Animal Feed Science and Technology 241 (2018) 210–222

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Amino acid profiles of nine seaweed species and their *in situ* degradability in dairy cows

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ARTICLE INFO

Keywords:
Macroalgae
Alternative protein source
In situ
Degradability
Total tract degradability
Ruminant

ABSTRACT

The potential of seaweeds as alternative protein source was investigated in relation to their amino acid (AA) profiles and the ruminal and total tract digestibility of these AAs. Three red (*Mastocarpus stellatus, Palmaria palmata,* and *Porphyra* sp.), four brown (*Alaria esculenta, Laminaria digitata, Pelvetia canaliculata,* and *Saccharina latissima*), and two green (*Cladophora rupestris.* and *Ulva* sp.) seaweed species were used in this study (hereafter, referred to by Genus name only). All seaweeds were collected in Bodø, Northern Norway, during Spring and Autumn in 2014 and 2015, except *Ulva*, which was only sampled in Autumn of both years, and *Saccharina* which was not sampled in Spring 2014. All the samples were studied for AA concentration. Six species (*Cladophora, Laminaria, Mastocarpus, Palmaria, Porphyra* and *Ulva*) were selected for the more resource demanding *in situ* study. Species and season interactively affected the content of total AA in crude protein in different seaweeds investigated ($P = 0.02$), with values ranging from 67.2 for *Laminaria* in Spring to 90.2 gAA/16 g N for *Ulva* in Autumn. *in situ* AA degradability was also species specific. The seasonality of total AA in crude protein of different seaweed species mostly did not affect their ruminal degradability, except for alanine, while species and season interactively affected proline's ruminal degradability. The total tract degradability showed that for *Laminaria* and *Mastocarpus*, methionine followed by leucine, isoleucine, histidine and lysine, were protected against rumen degradation. These protections seemed to be acid labile allowing digestion in the lower digestive tract. However, due to high indigestible fractions, these two seaweeds provided low amounts of AA to the intestines. Total tract AA digestibility values were the highest for *Porphyra* (906 g/kg) followed by *Palmaria* (843 g/kg) and the green seaweeds. To conclude, *Laminaria* and *Mastocarpus* are beneficial sources for bypass protein supply as they contain AA protected against rumen degradation. Based on their amount of AA and their AA degradability, *Porphyra*, followed by *Palmaria* and the green seaweeds (*Ulva* and *Cladophora*) can be considered as relevant sources of protein for ruminants.

Abbreviations: AA, amino acid; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; N, nitrogen; TAA, total amino acid; EAA, essential amino acid; NEAA, non-essential amino acid; SAA, sulfur amino acid; BCAA, branched chain amino acid; Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Val, valine

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https://doi.org/10.1016/j.anifeedsci.2018.05.003
Received 14 February 2018; Received in revised form 6 April 2018; Accepted 13 May 2018

1. Introduction

Edible seaweed biomass is a valuable alternative feed ingredient for livestock (Evans and Critchley, 2014; Makkar et al., 2016). The composition of seaweeds is highly variable, with large differences in proteins, minerals, lipids and fibers (Makkar et al., 2016), depending on the species, season, habitat, and prevailing proximate environmental conditions (desiccation, air and water temperature, light intensity, and nutrient concentrations) (Marinho-Soriano et al., 2006; Marsham et al., 2007). Most seaweeds have a high mineral content due to their capacity to absorb inorganic substances from their environment (Mišurcová, 2011). Seaweeds contain small amount of lipids (1 to 5% of DM), mainly polyunsaturated n-3 and n-6 fatty acids, while being rich in polysaccharides (Dawczynski et al., 2007). Some seaweeds like *Porphyra* and *Cladophora,* previously misclassified and reported as *Acrosiphonia* in Tayyab et al. (2016) and Molina-Alcaide et al. (2017), are rich in protein. For example, in Spring samples crude protein (CP) is of 372 and 333 g/kg dry matter (DM) respectively for *Porphyra* and *Cladophora*, which is comparable to oil seed by-products such as sunflower meal and rapeseed meal (Woods et al., 2003b). Biancarosa et al. (2017) reported that brown seaweeds had lower protein content than red and green seaweeds, and that true protein values varied widely between seaweeds species. Therefore, seaweeds could supply energy, minerals and protein to animal rations, and have potential as alternative protein source for ruminants (Tayyab et al., 2016). Balancing rations for individual amino acids (AA) has the potential to improve milk component concentrations, milk production (Bequette et al., 2000; Hanigan et al., 2001), protein utilization, and to lessen dairy's environmental impact (Hanigan et al., 2004), feed costs, and nutrient wastage (Børsting et al., 2003). However, little is known about AA degradability between species and seasons. Therefore, the objective of the present study was to evaluate the ruminal and total tract digestibility of AA in different seaweeds harvested in different seasons to determine whether all seaweeds species can be used as an alternative ingredient, source of protein, for dairy cows' ration. We hypothesized that ruminal and total tract digestibility of AA will be higher for red seaweeds as they have been found to contain more protein than green or brown seaweeds (Belghit et al., 2017).

2. Material and methods

2.1. Seaweeds species, collection and preparation

Three red seaweed species (*Mastocarpus stellatus, Palmaria palmata,* and *Porphyra sp.*), four brown (*Alaria esculenta, Laminaria digitata, Pelvetia canaliculata,* and *Saccharina latissima*), and two green (*Cladophora rupestris,* and *Ulva sp.*) were used in this study (hereafter: *Mastocarpus, Palmaria, Porphyra, Alaria, Laminaria, Pelvetia, Saccharina, Cladophora,* and *Ulva*, respectively). All seaweeds were collected in Bodø, Northern Norway, during Spring and Autumn in 2014 and 2015, except for *Ulva*, which was only sampled in Autumn of both years, and *Saccharina* which was not sampled in Spring 2014. After collection, the seaweeds were cleaned of sand, epiphytes, and associated fauna in seawater; thereafter, rinsed in 30% seawater, and swiftly in freshwater to remove surface salts. To prevent protein denaturation, the seaweeds were frozen at -20 °C until they were freeze-dried. Freeze-dried samples were milled through a 1.5 mm screen with a cutter mill (Pulverisette 15; Fritsch GmbH, Idar-Oberstein, Germany) for *in situ* analysis, and 0.5 mm screen for chemical analysis. All samples were studied for AA concentration, and samples from *Mastocarpus, Palmaria, Porphyra, Laminaria, Cladophora* and *Ulva* were used for the *in situ* study.

