations were at least twice as high in the studied brown seaweeds compared to the green seaweeds. Total As concentration was higher in *A. nodosum* and *S. latissima* compared to *Ulva* sp. (Table 2). In contrast, the inorganic As concentration was highest (8.3 mg/kg DM) in *Ulva* sp. harvested in August.

3.3. Crude fat and fatty acid composition

As shown in Table 3, the crude fat concentration was low in all seaweeds (1−3 g/100 g DM). Half of the FA in almost all seaweeds, except for *A. nodosum,* were made up of saturated fatty acids (SFA). Polyunsaturated fatty acids (PUFA) made up between 20−38 g/100 g total FA of the seaweeds. *Ascophyllum nodosum* had the lowest SFA concentration, which was half as high as the SFA concentration in the other seaweeds. Consequently, *A. nodosum* had the highest concentrations of monounsaturated fatty acids (MUFA) and PUFA. The most abundant SFA in all seaweeds were C14:0 (myristic acid) and C16:0 (palmitic acid). Additionally, C16:0 was the most abundant FA overall in all seaweeds except for *A. nodosum.* In *A. nodosum,* the most abundant FA was C18:1n9 (oleic acid; 33 g/100 g total FA). Oleic acid was considerably lower in *S. latissima* (10 g/100 g total FA) and in *Ulva* sp. (2 g/100 g total FA) compared to *A. nodosum*. *Saccharina latissima* and *Ulva* sp. contained high amounts of C16:1n7 (palmitoleic acid; 6.6–10.3 g/100 g total FA). The most abundant MUFA in the studied green seaweed was C18:1n7 (12−14 g/100 g total FA). The following PUFA were present at the highest concentrations in brown seaweeds; C18:2n6 (lineoleic acid), C20:4n6 (arachidonic acid) and C20:5n3 (EPA). In addition, the highest docosahexanoic acid (C22:6n3; DHA) concentration was observed in *A. nodosum*. *Ulva* sp. harvested in June contained mainly

Table 2
Crude ash, macromineral (mean ± SD[1]), micromineral and heavy metal (mean ± SD[2]) concentrations in dried and ground *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva* sp.

	Ascophyllum nodosum	*Saccharina latissima*	*Ulva* sp. (June)	*Ulva* sp. (August)
Crude ash (g/100 g DM)	29.5 ± 0.78	39.9 ± 0.00	48.2 ± 0.18	54.4 ± 0.01
Macrominerals (g/100 g DM)				
Calcium	5.23 ± 0.18	5.62 ± 0.18	6.56 ± 0.17	2.15 ± 0.04
Magnesium	0.89 ± 0.04	0.97 ± 0.04	2.11 ± 0.03	1.48 ± 0.04
Phosphorus	0.23 ± 0.01	0.43 ± 0.01	0.23 ± 0.00	0.36 ± 0.01
Potassium	2.37 ± 0.11	6.24 ± 0.30	1.79 ± 0.03	1.91 ± 0.05
Sodium	3.48 ± 0.17	3.71 ± 0.21	4.95 ± 0.10	3.24 ± 0.09
Microminerals (mg/kg DM)				
Iodine	1365 ± 54.6	2067 ± 82.7	47.1 ± 1.88	169 ± 6.77
Copper	6.54 ± 0.39	6.81 ± 0.41	7.76 ± 0.47	24.1 ± 1.45
Iron	2118 ± 127	3442 ± 207	5079 ± 305	8019 ± 481
Manganese	71.9 ± 4.31	962 ± 57.7	1675 ± 101	10,486 ± 629
Zinc	13.5 ± 0.81	62.2 ± 3.73	23.2 ± 1.39	57.3 ± 3.44
Selenium	2.47 ± 0.15	3.72 ± 0.22	11.1 ± 0.67	15.0 ± 0.90
Chromium	3.22 ± 0.19	14.5 ± 0.87	26.3 ± 1.58	43.7 ± 2.62
Cobalt	2.47 ± 0.15	2.34 ± 0.14	1.83 ± 0.11	5.92 ± 0.36
Heavy metals (mg/kg DM)				
Arsenic	31.1 ± 2.49	43.1 ± 3.45	5.95 ± 0.48	11.6 ± 0.93
Inorganic arsenic	0.88 ± 0.07	0.74 ± 0.06	0.65 ± 0.05	8.3 ± 0.66
Cadmium	1.00 ± 0.08	0.86 ± 0.07	0.16 ± 0.01	0.23 ± 0.02
Lead	0.28 ± 0.02	2.45 ± 0.20	2.69 ± 0.22	3.28 ± 0.26
Nickel	3.32 ± 0.27	7.13 ± 0.57	9.92 ± 0.79	21.5 ± 1.72
Strontium	674 ± 40.4	863 ± 51.8	360 ± 21.6	149 ± 8.95
Barium	7.61 ± 0.46	62.3 ± 3.74	26.2 ± 1.57	78.9 ± 4.73

[1] Standard deviation represents duplicate analysis of a representative seaweed sample.
[2] SD was calculated based on intra-coefficient of variance (intra-CV).

