



## Evaluation of the nutritional value of seaweed products for broiler chickens' nutrition

L. Stokvis<sup>a,b,\*</sup>, M.M. van Krimpen<sup>a,1</sup>, R.P. Kwakkel<sup>b</sup>, P. Bikker<sup>a</sup>

<sup>a</sup> Wageningen University & Research, Wageningen Livestock Research, 6708 WD, Wageningen, the Netherlands

<sup>b</sup> Wageningen University & Research, Animal Nutrition Group, 6708 WD, Wageningen, the Netherlands

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### ABSTRACT

The use of seaweed as feed ingredient is gaining interest but the high ash content, low nutrient digestibility, short shelf life and poor economic feasibility hamper practical application.

This study investigates the effect of washing, ensiling and extraction processes on the nutritional value of seaweed products for broilers, based on nutrient content and *in vitro* and *in vivo* digestibility. The effects of ensiling, washing and extraction processes were evaluated using *Saccharina latissima*, *Laminaria digitata* and *Ulva lactuca*, with 2, 4, and 6 h incubations in an *in vitro* simulated digestibility model, to obtain insight into the kinetics of digestibility. In an *in vivo* study, 160 Ross 308 male broilers were fed (day 14–22) a basal grower diet, or the basal grower diet with 100 g/kg of *S. latissima* silage or silage residue. Performance and ileal and total tract nutrient digestibility were determined. Washing and ensiling reduced the ash content, but also the *in vitro* organic matter digestibility (both  $P < 0.001$ ). Washing also reduced nitrogen digestibility ( $P < 0.001$ ). Extraction of seaweed decreased *in vitro* organic matter and nitrogen digestibility. Feeding seaweed diets to broilers resulted in a higher feed conversion ratio (1.62 versus 1.86 and 1.77 for broilers fed the basal, silage and silage residue diets respectively,  $P < 0.001$ ) without increase in final body weight. Feeding *S. latissima* silage residue compared to silage resulted in a slightly better broiler performance and a higher amino acid digestibility. In conclusion, washing, ensiling and extraction processes reduce the nutritional value of the seaweed products, and do not make seaweed suitable for inclusion in broiler diets. To create suitable seaweed products for inclusion in broiler diets, a further reduction in the ash content and increase in digestibility is needed.

### 1. Introduction

The increasing world population (United Nations, population division, 2017) and increasing demand for animal protein (Boland et al., 2013) have stimulated the exploration of novel feed sources, including seaweed for farm animals (Makkar et al., 2016; Buschmann et al., 2017). Advantages of seaweed production are the use of salt instead of fresh water, sea instead of arable land-based

**Abbreviations:** AA-N, amino acid nitrogen; AAs, amino acids; AME, apparent metabolizable energy; ANOVA, analyses of variance; DM, dry matter; FCR, feed conversion ratio; h, hour; *L. digitata*, *Laminaria digitata*; LSD, least significant difference; N, nitrogen; OM, organic matter; *S. latissima*, *Saccharina latissima*; SED, standard error of differences; *Spp.*, species; TiO<sub>2</sub>, titanium dioxide; *U. lactuca*, *Ulva lactuca*.

\* Corresponding author.

E-mail address: [lotte.stokvis@wur.nl](mailto:lotte.stokvis@wur.nl) (L. Stokvis).

<sup>1</sup> The author Dr. Van Krimpen sadly passed away.

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production, and the high productivity in terms of biomass produced per unit of surface area (Buschmann et al., 2017). Nonetheless, the inclusion of a substantial percentage of seaweed in animal diets is hampered by the high content of ash and poorly digestible carbohydrates (Sharma et al., 2018), the low digestibility (Bikker et al., 2016, 2020), the limited shelf life (Paul and Chen, 2008; Stévant et al., 2017) and the high cost of production (Van den Burg et al., 2013). To address some of these disadvantages, seaweed can be washed to reduce the ash (minerals + sand) content on the outside of the leaves (Neveux et al., 2014), and the product can be ensiled to extend the shelf life of fresh harvested seaweed (Herrmann et al., 2015). A biorefinery approach, using seaweed fractions for different purposes, can increase the economic feasibility of the use of seaweed (Torres et al., 2019). Co-products from the biorefinery of seaweed may be valuable and cost-effective ingredients in diets for farm animals, including broilers (Bikker et al., 2016; Torres et al., 2019). Although studies have been conducted with a low inclusion level of seaweed in broiler diets (Abudabos et al., 2013; Choi et al., 2014), little information is available on the effect of processing on the nutritional value of seaweed and seaweed products.

This study was conducted to investigate the effect of washing, ensiling and extraction processes on the nutritional value and digestibility of seaweed products for broilers. The nutritional value and digestibility were evaluated based on nutrient content, *in vitro* simulated digestibility and an *in vivo* study in broilers.

## 2. Material and methods

Within the framework of this study, *in vitro* and *in vivo* experiments were conducted. In the *in vitro* experiment, the effects of ensiling, washing and extraction processes on simulated nutrient digestibility of different species of seaweed (*Saccharina latissima* (*S. latissima*, sugar kelp), *Laminaria digitata* (*L. digitata*, oarweed) and *Ulva lactuca* (*U. lactuca*, sea lettuce)) were investigated. Subsequently, the nutrient digestibility of *S. latissima* silage and silage residue was determined in broilers. Because of the large variation in chemical composition between and within seaweed species (Biancarosa et al., 2017; Bikker et al., 2020), this study focussed on species common in marine waters in Northwestern Europe.