2.2. Animals

All experimental procedures complied with Danish Ministry of Justice Law no. 382 (June 10, 1987) Act no. 726 (September 9, 1993), concerning experiments with and the care of animals. Two rumen fistulated (#1; Bar Diamond Inc, Parma, ID, USA) non lactating Danish Holstein cows were used for rumen incubation. The cows were fed at maintenance level with a standard ration consisting of 67:33 forage to concentrate ratio. The diet consisted of 2 kg/d spring barley straw, 4 kg/d grass hay, 2.8 kg/d concentrate (400 g/kg barley, 400 g/kg oats, 100 g/kg soybean meal, 30 g/kg rapeseed meal, 30 g/kg sugar beet molasses and 40 g/kg mineral-vitamin pre-mix) and 150 g/week vitamin mixture. The ration chemical composition (in g/kg DM) was 139 CP, 465 aNDFom, and 137 starch.

Four lactating multiparous Danish Holstein cows fitted with a T-shaped duodenal and ileal cannula were used for intestinal incubations using mobile bags. The cows were fed a 60:40 forage to concentrate ratio (DM basis) diet during the intestinal incubations. The diet consisted of a total mixed ration of 305 g/kg DM maize silage, 305 g/kg DM grass-clover silage, 21.2 g/kg DM dried beet pulp, 63.6 g/kg DM barley straw, 127 g/kg DM rapeseed, 84.8 g/kg DM soybean meal and 1.3 g/kg DM vitamin-mineral premix. All cows were housed in tie stalls with free access to drinking water.

2.3. Chemical analysis

Freeze-dried seaweed samples were dried at 103 °C in a forced air drying oven for 17 h and then placed in a desiccator to cool for determination of DM content. The CP was estimated as N x 6.25 after nitrogen (N) analysis by the Dumas method (Hansen, 1989) using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany). Feed samples were analyzed for AA (alanine, arginine, asparagine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, and valine; thereafter Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, and Val, respectively) according to the method described by Mason et al. (1980) via exchange chromatography. Briefly, feed samples were mixed with an oxidation solution containing performic acid in a flask, and sealed with an airtight film in a refrigerator at 0 °C. After 16 h, a hydrolysis mixture was added to the flask, and boiled for 23 h at 110 °C in order for the hydrolysis to take place. The hydrolysis mixture was then filtered through a 0.22 µm membrane filter, and transferred to a Biochrom 30 A A analyzer (Laborservice Onken, Gründau, Germany) for analysis via ion exchange chromatography. Serine, Val, and Ile are prone to oxidation with the addition of acid during the hydrolysis step; therefore, they were corrected with a factor of 1.06 (Rudemo et al., 1980).

2.4. AA degradation

In situ AA studies. For both rumen and mobile bag studies, incubated sample sizes were fit to get a planned N residue of approx. 10 mg N, to allow for quantitative AA analyses on the residue. Weigh out amounts were estimated based on Tayyab et al. (2016).

Estimation of the rumen AA degradability. To estimate the rumen AA degradation, the seaweed samples were incubated in the rumen of two cows for 16 h (resembling a rumen passage rate of 6.25 h^{-1}) using the Dacron bags method according to the standard NorFor procedure (Åkerlind et al., 2011). The samples were placed in Dacron bags (11 x 8 cm) with a pore size of 38 µm (Saatfil, PES 38/31, 22080 Veniano, Como, Italy), and mounted on rubber stoppers. The bags were placed in the rumen at 16:00, and removed the following day at 08:00. After rumen incubation, the bags were washed, and the residues transferred to stomaching bags containing 60 mL distilled water. The bags were placed in a lab stomacher (Stomacher® 400 Circulator, Seward UK), and treated for 5 min to minimize microbial contamination from the residues as described by Hvelplund and Weisbjerg (2000). Thereafter, the residues returned to the Dacron bag, and were washed before being transferred to N free filter paper (retention value 2, Whatman AGF 607-90 mm) for DM and AA analysis.

Estimation of the intestinal degradability. Intestinal AA degradability was measured according to Hvelplund and Weisbjerg (2000) with slight modifications as indicated thereafter. The seaweed samples were placed in pre-weighed Dacron mobile bags (6 x 6 cm) with a pore size of 12 µm, labeled, and heat-sealed. For rumen pre-incubation, mobile bags were placed inside larger nylon bags with a pore size of 200 µm (6 mobile bags per larger nylon bag). The bags were mounted on rubber stoppers, and incubated in the rumen of one of the cows for 16 h. Following the pre-incubation, the samples were treated with pepsin-HCl (200 mg pepsine in 2 L of 0.004 M HCl at 39 °C for 2 h). Thereafter, bags were incubated by insertion into the small intestine of the cows through duodenal cannula. After passing through the intestinal tract, the bags were recovered from the feces, washed, and frozen (−18 °C) until all mobile bags were recovered, before transferring to N free filter and AA analysis (Hvelplund and Weisbjerg, 2000).

2.5. Statistical analysis

Calculations. Small intestinal AA degradability was calculated as total tract degradability minus rumen degradability. The intestinal degradability of the AA that escaped the rumen (IDER) was calculated as follows:

> IDER in g/kg = 1000 × (Total tract AA degradability in g/kg − Ruminal AA degradability in g/kg) / (1000 − Ruminal AA degradability in g/kg)

A conversion factor of 6.25 is usually used to estimate the protein content (N * 6.25) of a variety of feedstuffs based on a N analysis (Dintzis et al. 1988). As there is an actual debate on the value of this factor for seaweeds (Angell et al. 2016), the N-factor was also calculated as the sum of the weights of anhydrous AA residues to total N content (Sosulski and Holt, 1980) for the seaweed species in this study and for other feedstuffs for comparison. The AA molar mass was described by Misciattelli et al. (2002), and for seaweeds, the amount of the missing AA, tyrosine (Tyr), and tryptophan (Try), were estimated from Makkar et al. (2016) and Dawczynski et al. (2007) and included in the sums of AA.

Sums of AA were calculated by adding all the AA to obtain the total AA (TAA), all the essential AA (EAA) to obtain the sum of EAA, and all the non EAA (NEAA) to obtain the sum of NEAA. The sum of Met and Cys is reported as the sum of sulphur AA (SAA), and the sum of Leu, Ile, Val as the sum of branched chained AA (BCAA).

