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Evaluation of seaweeds from marine waters in Northwestern Europe for application in animal nutrition

P. Bikker[a], L. Stokvis[a,b,*], M.M. van Krimpen[a,†], P.G. van Wikselaar[a], J.W. Cone[b]

[a] Wageningen University & Research, Wageningen Livestock Research, 6708 WD, Wageningen, the Netherlands
[b] Wageningen University & Research, Animal Nutrition Group, 6708 WD, Wageningen, the Netherlands

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ABSTRACT

The increasing demand for animal protein by the growing world population intensifies the exploration of novel feed sources. This study evaluated the nutritional value of six intact seaweed species (*i.e.* the brown species *Saccharina latissima, Laminaria digitata,* and *Ascophyllum nodossum,* the red species *Palmaria palmata,* and *Chondrus crispus* and green species *Ulva lactuca*), collected from the coast of Ireland, Scotland and France as an ingredient for animal feed. The nutrient composition, *in vitro* digestibility, and *in vitro* gas production simulating rumen fermentation, were determined.

The nutrient contents (g/kg dry matter), both between and within species, were highly variable, ranging from 45–248 for crude protein, 351–691 for non-starch polysaccharides, and 173–445 for ash. Overall, the brown seaweeds had the highest non-starch polysaccharides content, whereas samples of the red and green seaweeds had an amino acid content up to 265 g/kg dry matter. All samples had a substantial non-protein nitrogen fraction, varying from 0.12–0.29 of nitrogen. The fibre fractions of brown seaweeds showed different properties than land-based plants, as illustrated by a lower analysed neutral detergent fibre than acid detergent fibre content. The ileal organic matter and nitrogen digestibility, as well as the total tract organic matter digestibility (mean digestibility coefficients: 0.81, 0.89 and 0.88, respectively) were lower for all seaweeds compared to soybean meal (digestibility coefficients: 0.84, 0.98 and 0.97, respectively). *S. latissima, L digitata, P. palmata* and *U. lactuca* had a higher maximum gas production than alfalfa, but lower than sugar beet pulp. Based on the protein content and amino acid-pattern, intact *P. palmata* and *U. lactuca* would be a valuable protein source for farm animals, with the high non-starch polysaccharides and non-protein nitrogen contents and a low *in vitro* digestibility potentially limiting their use as a feed ingredient for monogastric species. The fermentability of *L. digitata, S. latissima* and *P. palmata* indicate that these intact seaweeds may have a higher nutritional value in ruminants. The high ash content in all seaweed species hampers the use of intact seaweed for both ruminants and monogastrics. Extraction of protein and other favourable components, while reducing the ash content, seems an important step towards seaweed inclusion in animal diets. Further identification and characterisation of seaweed polysaccharides is required to understand and improve the digestibility of seaweed fractions.

Abbreviations: AA, amino acid; AA-N, amino acid nitrogen; K_A, nitrogen to protein conversion factor; N:P, nitrogen to protein ratio; NPN, non-protein nitrogen

* Corresponding author.
E-mail address: lotte.stokvis@wur.nl (L. Stokvis).
† deceased

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1. Introduction

The expected population growth to 9.7 billion in 2050 (United Nations, 2017) coupled with increased living standards and urbanisation are important drivers for the expected global increase in meat, egg and milk consumption in the next decades (Boland et al., 2013). Concomitant with this increase is the increase in demand for feed ingredients and as such the development of novel sources of biomass for feed ingredient production. The extension of marine biomass production by wild harvest and large scale cultivation of seaweed could significantly contribute to biomass production for feed as well as food. Advantages of seaweed production are the use of salt instead of fresh water, sea instead of arable land-based production, and the high productivity in terms of biomass produced per unit of surface area (Buschmann et al., 2014, 2017).

The chemical composition of multiple species of the three classes (brown, red and green seaweeds, based on their pigmentation) of seaweed shows large variation between, and also within species, depending on *e.g.* season of harvest, geographical characteristics and environmental factors (Holdt and Kraan, 2011; Schiener et al., 2015; Boderskov et al., 2016; Sharma et al., 2018). At present, seaweed is not used in large scale commercial animal feed production to any significant extent. Detailed knowledge of the nutritive value of seaweed for feed is, hitherto lacking with only a few studies reporting *in vitro, in situ* or *in vivo* digestibility of seaweed (*e.g.* Greenwood et al., 1983; Molina-Alcaide et al., 2017). This knowledge is essential for the successful application of seaweeds as an ingredient in the diet of various production as well as companion animals. In addition, the number of published performance studies using intact seaweed as a source of macronutrients in production animals is limited. Most of the studies used low inclusion levels of 0.3 up to 4 g of seaweed per kg of diet on an as-is weight basis (Gahan et al., 2009; McDonnell et al., 2010; Abudabos et al., 2013) or focussing on the effects of bioactive compounds of seaweed rather than its nutritional value (*e.g.* Novoa-Garrido et al., 2014). The aim of this study was to evaluate the nutritive value and the variation in nutritive value of intact seaweeds obtained from coastal waters in Northwestern Europe as a source of macro nutrients, for application in animal feeds, by analyses of the chemical composition and use of *in vitro* digestibility models.

2. Material and methods

2.1. Seaweeds

In total 13 different samples of six seaweed species classified as either Phaeophyta (brown), Rhodophyta (red) or Chlorophyta (green) were evaluated. The study include the brown species *Laminaria digitata*, *Saccharina latissima*, and *Ascophyllum nodosum*, red species *Palmaria palmata* and *Chondrus crispus* and green species *Ulva lactuca*. The seaweed species were collected, mainly from wild populations, along the coast of Ireland, Scotland and France in 2013 with Table 1 providing an overview of the seaweed species and their origin. All six Scottish samples were harvested in August at the coast of the island Bute (West coast of Scotland). After drying on the coast by wind, air and sun, they were milled in a blender into course particles ($\pm$ 10 mm^2). These seaweeds were harvested and processed by Justseaweed (Rothesay, United Kingdom). The Irish samples of *A. nodossum*, harvested in summer, and *C. crispus*, harvested in spring, and *U. lactuca*, harvested in May, were processed by Ocean Harvest Technology (Ocean Harvest Technology Ltd, Milltown, Ireland). After harvesting near the coast of Galway, Ireland, the seaweeds were dried by wind, air and sun, and milled with a hammer mill over a 300 and 100 µm screen, respectively. The French samples of *S. latissima*, *P. palmata* and *U. lactuca* were harvested in September at the coast of Plouarzel (nearby Brest, France). After drying on the coast by wind, air and sun, they were milled with a Subaru mill in rough parts ($\pm$ 2 cm^2). These seaweeds were harvested and processed by Teranga Sea Weeds (Brest, France). The Irish *L. digitata* was provided by North Seaweed (North Seaweed BV, Kapelle, the Netherlands) and originated from the coast of Ireland. All samples were stored at room temperature until the start of the experiment.

Table 1
Overview of the brown, red and green seaweed species harvested along the coastal regions of Northwestern Europe used in the experiment.

Seaweed classification	Species	Harvesting location	Harvesting date
Brown Algae	*Laminaria digitata*	Bute (S)	08-2013
	Laminaria digitata	Ireland (I)	2013
	Saccharina latissima	Bute (S)	08-2013
	Saccharina latissima	Plouarzel, Bretagne (F)	09-2013
	Ascophyllum nodosum	Bute (S)	08-2013
	Ascophyllum nodosum	Galway (I)	Summer 2013
Red Algae	*Palmaria palmata*	Bute (S)	08-2013
	Palmaria palmata	Plouarzel, Bretagne (F)	09-2013
	Chondrus crispus	Bute (S)	08-2013
	Chondrus crispus	Galway (I)	Spring 2013
Green Algae	*Ulva lactuca*	Bute (S)	08-2013
	Ulva lactuca	Plouarzel, Bretagne (F)	09-2013
	Ulva lactuca	Galway (I)	05-2013

S = Scotland, F = France, I = Ireland.

