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Protein value and health aspects of the seaweeds *Saccharina latissima* and *Palmaria palmata* evaluated with mink as model for monogastric animals

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ABSTRACT

The aim of the present work was to evaluate the protein value of products from the two seaweeds *Saccharina latissima* and *Palmaria palmata* grown in Norwegian waters and to characterize possible beneficial or detrimental effects in the intestine and other organs. Mink, a well-established model for comparison of nutrient digestibility in monogastric animals was used. Two products from each of the seaweeds, a dried whole biomass and a protein concentrate, were evaluated. Five diets were made; a reference diet based on fish meal, and one for each of the four seaweed products. In the latter four, seaweed supplied 200 g/kg of crude protein. Each diet was fed to four male mink for two weeks. The results showed that diets with seaweed were less palatable than the fish meal diet. The animals fed the whole *Saccharina* diet had a significantly higher water intake and urine production than the other animals, supposedly due to the very high ash content of this seaweed product. This diet also stood out regarding urine concentration of iodine, which was 300 times higher than for the fishmeal-based diet. Apparent digestibility coefficient (ADC) for total amino acids, estimated by difference, showed low values for all the seaweed products; 0.574 and 0.734 for the whole and protein concentrated *Saccharina* products, and 0.588 and 0.700 for the two *Palmaria* products, respectively. The apparent amino acid digestibility coefficients (ADC) showed greater variation and were particularly low for histidine, <0.000 and 0.271 for the whole *Saccharina* and *Palmaria* products, respectively. Also, the ADC of methionine was low for these products, and cysteine ADC showed negative values for both *Saccharina* products. The estimated chemical score of the proteins, based on the digestible amino acids profile, was 0 and 520 g/kg for

Abbreviations: Actb, beta actin; ADC, apparent digestibility coefficient; ANOVA, analysis of variance; ASE, accelerated Solvent Extractor; bps, base pairs; BW, body weight; *cDNA*, complementary DNA; CF, crude fat; CP, crude protein; *Cq*, quantification cycle; Cys, cysteine; DM, dry matter; DNA, deoxyribonucleic acid; dsDNA, double stranded DNA; FM, fish meal; *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase; His, histidine; *hprt1*, hypoxanthine phosphoribosyl transferase 1; IELs, intraepithelial lymphocytes; Ile, isoleucine; Lys, lysine; Met, methionine; N, nitrogen; NMSD, nonmetric multidimensional scaling; PalmP, protein concentrated *Palmaria palmata*; PalmW, whole, dried *Palmaria palmata*; PCR, polymerase chain reaction; Phe, phenylalanine; QIIME, quantitative insights into microbial ecology; qPCR, quantitative polymerase chain reaction; RNA, ribonucleic acid; SaccP, protein concentrated *Saccharina latissima*; SaccW, whole, dried *Saccharina latissima*; SD, standard deviation; *sdha*, succinate dehydrogenase complex A; Tyr, tyrosine.

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the whole product and protein concentrate of *Saccharina*, 260 and 520 g/kg for the whole product and the protein concentrate of *Palmaria palmata*, respectively. Expression of genes associated with digestive and immune functions showed minor effects in the jejunum, somewhat more pronounced effects in the colon. The latter effects were related to immune functions and lipid metabolism. No diet-related alterations in the histology of the jejunum and colon were observed. The histological investigation of liver and kidney structure showed some alterations in the seaweed fed animals. Regarding microbiota assemblage in mucosa of jejunum and colon, no clear diet effects were observed either in richness or diversity. In conclusion: the biological value of the seaweed proteins was low. Only one of the products, the *Palmaria* protein concentrate, might be considered of any use as a protein source. No clear beneficial or detrimental effects of the seaweed products were observed on gut health and function. The results regarding kidney structure and function, as well as the high iodine in the urine, indicating that further investigations are required to secure that animal health is not challenged by use of these seaweed ingredients in animal diets.

1. Introduction

Seaweeds have been used as sources of nutrients and health-promoting components for thousands of years and are still being used particularly in Asian countries (McHuge, 2003). Their potential to become important nutrient sources for animals is now under investigation in several laboratories. Some seaweed species contain proteins which, based on chemical analyses, have well-balanced amino acid composition at levels high enough to make them useful as protein sources for humans and animals (McHuge, 2003; Garcia-Vaquero and Hayes, 2016; Biancarosa et al., 2018; Pirian et al., 2018). Until now, the nutritional value of seaweeds has been described and evaluated based only on chemical analyses and some *in vitro* studies (Paiva et al., 2017; Sharma et al., 2018; Overland et al., 2019). However, to be useful as an ingredient in diets for monogastric animals, the nutrients must be available *in vivo*. Present knowledge on bioavailability of nutrients in seaweed is very limited and no data have been found regarding amino acid apparent digestibility (ADC). The results from the few available studies indicate that seaweed may be included in diets for monogastrics without effects on growth performance, but the nutritional value may be low due to low protein and energy ADC (Summarized by Øverland et al. (2019)). Results from an *in vivo* experiment with sheep, conducted within the same project as the experiment presented here, are

Table 1
Proximate composition of experimental ingredients.

	Fish meal	Whole *Saccharina*	*Saccharina* protein concentrate	Whole *Palmaria*	*Palmaria* protein concentrate
Dry matter, g/kg	893	926	918	936	936
Crude protein, g/kg	664	129	203	268	459
Crude fat, g/kg	74	4	8	8	19
NFE[a], g/kg	0	0	0	39	138
Other carbohydrates, g/kg	18	421	476	411	278
Ash, g/kg	139	373	231	210	43
Iodine, g/kg	0.01	6.3	2.9	0.29	<0.09
Estimated gross energy[b], MJ/kg	19.5	10.8	13.7	14.8	19.4
Amino acids (AA), g/kg					
Arginine	42.0	5.7	11.6	13.8	29.1
Histidine	12.8	0.8	3.4	3.0	7.2
Isoleucine	28.8	5.3	10.1	9.1	17.4
Leucine	49.1	9.0	17.5	15.2	28.1
Lysine	53.6	5.5	10.5	13.4	26.5
Methionine	17.4	2.2	4.8	3.5	7.9
Phenylalanine	25.7	4.9	11.4	9.6	19.4
Threonine	29.2	6.2	11.2	11.7	22.1
Valine	33.0	6.8	12.4	13.7	28.1
Tryptophan	5.6	2.0	3.7	2.7	5.5
Alanine	43.4	10.1	14.8	17.9	30.4
Aspartic acid	64.4	12.8	23.2	27.5	45.0
Cystine	5.6	1.8	3.1	5.7	12.0
Glutamic acid	96.2	14.2	26.8	34.1	44.0
Glycine	44.8	8.0	12.9	15.4	30.0
Proline	28.9	5.8	10.1	21.3	20.6
Serine	30.5	5.9	10.6	12.5	26.0
Tyrosine	5.4	–	–	–	10.6
Sum AA	616	107	198	230	410
Estimated Sum AA/N	5.80	5.18	6.10	5.37	5.58

[a] NFE: Nitrogen free extracts, estimate of digestible carbohydrates.
[b] Gross energy: estimated as (Crude protein (g) * 24.5 (kJ)+Crude fat (g) *39.5 (kJ)+Starch (g) * 17.7 (kJ) + NFE (g) * 17.7 (kJ)) (Maynard et al., 1983).

available (Özkan-Gülzari et al., 2019). The results indicate that, in ruminants, seaweeds as nitrogen source has low nutritional value and may affect rumen fermentation negatively (Özkan-Gülzari et al., 2019).

In Norway, seaweed is extensively harvested for alginate production, but has not been significantly appreciated as food or for other applications. Recently the cultivation of seaweed for a wider range of potential product applications has been promoted (Skjermo et al., 2014). Among the seaweeds growing in Nordic waters, *Saccharina latissima* and *Palmaria palmata* are of interest as two quite different species, brown and red, respectively, with very different chemical composition. Typical for brown seaweed is a high content of alginate, comprising up to 40 0 g/kg of the dry matter. They also contain some cellulose, laminaran, mannitol and fucoidan (Holdt and Kraan, 2011). In contrast, the main carbohydrates of red algae are xylans (Holdt and Kraan, 2011). The protein content of brown algae is generally lower than in red, 30–140 g/kg of dry matter in *Saccharina latissima*, 80–350 g/kg in *Palmaria palmata.* Additionally, the two seaweed also differ in their mineral content. Of importance is the very high content of iodine in brown algae (Roleda et al., 2018), which, at sustained high intakes, may induce goitre in predisposed individuals (Holdt and Kraan, 2011), and possibly other health challenges (Shoyinka et al., 2008).

The present study was conducted to evaluate the potential protein value and reveal possible functional properties of two products of *Saccharina latissima* and *Palmaria palmata*, a whole dried and a protein concentrated from each. The whole products were produced by drying of newly harvested material, whereas the protein enrichment was achieved by a water-extraction and carbohydrase treatment which reduced the content of salts and soluble carbohydrates. Adult male mink (*Neovison* vison) was used as model animal for the study. This model is well established, not at least for the study of nutritional aspects of novel feeds and ingredients, but also to produce accurate results with a limited number of animals (Ahlstrøm and Skrede, 1998; Skrede et al., 1998) under conditions, as in the present, when availability of the experimental feed ingredients is limited. The main goals of the work were to evaluate the protein quality of the products and to find possible effects on the health and function of the intestine and other organs.

2. Materials and methods

2.1. Seaweed products

The seaweed products evaluated in the present study were as follows: Whole, dried *Saccharina latissima*, protein concentrated *Saccharina latissima*, whole, dried *Palmaria palmata*, and protein concentrated *Palmaria palmata*. Table 1 shows the content of nutrients of these products as analysed.

Wild *Palmaria* biomass was collected outside Bodø, Norway, while cultivated *Saccharina* biomass was harvested at the coast of Trøndelag, Norway. Seawater was drained, and small stones and other impurities were removed manually. The biomass was stored in plastic bags at −20 °C until further processing. The protein concentrate ingredient of the *Palmaria* was produced by milling frozen material, stirring in a tank containing water at 30 °C, in a ratio of one part wet biomass to three parts water. Xylanase (Sigma X2629, St. Louis, MO, USA) was used, 5.44 g/kg dry weight, to help solubilize the biomass and release soluble compounds. After stirring at pH 4.5–5 and 30 °C for 5 h, the biomass slurry was centrifuged in a continuous centrifuge. The protein concentrate of *Saccharina* was produced by milling frozen material and heat treatment (70−80 °C, 10 min) before transferring to a stirring tank. The heat treatment was included in order to prevent bacterial growth during overnight incubation. Coldwater was added until the temperature reached 27 °C (wet biomass: water 1:1) and the pH was adjusted to 7.6. An alginate lyase (AL951, provided by CEVA, France) was added, 0.33 g/kg dry weight, to partly hydrolyse alginate and thereby facilitate solid-liquid separation by centrifugation. After incubation for 15 h at 22−25 °C, the biomass slurry was centrifuged in a continuous centrifuge. The centrifuge sludges from the two species were collected and air-dried (25−30 °C) to give the protein concentrated products.