Statistical analysis. Statistical analyses were done using the PROC GLM and MIXED in SAS (SAS vr 9.4, Institute Inc) with species, season, year as independent factors, and the interaction between species × season. For total tract degradability the model included cows as random effect. Results were presented as least square means with standard error of mean. Differences among means with $P < 0.05$ were accepted as statistically significant and differences with $P > 0.05$-0.10 as representing tendencies to significance.

3. Results

3.1. Seaweeds chemical composition

The CP content of the seaweeds is presented in Table 1 as well as the effect of the different factors (species, season, interaction, year) on CP. Seaweeds harvested in Spring compared to Autumn were richer in CP (on average 220 vs. 147 g/kg DM respectively, $P < 0.01$), and *Porphyra* had the highest CP content compared with the other species.

3.2. AA composition of seaweeds

The AA composition and sums of AA are shown in Tables 1 and 2. Glutamine was the most abundant AA with an average of

Table 1
Crude protein and amino acid composition of different seaweeds species collected in Spring and Autumn.

Species	Season	CP	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Val
		(g/kgDM)									(g/16 g N)							
Cladophora	Spring	341	4.15	4.62	11.6	3.30	11.0	5.18	1.11	2.84	5.11	4.54	1.38	3.29	4.53	3.37	3.73	4.43
	Autumn	272	4.25	4.21	12.1	3.53	10.6	5.38	1.13	2.96	5.33	4.74	1.45	3.54	4.13	3.46	3.83	4.55
Alaria	Spring	164	11.0	4.18	9.83	1.43	13.1	4.73	1.36	3.70	6.21	4.57	1.92	4.02	3.51	4.31	4.11	5.03
	Autumn	115	14.2	1.91	6.40	1.93	31.6	2.79	0.97	1.67	2.65	3.23	0.87	2.03	1.68	3.05	2.89	2.98
Laminaria	Spring	157	9.96	3.11	8.10	2.01	9.84	3.81	1.02	2.81	4.70	3.49	1.21	3.14	3.13	3.40	3.50	3.95
	Autumn	80	7.57	3.79	11.5	3.01	12.1	4.97	1.49	3.61	6.12	4.63	1.66	4.07	3.97	4.56	4.65	5.15
Mastocarpus	Spring	180	4.35	5.93	8.60	2.60	8.02	6.60	1.60	2.89	4.51	5.77	1.27	3.82	3.68	4.25	3.62	4.15
	Autumn	164	4.27	5.67	8.22	2.53	7.87	6.16	1.56	2.87	4.46	5.61	1.27	4.01	3.62	4.29	3.52	4.10
Palmaria	Spring	265	6.57	4.96	9.92	3.08	11.0	5.59	1.21	3.41	5.41	5.26	1.55	3.71	6.76	4.60	4.03	5.26
	Autumn	149	6.11	4.83	10.7	3.44	12.4	5.38	1.27	3.41	5.42	4.75	1.54	3.76	4.45	4.65	3.83	5.34
Pelvetia	Spring	110	5.15	3.33	7.94	1.18	22.8	3.83	1.12	3.01	5.10	3.63	1.75	3.37	2.82	3.58	3.43	4.14
	Autumn	68	5.34	3.67	8.49	1.43	13.0	4.33	1.34	3.50	5.88	4.14	2.04	3.90	3.34	4.10	3.97	4.79
Porphyra	Spring	397	12.3	5.54	8.75	1.59	10.7	5.59	1.13	3.50	6.33	4.59	1.83	3.49	3.71	4.46	4.91	5.71
	Autumn	271	8.90	5.40	8.46	1.71	9.92	5.73	1.16	3.51	6.32	4.55	1.84	3.64	3.90	4.48	5.01	5.84
Saccharina	Spring[a]	145	8.63	3.70	8.86	1.93	10.6	4.51	0.99	3.34	5.66	4.02	1.45	3.61	3.88	3.91	4.21	4.64
	Autumn	82	8.83	3.11	10.1	2.28	21.4	4.33	1.20	2.97	5.18	3.85	1.58	3.47	3.14	3.98	4.28	4.74
Ulva	Autumn	122	8.25	5.10	12.2	1.84	11.5	5.86	1.36	4.07	6.97	4.37	2.13	5.02	4.78	4.92	5.54	6.30
SEM		21	0.63	0.26	0.53	0.18[a]	0.93	0.20	0.06	0.17	0.28	0.19	0.09	0.19	0.33	0.16	0.22	0.22
P value	Specie	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Season	< 0.01	0.30	0.02	0.37	< 0.01	< 0.01	0.38	0.04	0.21	0.19	0.67	0.69	1.00	0.02	0.29	0.63	0.87
	S x S[b]	0.22	0.01	< 0.01	< 0.01	0.24	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	< 0.01
	Year	0.04	0.12	0.79	0.71	< 0.01	0.66	0.25	0.01	0.65	0.47	0.68	0.04	0.28	0.12	0.13	0.30	0.72

[a] SEM for *Saccharina* Spring is 1.44 due to missing 2014 Spring sample.

[b] S x S: interaction between specie and season, AA: Amino acids.

Table 2

Total amino acids, sum of amino acids nitrogen by nitrogen, sum of essential amino acids, sum of non-essential amino acids, sum of sulphur amino acids, sum of branched chain amino acids for different seaweeds species collected in Spring and Autumn.

Species	Season	TAA[a] (g/kg DM)	AAN/N[b] (g/kg)	TAA	EAA[c]	NEAA[d] (g /16 g N)	SAA[e]	BCAA[f]
Cladophora	Spring	253	0.64	74.3	29.7	44.6	4.69	12.4
	Autumn	205	0.64	75.2	31.1	44.1	4.98	12.8
Alaria	Spring	136	0.71	83.0	32.4	50.7	3.34	14.9
	Autumn	93.2	0.64	80.8	19.2	61.6	2.80	7.31
Laminaria	Spring	105	0.57	67.2	25.8	41.3	3.22	11.5
	Autumn	66.5	0.70	82.8	34.4	48.4	4.66	14.9
Mastocarpus	Spring	129	0.66	71.7	30.2	41.4	3.86	11.6
	Autumn	115	0.64	70.0	29.9	40.1	3.80	11.4
Palmaria	Spring	218	0.71	82.4	32.9	49.4	4.64	14.1
	Autumn	122	0.70	81.4	32.7	48.6	4.98	14.2
Pelvetia	Spring	83.7	0.61	76.2	26.7	49.5	2.93	12.3
	Autumn	49.5	0.61	73.3	30.9	42.3	3.48	14.2
Porphyra	Spring	334	0.74	84.1	33.1	51.0	3.42	15.5
	Autumn	219	0.71	80.4	33.6	46.8	3.55	15.7
Saccharina	Spring	108	0.63	73.9	29.8	44.0	3.38	13.6
	Autumn	69.5	0.68	84.4	29.5	54.8	3.86	12.9
Ulva	Autumn	113	0.77	90.2	37.6	52.6	3.98	17.3
SEM		20.4[g]	0.02	2.67	1.15	1.82	0.17	0.66
P value	Species	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Season	< 0.01	0.80	0.27	0.86	0.14	< 0.01	0.40
	S × S[h]	0.29	0.02	0.03	< 0.01	< 0.01	< 0.01	< 0.01
	Year	0.06	0.29	0.29	0.89	0.15	0.02	0.76

[a] TAA: Total AA.