Table 3
Crude fat and fatty acids (FAs) concentrations (mean ± SD[1]) in dried and ground *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva* sp.

Fat and FAs	Trivial name	Ascophyllum nodosum	Saccharina latissima	Ulva sp. (June)	Ulva sp. (August)
Crude fat (g/100 g DM)		3.00	1.50	1.30	1.00
Total FA, g/100 g DM		2.14 ± 0.03	0.68 ± 0.03	0.34 ± 0.04	0.49 ± 0.06
Saturated FA (g/100 g FA)		25.3 ± 0.27	51.5 ± 0.63	53.8 ± 1.09	46.8 ± 0.65
C4:0	Butyric acid	0.09 ± 0.01	0.42 ± 0.11	0.51 ± 0.23	0.62 ± 0.05
C8:0	Caprylic acid	0.06 ± 0.00	0.21 ± 0.03	0.41 ± 0.04	0.00 ± 0.00
C10:0	Capric acid	0.03 ± 0.01	0.15 ± 0.06	0.18 ± 0.02	0.06 ± 0.05
C11:0	Undecylic acid	0.01 ± 0.02	0.14 ± 0.01	0.18 ± 0.06	0.07 ± 0.03
C12:0	Lauric acid	0.1 ± 0.00	0.31 ± 0.00	0.08 ± 0.01	0.13 ± 0.04
C13:0		0.03 ± 0.00	0.21 ± 0.09	0.32 ± 0.05	0.42 ± 0.18
C14:0	Myristic acid	9.92 ± 0.06	9.92 ± 0.07	8.04 ± 0.27	3.59 ± 0.02
C14:1		0.17 ± 0.02	0.14 ± 0.04	0.23 ± 0.03	0.17 ± 0.03
C15:0	Pentadecylic acid	0.39 ± 0.00	0.91 ± 0.02	0.59 ± 0.02	0.88 ± 0.00
C16:0	Palmitic acid	12.8 ± 0.09	31.8 ± 0.08	38.1 ± 0.05	36.8 ± 0.03
C17	Margaric acid	0.24 ± 0.00	1.21 ± 0.01	0.66 ± 0.10	0.43 ± 0.01
C18:0	Stearic acid	1.01 ± 0.02	4.86 ± 0.02	3.19 ± 0.12	2.03 ± 0.01
C20:0	Arachidic acid	0.23 ± 0.01	0.83 ± 0.02	0.39 ± 0.01	0.43 ± 0.02
C22:0	Behenic acid	0.11 ± 0.01	0.45 ± 0.06	0.97 ± 0.08	0.85 ± 0.04
C24:0	Lignoceric acid	0.13 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.13
Monounsaturated FA (g/100 g FA)		36.8 ± 0.40	26.3 ± 0.53	25.6 ± 1.07	25.4 ± 0.19
C16:1n9	Hexadecenoic acid	0.06 ± 0.01	0.31 ± 0.09	0.18 ± 0.06	0.22 ± 0.01
C16:1n7	Palmitoleic acid	2.37 ± 0.04	10.32 ± 0.15	9.45 ± 0.38	6.62 ± 0.00
C17:1	Heptadecenoic acid	0.18 ± 0.02	0.42 ± 0.08	1.25 ± 0.05	3.5 ± 0.08
C18:1n9	Oleic acid	32.7 ± 0.27	10.3 ± 0.07	2.32 ± 0.06	1.49 ± 0.02
18:1n7		1.05 ± 0.03	4.5 ± 0.04	11.97 ± 0.32	13.5 ± 0.03
C20:1n9	Gondoic acid	0.05 ± 0.00	0.27 ± 0.09	0.12 ± 0.04	0.09 ± 0.04
C22:1n11	Cetoleic acid	0.37 ± 0.02	0.14 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
C22:1n9	Erucic acid	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.17	0.00 ± 0.00
Polyunsaturated FA (g/100 g FA)		37.9 ± 0.21	22.2 ± 0.51	20.6 ± 0.65	27.8 ± 0.81
C18:2n6	Linoleic acid	8.59 ± 0.05	5.34 ± 0.02	3.15 ± 0.08	5.70 ± 0.05
C18:3n6	γ-Linolenic acid	0.19 ± 0.00	0.65 ± 0.01	0.21 ± 0.04	0.37 ± 0.07
C18:3n3	α-Linolenic acid	3.72 ± 0.00	2.33 ± 0.01	6.15 ± 0.16	14.56 ± 0.06
C18:4n3	Stearidonic acid	3.30 ± 0.01	3.13 ± 0.04	6.78 ± 0.06	2.44 ± 0.03
C20:2n6	Eicosadienoic acid	1.91 ± 0.01	0.57 ± 0.12	0.46 ± 0.04	0.4 ± 0.16
C20:3n6	Dihomo-γ-linolenic acid	0.67 ± 0.01	0.2 ± 0.01	0.00 ± 0.00	0.49 ± 0.02
C20:4n6	Arachidonic acid	9.93 ± 0.03	4.09 ± 0.04	0.3 ± 0.01	1.2 ± 0.01
C20:3n3	Eicosatrienoic acid	0.45 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0;00
C20:5n3	Eicosapentaenoic acid	6.85 ± 0.04	4.67 ± 0.15	1.94 ± 0.02	1.41 ± 0.18
C22:5n6		0.18 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.41 ± 0.07
C22:5n3	Docosapentaenoic acid	0.15 ± 0.02	0.2 ± 0.01	1.07 ± 0.06	0.54 ± 0.14
C22:6n3	Docosahexaenoic acid	2.01 ± 0.02	1.03 ± 0.1	0.50 ± 0.17	0.27 ± 0.02
n6		21.3 ± 0.10	10.8 ± 0.2	4.12 ± 0.18	8.15 ± 0.32
n3		16.5 ± 0.08	11.4 ± 0.32	16.4 ± 0.47	19.2 ± 0.43
n-6:n-3		1.29	0.95	0.25	0.42