2.2. Chemical composition

Prior to analyses, all seaweeds were ground using a laboratory mill (Peppink 200 AN, Olst, the Netherlands) equipped with a 1-mm sieve. The samples were analysed using official methods to determine moisture (dry matter: DM, ISO 6496, 1999), nitrogen (N, ISO 5983, 2005), ether extract (crude fat; ISO 6492, 1999), ash (ISO 5984, 2002), the HCl-insoluble ash was determined as ash-HCl, crude fibre (ISO 6865, 2000), starch (ISO 15914, 2004), total sugar (EC 152/2009), tryptophan (ISO 13904, 2005) and other amino acids (AAs, ISO 13903, 2005). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to ISO 13906, 2008. Neutral detergent fibre (NDF) was analysed according to ISO 16472, 2006. Organic matter (OM) was calculated as 1000–ash. Crude protein (CP) was calculated as N × 5.00 (Angell et al., 2015). For this purpose, the tyrosine and proline content were calculated based on their ratio to phenylalanine for each seaweed species (ratio calculated from data by Biancarosa et al. (2017)). Non-protein N (NPN) was calculated, as well as the N to protein (N:P) conversion factors K_P and K_A. K_P is the ratio between the sum of the anhydrous AAs (g/kg DM) and N (g/kg DM), and K_A is the ratio between the sum of anhydrous AAs (g/kg DM) and amino acid N (AA-N; g/kg DM). True protein was calculated as the sum of anhydrous AAs (g/kg DM). The fraction of non-starch polysaccharides (NSP) was calculated as OM minus CP, crude fat, starch and total sugar. The commonly used feed ingredients soybean meal (SBM), alfalfa and sugar beet pulp were used as reference ingredients.

2.3. In vitro digestibility of DM, OM and N

In vitro incubations were performed according to a modified two- and three-step method for pigs according to the procedures as described by Boisen and Fernandez (1997) to analyse the *in vitro* ileal and total tract digestibility. Reduced substrate concentrations were used, as described by Bikker et al. (2016), because of the high viscosity of seaweed samples during incubation. The reference ingredient SBM was included in the *in vitro* digestibility studies. A two-step *in vitro* incubation was performed to simulate the digestive process in the stomach and small intestine to estimate the ileal digestibility of the substrates. The two-step *in vitro* incubations were conducted in fourfold, of which 2 replicates were used to determine N digestibility and 2 replicates to determine OM digestibility. The three-step *in vitro* incubation was performed including an 18 h incubation with Viscozyme (Viscozyme® L, Sigma Aldrich, Germany) to simulate the digestive process in the stomach, small intestine and large intestine of a pig to estimate total tract digestibility. The three-step *in vitro* incubations were conducted in duplicate to determine OM digestibility.

2.4. Rumen fermentation

Potential fermentation in the rumen of the seaweed samples was measured by the gas production technique during incubation in rumen fluid as described by Cone et al. (1996). As insufficient seaweed was available to acclimatise the cows to the substrate, rumen fluid was obtained from dairy cows kept on a ration of maize and grass silage. Each sample was incubated in triplicate and a blank run (rumen fluid without sample) in duplicate. The cumulative gas production over time was described using a two-phase model based on asymptotic maximum gas production (ml/g OM), time at which half of this gas production was reached and a parameter determining the shape of the curve for different substrates (Groot et al., 1996). The first phase describes the fermentation of the soluble fraction, whereas the insoluble fraction is fermented in the second phase. The duration of each of the two phases was not set at the length generally used for land-based plants, but was determined by the model to find the best possible fit. Maximum gas production was corrected for protein content with an increase of 2.5 ml per percent true protein in the OM of the analysed products as calculated based on total AA content, according to Cone and Van Gelder (1999). Data from Hulshof et al. (2016) and Cone et al. (2008) were used for comparison to SBM, sugar beet pulp and alfalfa meal as reference ingredients. These products were analysed in the same lab, using the same techniques as used for the seaweed samples in the current experiment.

2.5. Statistical analyses

The *in vitro* digestibility data were analysed as an unbalanced 3 × 6 × 3 factorial design, with the factors seaweed classification (brown, red and green), species within classification (brown species: *L. digitata*, *S. latissima* and *A. nodossum*, red species: *P. palmata* and *C. crispus* and green species: *U. lactuca*), and origin (Scotland, Ireland and France). Because of some variation in season of harvest among different locations (see Table 1), the effect of origin may include an influence of season of harvest. These effects cannot be separated. The residual maximum likelihood procedure (Genstat, 19th edition) was used to test the effects of classification, species, and origin on *in vitro* digestibility parameters and curve fitting parameters of the gas production test. Data are presented as means with standard error of difference per parameter. All statements of significance are based on testing at $P < 0.05$. A regression analysis was performed with a generalized linear model. First, an all-subset regression was conducted for *in vitro* ileal OM digestibility, ileal N digestibility, total tract OM digestibility and corrected maximum gas production as dependent variables. Possible explanatory variables included in the model were ash, CP, crude fat, sugar, starch, NSP, NDF, ADF and ADL (all expressed in g/kg DM). All possible combinations of a model with a maximum of three explanatory variables were compared to avoid over-parametrisation in the dataset with 13 seaweed samples. Non-significant variables were excluded and the significant model with the highest explained variation was selected. In addition, regression analysis was conducted with corrected maximum gas production as dependent variable and *in vitro* OM and N digestibility as independent variable to determine whether the results from the *in vitro* digestibility test had a predictive value for the gas production.

Table 2
Analysed nutrient content of selected seaweed species, harvested along the coastal regions of Northwestern Europe.

		g/kg	g/kg DM										
Seaweed classification	Species (origin)	DM	Ash	OM [a]	CP [b]	Cfat	Cfibre	Sugar	Starch	NSP [c]	NDF	ADF	ADL	HCl-ash
Brown	*L. digitata* (S)	891	275	725	92	16	73	2	2	618	120	200	36	7
	L. digitata (I)	923	367	633	82	11	69	1	1	545	91	164	28	7
	S. latissima (S)	874	243	757	74	10	71	6	1	668	122	185	23	11
	S. latissima (F)	902	273	727	117	12	62	1	1	603	96	171	7	6
	A. nodossum (S)	883	214	786	45	38	54	30	1	679	162	331	180	3
	A. nodossum (I)	910	411	589	57	10	88	1	1	530	152	298	48	11
Red	*P. palmata* (S)	939	209	791	141	12	35	35	17	594	312	50	6	7
	P. palmata (F)	949	228	772	134	13	28	48	22	555	347	42	5	4
	C. crispus (S)	883	176	824	98	17	31	6	26	691	392	40	9	2
	C. crispus (I)	899	445	555	125	7	45	3	90	351	190	53	14	152
Green	*U. lactuca* (S)	842	243	757	70	23	76	24	75	567	385	141	70	20
	U. lactuca (F)	880	260	740	168	35	76	12	73	457	329	143	69	8
	U. lactuca (I)	883	173	827	248	21	57	7	42	530	259	135	69	11
	Soybean meal [d]	900	73	927	531	28	ND	134	10	224	90.6	51.4	UD[f]	ND
	Sugar beet pulp [e]	917	79	921	88	14	ND	234	4	581	356	182	8	ND
	Alfalfa [e]	927	125	875	174	27	ND	54	19	601	419	318	77	ND

Cfat = crude fat, Cfibre = crude fibre, S = harvested in Scotland, F = harvested in France, I = harvested in Ireland, UD = under detection limit, ND = not determined. Each value in the table is based on one analysis in duplicate.
[a] Calculated as 1000 – ash.
b Calculated as nitrogen × 5.00.
c Calculated as 1000 – ash – CP – Cfat – starch – sugars.
d Values based on analysis conducted by Hulshof et al. (2016).
e Values based on analysis conducted by.Cone et al. (2008)
f Detected ADL content was 0, which is under the limit for accurate detection of ADL.