[b] AAN/N: AA nitrogen divided by total nitrogen.

[c] EAA: Essential AA.

[d] NEAA: Non-essential AA.

[e] SAA: Sulphur AA (Met and Cys).

[f] BCAA: Branched chained AA (Leu, Ile, Val).

[g] SEM for *Saccharina* Spring is 1.44 larger due to missing 2014 Spring sample.

[h] S × S: interaction between specie and season, AA: Amino acids.

13.4 g/16 g N, followed by Asp with 9.52 g/16 g N, and Ala with 7.64 g/16 g N. In general, the seaweeds were rich in Thr, Ser, Gly, Val, Leu, Lys, and Arg (on average 4.58 g/16 g N, range from 4.06 to 4.99 g/16 g N), and low in Cys, His, and Met (on average 1.70 g/16 g N, range from 1.24 to 2.28 g/16 g N). The AA concentrations and the sums of EAA, NEAA, SAA, BCAA, and TAA in CP varied with the seasons and species. In short, during Spring, *Laminaria* had the lowest TAA and EAA, while in Autumn this seaweed had one of the highest values of TAA and EAA. On the other side of the ranking, *Porphyra* had the highest values of TAA and EAA during Spring, while having average values during Autumn. Finally, during Autumn, *Ulva* had the highest values of TAA and EAA.

Table 3 presents the calculated N-factor for each seaweed and season, and for different other feedstuffs (fresh ryegrass, wheat silages, soypass, rapeseed meal and sunflower cake). On average, the calculated N-factor for the seaweeds was equal to 4.36 ± 0.41, and for the other feedstuffs it is in the same range, varying between 3.25 and 5.12.

3.3. AA degradability

The ruminal degradability of Pro was different between species and season ($P = 0.01$ for the interaction) while for the other AA this interaction was not significant (Table 4). For these other AA, ruminal degradability was affected by species ($P < 0.01$), and only Ala was also affected by season ($P = 0.01$). For all the seaweeds, Glu was highly degraded in the rumen while His and Met had the lowest ruminal degradability compared with the other AA. The sums of AA were not affected by year or season but varied between species: *Palmaria* followed by *Porphyra* had the highest ruminal degradability values, while *Mastocarpus* had the lowest values (Table 5).

Total tract AA degradability did not differ between years or seasons, only between species for Cys, Asp and Thr, and with a tendency for Met, Ser, Glu and Gly (Table 6). *Porphyra* had the highest total tract AA degradability for those seven AA while *Laminaria* and *Mastocorpus* had the lowest values. The total tract degradability of the sums of EAA and BCAA were similar between species, seasons, and years, while those of NEAA and SAA differed between species, *Porphyra* having the highest values and *Laminaria* and *Mastocorpus* the lowest values (Table 7).

Species collected in different seasons and years did not differ in their small intestinal AA degradability (Table 8). Only the intestinal degradability of Cys and of the sum of SAA were lower in Autumn compared with Spring. Most of the AA intestinal degradability varied with species, *Mastocarpus* and *Porphyra* having the highest values and *Palmaria* the lowest values. The same results were observed for the intestinal degradability of the sums of AA (Table 9). Concerning the intestinal degradability of the sums

Table 3
Crude protein, calculated total amino acids and N-factor, and corrected crude protein for each seaweed species collected in Spring and Autumn, and for other feedstuffs (fresh ryegrass, wheat silages, soypass, rapeseed meal, sunflower cake).

Species	Season or reference	CP[a] (g/kg DM)	Calculated TAA[c] (g/kg DM)	Calculated N-factor[b]	Corrected CP[d] (g/kg DM)
Seaweeds					
Cladophora	Spring	341	226	4.15	238
	Autumn	272	183	4.20	190
Alaria	Spring	164	121	4.60	114
	Autumn	115	83	4.50	80.2
Laminaria	Spring	157	94	3.75	109
	Autumn	80	59	4.60	55.8
Mastocarpus	Spring	180	115	4.00	125
	Autumn	164	103	3.92	114
Palmaria	Spring	265	194	4.57	185
	Autumn	149	108	4.52	104
Pelvetia	Spring	110	75	4.27	76.7
	Autumn	68	45	4.10	47.4
Porphyra	Spring	397	295	4.65	277
	Autumn	271	193	4.46	189
Saccharina	Spring	145	95	4.12	101
	Autumn	82	61	4.69	57.2
Ulva	Autumn	122	97	4.99	85.1
Other feedstuffs					
Fresh ryegrass	Skiba et al., 1996	223	161	4.50	
Wheat silages	Skiba et al., 1996	89	46.3	3.25	
Soypass[e]	Weisbjerg et al., 1996	486	398	5.12	
Rapeseed meal[f]	Dakowski et al., 1996	373	293	4.92	
Sunflower cake	Mupeta et al., 1997	281	197	4.38	

[a] CP = N x 6.25.
[b] Calculated N-factor using AA protein (anhydrous TAA) to all seaweeds and feedstuffs, and adding estimates for not analyzed AA (Tyr and Try) in the TAA sum for seaweeds.
[c] Calculated TAA = sum of anhydrous AA × CP / 100, AA: Amino acids.
[d] corrected CP = average corrected N-factor for seaweeds x CP / 6.25.
[e] Soypass: equivalent to soybean meal.
[f] Untreated Danish rapeseed meal (UD in the paper).

of AA escaping the rumen, *Porphyra* had the highest value in Spring and *Laminaria* the lowest values in Autumn (Table 10).

The amounts of AA degraded in the rumen and in the small intestine, or passed out in feces (indigestible), across both season and year, are summarized in Fig. 1.