[1] Standard deviation represents duplicate analysis of a representative sample.

C18:3n3 (α-linolenic acid) and C18:4n3 (stearidonic acid) in its PUFA fraction. The PUFA fraction of *Ulva* sp. from the August harvest was mainly made up of linoleic and α-linolenic acid. The ratio of omega-6 to omega-3 (n-6:n-3) fatty acids was the lowest in the green seaweeds, indicating higher abundance of omega-3 fatty acids. Omega-6 fatty acids were more abundant than omega-3 fatty acids in *A. nodosum.*

3.4. Content of total dietary fiber, non-starch polysaccharides and lignin-like substances

The total NCP concentration was higher in *A. nodosum and S. latissima* compared to that observed in *Ulva* sp. (Table 4). Furthermore, the ratio between soluble and total NCP in *A. nodosum and S. latissima* was higher than that in *Ulva* sp. (0.68, 0.92 and 0.30−0.54, respectively). Hence, the concentration of insoluble NCP (I-NCP) was the highest in *Ulva* sp. harvested in August and the lowest in *S. latissima*. Soluble NCP (S-NCP) derived uronic acid concentration was the most abundant S-NCP derived monosaccharide in *A. nodosum.* This monosaccharide was higher in *A. nodosum* and *S. latissima* than in *Ulva* sp.. The highest concentration of S-NCP derived fucose was observed in *A. nodosum* (Table 4). It was the second most abundant S-NCP derived monosaccharide in this seaweed. The concentration of S-NCP derived fucose was four times lower in *S. latissima* than in *A. nodosum,* and it was negligible in *Ulva* sp.. *Saccharina latissima* had the highest concentration of glucose derived from S-NCP. The S-NCP derived rhamnose concentration was the highest in *Ulva* sp. harvested in June. In general, rhamnose was the most abundant NCP derived monosaccharide in *Ulva* sp. harvested in June. The concentrations of I-NCP derived monosaccharides were below 1 g/100 g DM in brown seaweeds, except for fucose (i.e. 4 g/100 g DM in *A. nodosum*). Contrary to rhamnose derived from S-NCP, rhamnose derived from I-NCP concentration was the highest in

Table 4
Concentrations of NDF, ADF, ADL, non-starch polysaccharides and Klason lignin (mean ± SD) in dried and ground *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva* sp. Values are expressed in g/100 g DM.