3. Results

3.1. Chemical composition

Ash content of the seaweeds ranged from 173 in Irish *U. lactuca* to 445 g/kg DM in Irish *C. crispus* (Table 2). Variation in ash content occurred between and within species from different locations. Likewise, CP content varied substantially, with the highest content in *U. lactuca* from Ireland (248 g/kg DM) and France (168 g/kg DM), and the lowest content in Scottish *A. nodosum* (45 g/kg DM). Crude fat content among seaweeds varied between 7 g/kg DM (Irish *C. crispus*) and 38 g/kg DM (Scottish *A. nodossum*). In most brown seaweed species, sugar and starch content was very low, whereas they were higher in most of the red and green species. Most species contained high NSP levels (> 500 g/kg DM), with the highest value in Scottish *C. crispus* (691 g/kg DM), and the lowest NSP level in *C. crispus* of Irish origin (351 g/kg DM). Within the NSP fraction, substantial variation in the different types of fibre (NDF, ADF and ADL) was observed, both within and between species, depending on origin. In most species, the HCL-ash content ranged between 2 and 20 g/kg DM, but in *C. crispus* 152 g/kg DM HCL-ash was determined.

The AA profiles of the seaweed samples, together with the N content and the N:P conversion factors are presented in Table 3. The AA-N content was lower in seaweeds than in SBM (70.7–87.9 *vs*. 94.0 g/100 g N). Consequently, the AA content (in g AA-N/100 g N) of most essential AA, apart from methionine and threonine, was lower than in SBM. The NPN ranged from 12.1–29.4% of N compared to 6.0% of N for SBM. The conversion factor K_P ranged between 4.16 and 5.01 for the seaweed samples, and K_A ranged from 5.55–5.89.

3.2. In vitro simulated ileal digestibility

3.2.1. Organic matter digestibility

The simulated ileal OM digestibility coefficient of the seaweeds ranged from 0.44 (Irish *A. nodossum*) to 0.81 (French *S. latissima*; Table 4). The OM digestibility coefficient of SBM was 0.84. Both classification and species within classification affected the OM digestibility, but their effects depended on the origin as indicated by the interactions, with P = 0.008 and 0.005, respectively. The OM digestibility coefficient of Irish *A. nodossum* (0.44) was lower compared to the Scottish variety (0.62). In contrast, the OM digestibility coefficient of Irish *U. lactuca* was higher (0.67) than Scottish *U. lactuca* (0.54) and French *U. lactuca* (0.51). For the other species, origin did not have a significant effect on OM digestibility coefficients. The multiple regression analyses indicated that 79.5% of the observed variation was explained by the selected significant model ($p < 0.001$) including the variables (in g/kg DM) ash ($P = 0.012$), NDF ($P < 0.001$) and ADF ($P < 0.001$; Table 5). All three terms included in the model were negatively correlated with the ileal OM digestibility, with coefficients -0.0006, -0.0012 and -0.0011 for ash, NDF and ADF, respectively.

Table 3
Individual AA content (g/100 g N), true protein expressed as anhydrous AAs (g/kg DM) and N content (g/kg DM), non-protein nitrogen (% of N) and N to protein conversion factors of selected seaweed species compared to soybean meal (reference ingredient).

Component	Seaweed classification													
	Brown						Red							Green
	L. digitata		*S. latissima*		*A. nodosum*		*P. palmata*		*C. crispus*		*U. lactuca*			SBM [a]
g AA-N/100 g N	S	I	S	F	S	I	S	F	S	I	S	F	I	
Lysine	4.9	5.0	5.6	4.2	4.0	4.2	6.3	6.7	7.3	4.7	4.4	5.7	4.7	7.5
Methionine	0.9	0.8	1.2	0.8	1.2	1.2	1.1	1.1	0.9	0.8	1.0	1.1	1.1	0.8
Cysteine	1.5	1.9	1.6	1.1	1.0	1.0	1.7	2.2	1.8	1.0	1.4	0.9	0.7	1.0
Threonine	3.2	3.1	3.4	2.7	3.1	3.1	3.3	3.4	3.2	3.2	4.1	3.4	3.1	2.9
Tryptophan	0.8	0.8	1.0	0.7	1.1	1.0	0.9	0.9	0.9	0.7	0.9	1.4	1.0	1.1
Leucine	3.7	3.5	4.6	3.2	3.9	4.4	4.2	4.4	3.5	4.0	4.3	4.7	4.3	5.2
Isoleucine	2.0	2.0	2.4	1.8	2.1	2.5	2.4	2.5	2.0	2.8	2.5	2.4	2.2	3.1
Histidine	3.0	3.5	2.9	2.3	2.7	3.1	3.1	3.3	3.0	2.5	2.5	3.4	1.6	4.7
Phenylalanine	2.0	1.9	2.4	1.7	2.2	2.4	2.1	2.2	2.1	2.3	2.6	2.9	2.6	2.8
Arginine	7.4	6.9	8.5	5.9	6.8	8.2	10.0	11.1	11.5	11.1	8.8	9.9	12.9	14.7
Asparagine + Aspartic acid	9.0	9.7	10.5	7.8	8.9	8.9	9.8	10.8	8.9	8.9	11.6	12.8	11.8	11.3
Serine	3.3	3.3	3.7	2.8	3.0	3.2	4.0	4.2	4.1	3.6	4.6	4.1	3.7	4.1
Glutamine + Glutamic acid	10.9	11.5	12.9	22.4	13.0	9.5	10.5	11.3	7.6	7.7	9.7	8.5	9.6	16.2
Glycine	5.4	5.4	6.1	4.6	5.0	5.7	6.9	6.9	7.8	6.2	7.1	6.8	6.0	5.0
Alanine	17.1	14.0	9.2	12.2	5.6	5.1	7.3	7.5	4.9	5.6	9.0	7.4	6.8	4.4
Valine	3.4	3.2	3.9	3.3	3.3	3.6	4.2	4.4	3.4	3.6	4.7	4.1	3.8	3.7
Proline [b]	3.0	2.9	3.0	2.1	2.6	2.9	3.4	3.5	2.9	3.2	3.2	3.5	3.1	3.7
Tyrosine [b]	1.1	1.1	1.3	0.9	1.1	1.2	1.3	1.4	1.2	1.3	1.5	1.6	1.5	1.7
AA-N (g/100 g N)	82.7	80.2	84.1	80.8	70.7	71.2	82.5	87.9	76.9	73.2	83.9	84.5	80.4	94.0
AA (g/kg DM)	102.0	87.9	84.5	128.3	43.8	55.3	155.5	156.9	98.1	121.8	79.9	190.9	265.0	523.6
Anhydrous AA (g/kg DM) [c]	86.5	74.8	72.2	109.6	37.5	47.4	132.8	134.1	83.8	104.1	68.1	163.3	226.9	451.2
AA-N (g/kg DM) [d]	15.2	13.1	12.4	18.9	6.4	8.1	23.3	23.5	15.1	18.3	11.7	28.3	39.9	79.9
N (g/kg DM)	18.3	16.3	14.8	23.4	9.0	11.4	28.2	26.8	19.6	25.0	13.9	33.5	49.7	85.0
Non-protein N (% of N)	17.3	19.8	15.9	19.2	29.4	28.8	17.5	12.1	23.1	26.8	16.1	15.5	19.7	6.0
N:protein factor, K_P [e]	4.72	4.58	4.89	4.69	4.16	4.16	4.71	5.01	4.27	4.17	4.89	4.87	4.57	5.31
N:protein factor, K_A [f]	5.71	5.70	5.81	5.81	5.89	5.84	5.71	5.70	5.55	5.70	5.83	5.77	5.68	5.65

AA-N = amino acid nitrogen, S = harvested in Scotland, F = harvested in France, I = harvested in Ireland.
a Soybean meal values based on analysis conducted by Hulshof et al. (2016).
b Values calculated based on their ratio to phenylalanine from Biancarosa et al. (2017).
c Anhydrous AA (g/kg DM): weight as found when AAs are bound in protein.
d Based on N content of each individual amino acid.
e Ratio between sum of anhydrous AAs (g/kg DM) and N (g/kg DM) as described by Mariotti et al. (2008).
f Ratio between sum of anhydrous AAs (g/kg DM) and AA-N (g/kg DM) as described by Mariotti et al. (2008).