4. Discussion

4.1. Seaweeds chemical composition

The CP contents of different seaweed species ranged from 68 to 397 g/kg DM which is in the range of CP reported for the green and red seaweeds in the study of Fleurence (1999) (100 to 470 g/kg DM). In the present study, *Porphyra* had an average CP content of 334 g/kg DM (ranging from 271 to 397 g/kg DM), which is comparable to high protein seeds such as soybeans (CP being 350 g/kg DM in Randoin et al., 1987) indicating that *Porphyra* is a relevant substitute for soybeans in terms of CP content.

The CP content of seaweeds varied between species and season, which is in accordance with previous studies (Hori et al., 1990; Mabeau and Fleurence, 1993; Kaehler and Kennish, 1996; Galland-Irmouli et al., 1999; Wong and Cheung, 2000). The difference in protein content within species can be explained by the differing habitats where the seaweeds grow and the concentration of nitrogenous nutrients in seawater (Rødde et al., 2004) but it can also be due to an ash dilution depending on if and how the samples were washed. Taboada et al. (2013) found a CP content of 332 g/kg DM for *Porphyra*, similar to our result (334 g/kg DM on average), while Fleurence (1999) reported a maximum CP content for *Porphyra* species of 470 g/kg DM which is even higher than the CP concentration of sunflower meal and rapeseed meal (Woods et al., 2003a), and whole soybeans (Norziah and Ching, 2000). Apart from *Ulva*, for which we only had Autumn sampling (2 CP values), the green and red seaweeds showed higher CP content (above 170 g/kg DM) than the brown seaweeds (below 140 g/kg DM) as found in Mabeau and Fleurence (1993). The CP content of brown and red seaweeds has been found to be 50–150 g/kg DM (Burtin, 2003) and 350–470 g/kg DM (Rohani-Ghadikolaei et al., 2012), respectively, in line with our findings (range from 68 to 164 g/kg DM for the brown seaweeds and from 149 to 397 g/kg DM for the red seaweeds). The higher CP in spring samples compared to autumn samples is also reported by Rødde et al. (2004), Hori et al. (1990), and Galland-Irmouli et al. (1999). This result might be due to more sunlight, which favors photosynthesis and nutrient assimilation in plants, and to higher concentrations of nitrogenous nutrients in seawater found in spring than in autumn (Rødde et al., 2004).

Table 4
Ruminal degradability of each amino acid of different seaweeds collected in Spring and Autumn.

Species		Ala	Arg	Asp	Cys	Glu	Gly	His	Ile (g/kg)	Leu	Lys	Met	Phe	Pro	Ser	Thr	Val
Cladophora		328	473	455	179	400	282	190	279	257	268	228	263	422	304	292	311
Laminaria		493	93.0	217	194	338	171	111	60.0	67.0	98.0	[c]	80.5	149	139	138	118
Mastocarpus		105	61.0	80	42.0	214	81.0	26.0	54.0	37.0	9.00	[c]	196	139	169	67.0	73.5
Palmaria		522	439	571	627	639	466	443	449	465	373	384	424	655	467	482	453
Porphyra		521	289	344	464	477	373	237	241	230	285	191	230	289	287	387	271
Ulva		266	283	362	329	321	266	227	203	208	261	146	195	356	251	236	245
SEM		53	58	34	30	29	52	50	50	46	57	52	49	38	48	48	47
P value	Species	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Season	0.01	0.30	0.76	0.22	0.20	0.54	0.75	0.65	0.68	0.39	0.79	0.94	0.03	0.70	0.41	0.71
	S × S[a]	0.06	0.38	0.24	0.24	0.07	0.35	0.17	0.17	0.12	0.13	0.13	0.21	0.01[b]	0.17	0.23	0.19
	Year	0.29	0.15	0.02	0.01	0.02	0.14	0.19	0.23	0.21	0.22	0.06	0.09	0.94	0.13	0.13	0.19

[a] S × S: interaction between specie and season.

[b] Interaction between specie and season on Pro ruminal degradability (g/kg): in Spring, 144, 229, 159, 755, 229, respectively for *Cladophora, Laminaria, Mastocarpus, Palmaria, Prophyra,* and in Autumn, 403, 70, 119, 555, 350, 356, for the same species including *Ulva,* AA: Amino acids.

[c] Negative values.

Table 5
Ruminal degradability of the total amino acids, of the sum of essential amino acids, of the sum of non-essential amino acids, of the sum of sulphur amino acids, and of the sum of branched chain amino acids of different seaweeds collected in Spring and Autumn.

Species		TAA[a]	EAA[b]	NEAA[c]	SAA[d]	BCAA[e]
				(g/kg)		
Cladophora		342	261	397	193	282
Laminaria		206	91.5	281	66.5	83.0
Mastocarpus		95.0	60.0	121	17.5	54.5
Palmaria		518	458	559	551	456
Porphyra		351	279	400	321	248
Ulva		274	225	309	230	220
SEM		40	46	37	37	47
P value	Species	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Season	0.21	0.76	0.07	0.34	0.69
	S × S[f]	0.12	0.17	0.08	0.27	0.15
	Year	0.09	0.14	0.07	0.05	0.21

[a] TAA: Total AA.
[b] EAA: Essential AA.
[c] NEAA: Non-essential AA.
[d] SAA: Sulphur AA (Met and Cys).
[e] BCAA: Branched chained AA (Leu, Ile, Val).
[f] S × S: interaction between specie and season, AA: Amino acids.

Table 6
Total tract degradability of each amino acid of different seaweeds collected in Spring and Autumn.

Species		Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Val
								(g/kg)								
Cladophora		841	765	465	783	708	621	752	758	632	786	763	810	779	792	809
Laminaria		668	626	357	678	596	492	567	602	532	578	603	446	546	559	606
Mastocarpus		603	661	318	778	487	494	769	772	547	806	727	660	739	672	715
Palmaria		830	878	756	893	770	776	832	865	742	868	810	888	846	850	822
Porphyra		931	929	785	942	870	888	874	901	874	927	860	888	892	898	891
Ulva		859	822	710	817	758	795	736	780	832	769	738	856	745	742	772
SEM		124	59	54	73	107	158	95	92	137	93	83	159	100	89	91
P value	Species	0.15	< 0.01	< 0.01	0.05	0.05	0.13	0.10	0.09	0.13	0.05	0.13	0.11	0.07	0.03	0.11
	Season	0.17	0.06	0.07	0.13	0.15	0.17	0.11	0.13	0.13	0.13	0.14	0.15	0.13	0.11	0.12
	S × S[a]	0.41	0.12	0.12	0.21	0.36	0.35	0.33	0.37	0.29	0.37	0.39	0.36	0.27	0.32	0.31
	Year	0.62	0.94	0.82	0.69	0.67	0.6	0.63	0.54	0.68	0.59	0.70	0.44	0.66	0.66	0.64

[a] S × S: interaction between specie and season, AA: Amino acids.