The whole *Saccharina* product was obtained by drying in a hot-air dryer for 2 h at 70 °C until approx. 700 g/kg dry weight, followed by further drying at 30 °C until >900 g/kg dry weight. The whole Palmaria product was dried in a freeze-dryer.

2.2. Animals and feeding protocol

The mink trial was carried out at Centre of Animal Research of the Norwegian University of Life Sciences in Ås, Norway (National permission number: 2012-15-2934-00394 in accordance with the institutional and national guidelines for the care and use of animals (NMAF, 1996, 2009). The animals used in the study were adult male mink (*Neovison vison*) of the brown genotype. Mean body weight was 2.8 kg (Standard deviation (SD):0.2). Four healthy mink were assigned for each of five experimental diets. The animals were kept in individual cages equipped for controlled feeding and quantitative faecal collection, separating faeces and urine as described by Jørgensen and Hansen (1973). The experiment was conducted in a well-ventilated room with controlled temperature (18 °C) and lighting to adjust the day length to natural photoperiod. The experiment lasted for 14 days, of which the first three days were used for the adaptation of the mink to the feed and the following four days were used for the faecal collection (Skrede, 1979). The last 7 days gave additional time for studying possible functional effects of the algae products. Yttrium oxide was applied at 1.5 g/kg as an inert marker in the feed for digestibility measurements. Feed allowance was 62−65 g DM per day corresponding to the daily requirement of metabolizable energy, i.e. of 600 kJ/ kg $BW^{0.75}$ (Lassén et al., 2012). Feed was given once daily, and feed intake and faecal production were registered once every day in the faecal collection period. Faeces from each animal were pooled over the four-day collection period and freeze-dried, grounded and sifted to remove hair.

Table 2
Diet ingredients and planned macronutrient composition[a].

	Diets[b]				
	FM	SaccW	SaccP	PalmW	PalmP
Ingredient[c], g/kg dry ingredients					
Fish meal (LT quality)	386	271	289	301	313
Whole saccharina		354			
Saccharine conc.			232		
Whole palmaria				186	
Palmaria conc.					117
Corn starch	229	201	214	224	232
Soybean oil	193	170	181	188	195
Cellulose powder	187		80	96	139
Vitamins and minerals	2.9	2.5	2.7	2.8	2.9
Yttrium marker	1.4	1.3	1.4	1.4	1.5
SUM	1000	1000	1000	1000	1000
Planned chemical content, g/kg dry matter					
Ash	61	189	105	85	57
Protein	280	247	263	273	283
Fat	237	208	219	227	236
Carbohydrates	422	356	413	414	424
Analyzed chemical content					
Crude protein, g/kg dry matter	270	241	250	273	279
Crude fat, g/kg dry matter	195	183	146	214	195
Starch, g/kg dry matter	201	175	188	197	212
NFE[d], g/kg dry matter	472	385	498	420	464
Ash, g/kg dry matter	63	190	106	92	61
Gross Energy[d], MJ/kg dry matter	26.2	23.0	24.0	26.1	26.5
Iodine[e], g/kg dry matter	0.004	2.23	0.67	0.05	<0.01
Essential amino acids (EAA), g/kg dry matter					
Arginine	16.6	14.2	15.4	15.8	18.0
Histidine	5.0	4.2	4.7	4.5	5.3
Isoleucine	11.2	9.9	10.9	10.3	11.7
Leucine	19.5	17.6	19.2	18.6	20.6
Lysine	20.8	16.9	18.6	18.9	20.7
Methionine	6.8	5.1	6.4	5.8	7.0
Phenylalanine	10.1	9.8	10.4	10.0	11.3
Threonine	11.6	10.9	11.6	11.8	13.0
Valine	13.0	11.7	12.8	12.7	14.6
Tryptophan	2.7	2.7	3.0	2.7	2.9
Sum EAA	117.3	103.0	113.0	111.1	125.1
None essential (NEAA), g/kg dry matter					
Alanine	17.3	17.0	16.8	17.4	18.9
Aspartic acid	25.3	23.7	25.1	26.0	28.1
Cystine	2.2	2.2	2.4	2.9	3.5
Glutamic acid	37.8	33.1	35.5	37.1	38.6
Glycine	17.7	15.9	16.6	17.2	19.1
Proline	12.0	10.4	11.3	13.7	13.0
Serine	12.3	11.3	12.0	12.6	14.3
Tyrosine	5.8	6.5	5.5	6.2	6.4
Sum NEAA	130.4	120.1	125.2	133.1	141.9
Sum amino acids (AA)	247.7	223.1	238.2	244.2	267.0
Estimated ratio AA/N	5.73	5.78	5.96	5.59	5.98

[a] Before given to the animals, the diets were added water to obtain a suitable consistency.

[b] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein.

[c] Fish meal, Norse-LT 94, Norsildmel AS, Bergen, Norway; Corn starch, pregelatinized, Pregeflo ®CH 20, Roquette Freres, Lestrem, France; Soybean oil, Mills AS, Oslo, Norway, Cellulose powder, Arbocel ®; crude fibre concentrate, J. Rettenmaier & Sohne, GMBH, Rosenberg, Germany; Yttrium marker, Yttrium III oxide, 990 g/kg (Y_2O_3), Merck, NJ, USA; Vitamin and minerals, Vilomix AS, Hønefoss, Norway. Containing per g: 11 mg Cu, 115 mg Zn, 35 mg Mn, 1.5 mg I, 100 mg Fe, 1376 µg vitamin A, 10 µg vitamin D3, 100,000 µg vitamin E, 12,000 µg thiamin, 24,000 µg riboflavin, 150,000 µg niacin, 60,000 µg pantothenic acid, 30,000 µg vitamin B6, 64 µg vitamin B12, 4000 µg folic acid, 1500 µg biotin.

[d] NFE: Nitrogen free extracts, estimate of digestible carbohydrates. Gross energy: estimated as (Crude protein (g) * 24.5 (kJ)+Crude fat (g) *39.5 (kJ)+Starch (g) * 17.7 (kJ) + NFE (g) * 17.7 (kJ)) (Maynard et al., 1983).

[e] Estimated based on table values for fish meal and calculated based on analyses of content in the macroalgae ingredients.

2.3. Drinking water consumption and urine excretion

Drinking water was supplied from a 1000 mL plastic bottle with a semi-automatic nipple system operated by the animal, and consumption was measured by registration of the change in water volume in the bottle. Urine was collected in bottles connected to each tray under the cages and weighed and sampled after the collection period. Water intake and iodine balance were determined over the last seven days of the trial.

2.4. Feeds and feeding

Table 1 shows the nutrient content in the four seaweed products as analysed. Receipts and nutrient composition of the diets are presented in Tables 2 and 3. Precooked corn starch and soybean oil are standard ingredients and they were included at the same level in all five diets. Cellulose powder was added to most of the diets to balance carbohydrate level. Water was added to optimize feed consistency. In the four experimental diets with seaweed products 200 g/kg of the crude protein came from seaweed and 800 g/kg from fishmeal.

At termination of the feeding period, the animals were euthanized employing CO_2 gas, according to the standard procedures used by the fur animal industry in Norway. The animals were dissected, and samples collected from the jejunum, colon, liver, kidney and spleen for histological (preserved in buffered-formalin diluted in water (100 g/kg) and stored in alcohol diluted in water (700 g/kg)) and gene expression analyses (kept in RNA-later, at 4 °C for 24 h, and thereafter at -20 °C). In addition, samples for microbiota analyses were taken from mucosa of the jejunum and colon. This was done by skilled technicians wearing contamination protection and near to a gas burner to decrease bacterial contamination from the environment. After sampling from each of the animals, dissecting tools were cleaned and flamed. Mucosal tissue was washed thoroughly three times with sterile phosphate-buffer, placed in a sterile tube, snap-frozen in liquid nitrogen and then stored at −80 °C until DNA extraction.

2.5. Chemical analyses

Samples of the feed and faeces were analysed for dry matter (DM), ash, crude protein (CP), crude fat (CF) and amino acids at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway. Dry matter was determined by drying the samples to a constant weight at 103 °C. Determination of ash content in the samples was conducted by combustion at 550 °C for 10 h. Nitrogen was analysed by use of a Kjeltec 1015 Digester at 420 °C and a Kjeltec Auto 2400/2600 (Foss Tecator AB, Höganäs, Sweden), and CP was determined as Kjeldahl-N × 6.25. The analysis of amino acids followed the European Commission Directive 98/64/EC (1998). Crude fat was determined by extraction with petroleum ether and acetone in an Accelerated Solvent Extractor (ASE 200) from Dionex (Sunnyvale, CA, USA). Carbohydrates was calculated by difference: carbohydrates = DM – (CP + crude fat + ash). Iodine in the seaweed ingredients was analysed by Roleda et al. (2018) whereas urine iodine was analysed by VITAS – Analytical Services, employing a method developed by the company. In brief: Urine samples were diluted with an aqueous alkali solution and mixed before the sample were analysed for iodine content using an Agilent 7900 ICP-MS, including an ICP-MS auto sampler ASX-500 series, from Agilent Technologies, Waldbronn, Germany. Unknowns were calibrated against known standard from Sigma-Aldrich and

Table 3
Mean feed consumption, water intake and balance data the last 7-days, water intake per dry matter consumed, urine excretion per dry matter consumed and iodine excretion in urine[a].

	Diets						
	FM	SaccW	SaccP	PalmW	PalmP	Pooled SEM	P-(model)
Feed intake, wet, g/day	204	159	182	187	209	17.3	0.309
Feed intake, dry, g/day	61	48	57	55	61	5.3	0.404
Water from feed, g/day	143	111	125	133	147	12.1	0.260
Drinking water, g/day	26c	149a	54b	64b	40bc	9.4	0.001
Water intake, g/day	169b	260a	179b	196b	187b	17.0	0.012
Water intake g/g DM intake	2.8c	5.5a	3.2bc	3.6b	3.1bc	0.2	0.001
Urine excretion (g/day)	51b	133a	50b	66b	45b	9.2	0.001
Water balance (g/day)[b]	118	127	129	130	142	13.2	0.638
Urine, g/g DM intake	0.85b	2.84a	0.99b	1.19b	0.74b	0.2	0.001
Iodine intake[c], mg/day	0.24	107	39	2.2	1.6		
Iodine, µg/mL urine	0.8c	318a	258b	12.5c	14.6c	19.0	0.001
Iodine in urine, mg/day	0.04c	42.41a	12.65b	0.82c	0.65c	2.0	0.001

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).
[b] Indicating water evaporation from lungs and skin.
[c] Estimated based on analyses of content in macroalgae ingredients and table values for fish meal.

reported as ng/mL urine. Analyses of yttrium content in feed and faeces were carried out by pre-digestion with concentrated ultrapure HNO3 at 250 °C using a Milestone microwave UltraClave III (Milestone Srl, Sorisole, Italy). Samples were then diluted (to 100 g/kg HNO3), and yttrium was determined by inductively coupled plasma optical emission spectrometry (ICP-OES analysis) with a PerkinElmer Optima 5300 DV (PerkinElmer Inc., Shelton, CT, USA).