3.2.2. Nitrogen digestibility

The *in vitro* N digestibility coefficients of the seaweeds ranged from 0.25 (Scottish *A. nodossum*) to 0.89 (French *S. latissima*), while that of SBM was 0.98 (Table 4). Both classification, species within classification, and origin affected the N digestibility, but their effects were interdependent as indicated by the interactions (all with $P < 0.001$). The N digestibility coefficients of French *S. latissima* and *U. lactuca* (0.89 and 0.74) were higher compared to their Scottish varieties (0.71 and 0.69). The N digestibility coefficients of all seaweeds of Irish origin were higher than that of their respective Scottish varieties (*A. nodossum* (0.60 *vs*. 0.25), *C. crispus* (0.82 *vs*. 72), *L. digitata* (0.82 *vs*. 0.72) and *U. lactuca* (0.80 *vs*. 0.69)). Overall, *A. nodosum* had a lower N digestibility coefficient than the other species.

Multiple regression analyses indicated that 88.4% of the observed variation was explained by the selected significant model ($P < 0.001$) including the variables (in g/kg DM) CP ($P = 0.004$) and ADL ($P < 0.001$; Table 5). Crude protein was positively correlated (coefficient = 0.0012), whereas ADL was negatively correlated (coefficient = -0.0027) with the ileal N digestibility.

3.3. In vitro simulated total tract digestibility

3.3.1. Organic matter digestibility

Total tract OM digestibility coefficients ranged from 0.65–0.88 for the seaweed samples, and was 0.98 for SBM (Table 4). The factors classification, species within classification and origin effected the OM digestibility, but were interdependent as indicated by their interactions ($P < 0.001$). Mean total tract OM digestibility coefficients were highest for *L. digitata* (0.84), *S. latissima* (0.86) and *P. palmata* (0.85) and lowest for *A. nodossum* (0.69). The OM digestibility coefficients of seaweeds of Irish origin was higher than that of their respective Scottish varieties. The total tract OM digestibility coefficient of brown seaweeds from France were higher compared to their Scottish varieties (0.88 *vs*. 0.77).

Multiple regression analyses indicated that 82.1% of the observed variation was explained by the selected model ($P < 0.001$)

Table 4
In vitro digestibility coefficients of OM and N of selected seaweed species, determined with a modified Boisen method (Boisen and Fernandez, 1997) to simulate ileal and total tract digestibility.

Seaweed classification	Species	Origin	Ileal digestibility OM	Ileal digestibility N	Total tract digestibility OM
Brown	*L. digitata*	S	0.707^{bcd}	0.715^{cd}	0.807^{d}
		I	0.742^{abc}	0.820^{b}	0.869^{ab}
	S. latissima	S	0.755^{abc}	0.717^{cd}	0.843^{bcd}
		F	0.812^{a}	0.886^{a}	0.883^{a}
	A. nodossum	S	0.621^{de}	0.249^{f}	0.646^{f}
		I	0.441^{g}	0.597^{e}	0.739^{e}
Red	*P. palmata*	S	0.662^{cd}	0.826^{b}	0.860^{abc}
		F	0.625^{de}	0.821^{b}	0.842^{bcd}
	C. crispus	S	0.707^{bcd}	0.715^{cd}	0.764^{e}
		I	0.764^{ab}	0.824^{b}	0.807^{d}
Green	*U. lactuca*	S	0.540^{ef}	0.688^{d}	0.736^{e}
		F	0.514^{fg}	0.737^{c}	0.755^{e}
		I	0.672^{bcd}	0.799^{b}	0.828^{cd}
	Soybean meal		0.842	0.979	0.970
	Standard error of differences		0.0447	0.0145	0.0179
	P-value				
	Classification		< 0.001	< 0.001	0.002
	Species		< 0.001	< 0.001	< 0.001
	Origin		0.458	< 0.001	< 0.001
	Classification × Origin		0.008	< 0.001	< 0.001
	Species × Origin		0.005	< 0.001	0.256

S = harvested in Scotland, F = harvested in France, I = harvested in Ireland.
Each digestibility coefficient value is based on 2 replicate measurements.
[a−g] Means within a column without a common superscript differ significantly (P < 0.05).

Table 5
Multiple regression analyses performed for *in vitro* ileal OM and N digestibility, total tract OM digestibility (see Table 4) and the corrected maximum gas production (see Table 6).

	Model			P-value[a]						
	P-value	Adjusted R^2	SE	Ash	CP	Cfat	Sugar	NDF	ADF	ADL
Ileal OM digestibility	0.137	11.3	0.102			0.137				
	0.003	61.4	0.067					0.002	0.003	
	< 0.001[c]	79.5	0.049	0.012				< 0.001	< 0.001	
Ileal N digestibility	< 0.001	74.3	0.082							< 0.001
	< 0.001[c]	88.4	0.055		0.004					< 0.001
	< 0.001	88.9	0.054	0.256	0.003					< 0.001
Total tract OM digestibility	< 0.001	61.7	0.042							0.001
	0.001	68.1	0.038		0.015	0.001				
	< 0.001[c]	82.1	0.029		0.014			0.011		< 0.001
Corrected maximum gas production [b]	0.076	19.1	0.850							0.076
	0.051	33.8	0.769				0.093			0.034
	0.036[c]	46.1	0.694				0.024	0.103		0.014

Cfat = crude fat.
[a] The variables starch and non-starch polysaccharides were also included in the multiple regression analyses, but not selected in any model and thus not shown here.
[b] In each of the generated models for the gas production test one term did not significantly correlate to the variation in the gas production test. The model with three terms was selected, as the best and only significant model.
[c] Indicate the selected models, based on significance of the model and terms included as explanatory variables.

including the variables (in g/kg DM) CP (P = 0.014), NDF (P = 0.011) and ADL (P = < 0.001; Table 5). Only CP was positively correlated with total tract OM digestibility, with correlation coefficients of 0.0005, -0.0002 and -0.0011 for CP, NDF and ADL, respectively.

3.3.2. *In vitro* gas production

The results of the *in vitro* gas production test are shown in Table 6 and Fig. 1. The protein corrected maximum gas production of all seaweeds was below that of the reference ingredient sugar beet pulp (425.7 ml/g OM), but the gas production of *P. palmata*, *S. latissima* and *L. digitata* was above that of alfalfa meal (248.6 ml/g OM). The uncorrected maximum gas production of *P. palmata, S. latissima, L. digitata* and *U. lactuca* was significantly higher than that of the other seaweeds. Phase 1 asymptotic gas production was

Table 6
Curve fitting parameters of the two-phase model for selected seaweed species in the gas production (GP) test.