	Ascophyllum nodosum	Saccharina latissima	Ulva sp. (June)	Ulva sp. (August)
NDF[a]	34.5 ± 2.37	21.7 ± 3.04	13.0 ± 0.70	29.1 ± 0.54
ADF[b]	18.9 ± 1.08	8.00 ± 0.46	7.40 ± 0.26	7.50 ± 0.20
ADL[c]	12.9 ± 0.77	2.80 ± 0.25	3.10 ± 0.13	3.30 ± 0.23
S – NCP[1]				
Rhamnose	0.04	0.21	3.65	0.84
Fucose	3.08	0.72	0.10	0.03
Arabinose	0.01	0.04	0.01	0.01
Xylose	1.23	0.19	0.43	0.33
Mannose	0.50	0.56	0.17	0.15
Galactose	0.44	0.54	0.41	0.22
Glucose	0.50	8.50	0.32	0.75
Uronic acid	7.30	7.05	0.62	1.92
I-NCP[2]				
Rhamnose	0.02	0.03	0.75	2.80
Fucose	3.62	0.25	0.02	0.00
Arabinose	0.03	0.04	0.03	0.05
Xylose	0.71	0.14	1.01	2.25
Mannose	0.22	0.14	0.15	0.33
Galactose	0.31	0.16	0.21	0.47
Glucose	0.42	0.23	1.35	2.95
Uronic acid	0.93	0.62	1.27	1.43
Total S-NCP	13.1	17.8	5.70	4.25
Total I-NCP	6.26	1.60	4.79	10.3
T- NCP[3]	19.4	19.4	10.5	14.5
Cellulose	2.50	3.60	2.30	2.20
S-NSP[4]	13.1	17.8	5.70	4.30
I-NSP[5]	8.80	5.20	7.10	12.5
T-NSP[6]	21.8	23.0	12.8	16.8
Klason lignin	20.7	4.90	5.20	4.70
S-DF[7]	13.1	17.8	5.70	4.30
I-DF[8]	29.5	10.0	12.2	17.2
Total DF[9]	42.6	27.8	17.9	21.5

[a] Neutral detergent fiber.
[b] Acid detergent fiber.
[c] Acid detergent lignin.
[1] Soluble non-cellulosic polysaccharide.
[2] Insoluble non-cellulosic polysaccharide.
[3] Total non-cellulosic polysaccharide = S-NCP + I-NCP.
[4] Soluble non starch polysaccharide = S-NCP.
[5] Insoluble non starch polysaccharide = I-NCP + cellulose.
[6] Total non starch polysaccharide = S-NSP + I-NSP.
[7] Soluble dietary fiber = S-NCP.
[8] Insoluble dietary fiber = I-NCP + cellulose + lignin.
[9] Total dietary fiber = Total NSP + lignin.

Ulva sp. harvested in August. Glucose, uronic acid and xylose were the most abundant I-NCP derived monosaccharides in *Ulva* sp. Cellulose concentrations were similar among seaweeds. The content of lignin-like substances in *A. nodosum* was around 4 times higher than in other seaweeds (Table 4). Therefore, the insoluble dietary fiber concentration was higher than the soluble dietary fiber concentration in *A. nodosum*. Moreover, the total dietary fiber concentration was the highest in this seaweed. *Saccharina latissima* contained the second highest level of total dietary fiber, the majority of which was soluble.

4. Discussion

Seaweeds are known to have a highly variable chemical composition in terms of protein, polysaccharide, mineral, pigment and lipid contents (Makkar et al., 2016; Bikker et al., 2020). Extensive knowledge on the seaweed nutrient composition, which is affected by inter-, intra-species and seasonal differences, is essential for the use of seaweeds in feed rations. It is important to know if regional differences affect the seaweed composition due to environmental factors and level of pollution, but not possible to study herein. However, this paper will contribute to the increasing pool of knowledge on seaweeds suitable for animal nutrition and create the possibility for comparisons with other recent literature such as Bikker et al. (2020), who observed the suitability of the same seaweed species grown in Scottish, Irish and French waters, for animal nutrition.

4.1. Crude protein and amino acids

A nitrogen to protein conversion factor of 5 is often used for seaweeds according to Angell et al. (2016). However, a recent study by Gaillard et al. (2018) explains that, in terms of the calculated N-factor, seaweeds are not specifically different from that of forages and/or other common animal feedstuffs (i.e. soybean meal, rapeseed meal, etc.). Comparisons herein are made converting the published CP contents of seaweeds in literature, using a N conversion factor of 6.25.