2.6. Gene expression in gut and liver tissue

Real-time quantitative PCR assays were carried out following the MIQE guidelines (Bustin et al., 2019). Total RNA from jejunum, colon and liver was extracted on a Biomek® 4000 Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA, USA) using a custom-made Reliaprep simplyRNA HT protocol (Promega, Madison, WI, USA). The RNA extraction included a DNase treatment according to the manufacturer's protocol.

The integrity of the RNA samples was evaluated by RNA Nano chip using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA purity and concentration were measured using an Epoch Microplate Spectrophotometer (BioTeK Instruments, Winooski, USA). Total RNA was stored at −80 °C until use. First-strand complementary DNA was synthesized from 1.0 μg total RNA from all samples using SuperScript® IV VILO™ Master Mix (Invitrogen™). Negative controls were performed in parallel by omitting RNA or enzyme. The qPCR primers were designed and evaluated in silico for specificity using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Ye et al., 2012). All primer pairs were run in gradient reactions in order to determine optimal annealing temperatures. Specificity was checked by a melting curve after each qPCR assay and subsequent agarose gel electrophoresis to confirm the amplification of a single product with the expected molecular size and absence of primer-dimers. Amplification efficiency was determined for each primer set using a standard curve based on 2-fold serial dilutions of randomly pooled complementary DNA. Primer details are presented in Supplementary Table 1. The expressions of individual gene targets were analyzed using the LightCycler 96 (Roche Diagnostics, Basel, Switzerland). Each 10 μl DNA amplification reaction contained 2 μL PCR grade water, 2 μL of 1:10 diluted complementary DNA template, 5 μL LightCycler 480 SYBR Green I Master (Roche Diagnostics) and 0.5 μL (10 mM) of each forward and reverse primer. Each sample was assayed in duplicate, including a no-template control. The three-step qPCR run included an enzyme activation step at 95 °C (5 min), forty to forty-five cycles at 95 °C (10 s), 60 °C (10 s), and 72 °C (15 s) and a melting curve step. The candidate reference genes beta-actin (*actb*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), hypoxanthine phosphoribosyl transferase 1 (*hprt1*) and succinate dehydrogenase complex A (*sdha*) were evaluated for intra- and interspecific stability as described earlier (Kortner et al., 2011). Target gene expression was normalized to the geometric average of *actb* and *sdha* (jejunum) and *hprt1* (liver). For colon samples, the four candidate reference genes displayed systematic and similar diet-specific variation. Therefore, we chose to use non-normalized target gene expression levels for colon, i.e. normalization towards total RNA input in the cDNA synthesis. Mean normalized expression of the target genes was calculated from raw Cq values by relative quantification (Muller et al., 2002).

2.7. Histological appearance of the gut, liver, kidney, and spleen

The fixed tissue samples collected for histological assessment were processed according to standard techniques of the Histology laboratory at Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Ås, Norway, to produce haematoxylin and eosin-stained sections. Evaluation of the tissue sections was performed blindly and in randomized order using light microscopy following criteria established in our laboratory (Krogdahl et al., 2015).

Jejunum and colon were assessed for degenerative or inflammatory morphological changes in the mucosal structure. Cell size, cell composition, and cellular changes such as hyperplasia or atrophy of the intestinal crypt were evaluated. The mucosa folds were graded for changes in villus height, lamina propria width and cellular composition, as well as appearance of enterocytes, goblet cells, and intraepithelial lymphocytes (IELs).

The liver, spleen, and kidney were all evaluated for degenerative and/or inflammatory changes to their morphology such as vascular changes due to oedema or congestion, inflammatory cell infiltration, or change in the respective cell and parenchyma structure.

The degree of change in selected morphological features for each of the tissues was graded using a scoring system ranging from 0 (normal and healthy) to 4 (severe or extensive changes).

2.8. Microbiota analysis of intestinal mucosa

Total genomic DNA was extracted from 100 mg of intestinal tissue using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The standard procedure provided by the manufacturer was followed, except for adding a bead-beating step followed by heating at 95 °C for 7 min. at the beginning as suggested by Knudsen et al. (2016). DNA extraction controls i.e. a blank negative control and a positive mock control (ZymoBIOMICS Mock Community Standard, Zymo Research Corp, Irvine, CA, USA), were included in the DNA extraction protocol. Following the extraction, PCR amplification of the V1-V2 region of the 16S rRNA gene using 27 F and 338R primers (Roeselers et al., 2011), with the Illumina overhang adapters was performed for all the extracted DNA in duplicate, including a PCR negative control (molecular grade water instead of DNA template). The PCRs were carried out as described previously (Gajardo et al., 2017) in 25 μL reactions with 12.5 μL of Phusion® High-Fidelity PCR Master Mix (Thermo Scientific, CA), 11 μL molecular grade PCR water, 0.25 μL each of the forward and reverse primers (1 μM final concentration) and 1 μL DNA. After the PCR amplification, all the duplicate amplicons were pooled and run on 15 g/kg agarose gel. Samples with bright bands between 350 and 400 bp were considered suitable for library preparation.

PCR products clean-up, library quantification, normalization, and pooling were performed as outlined in the protocol by Illumina (Illumina, 2013). Briefly, the PCR products were cleaned using AMPure beads followed by index PCR using the Nextera XT Index kit and subsequently another round of purification with the AMPure beads. Prior to library normalization and pooling, cleaned PCR products were run on a Bioanalyzer using the Agilent DNA 1000 kit to assess the amplicon size and quantified using the Qubit® dsDNA HS assay kit (Thermo Scientific). The pooled library was then denatured, diluted to 6 pM, and the PhiX control was spiked into the final pool at 150 g/l, before 2 × 300 bp paired-end sequencing on the MiSeq platform using the MiSeq v3 reagent kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

2.9. Quantification of 16S rRNA gene by qPCR

The qPCR assays were performed using a universal primer set (forward, 5′−CCA TGA AGT CGG AAT CGC TAG-3′; reverse, 5′-GCT TGA CGG GCG GTG T-3′) as reported by Vandeputte et al. (2017). The assays were run in the LightCycler 96 (Roche Applied Science, Basel, Switzerland) in a 10 μL reaction volume, which contained 2 μL of PCR-grade water, 1 μL diluted DNA template, 5 μL LightCycler 480 SYBR Green I Master Mix (Roche Applied Science) and 1 μL (3 μM) of each primer. The thermal profile for the qPCR was 95 °C for 2 min, 45 three-step cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 15 s, and a melting curve analysis at the end. Quantification cycle (Cq) values were determined using the (Rasmussen, 2001). The specificity of qPCR amplification was confirmed by the band pattern on the agarose gel after electrophoresis.

2.10. Calculations

Nutrient ADC of the diets was calculated using the formula:

((Nutrient concentration in feed/yttrium concentration in feed) - (Nutrient concentration in faeces/yttrium concentration in faeces))/(Nutrient concentration in feed/yttrium concentration in feed).

Digestibility of CP and amino acids of the seaweed protein was calculated by difference using this formula:

Digestibility of protein from seaweed = ((Protein digestibility each diet - (Digestibility fishmeal x 0.8))/0.2. Amino acid digestibilities from seaweed were calculated applying the same formula, but as the contribution factors of single amino acids could deviate from that of CP (800 g/kg from fishmeal and 200 g/kg from seaweed) the actual contribution factors were applied.

2.11. Statistical evaluation

The microbiota results were evaluated as follows: The demultiplexed, pair-ended reads were analysed using the QIIME2 (version 2017.10) (Bolyen et al., 2019). Reads were trimmed off the primer sequence (forward reads, first 20 bps; reverse reads, first 18 bps), truncated where the sequence quality drops (forward reads, at position 250 bp; reverse reads, at position 190 bp). DADA2 algorithm was used to denoise and infer amplicon sequence variants (ASVs) (Callahan et al., 2016). After the sequence denoising, the taxonomy was assigned to representative sequences by a naive Bayes machine-learning classifier (Bokulich et al., 2018), using the Greengenes 13.8 as the reference database. Mitochondria and chloroplast sequences were removed from the analysis. In addition, contaminant sequences were removed based on their presence in both, positive mock controls and negative controls. Contaminants were also detected using the criteria suggested by Davis et al. (2018) i.e., contaminants with relative abundance inversely correlated with their DNA concentration.

In order to compute alpha and beta diversity, the feature table was rarefied at 1500 reads to have an even number of reads across all the samples. The alpha diversity was evaluated using the observed species and Shannon indices. To calculate beta diversity and plot nMDS graphs, tables with relative abundance of bacteria at genus level were imported to PRIMER v7 software (Clarke and Gorley, 2015). Kruskal-Wallis-pairwise test was performed to compare the differences in alpha diversity within QIIME2. In addition, PERMANOVA was performed in PRIMER v7 (Anderson et al., 2008) based on Bray-Curtis distance. Graphs for taxa distribution were generated using QIIME2 and EXCEL.

Differences in histological scores for the various evaluated morphological characteristics of the DI tissue were analysed for statistical significance using ordinal logistic regression run in the R 3.6.2 (R Project for Statistical Computing) using *ordinal* package (Christensen, 2015). Differences were examined based on odds ratios and confidence intervals of other diet groups being allocated higher histology scores than samples from the reference FM diet group.

Regarding the evaluation of gene expression results, all results were log-transformed and then tested for normality and variance homogeneity using the Shapiro–Wilk W goodness of fit test. Comparison among diets was performed employing one-way analysis of variance (ANOVA). When ANOVA was significant, the post-hoc Tukey–Kramer HSD test was performed to interpret and compare the mean values of each treatment and check the significant difference among different sampling groups. The statistical analyses were performed in JMP Pro 14.3.0 (SAS, 2018). For other data, one-way ANOVA employing the SAS 9.3 computer software (SAS, 2017). The results are presented as least-square means, and significant differences between means ($P < 0.05$) were found with the PDIFF option using the Tukey adjustment. The level of significance was set to $P < 0.05$ for all analyses.