Table 7
Total tract degradability of the total amino acids, of the sum of essential amino acids, of the sum of non-essential amino acids, of the sum of sulphur amino acids, and of the sum of branched chain amino acids of different seaweeds collected in Spring and Autumn.

Species		TAA[a]	EAA[b]	NEAA[c]	SAA[d]	BCAA[e]
				(g/kg)		
Cladophora		752	746	756	560	775
Laminaria		609	576	629	434	595
Mastocarpus		661	683	646	480	751
Palmaria		843	820	856	792	840
Porphyra		906	888	917	860	891
Ulva		786	768	798	742	767
SEM		89	100	83	65	92
P value	Species	0.06	0.11	0.03	< 0.01	0.11
	Season	0.12	0.13	0.09	0.08	0.12
	S × S[f]	0.26	0.33	0.22	0.17	0.34
	Year	0.69	0.65	0.72	0.97	0.63

[a] TAA: Total AA.
[b] EAA: Essential AA.
[c] NEAA: Non-essential AA.
[d] SAA: Sulphur AA (Met and Cys).
[e] BCAA: Branched chained AA (Leu, Ile, Val).
[f] S × S: interaction between specie and season, AA: Amino acids.

Table 8
Calculated small intestinal degradability[a] of each amino acid of different seaweeds collected in Spring and Autumn.

Species		Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Val
								(g/kg)									
Cladophora		464	368	309	286	382	426	431	473	500	364	558	500	387	499	475	498
Laminaria		264	575	409	162	340	425	380	507	535	433	731	522	297	421	406	488
Mastocarpus		654	542	581	276	563	406	468	715	735	538	838	531	521	605	570	642
Palmaria		348	392	307	129	253	304	333	382	400	368	484	386	233	368	378	369
Porphyra		424	641	584	320	464	497	652	632	671	589	736	630	599	510	605	619
Ulva		475	576	460	382	496	492	568	533	572	571	622	543	500	506	493	527
SEM		51	88	62	48	72	80	141	76	77	99	90	66	133	68	77	74
P value	Species	< 0.01	0.06	< 0.01	< 0.01	0.02	0.25	0.28	0.02	0.01	0.14	0.03	0.07	0.13	0.07	0.08	0.04
	Season	0.65	0.20	0.11	0.01	0.22	0.13	0.16	0.09	0.11	0.12	0.09	0.07	0.30	0.12	0.09	0.10
	S × S[b]	0.53	0.31	0.35	0.38	0.70	0.39	0.39	0.27	0.26	0.26	0.29	0.30	0.32	0.24	0.25	0.30
	Year	0.14	0.11	0.13	0.10	0.16	0.13	0.30	0.18	0.15	0.21	0.10	0.09	0.35	0.11	0.13	0.17

[a] Calculated as total tract degradability – rumen degradability.

[b] S × S: interaction between specie and season, AA: Amino acids.

Table 9
Calculated small intestinal degradability of the total amino acids, of the sum of essential amino acids, of the sum of non-essential amino acids, of the sum of sulphur amino acids, and of the sum of branched chain amino acids of different seaweeds collected in Spring and Autumn.

Species		TAA[a]	EAA[b]	NEAA[c]	SAA[d]	BCAA[e]
				(g/kg)		
Cladophora		410	484	359	366	493
Laminaria		403	484	348	367	512
Mastocarpus		566	623	526	462	697
Palmaria		325	362	297	241	384
Porphyra		554	609	517	539	643
Ulva		512	543	488	512	547
SEM		74	80	70	56	76
P value	Species	0.04	0.06	0.03	< 0.01	0.02
	Season	0.18	0.09	0.28	0.01	0.10
	S × S[f]	0.44	0.31	0.54	0.22	0.28
	Year	0.16	0.16	0.16	0.18	0.16

[a] TAA: Total AA.

[b] EAA: Essential AA.

[c] NEAA: Non-essential AA.

[d] SAA: Sulphur AA (Met and Cys).

[e] BCAA: Branched chained AA (Leu, Ile, Val).

[f] S × S: interaction between specie and season, AA: Amino acids.

The method used to measure N affects the CP values (Lourenço et al., 2002; Mišurcová, 2011). It seems that the conversion factor of 6.25 leads to an overestimation of protein content indicating that seaweeds contain higher amounts of these non-AA nitrogen compounds.

The calculated N-factor is in accordance with the 4.4 N-factor of Mariotti et al. (2008) for vegetables, mushrooms, and leaf proteins, and close to the seaweeds N-factor of 5 proposed by Angell et al. (2016). Nevertheless, when comparing this factor to other calculated N-factors of different common animal feedstuffs (Table 3), it shows clearly that seaweeds are not special, and do not have more requirements for special N-factor than forages, for which the 6.25 N-factor is traditionally used. It also has to be noticed that the way to calculate the N-factor should be taken into consideration when comparing studies. The N-factor in this study was calculated as anhydrous AA over N, which will estimate AA protein, however it does not take into account other N containing compounds, and thereby will underestimate total N containing compounds (Angell et al., 2016).

4.2. AA composition

Species-specific and seasonal variations in seaweeds AA concentrations have been reported (Galland-Irmouli et al., 1999; Dawczynski et al., 2007), and correlated to seasonal variations in solar irradiance and N concentration in seawater (Galland-Irmouli et al., 1999). Seaweeds are reportedly rich in Asp and Glu (Fleurence, 1999; Dawczynski et al., 2007; Biancarosa et al., 2017), and low in Met and His (Galland-Irmouli et al., 1999; Biancarosa et al., 2017) which is consistent with our findings. The abundance of Glu and Asp is responsible for the characteristic flavor and taste of seaweeds (Yaich et al., 2011). The AA concentrations reported in Table 1 are within the ranges reported in Makkar et al. (2016) for 5 different species of seaweeds (including *Ulva sp.* and *Saccharina japonica*). Compared with soybean, most of the seaweeds species were deficient in essential AA except for the SAA (Makkar et al., 2016). Proline concentrations were low, ranging from 1.68 to 4.78 g/16 g N, compared to those of Khairy and El-Shafay (2013) where

Table 10
Calculated small intestinal degradability of the total amino acids that escaped the rumen, of the sum of essential amino acids that escaped the rumen, of the sum of non-essential amino acids that escaped the rumen, of the sum of sulphur amino acids that escaped the rumen, and of the sum of branched chain amino acids that escaped the rumen of different seaweeds collected in Spring and Autumn.