### 2.1. *In vitro* experiment

The effects of ensiling and washing of seaweed on nutritional value were studied using fresh and ensiled *S. latissima*. The effect of extraction of valuable sugars on nutritional value were studied using *S. latissima*, *L. digitata* and *U. lactuca*.

The unwashed, washed, fresh and ensiled *S. latissima* samples included in the *in vitro* analyses were provided by Hortimare (Heerhugowaard, the Netherlands) from the production location Kverhella, Norway (61.00 °N, 4.70 °E). For the production of silage, cubic meter containers were filled with fresh *S. latissima*, covered with plastic sheets with a bag filled with water on top as an air tight seal. A drain was installed at the bottom of the container to drain remaining seawater. The silage was stored at room temperature for 4 weeks. Washed fresh and ensiled *S. latissima* samples were produced by placing the unwashed material on plastic sheets and flushing it with a substantial amount of tap water without immersing the seaweed material. After removal of superficial water with paper towel, the *S. latissima* was oven dried (Thermo Heraeus OMH750, Thermo Fisher Scientific, Breda, The Netherlands) at 39 °C for three days, until approximately 900 g/kg dry matter (DM) was reached.

A biorefinery approach with extraction of mannitol (*S. latissima* and *L. digitata*) or rhamnose (*U. lactuca*) was adopted as a method to improve the economic feasibility of the use of seaweeds. Unwashed *S. latissima* silage and fresh *L. digitata* (provided by Ocean Harvest, Galway, Ireland) were used to determine the nutritional value of the residue after aqueous extraction of mannitol by ECN (Petten, the

**Table 1**  
Analysed nutrient content of seaweed products as used in the *in vitro* digestibility study.

	Analysed nutrient composition			
	g/kg	g/kg DM		
Seaweed product	DM	Ash	OM <sup>1</sup>	N
<i>Saccharina latissima</i> fresh unwashed	924	465	535	10.8
<i>Saccharina latissima</i> fresh washed	913	425	575	13.6
<i>Saccharina latissima</i> silage unwashed	922	374	626	20.5
<i>Saccharina latissima</i> silage washed	907	290	710	21.6
<i>Saccharina latissima</i> silage residue <sup>2</sup>	925	266	734	18.0
<i>Laminaria digitata</i> unwashed fresh	940	241	759	11.9
<i>Laminaria digitata</i> residue <sup>3</sup>	909	226	774	13.4
<i>Ulva lactuca</i> unwashed fresh	861	363	637	23.3
<i>Ulva lactuca</i> residue <sup>4</sup>	926	83	917	43.4
Reference ingredient				
Soybean meal <sup>5</sup>	900	73	927	85.0

DM, dry matter, OM, organic matter, N, nitrogen.

<sup>1</sup> Calculated as 1000 – Ash.

<sup>2</sup> Residue after extraction of mannitol from *Saccharina latissima* silage unwashed material.

<sup>3</sup> Residue after extraction of mannitol from *Laminaria digitata* fresh unwashed material.

<sup>4</sup> Residue after extraction of rhamnose from *Ulva lactuca* fresh unwashed material.

<sup>5</sup> Values based on analyses conducted by Hulshof et al. (2016).

Netherlands) as described by Van Hal and Huijgen (2014). In short, seaweed was treated with water of a salinity of less than 20 g/kg and a pH between 3.0 and 9.0, which solubilizes the mannitol in the water. The water with mannitol is then drained, leaving the residue product. Fresh *U. lactuca*, cultivated by WUR-IMARES in Yerseke, the Netherlands, was used for rhamnose extraction using acid hydrolysis with HCl, in a 20 L autoclave (Kiloclaaf, Büchi Labortechnik AG, Flawil, Switzerland) at 100 °C for 60 min by ECN (Petten, the Netherlands). The residues of mannitol and rhamnose extraction were used in dried form. All seaweed samples were oven dried at 39 °C until approximately 900 g/kg DM was reached. The analysed nutrient composition of the seaweed products used in the *in vitro* study are given in Table 1.

All samples were ground to pass a 1 mm sieve. The *in vitro* simulated digestibility analyses were performed according to an adjusted Boisen two-step method (Boisen and Fernandez, 1997) as described in the study of Bikker et al. (2016). Briefly, 1 g samples were incubated with 75 mL 0.1 M phosphate buffer solution (pH 6.0) and 0.2 M HCl solution until a pH of 2.0 was reached. One mL pepsin solution (25 g/L, 2000 International Federation of Pharmaceuticals U/g) was added, and samples were incubated at 39 °C for 2 h under constant stirring. Thereafter, 30 mL 0.2 M phosphate buffer (pH 6.8) was added, plus NaOH until a pH of 6.8 was reached. One mL pancreatin (100 g/L) was added and the incubation was continued for 4 h under the same conditions. To obtain insight in the digestion kinetics of seaweed, the *in vitro* incubations were terminated after two, four or six hours, representing the gastric and small intestinal digestion, respectively. The *in vitro* incubations were conducted in four-fold, of which two replicates were used to determine nitrogen (N) digestibility and two replicates to determine DM and organic matter (OM) digestibility.

## 2.2. In vivo experiment

The *S. latissima* silage and silage residue used in the *in vivo* experiment were produced by Hortimare (Heerhugowaard, the Netherlands) and originated from the production location Kverhella, Norway (61.00 °N, 4.70 °E). This seaweed species was selected because of its high availability in this area, suitability for cultivation, and as part of a project investigating the long term storage as

**Table 2**

Analysed nutrient composition and calculated energy content of *Saccharina latissima* silage and silage residue<sup>1</sup> products as used in an *in vivo* study in broilers.