3. Results

3.1. Feed consumption, water and iodine balance

Feed containing whole *Saccharina* (SaccW) was less palatable for the mink compared to all the other diets, i.e. the diet containing whole *Palmaria* (PalmW), and the protein concentrate of *Saccharina* (SaccP) and *Palmaria* (PalmP). Two of the animals fed SaccW showed very low feed intake the first days and were replaced by two other animals. The new animals showed better appetite. Samples of faeces from these animals were collected the second week of the experiment, after a similar adaptation period as for the other diets. Mean feed consumption for the reference diet based on fish meal (FM), and the SaccW, SaccP, PalmW and PalmP diets were 950, 770, 870, 870 and 850 g/kg of the feed offered, respectively.

Table 3 shows results regarding feed and water intake, and urine excretion. Animals fed SaccW diet produced significantly more urine (2.8 g/g DM feed intake) and had higher urine iodine concentration (318 μg/mL), than those fed the SaccP diet (1.0 g/g DM and 258 μg/mL). In comparison, the FM fed control mink produced low urine amounts with low iodine level (0.9 g/g DM and 0.8 μg/mL), while the PalmW (1.2 g/g DM and 12.5 μg/mL) and PalmP (0.7 g/g DM and 14.6 μg/mL) fed mink showed intermediate levels. The SaccW and SaccP diets both resulted in extremely high ash and iodine content in the urine (Table 3).

Mean body weights decreased 5% during the 14 days period (data not shown). The reduction was numerically highest for animals fed the SaccW, 8%, but there were no significant dietary effects on performance as indicated by body weight and organo-somatic indices at the end of the study (Table 4).

3.2. Digestibility coefficient of crude protein and amino acids

Results regarding protein and amino acid ADC of the ingredients as such, i.e. estimated by difference, are shown in Table 5. The ADC results for protein are expressed both as ADC of crude protein (N x 6.25) and ADC of the sum of amino acids. The two ways of estimating protein ADC gave different results. Expressed as ADC of crude protein, the SaccP and PalmP showed lower ADC values than the SaccW and PalmW, whereas the opposite picture was seen when the results were expressed as ADC of the sum of amino acids. The cause of this difference was most likely a combination of two factors: an N content of the sum of amino acids deviating from 160 g/kg, the condition for using 6.25 as multiplication factor for estimation of crude protein and/or presence of non-protein nitrogenous compounds in the seaweed products, producing unpredictable errors in the evaluation of protein content in the faeces. The average N content of the seaweed ingredients and the diets are given in Tables 1 and 3, estimated based on the actual amino acid content. The estimates were lower than 6.25, mostly below 6.0, in agreement with the results of a screening of amino acids content of a great number of seaweeds (Angell et al., 2016). Thus, in the present study, protein ADC estimated based on the sum of amino acids is considered as the best estimate of protein digestibility.

Protein ADC did not differ significantly between the *Palmaria* and *Saccharina* products, but the processing to increase protein level improved the digestibility. The SaccW and PalmW showed quite low values, 0.588 and 0.574, respectively, whereas the SaccP and PalmP showed higher values, 0.734 and 0.700. The fish meal showed much higher protein digestibility, 0.862.

The differences between the seaweed products in observed ADC of the individual amino acids mostly reflected the differences in protein digestibility. However, some amino acids showed a picture deviating clearly from that of protein. Histidine ADC was negative for whole *Saccharina latissima* and low also for PalmW (0.271). Moreover, the two sulphur amino acids showed low values for the two *Saccharina* products. Methionine ADC for whole *Saccharina latissima* was 0.264 and for SaccP 0.620, clearly lower than for PalmW, 0.497, and PalmP, 0.783. Digestibility coefficient of cysteine was negative for both whole *Saccharina latissima* and SaccP. Cysteine ADC was quite low also for the *Palmaria* products, showing higher values for the PalmW (0.513) than PalmP (0.365). Lysine and Threonine, two amino acids often in shortage in animal feeds, also showed very low values for the *Saccharina* products.

Figs. 1 and 2 present the profile of digestible amino acids for the four seaweed products using recommended amino acid profiles for mink (Glem-Hansen, 1992; Børsting and Clausen, 1996) and pigs (Tybirk et al., 2019) as reference, respectively. For all products, histidine turned out to be the first limiting amino acid when mink requirement was used as the reference, with the following chemical scores: whole *Saccharina latissima* = 0%, protein concentrate of *Saccharina latissima* = 52 %, whole *Palmaria palmata* = 26 %, protein

Table 4
Body weight (BW) (g) and relative weights (somatic index, SI, g/100 g BW)) of liver (LISI), kidney (KISI), spleen (SPSI), heart (HESI) and adrenals (ADSI)[a].

	FM	SaccW	SaccP	PalmW	PalmP	Pooled SEM	P-(model)
BW	2936	2731	2816	2688	2876	97	0.594
LISI	2.26	2.38	2.32	2.37	2.62	0.158	0.584
KISI	0.60	0.55	0.60	0.55	0.62	0.042	0.691
SPSI	0.30	0.25	0.27	0.28	0.40	0.057	0.394
HESI	0.47	0.54	0.52	0.54	0.51	0.026	0.252
ADSI	0.012	0.011	0.013	0.009	0.007	0.003	0.618

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

Table 5
Apparent crude protein and amino acid digestibility coefficients (lsmeans) for the fishmeal and the seaweed products[a].

	Fish meal	Whole *Saccharina*	*Saccharina* protein concentrate	Whole *Palmaria*	*Palmaria* protein concentrate	Pooled SEM	P-(model)
Crude protein	0.824a	0.467c	0.416c	0.786a	0.642b	0.030	<0.001
Total amino acids[b]	0.862a	0.574c	0.734b	0.588c	0.700b	0.036	0.030
Sum of digestible amino acids, g/kg	531	61	145	135	287	–	–
Essential amino acids							
Arginine	0.924a	0.550d	0.729b	0.682c	0.767b	0.014	<0.001
Histidine	0.853a	neg	0.548b	0.271c	0.585b	0.033	<0.001
Isoleucine	0.908a	0.457d	0.634c	0.597c	0.715b	0.020	<0.001
Leucine	0.917a	0.562d	0.680c	0.700c	0.821b	0.014	<0.001
Lysine	0.926a	0.438d	0.658b	0.536c	0.568c	0.025	<0.001
Methionine	0.915a	0.264d	0.620c	0.497c	0.783b	0.044	<0.001
Phenylalanine	0.850a	0.479d	0.694c	0.549d	0.818a	0.026	<0.001
Threonine	0.791a	0.397c	0.702b	0.391c	0.710b	0.021	<0.001
Valine	0.851a	0.588d	0.745bc	0.711c	0.792b	0.017	<0.001
Tryptophan	0.810a	0.538d	0.662bc	0.608c	0.688b	0.018	<0.001
Non-essential amino acids							
Alanine	0.891a	0.659c	0.683c	0.696c	0.775b	0.015	<0.001
Aspartic acid	0.773a	0.432c	0.688ab	0.335d	0.675b	0.030	<0.001
Cystine	0.586a	neg	neg	0.513a	0.365b	0.041	<0.020
Glutamic acid	0.900a	0.513c	0.743b	0.690b	0.698b	0.021	<0.001
Glycine	0.843a	0.510c	0.690b	0.390d	0.532c	0.027	<0.001
Proline	0.849a	0.415c	647b	0.703b	0.653b	0.018	<0.001
Serine	0.824a	0.385d	729b	0.517c	0.729b	0.024	<0.001
Tyrosine[c]	0.792	0.617	0.665	0.654	0.832	0.065	<0.100

[a] The digestibility values for the seaweed products were obtained by difference calculation. Pooled SEM and P-value. The experimental unit was one individually caged animal, n = 4).
[b] Estimated apparent digestibility coefficient of sum of amino acids, i.e. weighted average digestibility.
[c] Tyrosine results were determined from calculated values.

concentrate of *Palmaria palmata* = 52 %. The second limiting amino acid was for whole *Saccharina latissima*=Met + Cys 31 %, protein concentrate of *Saccharina latissima*=Met + Cys 56 %, whole *Palmaria palmata*=Phe + Tyr 56 %, and protein concentrate of *Palmaria palmata*=Ile 84 %. When using the requirement for pigs (9−30 kg) as reference, the digestible amino acid profile showed the following chemical score: whole *Saccharina latissima*=His 54 %, protein concentrate of *Saccharina latissima*=Met + Cys, 56 %, whole *Palmaria palmata*=His 30 %, protein concentrate of *Palmaria palmata*=His 58 %. The second limiting amino acid was: whole *Saccharina latissima*=Met 31 %, protein concentrate of *Saccharina latissima*=His 59 %, whole *Palmaria palmata*=Phe + Tyr 62 %, protein concentrate of *Palmaria palmata*=Lys 67 %.

3.3. Gene expression

Expression levels of genes related to immune response and intestinal function were evaluated by quantitative PCR in colon, jejunum, and liver (Table 6). Overall, the most pronounced changes associated with the seaweed products were detected in the colon. The highest expression levels of most of the genes evaluated in the colon were observed in the mink fed the protein concentrated seaweed products PalmP group followed by SaccP group. Differences between PalmP and SaccP groups and all the other diet groups were significant mainly regarding genes related to immune response (*il1β*, *il10*, *tnfα*, and *infβ*) and lipid metabolism (*mttp*, *fabp2*, and *fasn*). Significant differences in expression levels in jejunum were only observed for two genes related to immune function *(il10* and *tnfα*), and was evident only for the comparison between the SaccW and FM groups. The liver did not show any significant diet effects.

3.4. Histological findings

The differences in frequency of intestinal histological changes between the groups were not found of statistical significance. See Table 7; Fig. 3a-b and Table 8; Fig. 3c-d.