Seaweed classification	Species	Origin	Asymptotic GP (ml/g OM)		Maximum GP (ml/g OM)	Corrected maximum GP (ml/g OM)[a]	T_{half} (h)		Shape	
			phase 1	phase 2			phase 1	phase 2	phase 1	phase 2
Brown	*L. digitata*	S	111.3^{b}	186.6^{a}	297.9^{abc}	327.9	1.7	16.5	2.14	1.91
		I	135.3^{b}	66.5^{bc}	201.8^{bcde}	231.4	2.8	27.1	1.74	7.15
	S. latissima	S	145.9^{b}	161.7^{ab}	307.6^{ab}	331.4	2.2	20.6	1.97	1.87
		F	84.0^{b}	191.4^{a}	275.5^{abc}	313.3	1.9	13.8	2.61	1.83
	A. nodossum	S	66.0^{b}	33.7^{c}	99.7^{de}	111.8	6.0	15.4	2.09	31.48
		I	53.8^{b}	64.9^{bc}	118.7^{de}	138.6	3.5	20.7	1.27	5.02
Red	*P. palmata*	S	282.1^{a}	52.0^{bc}	337.3^{a}	379.3	3.7	28.3	2.03	2.16
		F	306.5^{a}	15.1^{c}	321.7^{ab}	365.1	4.8	36.2	1.89	11.11
	C. crispus	S	49.1^{b}	40.8^{c}	89.9^{e}	115.4	2.1	22.4	1.58	3.86
		I	65.0^{b}	48.5^{bc}	113.4^{de}	160.2	4.0	25.4	1.37	25.78
Green	*U. lactuca*	S	133.7^{b}	78.5^{abc}	212.2^{bcd}	234.7	5.7	12.0	1.22	3.53
		F	103.8^{b}	82.7^{abc}	186.5^{cde}	241.6	5.5	27.7	1.28	3.28
		I	93.1^{b}	32.8^{c}	125.8^{de}	194.4	6.0	26.6	1.20	4.97
Sugar beet pulp			194.0	207.8	401.8	425.7		5.0	2.65	3.90
Alfalfa			202.5	0.0	198.9	248.6	7.2	0.0	1.09	0.00
Standard error of differences			45.64	53.89	54.97		4.17	9.65	0.777	16.89
P-value										
Classification			< 0.001	0.004	0.247		0.413	0.117	0.165	0.780
Species			< 0.001	0.009	< 0.001		0.743	0.568	0.573	0.460
Origin			0.817	0.422	0.356		0.993	0.238	0.623	0.966
Classification × Origin			0.600	0.824	0.708		0.987	0.544	0.894	0.623
Species × Origin			0.575	0.046	0.137		0.542	0.695	0.701	0.182

S = harvested in Scotland, F = harvested in France, I = harvested in Ireland.
Each digestibility value in the table is based on 3 replicate measurements.
Sugar beet pulp and alfalfa were included as reference ingredients.
[a] Maximum gas production corrected with an increase of 2.5 ml per percent TP in OM of the seaweed and reference ingredients, according to Cone and van Gelder (1999).
[a−g] Means within a column without a common superscript differ significantly (P < 0.05).

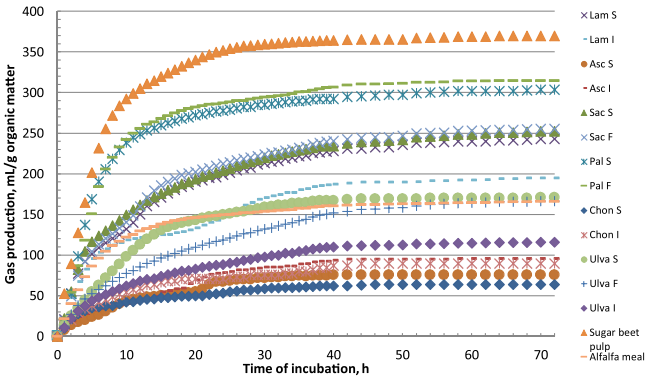

Fig. 1. Cumulated gas production (ml/g OM) over a 72 h incubation period of the selected seaweed species, and sugar beet pulp and alfalfa meal as reference ingredients.
S = Harvested in Scotland, F = Harvested in France, I = Harvested in Ireland.
Lam = Laminaria digitata, Asc = Ascophyllum nodosum, Sac = Saccharina latissima, Pal = Palmaria palmata, Chon = Chondrus crispus, Ulva = Ulva lactuca.

affected by classification (P < 0.001) and species (P < 0.001), with *P. palmata* showing a significantly higher gas production than all other seaweeds. In the second phase, asymptotic gas production was also affected by classification (P = 0.004) and species (P = 0.009), but these effects were interdependent because of the interaction between species and origin. The gas production was only affected by species (P < 0.001). No differences between seaweeds were found for rate of gas production, or switching characteristics (shape) between phase 1 and 2.

The multiple regression analyses showed that there was no significant model only including terms that significantly correlated with the corrected maximum gas production. The selected significant model (P = 0.036) indicated that 46.1% of the observed

variation was explained including the variables (in g/kg DM) sugar (P = 0.024), NDF (P = 0.103) and ADF (P = 0.014; Table 5). Only sugar was positively correlated with the corrected maximum gas production, with coefficients 0.0434, -0.0039 and -0.0131 for sugar, NDF and ADL, respectively.

The multiple regression analyses relating the *in vitro* OM and N digestibility analyses to the corrected maximum gas production showed that total tract OM digestibility was positively correlated (correlation coefficient: 9.63) with the gas production test (P = 0.010), explaining 42.4% of the variation.

4. Discussion

The aim of this study was to evaluate the nutritive value of intact seaweeds, common in marine waters around Northwestern Europe, for application in animal feed as source of macro nutrients.

4.1. Chemical composition

The chemical composition of seaweeds as observed in this study showed a varying protein content, with an interesting AA pattern, together with relatively high levels of NPN. High NSP contents were observed and sugar, starch and fat were only present at low levels, together with a very high ash content in all seaweeds. The obtained results, including the large variation in chemical composition within and among seaweed species, are largely in line with results for brown, red and green species as described in literature (Øverland et al., 2019; Makkar et al., 2016 and Biancarossa et al., 2017).

The CP content ranged between 45 and 248 g/kg DM, with the relatively high values for the red and green compared to the brown species, which generally agrees to values in the literature (*e.g.* Øverland et al., 2019). Nonetheless, the higher levels within the range of CP as reported in literature for brown, red and green algae (up to 168, 376 and 352 g/kg DM respectively) were not observed here. This could be due to the inclusion of seaweeds from other regions in those studies, with seaweeds grown under other environmental conditions like water temperature, availability of nutrients, and another stage of maturity at harvest. The CP values as reported by Øverland et al. (2019) were based on a N:P conversion factor of 5.00. Thus the observed differences cannot be explained by the use of different conversion factors.

For seaweed, a conversion factor of 5.00 is proposed (Angell et al., 2015), based on the higher NPN in seaweed compared to standard land-based feed stuffs, for which a factor of 6.25 is commonly used. We observed an average NPN content of 200 g/kg N compared to 60 g/kg N in SBM. Two conversion factors were calculated based on the AA content, one calculating the ratio of anhydrous AA (g/kg DM) to AA-N (g/kg DM; K_A), and the other also taking into account the NPN by calculating the ratio of anhydrous AA (g/kg DM) to N (g/kg DM; K_P) both based on Mosse (1990). Because of the potentially high NPN content of seaweed, the conversion factor K_P is most applicable when evaluating intact seaweed as protein source for animal diets (Lourenço et al., 2002; Templeton and Laurens, 2015; Biancarosa et al., 2017; Angell et al., 2015).

A proposed conversion factor of 5.00 provided a more accurate calculation of true protein than a factor of 6.25. Nonetheless, the variation in actual conversion factors as calculated from our data, ranged from 4.16–5.01, showing that one factor for seaweed in general, as well as for seaweed classification, does not accurately reflect the true protein content. Because the K_P factors of samples within species are similar, we suggest using a conversion factor per species, which could be based on literature values and data mining, using already available data on true conversion factors.

The observed AA content, AA-N and N of the brown, red and green seaweed species showed a pattern, with the highest AA-N content in *P. palmata*, and *U. Lactuca* and the lowest content in *A. nodosum*. However, due to the large variation, even within species, provision of average contents has little value. The NPN can be utilized by rumen microbiota, but not by monogastric animals. This indicates that intact seaweed is a better protein source for ruminants, although the nitrogen content of most studied seaweed species was relatively low for using intact seaweed as protein supplement in ruminant diets.