Nutrient content (g/kg dry matter)	Seaweed products	
	<i>S. latissima</i> silage	<i>S. latissima</i> silage residue
Dry matter (g/kg)	896	897
Ash	242	263
Organic matter <sup>2</sup>	758	737
Nitrogen	23.8	26.2
Fat	20	20
Crude fibre	157	196
Sugar	<1	0
Starch	5	4
Non-starch polysaccharides <sup>3</sup>	583	550
Calcium	35.7	35.4
Phosphorous	1.4	1.7
Sodium	17.8	41.1
Chloride	46.1	26
<i>Amino acids (g AA-N/100 g nitrogen)</i>		
Lysine	4.8	5.9
Methionine	1.4	1.4
Cysteine	1.3	1.1
Threonine	3.3	3.5
Isoleucine	2.8	2.9
Arginine	8.5	9.5
Phenylalanine	2.7	2.6
Histidine	2.8	3.1
Leucine	4.9	5.1
Valine	4	4.2
Alanine	7.4	6.2
Asparagine + Aspartic acid	9.4	9.9
Glutamine + Glutamic acid	9.2	10.5
Glycine	7.1	6.6
Serine	3.6	3.8
Sum of AA-N	73	76.2
Calculated energy content (kJ/g dry matter) <sup>4</sup>	11.88	11.23

AA-N, nitrogen from amino acids.

<sup>1</sup> Residue after the aqueous extraction of *Saccharina latissima* silage.

<sup>2</sup> Calculated as 1000 – ash.

<sup>3</sup> Calculated as organic matter – crude protein (as N × 6.25) – fat – starch – sugar.

<sup>4</sup> Calculated as gross energy = 22.6 × crude protein (as N × 6.25) + 38.8 × fat + 17.5 × starch + 16.7 × sugar + 18.6 × residue (as organic matter – crude protein (as N × 6.25) – fat – starch – sugar) (Milgen et al., 2018).

*S. latissima* silage and subsequent biorefinery. The silage residue was produced by first crushing the silage, to improve the efficiency of the extraction process. Thereafter the silage was soaked in fresh water under alkaline conditions (pH > 9, by adding Na<sub>2</sub>CO<sub>3</sub>) for 24 h without stirring. The silage residue was the remaining product after draining the water for subsequent extraction of the soluble components from the drained water. The seaweed products were oven dried at 39 °C for 3 days until a DM content of approximately

**Table 3**

Ingredients and calculated and analysed nutrient composition of the basal diet and diets containing 100 g/kg *Saccharina latissima* silage and silage residue, as fed to broilers to study the digestibility of the processed seaweed products.

Ingredient (g/kg)	Diet		
	Basal diet	Basal with <i>S. latissima</i> silage	Basal with <i>S. latissima</i> residue <sup>1</sup>
Maize starch	600.00	538.65	538.65
Soybean meal	120.00	107.73	107.73
Oat hulls	80.00	71.82	71.82
Casein	66.10	59.33	59.33
Dextrose	51.89	46.58	46.58
Soybean oil	23.33	20.94	20.94
Limestone	11.38	10.22	10.22
Mono-calcium phosphate	13.38	12.01	12.01
Magnesium oxide	1.00	0.89	0.89
L-Lysine HCl	1.38	1.24	1.24
DL-Methionine	3.13	2.81	2.81
L-Threonine	1.52	1.36	1.36
L-Arginine	3.36	3.02	3.02
L-Isoleucine	0.60	0.54	0.54
L-Valine	0.95	1.00	1.00
Salt	3.33	0.00	0.00
Sodium bicarbonate	1.74	0.37	0.00
Potassium carbonate	6.91	1.03	0.00
Diamol	0.00	10.58	11.98
Vitamin and mineral premix	5.00	5.00	5.00
Titanium dioxide	5.00	5.00	5.00
Saccharina silage	0.00	100.00	0.00
Saccharina silage residue	0.00	0.00	100.00
Apparent metabolizable energy (MJ)	12.80		
Retainable phosphorous	3.20		
Dry matter (g/kg)	878	880	868
Ash	56	75	73
Organic matter	944	925	927
Nitrogen	25	24	24
Fat	32	30	25
Crude fibre	23	38	37
Sugar	66	57	59
Starch	620	555	283
Non-starch polysaccharides	70	133	410
Calcium	8.4	11.0	11.2
Phosphorous	5.4	5.0	4.9
Sodium	2.2	2.2	4.1
Chloride	2.0	4.6	2.7
Amino acids (g AA-N/100 g nitrogen)			
Lysine	10.9	10.6	11.5
Methionine	6.2	6.0	6.0
Cysteine	1.1	1.3	1.4
Threonine	7.3	7.3	7.7
Isoleucine	7.4	7.3	7.8
Arginine	9.9	9.9	10.5
Phenylalanine	7.4	7.5	7.9
Histidine	3.8	3.6	3.9
Leucine	12.3	12.2	13.0
Valine	9.6	9.7	10.1
Alanine	5.4	5.9	6.2
Asparagine + Aspartic acid	13.0	13.1	14.1
Glutamine + Glutamic acid	28.0	26.5	28.5
Glycine	4.4	4.8	5.1
Serine	7.6	7.5	8.0
Sum of AA-N	134.3	133.2	141.7

AA-N, nitrogen from amino acids.

<sup>1</sup> Residue after the aqueous extraction of *Saccharina latissima* silage.

900 g/kg was reached. The chemical composition of the *S. latissima* products is included in Table 2, showing a somewhat higher ash, N and crude fibre content and lower non-starch polysaccharide content in *S. latissima* silage residue compared to the silage. In the silage residue product, the observed sodium content was twice as high compared to the silage product, whereas the chloride content was twice as low. In addition, small differences were observed in amino acid content expressed per 100 g N and a higher sum of amino acid nitrogen (AA-N) in the silage residue.