Liver sections (Fig. 4) exhibited hepatocyte vacuolization of both the macro-vesicular (Fig. 4b) and the micro-vesicular (Fig. 4c) type. The former is typically lipid in nature (fatty change) and the latter representing glycogen storage, based on our previous findings (Krogdahl et al., 2015). No special staining to characterize the vacuolization was conducted in the present study. Fig. 4f shows that the occurrence of the morphological change was comparable between the groups. Mild to moderate congestion (Fig. 4d) of the liver parenchyma was also observed in most of the sections assessed (15 of 20; Fig. 4e). All individuals from the SaccW and PalmP exhibited mild to moderate congestion changes while the FM and the SaccP groups each had half of the animals with mild to moderate changes. In addition, two individuals (one each from FM and SaccW) presented with multifocal lymphocytic infiltrations of the liver

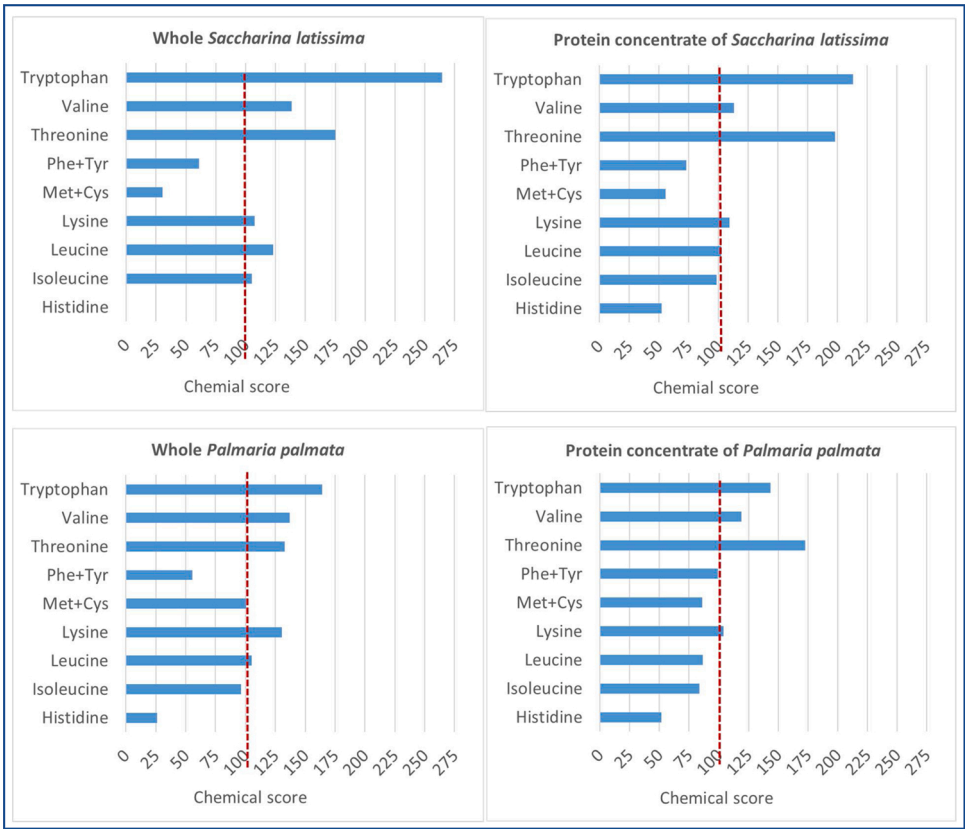

Fig. 1. Chemical score for content of digestible essential amino acids in the four products of seaweed evaluated with mink as model animal using recommendations given for growing mink (Glem-Hansen, 1992; Børsting and Clausen, 1996). The experimental unit was one individually caged animal, n = 4).

parenchyma.

Spleen tissue did not show any morphological changes deviating from the normal.

The kidneys showed notable changes in vacuolization of tubular epithelium that appeared to affect, predominantly, the proximal convoluted tubule (see Fig. 5). The changes were not observed in any of the individuals from the FM and PalmP groups, but in one individual from each of the SaccP and PalmW groups, and from two animals from the SaccW group (Table 9).

3.5. Microbiota of intestinal mucosa

The plan was to collect samples from both the digesta and mucosa from jejunum as well as colon. However, the digesta samples varied greatly in amount and consistency and were considered unsuitable for microbiota analyses. They were therefore not collected. As expected, the colon samples showed a higher average richness (Observed species 54) than the jejunum samples (Observed species 27). No significant differences among dietary groups were observed in alpha diversity. Fig. 6 shows the results of nonmetric multi-dimensional scaling (NMDS) ordination and PERMANOVA based on Bray-Curtis distance, visualizing the differences in the bacterial community structure between dietary groups. Overall, a clear clustering pattern by diet was not apparent, neither in the jejunum nor in colon. We identified 12 different phyla in both tissues, 11 phyla were shared between the two. On the other hand, the phyla *Tenericutes* and *Fusobacteria* were only found in the jejunum and colon, respectively. The most abundant genera in the jejunum and colon for each experimental group are displayed in Table 10. In jejunum the most abundant genera were *Ralstonia* (abundance ranged from 7.9 % to 21.5 %) and *Curvibacter* (abundance ranged from 5.1 % to 12.9 %) which belonged to the phylum Proteobacteria. In colon the most abundant genus was an unidentified taxon from family *Peptostreptococcaceae* belonging to the phylum Firmicutes (abundance >28 %). In agreement with the alpha and beta diversity results, no significant differences were observed in the relative abundances of genera among experimental groups. The qPCR assay, targeting a region of the 16S rRNA gene, an indirect method to evaluate total bacteria in the intestinal mucosa, indicated that there were no significant differences in the total bacterial DNA between dietary groups in any of the intestinal regions (Fig. 6c).

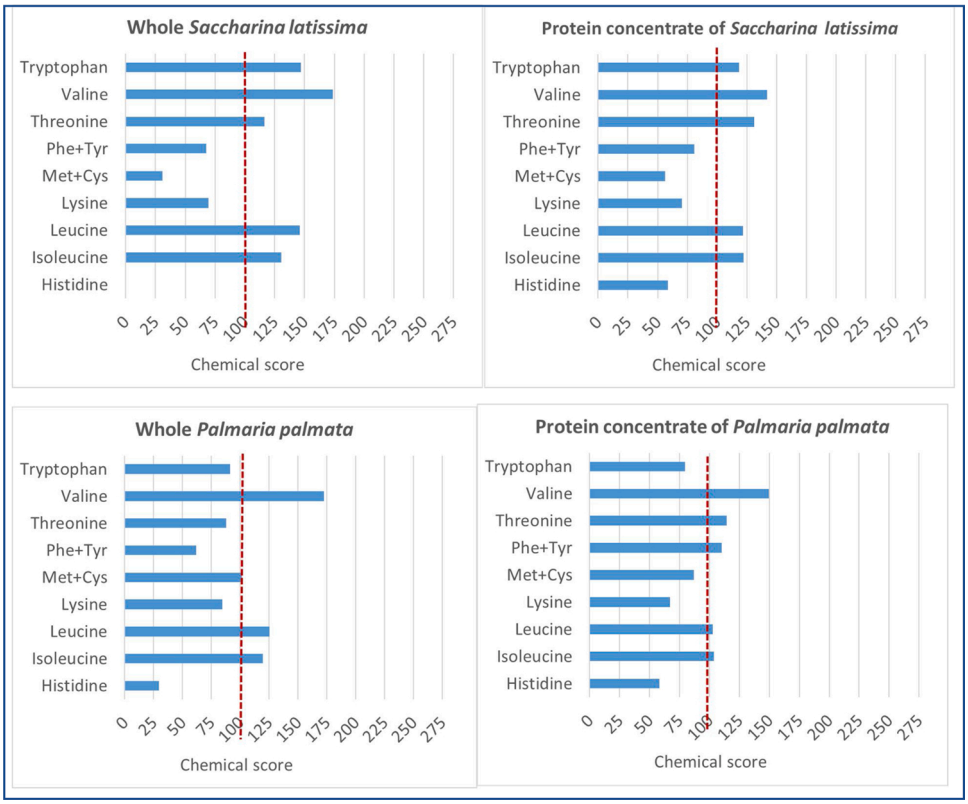

Fig. 2. Chemical score for content of digestible essential amino acids in the four products of seaweed evaluated with mink as model animal using recommendations for pigs (9-30 kg) given by Tybirk et al (2019). The experimental unit was one individually caged animal, n = 4).

4. Discussion

4.1. Water intake, urine and iodine excretion

The water intake observed for the mink fed the FM diet, 2.8 g/g DM was in agreement with earlier studies showing water intake of about 3.3 g water g/g DM consumed of a normal diet (Tauson, 1999). The increased water intake of animals fed the diets with whole seaweed products, was most likely due to the high mineral content of the seaweed products, i.e. 370, 230, 210, 40 g/kg for the SaccW, SaccP, PalmW and PalmW, respectively compared to 140 g/kg for the fish meal. These results are in line with the observations of Eriksson et al. (1984) feeding mink with diets added NaCl.

Urine excretion reflected the water intake of the animals and corresponded to the ash content of the diets. The same applied to the urine iodine level. Dietary iodine level was very high for all diets containing seaweed, in particular for SaccW with 2.2 g iodine/kg, an extremely high level compared to the suggested requirement level of 0.2 mg/kg dry feed (NRC, 1982), i.e. more than 10 000 times the recommended level for mink. The recommended level of iodine for cats and dogs is about 1.5 mg/kg (FEDIAF, 2018). For humans, urine iodine level of 0.3 µg/mL is considered excessive (WHO/UNICEF/ICCIDD, 2007), which means that the mink fed the SaccW diet, showing 318 µg/mL in the animals fed the SaccW diet, had 1000 times higher iodine concentration in the urine than considered acceptable for humans. Possible consequences of excessive iodine levels have, for most animals, not been investigated. An experiment with mink studying effects of iodine levels between 10 and 320 mg/kg DM indicated that a level in the range of 10−20 mg/kg is compatible with good health and secures high reproduction and growth. However, enlargement of the thyroid gland has been reported for young female mink at dietary levels above 20 mg, and for adult females at levels above 80 mg (Jones et al., 1982). Temporary effects in the thyroid tissue are often observed in other animals, including humans. In some, particularly vulnerable animals and humans, the effects may become long-lasting (Sundick et al., 1992; Tartellin and Ford, 1994; Markou et al., 2001; Shoyinka et al., 2008; Aakre et al., 2017). Moreover, other organs than the thyroid could also be affected (Aakre et al., 2017). Experiments with rats fed diets with iodine level of 3 mg/kg, have shown reduced growth rate as well as the reduced weight of testis and low sperm cell counts (Shoyinka et al., 2008). It is, therefore, reasonable to be careful with long term inclusion of high levels of seaweed in animal diets until more information is available on possible health consequences.

Table 6
Gene expression in colon, jejunum and liver of mink fed the four seaweed products[a].