In general, the green seaweed *Ulva* sp. has higher CP content than the brown seaweed *A. nodosum* (Vieira et al., 2018), which was also seen in the present study. The CP content of *A. nodosum* observed in the present study was similar to the observations by Tibbetts et al. (2016) and Tabassum et al. (2016). The CP content of *Ulva* sp. in the present study is in accordance with the observations by Gaillard et al. (2018). The observed CP concentration of *S. latissima* is similar to the values observed by Mols-Mortensen et al. (2017) and Bruhn et al. (2019) for the same species harvested in the same time of the year from Faroese waters. On the contrary, Nielsen et al. (2016) found lower CP concentrations in *S. latissima* harvested from Danish waters. There is a large variation among different studies in the reported CP concentrations. *Ulva* sp. has been reported to contain between 9 and 27 % CP on DM basis (Ortiz et al., 2006; Yaich et al., 2011). The reported CP concentration in *S. latissima* ranges from 0.88 to 32 % on DM basis (Nielsen et al., 2016; Sharma et al., 2018). Heterogeneity in laboratory methods used for CP analysis in these studies is one of the major limitations in comparing CP contents of these seaweeds (Manns et al., 2017; Mols-Mortensen et al., 2017; Harrysson et al., 2018). In addition, confusions may also arise from the use of different nitrogen to protein conversion factors (i.e. Nx6.25 *vs.* 5.0).

Due to the low protein levels, whole seaweeds will supply very little protein in ration formulations for non-ruminant livestock. Moreover, the digestibility may be low for non-ruminant animals due to encapsulation of protein in the cellular matrix making protein non-accessible for digestive enzymes (Øverland et al., 2019). Indeed, Bikker et al. (2020) observed that fiber was negatively correlated with *in vitro* digestibility of seaweeds. Therefore, whole seaweeds are not suitable as a protein supplement for non-ruminant animals, but an extract of seaweed proteins may provide a product with high protein quality.

Despite the low levels of CP in these seaweeds, the observed EAA:NEAA ratio in the studied seaweeds is only slightly lower than that in soybean meal (Table 1). Soybean meal can be used as a reference for a high quality protein source. Monogastric animals and pre-ruminant calves cannot synthesize EAA, therefore they depend on the daily supply of EAA (Wu, 2014). Similar to our results, Vieira et al. (2018) observed that tryptophan and methionine were the least abundant EAA in *A. nodosum* and *Ulva* sp.. Tryptophan plays a role in the immune system functioning of the neonatal calves (Hernandez-Castellano et al., 2018). The observed tryptophan concentrations in seaweeds were slightly lower than the concentrations found in soybean meal. In accordance with the present study, other authors also found leucine to be the most abundant EAA in seaweeds from Nordic waters (Nielsen et al., 2012; Marinho et al., 2015; Gaillard et al., 2018; Sharma et al., 2018). The leucine concentrations in seaweeds were slightly below those in soybean meal in which leucine is also often the most abundant EAA. In the present study, the lysine concentrations observed in seaweeds were at least 1.2 times lower than lysine concentration in soybean meal. Among EAA, leucine and lysine are required at highest concentrations in diets for pigs and the latter is often first limiting (National Research Council, 2012). Furthermore, *A. nodosum* had a 1.4 times higher methionine concentration compared to soybean meal (Table 1). Lysine and methionine are the most limiting AA in the diets for dairy cows (Schwab and Broderick, 2017).

4.2. Crude ash, minerals and heavy metals content

Generally, seaweeds have high concentrations of crude ash, which can be as high as 55 g/100 g DM (Holdt and Kraan, 2011; Makkar et al., 2016; Øverland et al., 2019). This is mainly due to their tendency to accumulate minerals from the seawater. Similar to the present study, Tayyab et al. (2016) observed high concentration of crude ash in *U. lactuca* (48.3 g/100 g DM) harvested from Norwegian waters during autumn. The ash content in seaweeds commonly ranges from 20.0 to 35.0 g/100 g DM (Holdt and Kraan, 2011; Corino et al., 2019; Øverland et al., 2019). High mineral contents in seaweeds make them an alternative source of inorganic minerals for livestock (Holdt and Kraan, 2011; Makkar et al., 2016; Øverland et al., 2019). Therefore, seaweeds can be a sustainable source of essential minerals such as Zn, Cu, Se, and Co for weanling piglets and calves. Selenium, which is an essential mineral, has immunomodulatory and antimicrobial properties, and is of particular interest for calves because it can often be deficient in milk (Mehdi and Dufrasne, 2016). Furthermore, whole dried *A. nodosum* supplementation to weaned piglet diets improved the I retention in muscle and fat tissues of these animals (Dierick et al., 2009), suggesting that this mineral was bioavailable. The brown seaweeds and green seaweed in the present study had high concentrations of I and Fe, respectively. Therefore, bioavailability of these minerals from whole seaweeds or seaweed mineral extracts to livestock can be an interesting area of research, promoting sustainability in utilization of mineral resources. Extraction of the mineral fraction of seaweeds may alter bioavailability and will allow it to be used as an ingredient for vitamin and mineral premixes used in animal nutrition. However, as also observed in the present study, inter- and intra-species variability in mineral concentrations may become a challenge for the future use of seaweeds as a mineral supplier, due to both nutritional and environmental concerns (i.e. excess minerals in excreta). Wells et al. (2017) and Nielsen et al. (2016) observed that the mineral and heavy metal composition of the seaweeds is affected by harvest location and season, which was also observed in the current study regarding the *Ulva* sp.. Water quality monitoring and enhanced knowledge of changes in mineral composition along the year will contribute to obtain a more constant seaweed mineral composition for animal feeds.