### 2.2.1. Animal housing and experimental design

The experiment was conducted at the experimental animal facility Carus of Wageningen University and Research (Wageningen, the Netherlands). All experimental procedures were approved by the Animal Care and Use Committee of Wageningen University and Research, the Netherlands (AVD401002015196). A total of 160 one-day-old male Ross 308 broiler chickens (Hatchery Morren BV, Lunteren, the Netherlands) were housed in 4 pens with concrete floors and wood shavings (2 kg/m<sup>2</sup>) as bedding material during the pre-experimental period from 1 to 14 days of age. All birds received a commercial starter diet. All chickens were vaccinated against infectious bronchitis at arrival at the experimental facility, and against Newcastle disease at day 11. At day 14, the 160 chickens were distributed over 16 pens (0.87m × 1.10m, slatted floors) with 10 birds per pen, while pen weight was kept within a 3 % difference from mean pen weight. Three dietary treatments, a basal diet and a basal diet with either *S. latissima* silage or silage residue, were allocated to pens in a completely randomized design. From day 14–22, the basal diet was fed to 6 replicate pens and each seaweed diet was fed to 5 replicate pens. A standard temperature and lighting schedule was applied. During the entire experimental period, birds had *ad libitum* access to feed and water. At the end of the experiment (day 22) all chickens were euthanized by 0.5 mL T61 injection to the wing vein.

### 2.2.2. Experimental diets

All chickens received a standard broiler starter feed (AME 12.3 MJ/kg, dig. Lys 11.9 g/kg) during the first 13 days and the experimental diets from day 14 to day 22. The experimental diets were formulated to meet the requirements for broilers in the grower phase (CVB, 2016). All experimental grower diets were supplemented with 5 g/kg titanium dioxide (TiO<sub>2</sub>) as indigestible marker. The seaweed diets were supplemented with 100 g/kg dried *S. latissima* silage or *S. latissima* silage residue before mixing and pelleting at a diameter of 3.2 mm dye. The dietary ingredients and calculated nutrient content of the diets are given in Table 3.

### 2.2.3. Measurements

Feed intake was monitored on a weekly basis. All chickens were weighed at arrival at the experimental facility. Body weight was determined per pen after allocation to the treatments at day 14 and determined again at day 22. Excreta were collected from day 20 to day 22, after which all animals were euthanized and ileal contents were collected per pen.

### 2.2.4. Chemical analyses

All seaweed products were analysed using official methods described to determine moisture (DM; ISO 6496, 1999), ash (ISO 5984, 2002), and N (ISO 5983, 2005). The seaweed products and diets used in the *in vivo* study were also analysed for amino acids (AAs; ISO 13903, 2005), ether extract (fat; ISO 6492, 1999), crude fibre (ISO 6865, 2000), sugar (EC, 2009), starch (ISO 15914, 2004), Ca (ISO 6869, 2000), P (ISO 6491, 1998), Na (ISO 27085, 2009), and Cl (ISO 6495, 2015). The ileal digesta were analysed for DM, N, AAs, Ca and P as described above and for TiO<sub>2</sub> as marker for digestibility (Short et al., 1996). The OM was calculated as 1000 minus ash. Non-starch polysaccharide content was calculated as OM minus crude protein, crude fat starch and total sugars (CVB, 2016). Excreta were analysed for DM, ash, fat and crude fibre.

### 2.2.5. Calculations and statistical analyses

Performance parameters were calculated using feed intake and body weight measurements over time. Apparent pre-caecal digestibility and apparent total tract digestibility of nutrients in the experimental diets were calculated, using Ti as indigestible marker according to the following equation:

$$D(X) = \left( 1 - \frac{[Ti]_{\text{diet}} \times [X]_{\text{sample}}}{[Ti]_{\text{sample}} \times [X]_{\text{diet}}} \right)$$

where D(X) is the digestibility of nutrient x and [Ti]<sub>diet</sub>, [Ti]<sub>sample</sub>, [X]<sub>diet</sub>, and [X]<sub>sample</sub> are the concentrations of Ti and nutrient X in the diet and digesta or faecal sample, respectively. The apparent pre-caecal digestibility and apparent total tract digestibility of nutrients in the seaweed co-products were calculated applying the difference method (Kong and Adeola, 2014), ascribing differences in digestibility between a basal diet and a basal diet including a component of investigation, assuming additivity.

All statistical analyses were performed using Genstat statistical software (VSN International, 2020). The effect of ensiling and washing on *in vitro* OM and N digestibility of *S. latissima*, in dependence of incubation time, was analysed with ANOVA using a 2 (no ensiling or ensiling) × 2 (no washing or washing) × 3 (2, 4 or 6 h incubation time) factorial arrangement. The effect of species and mannitol or rhamnose extraction on the *in vitro* simulated OM and N digestibility of included seaweed products, in dependence of incubation time, was analysed with ANOVA using a 3 (*S. latissima*, *L. digitata* or *U. lactuca*) × 2 (mannitol or rhamnose extraction) × 3 (2, 4 or 6 h incubation time) factorial arrangement. The experimental unit for all *in vitro* analyses were separate runs in the simulated digestibility analyses. An ANOVA was used to determine differences in growth performance, and *in vivo* pre-caecal and total tract digestibility between *S. latissima* silage and silage residue. Pen was the experimental unit for BW, body weight gain, feed intake, feed conversion ratio (FCR) and the ileal and faecal digestibility. All data are presented as least square means. Differences were considered

significant at  $P < 0.05$ .