Tissue	Gene	FM	SaccW	SaccP	PalmW	PalmP	P (model)
	il1β	0.0060 a	0.0073 a	0.0100 ab	0.0070 a	0.0230 b	0.007
	il6	0.016	0.033	0.027	0.009	0.045	0.386
	il10	0.0032	0.0067	0.0051	0.0023	0.0126	0.049
	tnfα	0.0035 a	0.0050 ab	0.0066ab	0.0035 a	0.0131b	0.009
	ifnβ	0.00041a	0.00036 a	0.00077 ab	0.00034 a	0.00132 b	0.003
	mttp	0.399 ab	0.121 a	0.481 b	0.279 ab	0.752 b	0.006
Colon	*fabp2*	20.49 ab	6.52 a	29.13 b	13.91 ab	25.92 ab	0.029
	fasn	0.073 a	0.065a	0.127 ab	0.075a	0.201 b	0.006
	pcna	0.356	0.416	0.436	0.358	0.782	0.076
	hspa1	0.612	0.561	0.626	0.542	0.99	0.186
	cat	0.184	0.134	0.216	0.173	0.258	0.091
	zo1	0.289	0.219	0.321	0.347	0.462	0.052
	ecad	0.361 ab	0.258 a	0.472 ab	0.459 ab	0.760 b	0.012
	il1β	0.0035	0.005	0.004	0.0057	0.004	0.371
	*il6**	0.0016	0.0058	0.0015	0.002	0.0016	0.594
	il10	0.0012 b	0.0026 a	0.0014 ab	0.0019 ab	0.0019 ab	0.038
	tnfα	0.0011 b	0.0027 a	0.0012 b	0.0017 ab	0.0017 ab	0.002
	ifnβ	0.00015	0.00039	0.0003	0.00027	0.0003	0.243
	mttp	0.613	0.697	0.534	0.678	0.56	0.325
Jejunum	*fabp2*	13.4	20.31	18.42	18.04	16.95	0.185
	fasn	0.034	0.034	0.035	0.034	0.027	0.621
	pcna	0.146	0.202	0.154	0.174	0.148	0.172
	hspa1	0.217	0.269	0.243	0.29	0.27	0.713
	cat	0.115	0.116	0.131	0.16	0.122	0.24
	zo1	0.11	0.113	0.102	0.134	0.109	0.349
	ecad	0.151	0.178	0.163	0.164	0.141	0.855
	il1β	0.0069	0.0034	0.0023	0.0013	0.0031	0.124
	il6	0.0038	0.0042	0.0055	0.0047	0.0054	0.787
	il10	0.0062	0.0078	0.0074	0.0055	0.0073	0.792
	tnfα	0.0035	0.0016	0.0011	0.0011	0.0017	0.444
	mttp	0.195	0.194	0.201	0.176	0.237	0.321
Liver	*fasn*	0.062	0.117	0.133	0.151	0.078	0.253
	Pcna[b]	0.469	0.381	0.335	0.355	0.407	0.507
	hspa1	0.914	0.828	1.04	0.672	1.02	0.477
	cat	4.515	4.89	5.208	5.04	4.676	0.519
	zo1	0.245	0.288	0.278	0.24	0.288	0.431
	ecad	0.331	0.426	0.345	0.429	0.464	0.929

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. For explanation of gene abbreviation, se Supplementary Table 1. The experimental unit was one individually caged animal, n = 4).
[b] Kruskal-Wallis test.

Table 7
Frequency of selected mucosal morphological features of the jejunum observed in the evaluated mink tissue sections. Numbers represent the number of animals with the finding, and n represents the total number of mink individuals assessed per diet group[a].

	n	None (normal)	Mild	Moderate	Marked
Increase in lymphocytic and plasma cell cellularity of lamina propria					
FM	4	4	0	0	0
SaccW	4	2	2	0	0
SaccP	4	3	1	0	0
PalmP	4	4	0	0	0
PalmW	4	3	1	0	0
Increase in intraepithelial lymphocyte count					
FM	4	2	2	0	0
SaccW	4	1	3	0	0
SaccP	4	2	2	0	0
PalmW	4	3	1	0	0
PalmP	4	2	2	0	0

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

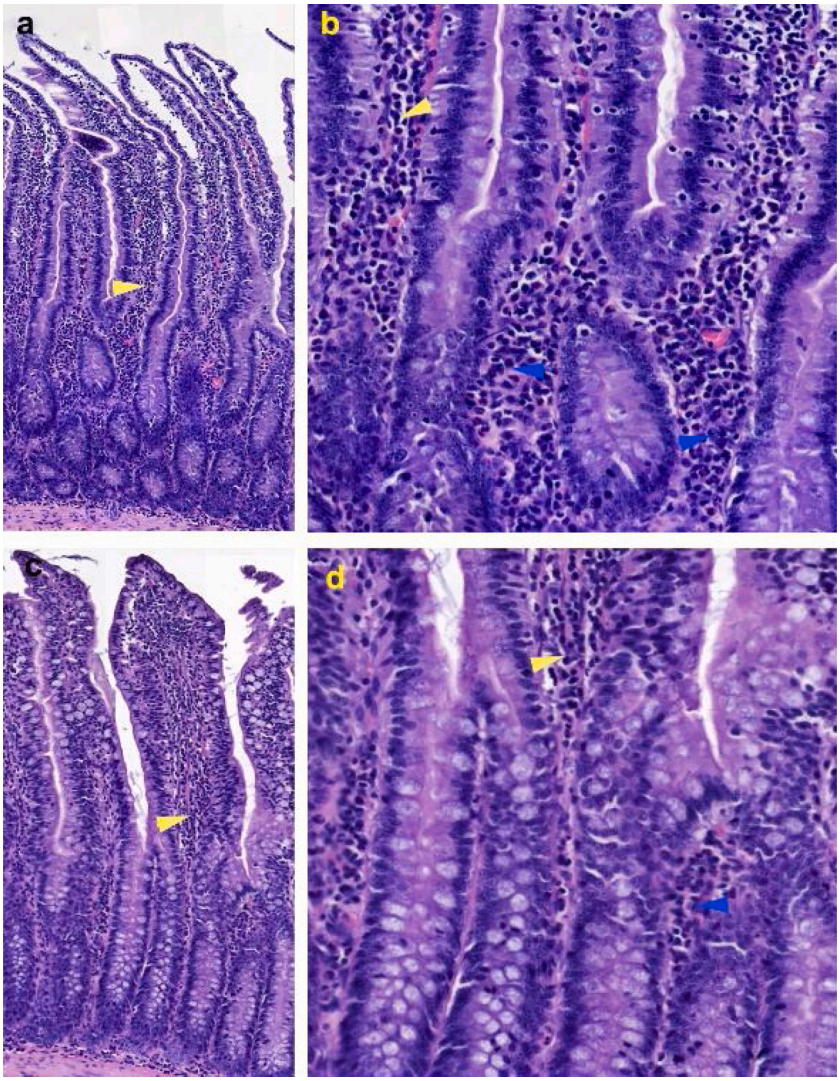

Fig. 3. Depictions of a mild increase in leucocytic content of the inter-crypt space (blue arrowheads) and the villi lamina propria (yellow arrowheads) in the jejunum (picture a and b) and colon (picture c and d) of the mink assessed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 8
Frequency of selected renal morphological features observed in the evaluated mink tissue sections. Numbers represent the number of animals with the finding, and n represents the total number of mink individuals assessed per diet group[a].

	n	None	Mild	Moderate	Marked
FM	4	1	3	0	0
PC	4	1	2	1	0
SC	4	1	1	2	0
WP	4	2	0	2	0
WS	4	2	1	1	0

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

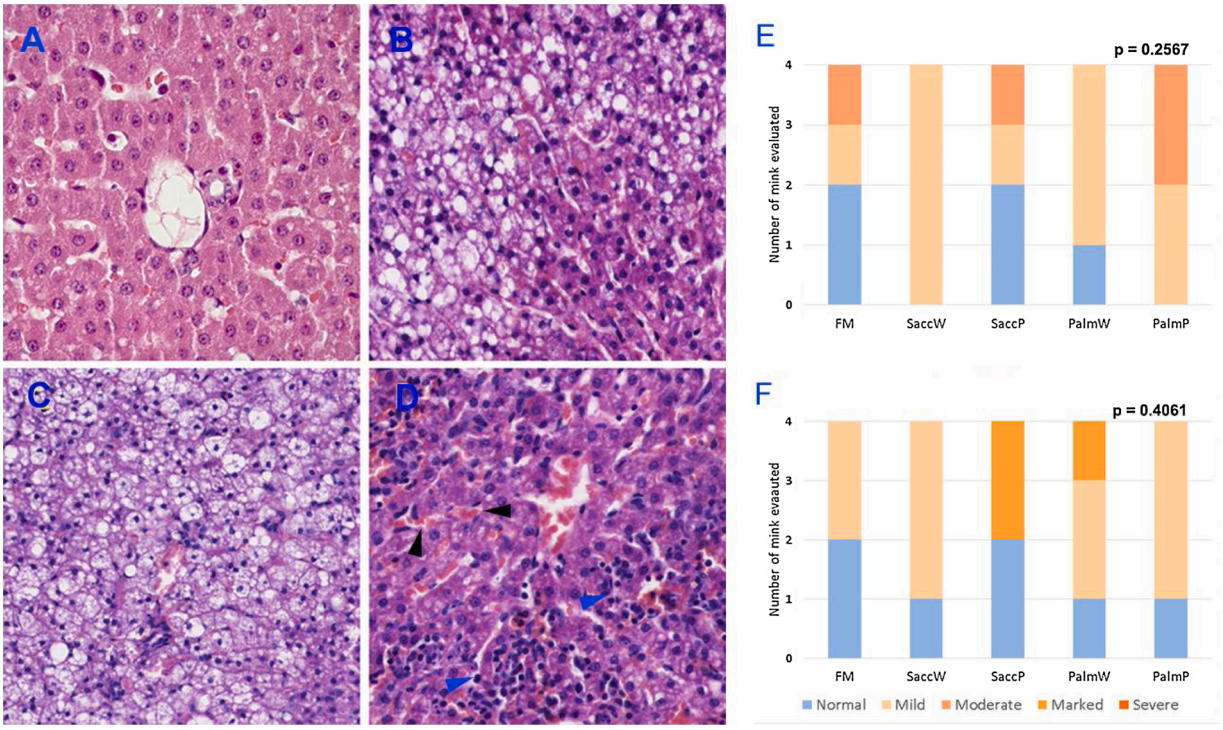

Fig. 4. Representative images of histo-morphological changes observed in liver tissue. A – normal liver appearance; B – liver section with predominantly macro-vesicular hepatocyte vacuolization typical of fatty change; C – liver section with largely micro-vesicular vacuolization pattern of hepatocytes, possibly representing glycogen storage; D – mild congestion (black arrowheads) and lymphocytic infiltration (inflammation) of the parenchyma (blue arrowheads); E and F – counts of mink liver sections observed with changes of congestion and hepatocyte vacuolization (as observed in C) during the histological assessment. Explanation of abbreviations in the figure: FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 80= g/kg of protein. The experimental unit was one individually caged animal, n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4.2. Nutrient composition and availability

Based on the proximate analyses of macronutrients, a clear characteristic shown by all the seaweed products was high level of "other carbohydrates". The detailed carbohydrate composition of this fraction has not been characterized for the products used in the present experiment. However, the major carbohydrates of whole *Saccharina,* harvested in spring are alginate and mannitol (Stevant et al., 2017), and of whole *Palmaria* xylans (Schiener et al., 2017). None of these carbohydrates are available substrates for endogenous enzymes in monogastric animals. Accordingly, they have very low, if any, energy value for monogastric animals. The lipid level of the seaweed products was very low for all, between 4 and 190 g/kg, a level of marginal value in an animal nutrition context, even though long-chain polyunsaturated fatty acids may comprise as much as 250 g/kg of total fatty acids in both species. See review by Holdt and Kraan (2011). Hence, among the energy-yielding nutrients, only the protein, i.e. the amino acids, may be of quantitative importance.