On the contrary, high crude ash concentrations can limit the inclusion level of whole seaweeds in feed rations, as this can dilute the concentration of other nutrients in the diet (Tayyab et al., 2016). In addition, high concentrations of some macrominerals in the seaweed meals, such as Na and K observed in the present study, can increase water consumption by the animals (Makkar et al., 2016). Growth impairment or toxicity may arise from misbalanced or excess mineral concentrations in feed rations (National Research

Council, 2012). Moreover, toxicities due to high heavy metal concentrations could limit seaweed inclusion (i.e. As and Pb) in feed rations (Øverland et al., 2019).

The European Union (EU) has established maximum levels (MLs) for a wide range of undesirable substances in animal feeds and feedstuffs, including heavy metals such as As, Pb, Cd and Hg (European Commission, 2002). These regulations, therefore the following discussions, are based on a DM content of 88 g/100 g. Considering the European directive, As concentrations in seaweeds can be a limiting factor for their inclusion rate in a feed ration. For a given seaweed meal (i.e. as a feedstuff), the ML for As is 40 mg/kg DM. In the present study, *S. latissima* had the highest As concentration (37.9 mg/kg), which is just below the ML for seaweed meal. The ML for As in complete feed rations is 2 mg/kg. According to the present study, at a 5.27 % inclusion rate, *S. latissima* would reach the ML for As concentration in a complete feed. Considering these regulations on As concentration in the complete feed, maximum inclusion rates for early and late harvested *Ulva* sp. as well as for *A. nodosum* are 38, 20 % and 7 %, respectively. However, As is only toxic in its inorganic form (Wells et al., 2017). The EU directive states that for seaweeds used as feed materials, the level of inorganic As concentration should be below 2 mg/kg DM. Results from the present study are in compliance with this limit for *A. nodosum*, *S. latissima* and *Ulva* sp. harvested in June. However, *Ulva* sp. harvested in August had a concentration of inorganic As above the ML (8.3 mg/kg DM). Hence, this seaweed may not be considered as an ingredient in a complete feed. These results emphasize the need for analysing inorganic As concentrations in seaweeds, before using them in feed rations. For complete feed rations, the ML for Cd is 0.5 mg/kg DM. Therefore, up to 50 % of the complete feed could consist of seaweeds from this study without compromising the ML. In addition, ML for Pb concentration in a complete feed ration is 5 mg/kg DM. Consequently, up to 67 % of a complete feed could consist of seaweed before ML is reached. Hence, Pb and Cd concentrations in the studied seaweeds are not limiting their inclusion in a feed. In all above incidences, potential sources of these toxic heavy metals coming from other feedstuffs in the complete feed should be considered as a whole, during the compliance assessment.

4.3. Crude fat and fatty acids composition

As seen in the present study, the crude fat content in seaweeds is generally below 4–5 g/100 g DM (Holdt and Kraan, 2011; Øverland et al., 2019), however this can vary depending on the species (Corino et al., 2019). In agreement with the present study, Harrysson et al. (2018) found palmitic acid to be the most abundant FA in *Ulva lactuca* harvested from Swedish waters. Furthermore, similar to the present study, oleic acid was the most abundant FA in *A. nodosum* (Peinado et al., 2014; Lorenzo et al., 2017). In addition, Peinado et al. (2014) observed that palmitic, myristic and oleic acid are the most abundant FA in the studied seaweeds, including *A. nodosum*. Such a trend was also observed in the seaweeds in the present study.