The mean contents of the AAs methionine, cysteine, threonine, and valine (g AA-N/100 g N) were 20–50% higher than in SBM, whereas the contents of some other essential AAs, *i.e.* lysine, leucine, isoleucine, histidine, and arginine were 10–30% lower. The AA pattern and levels of individual AAs, as well as the variation observed, correspond with data reported in the literature (Øverland et al., 2019; Biancarosa et al., 2017), although most of the AA levels observed in our study are slightly higher than the values reported for the same seaweed species harvested around the coastal areas of Norway. Thus, with adequate supplementation with other protein sources or individual AA, the AA pattern of a seaweed containing feed could meet the requirements of both monogastric and ruminant species. Nonetheless, the variation in AA profile within and between species was quite substantial. Therefore, it is recommended to evaluate the protein and AA content of each batch of seaweed before inclusion in animal diets, and to build a database to obtain more quantitative insight in the variation in AA content and pattern among and within seaweed species.

The NSP content was high in all seaweed samples, in accordance with data in the literature (Øverland et al., 2019). The polysaccharides in the NSP, NDF and ADF fractions are not enzymatically digestible, and thus of low nutritive value for poultry, whereas they may be potentially fermentable in the large intestine of pigs (Nielsen et al., 2014; Metzler-Zebeli et al., 2010). For ruminants, the NSP content contributes as a substrate for rumen microbiota, thus potentially contributing to the short chain fatty acids and protein availability (Belanche et al., 2012).

Seaweeds contain different polysaccharides compared to SBM or other land plants (Rupérez and Toledano, 2003; Jiménez-Escrig and Sánchez-Muniz, 2000; CVB, 2018), and it is not known in which analysed dietary fraction these polysaccharides are included. Although useful for evaluation of digestibility in animals, the analyses of NSP, NDF, ADF and ADL do not provide detailed information regarding specific polysaccharides and the actual nutritional value of the seaweeds. A striking example was observed in the

brown seaweeds, in which the analysed ADF fraction was up to twofold greater than the NDF fraction. To our knowledge, this has not been reported elsewhere in literature, although the NDF and ADF of seaweeds are not often analysed. We speculate that the higher ADF than NDF fraction might be caused by the presence of specific polyphenolic compounds, which can precipitate in an acidic environment, but not in an neutral environment (Manev et al., 2013; Soest van et al., 1991). Further analyses are required for a better identification and quantification of the specific polysaccharides of seaweed, their inclusions in different analytical fractions and their contribution to the nutritional value.

The analyses of the carbohydrates after complete hydrolyses of the seaweeds (Appendix 1) showed a species specific carbohydrate composition, in agreement with species specific polysaccharide fractions as reported by Øverland et al. (2019). Again a large variation in composition was observed between and within species, for example with galactose mainly observed in red species, fucose in brown species and rhamnose in green species.

Regarding the energy content of seaweed, all seaweeds were very low in sugar, starch and fat content, which is in line with data in the literature (Holdt and Kraan, 2011; Øverland et al., 2019). Because of the low fat content, the contribution to the nutritional value of seaweeds is low and fatty acid composition (Appendix 2) will not be further discussed within the scope of this paper. Nonetheless, the fatty acid content and composition might be relevant for the study of potential bioactive properties.

The high ash content in all seaweed species in this study hamper the inclusion of intact seaweed in animal diets, and indicates that the composition of the ash fraction needs to be taken into account (Appendix 3). Especially poultry are susceptible to high mineral concentrations, as for example indicated by a maximum tolerable level of sodium chloride of 17, 30 and 45 g/kg DM for poultry, pigs and growing beef cattle (NRC, 2005). A too high mineral content in pigs and poultry feed may cause wet litter and diarrhoea, which may result in a decrease in performance (Guiry and Blunden, 1991; Koreleski et al., 2010). Studies in sheep and goats showed that a high ash content of the diet due to inclusion of seaweed also resulted in a higher water intake and urine excretion in these animal species, but it had no negative effect on the digestibility of the diets (Marin et al., 2009; Castro et al., 2009). These results may suggest that the impact may be more detrimental in monogastric species. A potential method of reducing ash content of seaweed, is to rinse the intact seaweed with fresh water. This would reduce the ash content, and consequently increase the relative content of other nutrients, but also other soluble nutrients might be lost during washing (Magnusson et al., 2016). Based on the high mineral content of all seaweed samples, these intact seaweeds are not suitable to be use in animal diets at high inclusion level.

This paper does not address the heavy metal content in the seaweeds. Nonetheless, these do need to be taken into account when formulating diets. Heavy metals can accumulate in large amounts in seaweed, which limits the use of intact seaweed in animal feed (Øverland et al., 2019).

The large variation in chemical composition among and within seaweed species hampers seaweed inclusion in animal diets. In the literature, this variation is attributed to multiple environmental factors, like season of harvest (Holdt and Kraan, 2011; Schiener et al., 2015; Øverland et al., 2019; Sharma et al., 2018), depth of the seaweed in the water column (Sharma et al., 2018), and light and nutrient availability in the water (Boderskov et al., 2016). Due to the characteristics of the seaweed samples in our study, the origin of the samples was to some extend confounded with the time of harvest. Hence, these two factors could not be fully separated. The variation also depends on the stage of the reproductive cycle, where results can be contradictory between species regarding changes in levels of *e.g.* main structural component during the reproductive phase (Skriptsova et al., 2012). The variation in our results illustrate that dried seaweed meal is not a standardized feed material with a predictable chemical composition.

Within land-based feedstuffs, variation in nutrient levels also occurs (CVB, 2018), but to a lesser extent than in seaweed. Whereas each batch of land-based feedstuffs is preferably analysed for its nutrient composition before diet formulation, this practice will be even more required for effective inclusion of seaweeds in animal diets. Also within species grown at the same location, the variation is considerable (*e.g.* Marinho-Soriano et al., 2006 and Schiener et al., 2015). Cultivation of seaweed might be of interest, since it has been shown that there is a potential for the selection of seaweed, possibly leading to a higher yield of a more preferred, and possibly more constant or predictable, chemical composition (Li et al., 2008; Westermeier et al., 2010).

4.2. *In vitro digestibility*

The Boisen *in vitro* digestibility test has been developed to simulate the digestibility in the stomach, small intestine and large intestine of pigs in three distinct steps. Although developed for pigs, this method has been used for poultry without using the third step, since the contribution of large intestinal fermentation in birds is negligible (Losada et al., 2010).

The *in vitro* simulated ileal OM digestibility of the seaweeds ranged from very low to reasonably good (OM digestibility coefficients: 0.44 - 0.81), The highest values were observed for *L. digitata*, *S. latissima*, and *C. crispus* but the mean digestibility was still about 10%-points lower than in SBM (0.84). The mean simulated ileal N digestibility coefficients of all seaweed species apart from *A. nodosum* was relatively high, generally above 0.75, but well below the N digestibility of SBM of 0.98. This could be explained by part of the N being bound to, or entrapped in, poorly digestible polysaccharides, *e.g.* cell wall structures (Lahaye and Robic, 2007). This would also explain the low OM digestibility (MacArtain et al., 2007). In the multiple regression analyses performed on the *in vitro* digestibility, the fibre fractions NDF, ADF and ADL were included in the selected regression models and correlated negatively with the ileal and total tract digestibility. This emphasises the importance of further identification and characterisation of seaweed polysaccharides in different fibre fractions, to understand, predict and improve the digestibility of seaweed fractions in farm animals. Extraction of protein or other favourable components might be a good step forward towards seaweed inclusion in animal diets (Øverland et al., 2019; Bjarnadóttir et al., 2018; Bikker et al., 2016). Indeed, Bikker et al. (2016) observed a 5% increase in an *in vitro* protein digestibility of *U. lactuca* extracted fraction compared to the intact seaweed.