Species	Season	TAA[a]	EAA[b]	NEAA[c]	SAA[d]	BCAA[e]
			(g/kg rumen escape)			
Cladophora	Spring	659	693	629	486	726
	Autumn	587	616	561	420	643
Laminaria	Spring	721	736	706	551	742
	Autumn	334	352	314	244	393
Mastocarpus	Spring	645	686	617	500	758
	Autumn	606	641	580	443	716
Palmaria	Spring	669	669	663	537	701
	Autumn	678	667	681	537	711
Porphyra	Spring	877	867	885	819	876
	Autumn	832	821	839	764	832
Ulva	Autumn	705	701	708	665	701
SEM		10.2	4.54	5.69	0.46	2.03
P value	Species	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Season	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	S × S[f]	0.03	0.03	0.03	0.06	0.01
	Year	0.02	0.02	0.01	0.01	0.02

[a] TAA: Total AA.
[b] EAA: Essential AA.
[c] NEAA: Non-essential AA.
[d] SAA: Sulphur AA (Met and Cys).
[e] BCAA: Branched chained AA (Leu, Ile, Val).
[f] S × S: interaction between specie and season, AA: Amino acids.

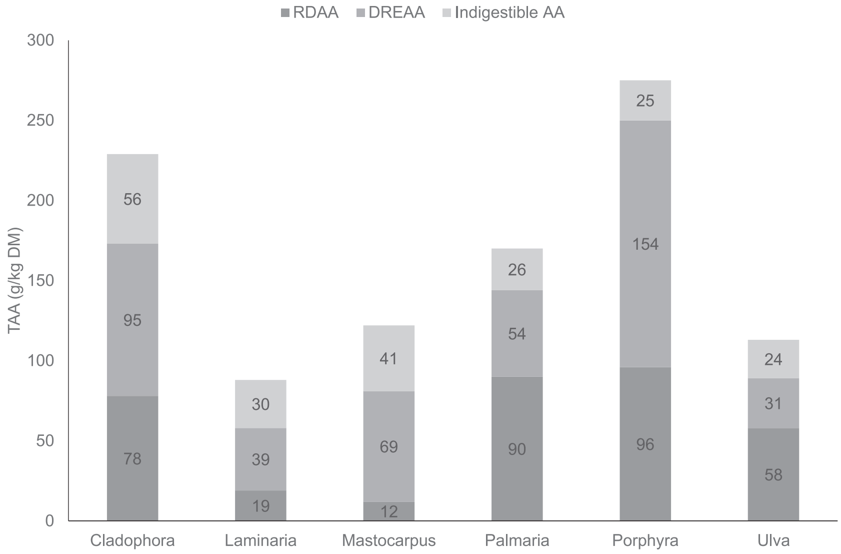

Fig. 1. Total amino acid fractions (TAA, in g/kg DM) degraded in the rumen (RDAA) and the small intestine (DREAA = digestible rumen escape AA), and indigestible amino acids fraction of different seaweed species (average across seasons and years).

Pro was the major AA in *Ulva lactuca*. Proline is an interesting AA and it is known to accumulate in large quantities in response to environmental stressors (Ashraf and Foolad, 2007), so many plants accumulate Pro as a nontoxic and protective osmolyte under saline conditions (Bohnert and Jensen, 1996). The BCAA of soymeal was within the range of the seaweeds (16.2 and 7.30 to 17.3 g/16 g N respectively). The TAA range (49–334 g/kg DM) is below the CP range (80–397 g/kg DM) ($P < 0.01$) indicating that the amount of non-protein nitrogenous materials in these seaweeds is significant (average of 33 g/kg). However, it is important to notice that we did not analyze for all the AA. Tryptophan and Tyr were not analyzed so, based on the Makkar et al. (2016) and Dawczynski et al. (2007), we estimated these AA to be in the amount of 0.60 and 2.7 g/16 g N respectively, as rough estimates for all species. This resulted in the 4.36 N-factor.

The seaweeds contained all the EAA in different proportions, contributing for 24–42% of TAA which is in accordance with results reported in different species of seaweeds by Behairy and El-Sayed (1983) and Wong and Cheung (2000). However, as noticed earlier, most seaweeds have lower EAA proportions compared to soybean meal, except for the SAA, but the AA composition among seaweeds of the same species is also highly variable (Mišurcová, 2011). For instance, Galland-Irmouli et al. (1999) reported the AA composition of *Palmaria palmata* and found that some AA such as Lys, Glu, Ser and Ala strongly decreased during summer and early winter months of the year. The ratio of EAA/NEAA for the red and green seaweeds was around 0.7, which was similar to the results of Dawczynski et al. (2007), while brown seaweeds had a lower ratio.

In summary, *Porphyra* is generally a good AA source but should be preferentially harvested during Spring when higher amounts of AA were measured. *Ulva* had the highest values of TAA and EAA so, in terms of AA content, it is valuable seaweed to harvest at least during Autumn.

4.3. AA degradability values

4.3.1. Ruminal AA degradability

Knowing AA degradability is necessary to determine the amount of nutrients digested in various part of the gastro-intestinal tract, which later can be absorbed and utilized by animals (Mertens, 2005). In the present study, the ruminal degradability of individual AA differs between seaweeds species, probably due to seaweeds physical properties and variation in AA composition (Clark et al., 1987). Indeed, thallus morphology and anatomy differ with seaweed species. For example, the external morphology can be thin and sheet like (e.g. *Ulva* and *Porphyra*), coarsely branched (e.g. *Pelvetia* and *Mastocarpus*), and thick blades (e.g. *Laminaria* and *Saccharina*) with texture that are soft, fleshy-wiry, and leathery-rubbery, respectively. The corresponding internal anatomy can be uncorticated, one to several cell layers thick; corticated; and differentiated, heavily corticated and thick-walled, respectively (Littler et al., 1983). These differences most likely contributed to the digestibility of raw seaweeds. Aside from *Mastocarpus,* the rest of the red seaweeds (*Palmaria* and *Porphyra*) had high ruminal TAA and EAA degradability while the brown seaweed *Laminaria* had the lowest values. Although the concentration of phenolic compounds was not measured, seaweeds vary in polyphenol content which influence protein digestibility (Hurrell and Finot, 1985; Molina-Alcaide et al., 2017) and may be one of the causes of the varying rumen AA degradability among species seen in this study. Indeed, phenolic compounds are thought to form insoluble complexes with protein, protecting protein from degradation resulting in a less efficient utilization of dietary proteins (Shahidi and Naczk, 1995). More specifically, the lowest ruminal TAA and EAA degradability in brown seaweeds may be attributed to the presence of phlorotannins, a class of phenolic compounds present only in brown seaweeds and reported to influence ruminal bacterial population that in turn affects fermentation parameters (Wang et al., 2009). Moreover, cell wall polysaccharides differ between seaweed species, which may participate in the differences in ruminal degradability (Belghit et al., 2017).