A nutrient must be biologically available to be of importance for an animal, i.e. first of all, of high digestibility. The present mink experiment showed very low digestibility, < 0.500, of protein from both *Saccharina* products. Crude protein ADC values of less than 0.500 are too low for a protein source to be considered useful for monogastrics. The picture was even less favourable when taking amino acid ADC into account. For whole *Saccharina latissima* the estimated apparent histidine ADC was negative which means that the protein as a sole protein source in a diet has no value. The cause of this low ADC may be low absorbability of the histidine present in the ingredient, or increased loss of endogenous compounds containing histidine. The result for the protein concentrate of *Saccharina latissima* was higher, but still very low, 0.548. Also, the whole *Palmaria palmata* showed low histidine digestibility, 0.271. These three products would require combination with histidine-rich protein sources and/or supplementation with histidine to reach the required amino acid balance. The cysteine ADC also deserves consideration. This amino acid is not considered essential as it can be synthesized from methionine. However, as methionine is the first limiting amino acid in many protein sources, e.g. legumes, the sum of the two should be taken into account. In whole *Saccharina latissima*, cysteine ADC was negative and methionine ADC was 0.264. Also, for protein concentrate of *Saccharina latissima*, cysteine ADC was negative, but methionine ADC was much higher than for whole *Saccharina latissima*, although still quite low in a nutritional context. The corresponding results for the whole *Palmaria palmata*, showed

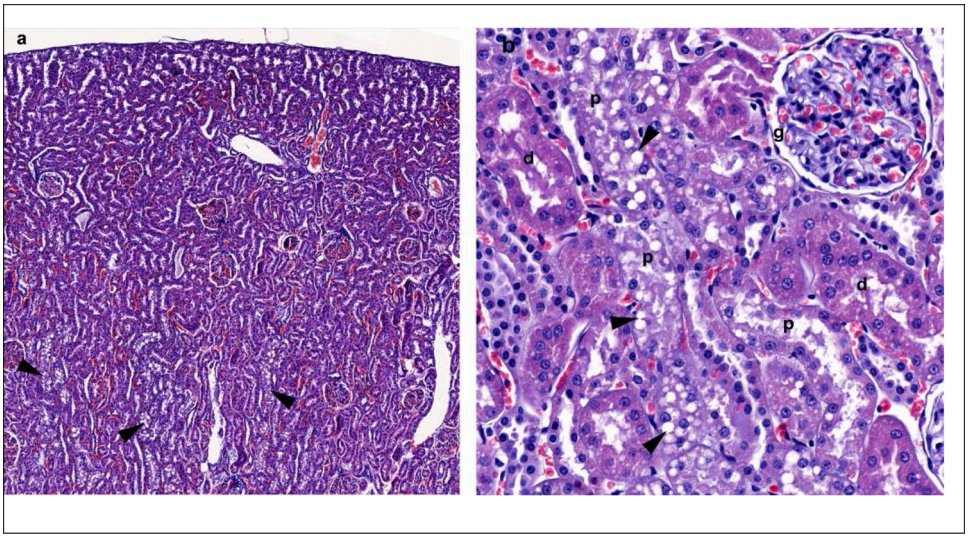

Fig. 5. Vacuolization of tubular epithelium observed in kidney sections. a - cortical region of the kidney showing regions with epithelial vacuolization (black arrowheads); b - close-up view of the tubular epithelial vacuolization (black arrowheads) affecting the proximal convoluted tubules (p). d – distal convoluted tubule; g – glomerulus. FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole Saccharina latissima comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of Saccharina latissima comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole Palmaria palmata comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of Palmaria palmata comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

Table 9
Alpha diversity of jejunum and colon microbiota comparing dietary groups (n = 4)[a].

Tissue	Alpha diversity index	FM	SaccW	SaccP	PalmW	PalmP	P-(model)
Jejunum	Observed species	24.8	25.5	27.5	24.0	33.0	0.719
	Shannon	4.1	3.9	4.1	3.8	4.3	0.573
Colon	Observed species	54	43.3	45.3	40	40.3	0.442
	Shannon	4.7	3.8	4.5	4.4	3.8	0.287

[a] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

higher results for both cysteine and methionine compared to whole *Saccharina latissima.* The protein concentrates of the two seaweeds showed higher, and quite similar values, indicating that the processing either removed components with a negative impact, or increased the ADC of the protein in a more direct way. Soluble compounds reduced by the processing would, in addition to minerals, also include soluble protein and free amino acids, and the carbohydrates mannitol and fucoidan of the *Saccharina* and the xylans of the *Palmaria*.

Low ADC was also observed for other amino acids, but not as critical as for histidine and methionine plus cysteine. Altogether, the results from these amino acids indicate that the potential of *Saccharina latissima* to become an important protein source for monogastrics, seems small. For *Palmaria palmata*, the protein concentrate may have a potential for use with 290 g/kg digestible protein, if it can be produced at a price the feed manufacturers can pay. As the remaining 710 g/kg of the *Palmaria palmata* has very little energy value for monogastric animals, the product will lower the concentration of digestible energy of the feed.

4.3. Expression of functional genes in gut tissue

Modulation of gene expression by different diets was detected mainly in the colon with small or no effects in the jejunum and liver. This finding, together with the relatively subtle changes in gene expression observed in the colon suggests that the overall effect of inclusion of seaweed products in the diet of mink had a small effect on the immune function and lipid metabolism and that this effect was restricted to the colon. We hypothesized that the diets with more potential to modulate the immune response would be the ones that had whole seaweed products, i.e SaccW and PalmW, due to the presence of bioactive compounds such as alginate, laminaran, fucoidan, mannitol, and phlorotannin in *Saccharina latissima* and of xylan, floridoside, kanoic and domoic acid, desmosterol, phycobiliprotein, phlorotannins in *Palmaria palmata* (Holdt and Kraan, 2011).

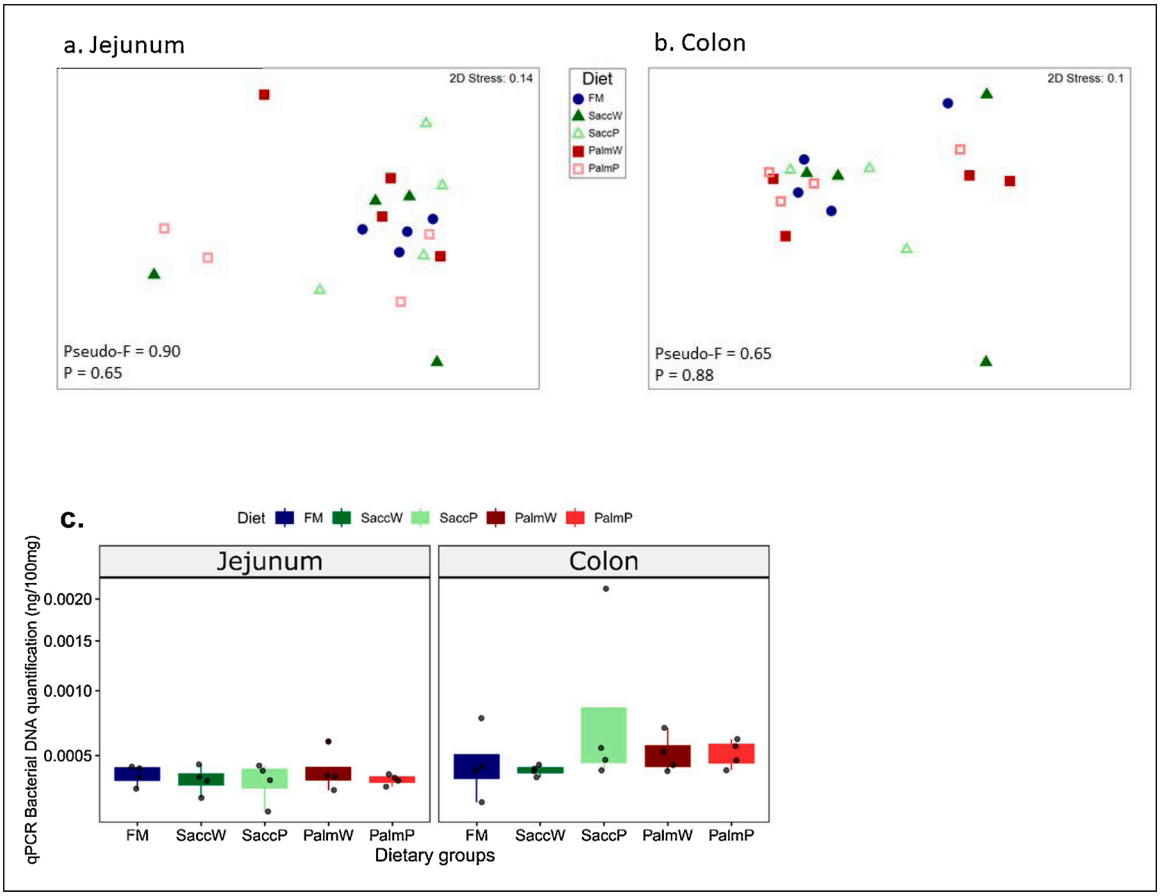

Fig. 6. Bacterial microbiota evaluation. a. Beta diversity of Jejunum samples based on Bray-Curtis (n = 4); b. Beta diversity of Colon samples based on Bray-Curtis (n = 4 for all groups but SaccP which has n = 3); c. Bacterial DNA quantification based on qPCR for samples from the mucosa of Colon and Jejunum. No significant differences among dietary groups were detected in beta diversity or bacterial DNA quantification analysis. FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

However, contrary to our hypothesis, the diets containing SaccP and PalmP were the ones that induced the clearest changes in gene expression in the colon, indicating mild but significant activation of elements of the immune apparatus (increased expression *il1β, tnfα*, *infβ*). These responses in the groups fed the protein-concentrated seaweed products occurred concomitantly with a mild increase in the expression of genes related to lipid metabolism (*mttp*, *fabp2,* and *fasn*). It seems possible that these results may have a causal connection, but the mechanisms behind are unknown. The processing steps of the protein concentrated products, by removing the soluble components, increased the relative amounts of the insoluble constituents, such as alginate, the major fraction of the protein, as well as lipids. To answer the question whether the increase the level of these compounds may explain the activation of the immune genes, would require further studies.