We acknowledge that the *in vivo* validation of the observed *in vitro* results is lacking, since there are only few *in vivo* studies

published with intact seaweed added at a substantial level, *e.g.* > 50 g/kg DM or more, since most studies focussed on the effects of the bioactive compounds of seaweed rather than the nutritional value (*e.g.* Abudabos et al., 2013; McDonnell et al., 2010). Nonetheless, when taking into account the low protein content, the high NPN content, and low N digestibility relative to SBM, neither of the seaweeds investigated here would seem to be a high quality protein source for monogastric animals.

4.3. In vitro rumen fermentation

The gas production model for land-based feed materials generally includes predefined time periods for each phase (Van Gelder et al., 2005). However, seaweed consists of very different polysaccharides and sugars compared to land-based plants, and the behaviour of the different fractions in the gas production test is not well known. Therefore, a basis to predefine the duration of the phases to set time periods was not available. The seaweeds *L. digitata*, *S. latissima*, *P. palmata* had the highest mean corrected maximum gas production in the *in vitro* gas production test. The values varied from approximately 65–90% of the corrected maximum gas production of sugar beet pulp, and were well above the result for alfalfa. This indicates their potential value in ruminant diets.

The maximum gas production as recorded for seaweeds in this experiment was consistently about a factor 10 higher than in the study of Molina-Alcaide et al. (2017), who determined the asymptotic gas production of the species *L. digitata* (18 ml/g OM) and *P. palmata* (38 ml/g OM). Although a large variation in chemical composition and consequently in gas production may be expected between and within seaweed species in these two studies, we suspect the large differences between studies to be due to methodological differences. Nonetheless, the ratio between the maximum gas production of the two species is quite similar between the two studies.

The difference in the time at which half of the gas was produced between the seaweeds and sugar beet pulp, especially in the second phase, was likely caused by differences in the fermentability of specific polysaccharides in the sugar beet pulp *versus* those in the seaweeds. The behaviour of the specific NSP components of the seaweed during fermentation is important to understand the kinetics of the fermentation of seaweed in ruminant diets, but this has not been studied comprehensively. The lower gas production of the seaweeds *A. nodossum* and *U. lactuca* might be caused by a relatively high lignin content, which is poorly fermentable, although *C. crispus* does not contain a high lignin content and also had a notably low gas production. The negative impact of lignin was confirmed by the results of the multiple regression analyses of the *in vitro* data, with the highest negative impact of the ADL fraction. The ADL fraction was also shown to negatively correlate to digestibility of land-based plants using an *in vitro* rumen fermentation approach (Buxton and Russell, 1988). The negative correlation enhances the importance of further identification and characterisation of seaweed polysaccharides in different fibre fractions. Characterisation of the fermentability of individual NSP components is also of importance for the inclusion of seaweed in ruminant diets, where the intact seaweeds with high NSP content might be a nutritionally valuable feed stuff (Choct, 1997; Metzler-Zebeli et al., 2010; Susmel and Stefanon, 1993).

Fermentability might be higher when using rumen fluid from animals that are adapted to a seaweed diet. Relative high OM digestibility coefficients of 0.84-0.97 (Tilley and Terry method) were observed for several seaweed species among which the brown *L. digitata* and *S. latissima* and the red species *P. palmata* using rumen fluid of seaweed fed Orkney sheep (Greenwood et al., 1983). When sheep were fed seaweed diets, the microbial composition of the rumen changed towards more favourable microbial composition for seaweed fermentation, leading to an up to twofold higher digestibility of nutrients (Orpin et al., 1985).

A regression analysis was performed relating the *in vitro* digestibility to the corrected maximum gas production, to determine the agreement between the *in vitro* models. Only the total tract OM digestibility correlated significantly with the gas production, explaining 43% of the variation. This correlation was expected since the *in vitro* total tract digestibility analyses included an enzyme step containing a range of carbohydrates including arabinase, cellulase, beta-glucanase, hemicellulase and xylanase activity, mimicking the fermentation in the large intestine of pigs. This would come closest to the fermentation determined in the *in vitro* gas production test. The low percentage of variation in corrected maximum gas production explained by total tract OM digestibility (43%) indicates that the *in vitro* total tract OM digestibility is not a sufficient indicator for maximum gas production.

5. Conclusion

The results of this study demonstrate a large variation in nutrient composition, *in vitro* digestibility, and nutritional value, both between and within seaweed species. Therefore, use of mean nutrient contents have little value and the rational use in animal diets will require adequate analysis of each batch of product. Based on the protein content and AA pattern, some of the red and green seaweed species (*e.g. P. palmata* and *U. lactuca*) would be a valuable protein source for farm animals. However, several characteristics including a high NSP and NPN content and a low *in vitro* digestibility relative to SBM limit the use of intact seaweed in monogastric species, especially poultry. Since NPN can be used by rumen microbiota and some species (*e.g. L. digitata*, *S. latissima* and *P. palmata*) demonstrated a relatively high *in vitro* fermentability, intact seaweed may have a higher nutritional value for ruminants. Nonetheless, the high ash content in all seaweed species, potentially including heavy metals, hampers the use of intact seaweed for both ruminants and monogastrics. Washing seaweeds may reduce this high ash content, but also result in a loss of other soluble components. Extraction of protein and other favourable components seems an important step forward towards seaweed inclusion in animal diets. This would also allow to drastically reduce the high ash and mineral content in the intact seaweed. Further identification and characterisation of seaweed polysaccharides are required to understand, predict and improve the digestibility of seaweed fractions in farm animals, especially since the fibre fractions comprised up to 40% of DM in the selected seaweed samples. *In vivo* studies are required to validate the value of future seaweed products.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix 1

Content of total glucose and individual sugars (g/kg DM) of the selected seaweed species, after hydrolysis of carbohydrates.

	Brown						Red				Green		
Sugar	*L. digitata*		*S. latissima*		*A. nodosum*		*P. palmata*		*C. crispus*		*U. lactuca*		
(g/kg DM)	S	I	S	F	S	I	S	F	S	I	S	F	I
Total glucose	2	1	5.6	1.2	26.6	1.3	32.9	46	5	2.3	20.1	10.6	6.5
Fucose	30.2	21.8	14.8	12.3	63.7	22.4	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Rhamnose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	66.2	42.9	22.5
Arabinose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	6.1
Galactose	7.5	6.1	7.8	7.1	6.56	10.7	106.85	146.7	263.1	98.6	4.37	8.4	8.4
Glucose	54.6	43.5	76.17	74.3	41.31	5.5	18.17	25.5	51.31	63.7	164.15	110.9	48.7
Xylose/Mannose	6.7	13.4	5.13	3.8	25.33	8.4	341.21	289.5	10.37	12.5	73.09	31	10.2
Fructose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Saccharose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Lactose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Raffinose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Stachyose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Maltose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Verbascose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00
Maltotriose	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00	< 1,00

S = harvested in Scotland, F = harvested in France, I = harvested in Ireland.