4.3.2. Total tract and small intestinal AA degradability values

The AA in most feedstuffs are mainly degraded in the rumen, and therefore the *in situ* rumen protein degradation and amount of AA which reach the small intestines after escaping the rumen are important characteristics of a feedstuff (Martillotti et al., 1995; Hvelplund and Weisbjerg, 2000). To our knowledge, no work has been conducted on the degradability of AA content of seaweeds in the small intestine of dairy cows.

In the present experiment, despite the non-significant effect of season it has to be noticed that *Laminaria* had very low AA degradability values during Autumn compared with Spring or with other species. These low autumnal values lower the NEAA or SAA digestibility averages, and position our brown seaweed *Laminaria* at the bottom of the total tract digestibility ranking. Red seaweeds provided a better source of digestible AA than green seaweeds. This classification is in accordance with Mabeau and Fleurence (1993).

The results obtained from the total tract AA digestibility also show that for *Laminaria* and *Mastocarpus*, some of the AA, like Met, followed by Leu, Ile, His and Lys, seemed to be protected against rumen degradation, and became degradable in the small intestine after passing through enzymatic digestion and acidic conditions in the abomasum. A similar result was reported by Mora et al. (2009) and Zitouni et al. (2014) and may be explained by the presence of phenolic compounds in the seaweeds protecting the proteins against degradation in the rumen. Indeed, via hydrogen bonds, tannins-protein complexes are built and kept stable between pH 3.5 and 8 (approximately). These stable complexes at rumen pH, dissociate in the abomasum where pH falls below 3.5 (Frutos et al., 2004). An *in vitro* analysis of protein degradability by bovine trypsin conducted by Gojon-Báez et al. (1998) reported that up to 90% protein of brown seaweed *Macrocystis pyrifera* may be degraded within the abomasum, which indicated that bypass proteins of seaweeds may have a higher nutritional value than terrestrial feeds. In general, feeds with lower ruminal AA degradability values are used to increase the AA supply to the small intestine (Woods et al., 2003a); however in our case, the seaweeds *Laminaria* and *Mastocarpus* with low ruminal AA degradability (21 and 10% TAA respectively) contained high indigestible fractions (34% of TAA for both) and consequently provided low amounts of AA to the intestines.

4.3.3. Seaweed rankings based on CP, AA profile and their degradability

In the present study, we reported the chemical composition and AA degradability values of six seaweed species harvested along the coast in Northern Norway to determine their suitability as potential sustainable and local protein-rich ingredients in ruminant diets. The AA degradability values varied between seaweed species. Based on our results, the red seaweed species *Porphyra* followed by *Palmaria* and the green species *Ulva* and *Cladophora* are potential sources of AA for ruminants based on their TAA concentrations and degradability. Our results are in accordance with Fleurence (1999) reporting that red and green seaweeds have high nutritional values. Compared with the degradability values of soybean meal (677 g/kg CP for ruminal CP degradability, 970 g/kg rumen undegraded protein (RUP) for intestinal RUP degradability) presented in Moujn et al. (2010), the digestibility values of the seaweeds are lower. However, there are high variations among seaweeds, the TAA ruminal digestibility varying between 95 and 518 g/kg of TAA, respectively for *Mastocarpus* and *Palmaria*, and between 334 and 877 g/kg TAA rumen escape for the intestinal degradability of *Laminaria* and *Porphyra* respectively. Based on these results, the authors recommend red and green seaweeds as an additive protein source in dairy cows' ration. From the present results, we do not recommend the use of *Mastocarpus* and *Laminaria* as, compared to other seaweeds, their AA degradability values are low and they contain a high amount of indigestible AA.

4.4. Considerations

Larger use of seaweeds in animal feeding would require massive cultivation or harvest of wild biomass. At present, the production capacity of seaweed in Europe is limited and cultivated seaweeds are mainly brown seaweeds of the genus Laminariales (Stévant et al., 2017) with relatively lower protein concentrations compared to other green and red seaweeds (Fleurence, 1999). Moreover, seaweeds can have high ash concentrations (Tayyab et al., 2016), and the concentration of arsenic, cadmium and iodine (Besada et al., 2009; Nitschke and Stengel, 2015) especially in brown seaweeds could limit their inclusion in animal diets. Furthermore, post harvesting processes, e.g. drying, chemical treatment and freezing to increase shelf life, storage and transport, are challenges that still need to be addressed to economically and sustainable use seaweed as bulk material and/or ingredient for feed. But some of the seaweeds examined in this study seem to have a great potential as protein and mineral supplement when included in an adequately defined amount in the diet.

5. Conclusion

The AA composition of the seaweeds in the present study varied with species and harvesting season (Autumn and Spring). The amino acid nitrogen proportion of total nitrogen was comparable with terrestrial forages. The AA degradability values in rumen, small intestine, and total tract were affected by the species only. For some seaweed species like *Laminaria* and *Mastocarpus*, some AA were protected against rumen degradation making these seaweeds interesting sources for bypass protein supply. To conclude, *Porphyra* had the highest amount of AA and the highest AA degradability values in rumen and small intestine, which therefore make this seaweed a relevant feed ingredient as a protein source. *Palmaria* takes the second place of the ranking with high amount of AA, even though one third lower than *Porphyra*, and high AA degradability values in rumen and small intestine. Finally, the green seaweeds are also relevant as protein feed, with high amount of AA for *Cladophora* and a high intestinal AA degradability for *Ulva*.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

This research was part of a project titled "Legumes and seaweeds as alternative protein for sheep (AltPro)" at Norwegian Institute for Bioeconomy Research (NIBIO), Norway funded by The Research Council of Norway (Project no.: 233682).

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