4.4. Microbiota analysis of intestinal mucosa

The scientific literature regarding effects of seaweed on gut microbiota in animals is limited. However, the available information indicates that seaweed inclusion in diets for terrestrial as well as aquatic animals can induce changes in the intestinal bacterial microbiota (De Jesus Raposo et al., 2016; Sardari and Karlsson, 2018; Tapia-Paniagua et al., 2019; You et al., 2019). In this study, we investigated whether the mucosa-associated bacterial communities of the intestine in mink might be modulated by the inclusion of whole dried or protein concentrated seaweed products in the diet. The results of the present study may suggest that the seaweed products had small effects on the bacterial communities associated with the intestinal mucosa in mink. One explanation might be the high rate of passage of feed through the digestive tract in mink, which may prevent major effects of diet. Another potential explanation is that the experimental feeding time (14 days) might have been too short to cause clear effects on the microbiota associated to the intestinal mucosa. However, the amount of seaweed material available for the present study did not allow for a longer experimental

Table 10
Bacterial taxa abundance, mean ± SD, of 10 most abundant genera in each dietary group[a] for Jejunum and colon[b].

Tissue	Phylum	Genus	FM**	SaccW	SaccP	PalmW	PalmP
Jejunum	Actinobacteria	*Arthrobacter*	2.9 ± 2.2	0 ± 0	1.7 ± 2	2 ± 3.5	1.1 ± 1.6
		Nocardioides	7.9 ± 4.8	2.6 ± 4.6	5.6 ± 4.4	5.8 ± 2.8	5.4 ± 4.6
		Cutibacterium	4.2 ± 6.5	9.3 ± 13.6	4.8 ± 6.8	1.3 ± 2.5	3.2 ± 5.8
		Pseudonocardia	2.3 ± 1	2.8 ± 5.3	4.8 ± 4.2	2.5 ± 3.3	1 ± 1.2
		Streptomyces	2.1 ± 2.8	1.4 ± 2.4	0.7 ± 1	1.5 ± 2.9	2.6 ± 3.6
	Bacteroidetes	*Porphyromonas*	0 ± 0	0 ± 0	0 ± 0	5.6 ± 11.2	0.7 ± 1.4
		Family *Chitinophagaceae*	3 ± 4.2	1.7 ± 3.5	2.8 ± 3.9	0.8 ± 1	2.2 ± 3.3
		Sediminibacterium	3.6 ± 1.3	0.5 ± 0.6	3 ± 4.3	2.4 ± 3.5	4.8 ± 5.2
		Flavobacterium	4.2 ± 2	5.3 ± 4.2	7.8 ± 4.7	7.5 ± 7.1	3.3 ± 2.9
	Firmicutes	Family *Aerococcaceae*	0 ± 0	1.4 ± 2.8	0 ± 0.1	4.5 ± 5.6	0 ± 0
		Clostridium sensu stricto 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9.1 ± 17.6
		Family *Peptostreptococcaceae*	2.7 ± 5.4	9 ± 17.8	0 ± 0	0.3 ± 0.6	19.3 ± 24.1
		Family *Ruminococcaceae*	0 ± 0	3.1 ± 3.9	1.7 ± 3.4	0.2 ± 0.3	0.2 ± 0.4
	Patescibacteria	Order *Candidatus Peribacteria*	0.9 ± 1.9	0.2 ± 0.5	0 ± 0	1.6 ± 2	3.1 ± 6.1
	Proteobacteria	Family *Caulobacteraceae*	2.7 ± 2.8	2.5 ± 2.5	3.9 ± 5.3	1.3 ± 2.7	1 ± 2
		Bradyrhizobium	1.1 ± 2.3	5.2 ± 4.2	0.8 ± 1	3.3 ± 4.7	2.5 ± 4.4
		Curvibacter	12.9 ± 6.3	9.8 ± 7.1	5.1 ± 5.7	8.7 ± 6.4	11 ± 7.4
		Ralstonia	21.5 ± 5.1	19.3 ± 21	16.8 ± 4.8	20.6 ± 12.4	7.9 ± 7
		Citrobacter	0 ± 0	0 ± 0	3.2 ± 6.4	0 ± 0	0 ± 0
		Photobacterium	0.7 ± 1.3	4.9 ± 7.8	9.1 ± 17.2	1.2 ± 1.5	0 ± 0
		Luteimonas	0 ± 0	0 ± 0	0 ± 0	8.4 ± 16.7	0.2 ± 0.5
	Tenericutes	*Mycoplasma*	0.6 ± 1.1	4.1 ± 6.3	0.7 ± 1.3	0.4 ± 0.7	0 ± 0
	Actinobacteria	*Nocardioides*	0.3 ± 0.3	0.6 ± 0.4	1.9 ± 1.6	1.5 ± 2	1.3 ± 2.3
	Bacteroidetes	*Porphyromonas*	1.8 ± 3.5	0 ± 0	6 ± 10.3	0 ± 0	0 ± 0
		Flavobacterium	2.1 ± 3	1.2 ± 1.7	2.2 ± 1.1	4.3 ± 5.5	1.1 ± 1.6
	Cyanobacteria	Order *Obscuribacterales*	1.3 ± 2.6	0 ± 0	3.6 ± 6.2	1.5 ± 3.1	0 ± 0
	Epsilonbacteraeota	*Helicobacter*	0.5 ± 1	0 ± 0	1.5 ± 2.6	0.6 ± 1.2	0 ± 0
Colon	Firmicutes	*Bacillus*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 2.6
		Family *Aerococcaceae*	3.5 ± 3.3	11.5 ± 13.6	0.6 ± 1	0.1 ± 0.1	0.9 ± 1.8
		Carnobacterium	0 ± 0	1.6 ± 3.3	0 ± 0	0 ± 0	0 ± 0.1
		Streptococcus	7.4 ± 9	0.5 ± 0.8	1.7 ± 2.5	0.7 ± 0.5	0.9 ± 1.2
		Clostridium sensu stricto 1	3.2 ± 2.7	0.3 ± 0.7	0 ± 0	6.4 ± 12.3	2.5 ± 1.7
		Epulopiscium	4.7 ± 9.4	0 ± 0	0 ± 0	6.8 ± 13.4	3 ± 5.9
		Family *Peptostreptococcaceae;_*	39.3 ± 27.4	28.8 ± 35.4	44.5 ± 34.5	33.4 ± 41.3	59.6 ± 39.5
		Family *Peptostreptococcaceae*	4.4 ± 5.2	0.8 ± 1	0 ± 0	1.3 ± 2.6	2 ± 2.3
		Terrisporobacter	1.8 ± 2.9	2.3 ± 2.8	2 ± 3.5	3 ± 5.8	3.8 ± 4.3
	Proteobacteria	*Bradyrhizobium*	0.3 ± 0.5	1.7 ± 3	1.4 ± 2.1	3.3 ± 4.2	0.5 ± 0.6
		Curvibacter	0 ± 0	1.5 ± 1.6	0 ± 0	0.5 ± 1.1	1.9 ± 3.8
		Undibacterium	7.2 ± 6.7	7.6 ± 9	7.2 ± 6.4	10.7 ± 14.5	7.7 ± 10.9
		Family *Enterobacteriaceae*	0 ± 0	5.5 ± 10.9	0 ± 0	0.8 ± 1.1	0 ± 0
		Citrobacter	0.1 ± 0.3	15.6 ± 31.2	0.4 ± 0.6	0 ± 0	0.1 ± 0.1
		Salmonella	0 ± 0	0 ± 0	2.2 ± 3.8	0 ± 0	1.6 ± 3.2
		Acinetobacter	0 ± 0	4.2 ± 7.3	0 ± 0	2.8 ± 5.6	0 ± 0
		Moraxella	2 ± 3.2	0 ± 0	0.7 ± 1.2	0 ± 0	0 ± 0

[a] No significance differences were found among the dietary groups.
[b] FM: Reference diet with fish meal as the only protein source; SaccW: Diet with whole *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; SaccP: Diet with a protein concentrate of *Saccharina latissima* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmW: Diet with whole *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein; PalmP: Diet with a protein concentrate of *Palmaria palmata* comprising 200 g/kg of protein, fish meal 800 g/kg of protein. The experimental unit was one individually caged animal, n = 4).

setup. Further studies should focus on studying if seaweed products influence the luminal microbiota.

4.5. Histological observations

Histological assessment revealed mild to moderate changes in selected morphological features in most of the tissues assessed, but without any clear association of the changes with the dietary treatments. Notable group differences were only found regarding jejunal lymphocytic infiltration into the lamina propria for animals fed SaccW, when compared to those fed the FM diet. The implications of these subtle alterations are unclear but may be related to the immune responses seen for animals in this group. Whether they are indications of malfunction, cannot be stated based on the present study.

Vacuolization was observed in the kidneys, predominantly in the proximal convoluted tubule of the tubular epithelium, in animals fed the SaccW, SaccP, and PalmW, but not in animals from fed the PalmP and FM. No scientific literature has been found which can help explain the observation. The observations, however, call for further studies to strengthen knowledge on the relationship between seaweed intake and kidney function, in particular in light of the short duration of the present study.

4.6. Conclusions

The present work shows that products of *Saccharina latissima* and *Palmaria palmata* have low protein quality due to low apparent digestibility of most essential amino acids, in particular histidine and methionine. The very low apparent digestibility of cysteine, which in an animal body may be produced from methionine, aggravates the methionine deficiency. The protein concentrated products, overall, showed higher digestibilities of the available macronutrients, but only the protein concentrate from *Palmaria palmata*, could be considered a potential protein source for monogastric animals. It must, however, be used in combination with high-quality protein sources or fortified with amino acids to be useful as a protein source. Only minor effects were observed on gut function and gut health. Structure alterations in the kidneys were observed in animals fed the *Saccharina* products and the whole *Palmaria* product. Before long-term use of the two seaweeds investigated in the present study is recommended, further research of effects on internal organs, in particular, related to the high iodine level, is required.

CRediT authorship contribution statement

Åshild Krogdahl: Conceptualization, Data curation, Investigation, Writing original draft preparation. **Alexander Jaramillo-Torres**: Data curation, Investigation, Visualization, Writing and reviewing. **Øystein Ahlstrøm**: Conceptualization, Data curation, Investigation, Writing and reviewing. **Elvis Chikwati**: Investigation, Data curation and Visualization, Writing and reviewing. **Inga-Marie Aasen**: Conceptualization, Ingredient processing, Investigation, Writing and reviewing. **Trond M. Kortner**: Data curation, Investigation, Writing and reviewing.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2021.114902.

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