### 3. Results

#### 3.1. *In vitro* experiment

Washed fresh and silage products from *S. latissima* had a lower ash content and a higher OM and N content than unwashed fresh and silage products (Table 1). Washed and unwashed silage products had a lower ash content and a higher OM and N content compared to washed and unwashed fresh *S. latissima*. The *S. latissima* and *L. digitata* residues were slightly different from the original products, with a lower ash and higher OM content. Additionally, *L. digitata* residue had a marginally higher N content compared to the unwashed fresh *L. digitata*. The *U. lactuca* residue on the other hand, had a substantially lower ash content, and consequently higher OM and N content compared to the unwashed fresh *U. lactuca*.

As summarized in Table 4, both ensiling and washing reduced the *in vitro* OM digestibility of *S. latissima* products ( $P < 0.001$  for both ensiling and washing). Washing, but not ensiling, reduced the *in vitro* N digestibility ( $P < 0.001$  and  $P = 0.057$  for washing and ensiling respectively). The *in vitro* OM and N digestibility of all *S. latissima* products increased significantly during incubation from 2 to 4 h, with a small and largely insignificant increase during incubation from 4 to 6 h.

As summarized in Table 5, extraction reduced the *in vitro* OM and N digestibility of all seaweed products ( $P < 0.001$ ). The biggest reduction in OM and N digestibility was observed in the *U. lactuca* residue (Interaction Species  $\times$  Extraction,  $P < 0.001$ ). For *L. digitata* the highest OM and N digestibility was observed, with the lowest values observed for *U. lactuca* (OM digestibility: 0.68, 0.59 and 0.46, Species  $P < 0.001$ , and N digestibility 0.62, 0.51 and 0.45, Species  $P < 0.001$  for *L. digitata*, *S. latissima* and *U. lactuca* respectively). The *in vitro* OM and N digestibility of all seaweed products increased significantly during incubation from 2 to 4 h, with a small and largely insignificant increase during incubation from 4 to 6 h ( $P < 0.001$  for OM and N digestibility). A larger increase in OM and N digestibility was observed for *Laminaria* and *S. latissima* with an increasing incubation time compared to *U. lactuca* (Interaction Species  $\times$  Incubation Time  $P < 0.001$  for OM and N digestibility).

#### 3.2. *In vivo* experiment

Inclusion of *S. latissima* silage and silage residue in the broiler diets enhanced feed intake (Table 6;  $P < 0.001$ ) without an effect on body weight gain. Consequently, an impaired FCR was observed in birds fed the seaweed supplemented diets ( $P < 0.001$ ). Birds fed the *S. latissima* residue diet showed a lower FCR compared to birds fed the *S. latissima* silage diet (1.77 and 1.86 for the residue and silage diet fed birds respectively). Inclusion of *S. latissima* silage and silage residue in the diet resulted in a lower pre-caecal digestibility of N ( $P < 0.001$ ) and most AAs ( $P = 0.022$  to  $P < 0.001$ ) compared to the basal diet (Table 7). Inclusion of *S. latissima* silage and silage residue in the diet also resulted in a lower total tract digestibility of OM ( $P < 0.001$ ) and ash ( $P < 0.001$ ) compared to the basal diet. No differences were observed in the calculated N, Ca and P digestibility between the *S. latissima* silage and silage residue product (Table 8). The average pre-caecal N digestibility was 0.68. The pre-caecal digestibility of all AAs, apart from methionine, was lower for the *S. latissima* silage than for the *S. latissima* silage residue.

**Table 4**

Effect of ensiling and washing on *in vitro* organic matter and nitrogen digestibility of *Saccharina latissima*, determined with a modified Boisen method (Boisen and Fernandez, 1997) to simulate digestibility in the stomach and small intestine of monogastric species (*in vitro* experiment).

Ensiling	Washing treatment	OM digestibility			N digestibility		
		2 h	4 h	6 h	2 h	4 h	6 h
Fresh	Unwashed	0.53 <sup>cA</sup>	0.73 <sup>bA</sup>	0.75 <sup>aA</sup>	0.49 <sup>bA</sup>	0.81 <sup>aA</sup>	0.79 <sup>aA</sup>
	Washed	0.41 <sup>cC</sup>	0.64 <sup>bB</sup>	0.66 <sup>aB</sup>	0.41 <sup>bB</sup>	0.75 <sup>aB</sup>	0.75 <sup>aAB</sup>
Silage	Unwashed	0.42 <sup>cB</sup>	0.64 <sup>bC</sup>	0.66 <sup>aC</sup>	0.51 <sup>bA</sup>	0.76 <sup>aB</sup>	0.80 <sup>aA</sup>
	Washed	0.29 <sup>cD</sup>	0.59 <sup>bD</sup>	0.59 <sup>aD</sup>	0.40 <sup>bB</sup>	0.70 <sup>aC</sup>	0.72 <sup>aB</sup>
Soybean meal (reference)		0.76	0.86	0.84	0.89	0.98	0.98
LSD		0.045			0.047		
		P-value		SED	P-value		SED
Ensiling		<0.001		0.008	0.057		0.009
Washing		<0.001		0.008	<0.001		0.009
Incubation time		<0.001		0.010	<0.001		0.011
Ensiling $\times$ Washing		0.325		0.012	0.146		0.012
Ensiling $\times$ Incubation time		0.176		0.015	0.090		0.015
Washing $\times$ Incubation time		0.051		0.015	0.227		0.015
Ensiling $\times$ Washing treatment $\times$ Time		0.595		0.021	0.628		0.022

OM, organic matter, N, nitrogen, LSD, least significant difference, SED, standard error of differences.