Appendix 2

Content of fat and fatty acids (g/kg fat) of the selected seaweed species

		Brown						Red				Green		
Fatty acid		*L. digitata*		*S. latissima*		*A. nodosum*		*P. palmata*		*C. crispus*		*U. lactuca*		
(g/kg fat)	Trivial name	S	I	S	F	S	I	S	F	S	I	S	F	I
Elutable		14.2	10.2	9	10.6	33.5	9.1	11.6	12.6	15.1	6.3	19.7	30.9	6.1
Fat		9	7	2.4	6.1	22.5	3.2	11	9.9	18.1	4.1	10.3	21.9	2.8
SFA		4.4	2.7	3.9	3.7	7.6	5.9	5.3	5.3	5.6	5.2	7.6	8.6	4.1
MUFA		3	2.3	1.9	2	13.8	2.1	1.2	0.8	4	0.9	3.7	3.5	1.3
PUFA		6.8	5.2	3.2	4.8	12.1	1.1	5.1	6.5	5.4	0.2	7.5	14.8	0.6
Trans FA		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.8	4.1	< 0,1
C4:0	Butyric acid	0.1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1
C5:0	Valeric acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C6:0	Caproic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C7:0	Enanthic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C8:0	Caprylic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C9:0	Pelargonic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C10:0	Capric acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C10:1	Decenoic Acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C11:0	Undecylic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C12:0	Lauric acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C12:1	Lauroleic Acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
iso-C14:0	isomyristic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1

C14:0	Myristic acid	1.1	0.6	1.1	1.1	2.8	0.6	1.1	1.1	0.5	0.4	0.2	0.1	0.3
C14:1n5	Myristoleic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C14:1n9	Physeteric acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
iso-C15:0	isopentadecanoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
anteiso-C15:-0	anteisopentadecanoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C15:0	Pentadecylic acid	0.1	< 0,1	0.1	0.1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C15:1	Pentadecenoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
iso-C16:0		0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	< 0,1	0.7	0.1
anteiso-C16:-0		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C16:0	Palmitic acid	2.7	1.8	2.3	2.2	3.8	4.7	3.7	3.7	4.6	4.4	6.9	7	3.2
C16:1n7	Palmitoleic acid	0.4	0.4	0.4	0.4	0.5	0.5	0.2	0.1	0.4	0.1	0.2	0.2	0.2
C16:1n9	Hexadecenoic Acid	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 0,1	< 1,0	< 1,0
iso-C17:0		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
anteiso-C17:-0		< 0,1	< 1,0	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1
C17:0	Margaric acid	0.1	< 0,1	< 0,1	0.1	0.1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C17:1	Heptadecenoic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
iso-C18:0		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.2	0.1	< 0,1
C18:0	Stearic acid	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
C18:1 trans	Elaidic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C18:1n9	Oleic acid	2.4	1.7	1.4	1.6	12.9	1.2	0.5	0.3	3.1	0.6	0.7	0.9	0.3
C18:1n other isomers	Other isomers	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.1	0.3	0.2	0.3	0.2	2.8	2.4	0.8
C18:2 trans	Linolelaidic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	0.8	4.1	0.1
C18:2n6	Linoleic acid	0.8	0.7	0.7	1	3.2	0.4	0.1	0.1	0.2	< 0,1	2.8	1.5	0.2
C18:3n3	α-Linolenic acid	0.9	0.8	0.3	0.4	1.1	0.2	0.1	0.1	0.1	< 0,1	2.4	6.2	0.2
C18:3n6	γ-Linolenic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C18:4n3	Stearidonic acid	1.6	1	0.5	0.9	0.8	0.1	0.2	0.1	< 0,1	< 0,1	1.1	4.8	0.1
CLA 9cis 11-trans		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
CLA 10trans 12cis		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C19:0	Nonadecylic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C20:0	Arachidic acid	0.1	0.1	0.1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C20:1n9	Gondoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1
C20:1n11	Gadoleic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C20:2n6	Eicosadienoic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.4	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C20:3n3	Eicosatrienoic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.2	< 0,1	< 0,1
C20:3n6	Dihomo-γ-linolenic acid	0.1	< 0,1	< 0,1	0.1	0.3	< 0,1	< 0,1	< 0,1	0.1	< 0,1	0.1	0.1	< 0,1
C20:4n3		0.1	< 0,1	0.1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.6	< 0,1
C20:4n6	Arachidonic acid	1.5	1.1	0.8	1.6	4.4	0.4	0.2	0.4	2.7	0.1	0.2	0.1	< 0,1
C20:5n3	Eicosapentaenoic acid	1.8	1.6	0.8	0.8	1.7	0.1	4.3	5.7	2.2	< 0,1	0.2	0.3	< 0,1
C21:0	Heneicosylic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:0	Behenic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	0.2	0.3	0.1
C22:1n9	Erucic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1
C22:1n11	Cetoleic acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:2n6		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:3n3	Eranthic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.2	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:4n6		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:5n3	Docosapentaenoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.1	< 0,1	0.3	0.9	< 0,1
C22:5n6		< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C22:6n3	Docosaheptaenoic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C23:0	Tricosylic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C24:0	Lignoceric acid	< 0,1	< 0,1	< 0,1	< 0,1	0.1	0.1	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
C24:1n9	Nervonic acid	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	0.2	< 0,1	0.1	< 0,1	< 0,1	< 0,1	< 0,1

S = harvested in Scotland, F = harvested in France, I = harvested in Ireland, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, FA = fatty acid.

Appendix 3

Content of crude ash, minerals and trace elements (g/kg DM) of seaweed samples, compared to the reference ingredient soybean meal.

Ash	Brown						Red				Green			Reference ingredient SBM[a]
	L. digitata		*S. latissima*		*A. nodosum*		*P. palmata*		*C. crispus*		*U. lactuca*			
(g/kg DM)	S	I	S	F	S	I	S	F	S	I	S	F	I	
Ash (g)	245.0	338.7	212.3	246.3	189.2	373.8	195.8	216.5	155.0	400.1	204.3	228.9	507.4	65
HCl-ash (g)	6.3	6.5	9.7	5.2	3.0	10.4	6.8	3.9	1.8	136.4	16.6	7.2	235.4	
Macro minerals														
P (g)	2.33	3.15	2.24	1.35	0.62	0.85	3.36	2.22	1.45	2.59	0.96	1.51	0.92	7.3
Ca (g)	8.67	21.4	9.13	10.7	10.3	48.6	2.59	2.76	6.53	17	6.52	15.5	45.8	3.2
K (g)	50.2	76	39.6	51.7	19.1	64.9	63.7	70.8	14.5	59.2	16.7	17.7	17.4	25.2
Mg (g)	6.87	7.43	7.31	7.72	8.19	11.3	3.15	2.99	7.34	6.15	16.5	17.2	17.3	3.4
Na (g)	34.3	38	32.6	38.3	33.4	32.8	19.1	20.6	29.6	15.3	25.8	40.9	28.1	0.2
Cl (g)	66.9	98.2	70.9	79.4	29.6	82.4	72.9	87.4	10.9	38.1	35.8	59.8	44.8	0.3
S (mg)	13950	14170	8161	8390	22170	14170	7425	6432	67440	30670	44200	39570	30970	401
Trace elements														
Cu (mg)	< 5	< 5	< 5	< 5	< 5	< 5	5	< 5	< 5	5.32	< 5	< 5	< 5	17
Fe (mg)	93.7	697	290	235	96.5	1210	166	120	96.5	8520	537	214	3460	270
Mn (mg)	14.9	16.3	17.7	< 5	20.9	112	81.8	6.56	29.3	2460	78.2	11.4	441	46
Zn (mg)	28.9	14.2	31	31.8	89.7	15	165	28.8	103	38	54	11.9	17.9	55
Ni (mg)	0.8	1.7	1.2	1.4	1.1	3.5	4	3.5	2.7	13	6.58	3.7	21	n.a.
As (µg)	52845	83712	69195	73663	18595	93425	10764	12056	8771	11706	3400	5424	8198	n.a.
Co (µg)	< 100	503	107	< 100	923	2090	251	< 100	105	7000	293	119	1260	344
Se (µg)	< 100	410	< 100	< 100	< 100	370	< 100	195	< 100	1023	< 100	161	643	n.a.
Cd (µg)	160	445	221	449	132	1683	124	247	87	182	60	149	150	n.a.
Pb (µg)	186	< 100	314	183	150	712	855	162	307	6604	1510	167	3585	n.a.
Hg (µg)	19	46	30	< 10	14	16	14	30	< 10	24	36	11	28	n.a.

S = harvested in Scotland, F = harvested in France, I = harvested in Ireland.

[a] Values based on analysis conducted by Hulshof et al. (2016).

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