Polyunsaturated fatty acids (PUFA) are divided into 2 groups as omega (n); n-6 and n-3 groups, based on the position of the terminal double bond (Harris, 2018). Alpha-linolenic acid, EPA and docosahexaenoic acid (DHA) comprise the n-3 FA group. Linoleic acid and arachidonic acid comprise the n-6 FA group. The n-6:n-3 ratio is commonly used as an indicator of functional quality of a lipid source (Harris, 2018). Similar to the present study, van Ginneken et al. (2011) observed the highest concentration of α-linolenic acid (i.e. 20 % of total FAs) in *U. lactuca* among other studied seaweeds. These authors also observed similar concentrations of EPA in *U. lactuca* and *A. nodosum*. In addition, Peinado et al. (2014) observed similar concentrations of EPA and DHA in *A. nodosum*. Eicosapentaenoic acid, DHA and other unsaturated FAs in the diet are vital for improved health in humans (Calder and Yaqoob, 2009). Therefore, enrichment of food animal tissues (i.e. muscle, milk etc.,) with such FAs is beneficial.

Although other bioactive components of the lipid fraction were not analyzed in the present study, seaweeds are known to contain bioactive compounds such as antioxidants and phenols (Holdt and Kraan, 2011). In order to evaluate the quality of the fat fraction, up-concentrated seaweed fat extracts could be tested using animal models such as pigs and calves. However, extraction procedure could be too expensive for its use in feed rations.

4.4. Total dietary fiber, non-starch polysaccharides and lignin-like substances

The nutritional value of NSPs in seaweeds is dependent on their fermentability. Fermentation of NSPs by colonic bacteria provides a certain proportion of the energy requirement of host animal. Moreover, fermentation of specific seaweed NSPs has been associated with prebiotic and health promoting effects, as NSPs stimulate growth of beneficial microbes at the intestinal level. Laminarin and fucoidan derived from brown seaweeds are of particular interest as prebiotic substrates, as these stimulate the growth of *Lactobacilli* spp. while reducing pathogenic *E. coli* in faeces from weaned piglets (O'Doherty et al., 2010). The fermentation by the beneficial bacteria results in production of short chain fatty acids (SCFA) including butyrate in last part of the intestine. Butyrate is the main energy source for epithelial cells in colon and caecum (Bach Knudsen et al., 2018). Moreover, increased butyrate production and reduced pathogenic *E. coli* population improves the intestinal epithelial integrity, hence avoiding leaky gut condition and related diarrhea in piglets and calves (Bach Knudsen et al., 2018). Furthermore, increased production of SCFA and reduced pathogenic bacteria contribute to improvement of the villous architecture in small intestinal epithelium (Heo et al., 2013), increasing the surface available for nutrient absorption. All these factors together contribute to reduced diarrhea incidence and improved growth and health of piglets and calves. However, it is noteworthy that most of these beneficial effects were observed only when the animals were fed with specific seaweed extracts (i.e. laminarin and/or fucoidan) and such effects were not clearly visible when whole seaweeds were fed (Dierick et al., 2009; Michiels et al., 2012; McDonnell et al., 2016).

The fermentability and associated health promoting properties mentioned above may vary depending on the type and complexity (i.e. monomeric units, bonds and degree of branching, etc.) of the specific polysaccharides available in the seaweeds (Holdt and Kraan, 2011). This influences the physical properties including the degree of solubility, viscosity and lignin-like substances content of these

polysaccharides (Knudsen et al., 2013). For an example, soluble dietary fiber fermentation already starts in the ileum and caecum, whereas insoluble dietary fiber is degraded slowly, mainly in the colon (Knudsen et al., 2013).

Knowledge on the exact NSP composition of the seaweeds (i.e. laminarin, fucoidan, alginates, etc.) is useful to predict its fermentability in the distal intestine, although it was not possible to measure in the present study. Fiber derived monosaccharides (i.e. fucose, glucose, rhamnose and uronic acid) can provide an indirect indication about the type of NSP present in these seaweeds. Both brown seaweeds were rich in mostly soluble uronic acid. Uronic acid in seaweeds is present in the forms of β-mannuronic acid and α-guluronic acid, which are the monosaccharides that form the structural polysaccharide, alginate (Stiger-Pouvreau et al., 2016; Okolie et al., 2017).