Each digestibility value is based on 2 replicate measurements.

<sup>a-c</sup> Means within a row without a common superscript differ significantly ( $P < 0.05$ ) in OM or N digestibility.

<sup>A-D</sup> Means within a column without a common superscript differ significantly ( $P < 0.05$ ) in OM or N digestibility.



**Table 5**

Effect of extraction processes of fresh and ensiled seaweed on *in vitro* organic matter and nitrogen digestibility of selected seaweed products, determined with a two-step method as described by Boisen and Fernandez (1997) to simulate digestibility in the stomach and small intestine (*in vitro* experiment).

Seaweed species	Extraction	OM digestibility			N digestibility		
		2 h	4 h	6 h	2 h	4 h	6 h
<i>S. latissima</i> (ensiled)	Starting material	0.42 <sup>ba</sup>	0.64 <sup>ac</sup>	0.66 <sup>ab</sup>	0.51 <sup>ca</sup>	0.76 <sup>bb</sup>	0.80 <sup>aa</sup>
	Residue	0.22 <sup>bc</sup>	0.57 <sup>ad</sup>	0.55 <sup>ac</sup>	0.28 <sup>bd</sup>	0.61 <sup>ad</sup>	0.58 <sup>ad</sup>
<i>L. digitata</i> (fresh)	Starting material	0.44 <sup>ba</sup>	0.79 <sup>aa</sup>	0.80 <sup>aa</sup>	0.44 <sup>bb</sup>	0.83 <sup>aa</sup>	0.83 <sup>aa</sup>
	Residue	0.33 <sup>cb</sup>	0.70 <sup>ab</sup>	0.65 <sup>bb</sup>	0.40 <sup>cc</sup>	0.80 <sup>aa</sup>	0.76 <sup>bb</sup>
<i>U. lactuca</i> (fresh)	Starting material	0.41 <sup>ba</sup>	0.64 <sup>ac</sup>	0.64 <sup>ab</sup>	0.41 <sup>bbc</sup>	0.71 <sup>ac</sup>	0.69 <sup>ac</sup>
	Residue	0.20 <sup>bc</sup>	0.39 <sup>ae</sup>	0.41 <sup>ad</sup>	0.16 <sup>ce</sup>	0.39 <sup>be</sup>	0.43 <sup>ae</sup>
Soybean meal (reference)		0.76	0.86	0.84	0.89	0.98	0.98
LSD		0.051			0.036		
		<i>P</i> -value		SED	<i>P</i> -value		SED
Species		<0.001		0.010	<0.001		0.007
Extraction		<0.001		0.008	<0.001		0.006
Incubation time		<0.001		0.010	<0.001		0.007
Species × Extraction		<0.001		0.014	<0.001		0.010
Species × Incubation time		<0.001		0.017	<0.001		0.012
Extraction × Incubation time		0.149		0.014	0.617		0.010
Species × Extraction × Incubation time		0.015		0.025	0.002		0.017

OM, organic matter, N, nitrogen, LSD, least significant difference, SED, standard error of differences.

Each digestibility value is based on 2 replicate measurements.

<sup>a-c</sup> Means within a row without a common superscript differ significantly ( $P < 0.05$ ) in OM or N digestibility.

<sup>A-E</sup> Means within a column without a common superscript differ significantly ( $P < 0.05$ ) in OM or N digestibility.

**Table 6**

Effect of inclusion of 100 g/kg *Saccharina latissima* silage and silage residue on growth performance of broilers in the period that the experimental diets were fed (day 14 – 22; *in vivo* experiment).

Performance parameters	Diet			SED	<i>P</i> -value
	Basal diet	Basal with <i>S. latissima</i> silage	Basal with <i>S. latissima</i> residue <sup>1</sup>		
Initial body weight day 14 (g/bird)	504.4	507.0	511.3	5.20	0.435
Final body weight day 22 (g/bird)	1044	1020	1061	14.7	0.054
Day 14 to 22					
Feed intake (g)	890 <sup>b</sup>	954 <sup>a</sup>	974 <sup>a</sup>	14.6	<0.001
Body weight gain (g/bird)	540	513	550	13.5	0.052
Feed conversion ratio (g/g)	1.65 <sup>c</sup>	1.86 <sup>a</sup>	1.77 <sup>b</sup>	0.024	<0.001

SED, standard error of differences.

Each value is based on 6 (basal diet) or 5 (seaweed diets) replicate pens with 10 birds each.

<sup>1</sup> Residue after the aqueous extraction of *Saccharina latissima* silage.

<sup>a-c</sup> Means within a row without a common superscript differ significantly ( $P < 0.05$ ).

#### 4. Discussion

This study investigated the effect of washing, ensiling and extraction of valuable components from seaweed on the nutritional value of seaweed and seaweed residues for broilers.

As generally observed (e.g. Øverland et al., 2019; Bikker et al., 2020), the intact seaweed samples in this study had a high ash content, over 250 g/kg DM. Poultry are sensitive to high dietary mineral levels (National Research Council, 2005), which may lead to water overconsumption and result in diarrhoea and a reduced performance (Guiry and Blunden, 1991; Koreleski et al., 2010). The high ash content in seaweed hampers the inclusion of seaweed in poultry diets at nutritionally significant levels (e.g. > 50 g/kg). Washing seaweed can reduce the ash content as observed by Neveux et al. (2014), who reduced the ash content of *Ulva ohnoi* by 43 % and that of *Derbesia tenuissima* by 83 % with washing 3 times for 1 min by immersion in tap water, stirring and draining the water at each wash. Milledge et al. (2018) found a reduction in ash content of 21 g/kg in *Sargassum muticum*, after washing freshly harvested seaweed once in running tap water for 30 s. The ash composition in the latter study showed that sodium chloride in the ash fraction was reduced from 515 g/kg ash to 425 g/kg ash due to washing. In this study, a relative reduction in ash content of 9% in fresh *S. latissima* and 22 % in *S. latissima* silage was observed. This smaller reduction compared to the study of Neveux et al. (2014) might be explained by difference in seaweed species, but also by the more gentle washing treatment applied in the current study. Other studies addressed the effect of washing *Saccharina* spp. on specific minerals, applying different temperatures (16 ° C and 32 ° C) and durations (1, 2, 6 and 22 h) of washing (Stévant et al., 2018). From their study, the authors could not conclude whether the measured loss of dry weight due to tap water soaking treatments was due to nutrients being removed from the seaweed or water being taken up by the seaweed due to

**Table 7**

Effect of inclusion of 100 g/kg *Saccharina latissima* silage and silage residue in broiler diets on the *in vivo* pre-caecal and total tract nutrient digestibility of the diets (*in vivo* experiment).

Apparent pre-caecal digestibility	Diet			SED	P-value
	Basal	Basal with <i>S. latissima</i> silage	Basal with <i>S. latissima</i> residue <sup>1</sup>		
Nitrogen	0.936 <sup>a</sup>	0.917 <sup>b</sup>	0.925 <sup>b</sup>	0.003	<0.001
Calcium	0.787	0.761	0.765	0.029	0.623
Phosphorus	0.864	0.861	0.871	0.019	0.884
Amino acids					
Lysine	0.960 <sup>a</sup>	0.947 <sup>b</sup>	0.954 <sup>ab</sup>	0.003	0.003
Methionine	0.976 <sup>a</sup>	0.970 <sup>b</sup>	0.971 <sup>b</sup>	0.002	0.006
Cysteine	0.831 <sup>ab</sup>	0.815 <sup>b</sup>	0.836 <sup>a</sup>	0.006	0.022
Threonine	0.927 <sup>a</sup>	0.917 <sup>b</sup>	0.929 <sup>a</sup>	0.003	0.009
Isoleucine	0.946 <sup>a</sup>	0.925 <sup>b</sup>	0.939 <sup>a</sup>	0.003	<0.001
Arginine	0.951 <sup>a</sup>	0.943 <sup>b</sup>	0.951 <sup>a</sup>	0.002	0.002
Phenylalanine	0.962 <sup>a</sup>	0.930 <sup>c</sup>	0.945 <sup>b</sup>	0.003	<0.001
Histidine	0.955 <sup>a</sup>	0.933 <sup>c</sup>	0.944 <sup>b</sup>	0.003	<0.001
Leucine	0.954 <sup>a</sup>	0.928 <sup>c</sup>	0.942 <sup>b</sup>	0.003	<0.001
Valine	0.946 <sup>a</sup>	0.934 <sup>b</sup>	0.944 <sup>a</sup>	0.003	0.001
Alanine	0.872 <sup>a</sup>	0.732 <sup>b</sup>	0.768 <sup>b</sup>	0.018	<0.001
Aspartic acid	0.928 <sup>a</sup>	0.898 <sup>c</sup>	0.918 <sup>b</sup>	0.004	<0.001
Glutamic acid	0.956 <sup>a</sup>	0.935 <sup>c</sup>	0.948 <sup>b</sup>	0.002	<0.001
Glycine	0.905 <sup>a</sup>	0.881 <sup>b</sup>	0.896 <sup>a</sup>	0.005	<0.001
Serine	0.927 <sup>a</sup>	0.911 <sup>b</sup>	0.928 <sup>a</sup>	0.003	<0.001
Apparent total tract digestibility					
Ash	0.413 <sup>a</sup>	0.262 <sup>b</sup>	0.253 <sup>b</sup>	0.015	<0.001
Organic matter	0.821 <sup>a</sup>	0.726 <sup>c</sup>	0.751 <sup>b</sup>	0.009	<0.001
Fat	0.888 <sup>a</sup>	0.794 <sup>b</sup>	0.907 <sup>a</sup>	0.016	<0.001
Crude fibre	-0.029	0.072	0.013	0.062	0.293

SED, standard error of differences.

Each value is based on 6 (basal diet) or 5 (seaweed diets) replicate pens with 10 birds each.

<sup>1</sup> Residue after the aqueous extraction of *Saccharina latissima* silage.

<sup>a-b</sup> Means within a row without a common superscript differ significantly ( $P < 0.05$ ).

osmosis. Although some of their washing procedures did sufficiently reduced cadmium and iodine content in the *Saccharina spp.* for human consumption, they only did so to an extent that allows for ingestion of very small quantities of seaweed (3.3 g dry weight per day). Nonetheless, the reduction in ash content in this study was not adequate to substantially increase the seaweed inclusion in broiler diets.

Moreover, washing also drastically reduced the *in vitro* OM and N digestibility. The applied *in vitro* digestibility model is based on the solubility of the tested materials, hence, the reduction in soluble nutrients in washed samples automatically resulted in a lower *in vitro* digestibility. Other studies report a loss of nutrients like soluble fibres during washing, due to the difference in osmotic pressure between salt and fresh water, although digestibility was not taken into account (Stévant et al., 2018). It should be noted that not all soluble material is necessarily digestible (Choct et al., 2010), hence the *in vitro* digestibility based on solubility might overestimate the digestibility of (intact) seaweed with a high content of soluble nutrients.