In the present study, the total fucose concentration in *A. nodosum* was comparable to that observed by Dierick et al. (2009) in dried *A. nodosum* (7.6 g/100 g DM). Similar fucose concentrations were obtained by Bikker et al. (2020) in *A. nodosum* carbohydrates harvested from Scottish waters (6.4 g/100 g DM). Conversely, *A. nodosum* harvested from Irish waters contained only 2.24 g fucose/100 g DM (Bikker et al., 2020). Unlike green seaweeds, brown seaweeds contain fucoidan, which is a cell-wall polysaccharide primarily composed of sulphated L-fucose (Holdt and Kraan, 2011; Stiger-Pouvreau et al., 2016). Usually, *A. nodosum* has high concentrations of fucoidan (i.e., 4−10 g/100 g DM) (Holdt and Kraan, 2011), which might explain the high concentration of fucose in *A. nodosum* measured in the present study. Based on its fucose content, the brown seaweed *S. latissima* seemed to have lower fucoidan content in comparison to *A. nodosum*. In *S. latissima* harvested from Danish waters the fucoidan content varied between 2.3–6.2 g/100 g DM over the year (Bruhn et al., 2017). Sharma et al. (2018) observed fucose concentrations of 1.31–2.85 g/100 g DM in *S. latissima* harvested from Norwegian waters in August and May, respectively. Bikker et al. (2020) observed low levels of fucose in *S. latissima* from Scottish and Irish waters (1.5 and 1.2 g/100 g DM, respectively).

Laminarin is a β-glucan made up of glucose molecules (Stiger-Pouvreau et al., 2016; Okolie et al., 2017). Non-cellulosic glucose was the main monosaccharide derived from the fiber fraction of *S. latissima* in the present study. Therefore, this seaweed probably contained higher concentrations of laminarin than fucoidan. Stévant et al. (2017) observed 5% methanol-acid hydrolysed glucose in *S. latissima*, which was hypothesized to be derived from laminarin. Furthermore, Manns et al. (2017), found around 5−20 g/100 g DM glucose derived from glucan in *S. lastissima* harvested from Danish waters. *Saccharina latissima* in the present study had a soluble glucose concentration of around 8.5 g/100 g DM, which was comparable to the lower range in the latter mentioned study.

Ulvan is a major structural polysaccharide found in cell-walls of *Ulva* sp. Ulvan is a highly branched polymer consisting of monomeric units of predominantly rhamnose, glucuronic acid and xylose (Kidgell et al., 2019). These monosaccharides were indeed highly prevalent in the studied seaweeds. The high concentrations of rhamnose in *Ulva* sp. might indirectly indicate high concentrations of ulvan in this seaweed (Holdt and Kraan, 2011).

Ascophyllum nodosum was composed by a high concentration of lignin-like substances. Similarly, Dierick et al. (2009) observed high concentrations of lignin-like substances (21.4 g/100 g DM) in *A. nodosum* from Irish waters. The lignin-like substances analysed in the present study were insoluble in sulphuric acid, therefore, were expected to have similar properties to lignin regarding its effects on feed digestibility. Lignin cannot be digested by the endogenous enzymes of the host animal and cannot be fermented by the gut microbiota. Therefore, lignin does not supply nutritional or functional benefits to the animal. Moreover, at high levels, lignin impairs the fermentability of polysaccharides due to the formation of cross linkages (Knudsen et al., 2016). In accordance with this, Bikker et al. (2020) found that high ADL concentrations negatively impacted *in vitro* gas production from seaweeds.

5. Conclusion

The chemical composition of *A. nodosum, S. latissima* and *Ulva* sp. harvested from Danish and Icelandic waters was mainly made up of crude ash and fiber. The crude ash portion comprised of high concentrations of essential and valuable microminerals including I, Cu, Fe, Mn, Se. On the contrary, high total As and inorganic As concentrations might limit the use of *S. latissima* and *Ulva* sp. in the feed ration. Furthermore, the high crude ash concentrations may limit the inclusion of whole seaweeds in feed rations. The total dietary fiber concentration was the highest in *A. nodosum* followed by *S. latissima* and *Ulva* sp., where *S. latissima* had the highest soluble dietary fiber concentration. Soluble dietary fiber could be of interest as a fermentable substrate. *Ascophyllum nodosum* contained a high concentration of lignin-like substances, which might impair its fermentability. Compared to other components, crude protein and fat contents were low in the studied seaweeds. However, the protein fraction of the studied seaweeds was characterized by a high concentration of EAA. The evaluated chemical composition of the batches of *A. nodosum*, *S. latissima* and *Ulva* sp., suggests that the use of whole seaweeds in feed rations could be problematic, however, specific seaweed extracts (i.e. laminarin, fucoidan, DHA, EPA) can be of interest as health promoting functional feed ingredients for farm animals.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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