In this study the impact of ensiling *S. latissima* on composition and nutritional value for broiler chickens was determined. Ensiling reduced the ash content, and increased the protein content by approximately a twofold, with a lesser increase in OM content. Furthermore, a substantial decrease in OM digestibility was observed, as well as a small decrease in N digestibility.

In literature, a decrease in fibre and an increase in protein content was observed as a result of a seaweed fermentation process using *U. lactuca* (Felix and Brindo, 2014), which is in line with our observations for *S. latissima* silage. The latter authors hypothesised that the increase in protein content was caused by microorganisms utilizing fibres, consequently increasing microbial biomass. The decrease in ash content and extra protein available in ensiled seaweed improved the nutritional value of seaweed silage. Nevertheless, the strong decrease in OM digestibility indicates that the nutrients in the OM of seaweed silage cannot be utilized by broilers as well as the OM in fresh seaweed. The changes in digestibility might be largely explained by the process of ensiling. During ensiling, a part of the fluid from the silage was drained, and with that fluid also soluble nutrients will have drained. This might explain the decrease in OM digestibility, since the simulated digestibility model is based on solubility of the samples.

The nutritional value of seaweed residues was evaluated after extraction of components that can be used for food or chemical application to contribute to the economic value of seaweed production. Because of the high costs of production, harvest and processing of seaweed, such a biorefinery approach may be required for the economic feasibility of seaweed production (Van den Burg et al., 2013; Torres et al., 2019). After extraction, a higher ash and OM content, and specifically in *U. lactuca* a lower N content were observed, in combination with a lower OM and N digestibility compared to the original material. The reduction in ash content and increase in OM and N content due to extraction should, in theory, improve the nutritional value. However, the lower digestibility negatively affected the nutritional value of the seaweed residue products for broilers. Extraction of both *S. latissima* and *L. digitata* did



**Table 8**  
Pre-caecal amino acid and mineral digestibility of *Saccharina latissima* silage and silage residue in broilers (*in vivo* experiment).

Apparent pre-caecal digestibility	<i>Saccharina latissima</i>		SED	P-value
	Silage	Residue <sup>1</sup>		
Nitrogen	0.66	0.69	0.062	0.587
Calcium	0.71	0.72	0.089	0.891
Phosphorus	0.71	-0.53	2.255	0.597
Amino acids				
Lysine	0.79	0.91	0.040	0.014
Methionine	0.90	0.90	0.030	0.863
Cysteine	0.74	0.85	0.022	0.001
Threonine	0.84	0.95	0.030	0.006
Isoleucine	0.72	0.90	0.030	<0.001
Arginine	0.88	0.96	0.016	0.001
Phenylalanine	0.67	0.85	0.022	<0.001
Histidine	0.57	0.86	0.046	<0.001
Leucine	0.67	0.87	0.035	<0.001
Valine	0.83	0.93	0.024	0.003
Alanine	0.10	0.35	0.098	0.033
Asparagine + aspartic acid	0.64	0.86	0.033	<0.001
Glutamine + glutamic acid	0.54	0.88	0.045	<0.001
Glycine	0.78	0.86	0.024	0.007
Serine	0.75	0.94	0.026	<0.001
Apparent total tract digestibility				
Ash	-0.04	-0.12	0.058	0.215
Organic matter	0.02	0.13	0.106	0.334
Fat	-2.04	0.77	0.403	<0.001
Crude fibre	0.20	0.07	0.173	0.474

SED, standard error of differences.

Each value is based on 5 replicate pens with 10 birds each.

<sup>1</sup> Residue after the aqueous extraction of *Saccharina latissima* silage.

not only resulted in removal of mannitol, but also of a substantial portion of other nutrients and minerals, presumably due to washing and cell disruption caused by osmosis (Van Hal and Huijgen, 2014). This loss of soluble nutrients might have caused the decrease in digestibility as observed for the residues of *S. latissima* and the *L. digitata*, and showed a similar effect on digestibility as washing *S. latissima*. In *U. lactuca* a large increase in N content was observed, although the digestibility was largely decreased. The extraction process for *U. lactuca* was performed in an acidic environment at 100 °C. A large part of the minerals, and the polysaccharides rhamnose, glucuronic acid (both part of Ulvan, the main polysaccharide in *Ulva spp.*), glucose and xylose were extracted (Groenendijk et al., 2016), as also described in literature (Kidgell et al., 2019). Potentially, the more severe acidic extraction at a higher temperature solubilized and removed more nutrients than the watery extraction did in *S. latissima* and *L. digitata*, explaining the differences in remaining soluble nutrients, thus in chemical composition, and with that digestibility. For example, Ulvan (main structural component of *Ulva spp.*) extraction was dependent on temperature and duration of the extraction procedure (Kidgell et al., 2019). Other authors observed a relative increase of 20 % and 5 % in OM and N digestibility, respectively, of an *U. lactuca* product resulting from a bio-refinery approach (Bikker et al., 2016). These authors hypothesised that the enzymatic hydrolysis applied in their study degraded a large portion of the ileal indigestible carbohydrates, improving digestibility for monogastrics. This indicates that such an enzymatic hydrolysis, either as part of the extraction process or by itself, might be a good treatment to increase digestibility of seaweed products.

To quantify seaweed digestion in broilers, an *in vivo* experiment was carried out with *S. latissima* silage and silage residue added to broiler diets. In the residue product, sugars and other soluble nutrients were extracted with a watery alkaline extraction process by bruising and thereafter soaking and draining the seaweed silage. Nonetheless, the chemical composition did not show large differences between the silage and silage residue product. During the ensiling process, excess fluid was drained from the silage, already leading to a loss of soluble nutrients from the silage product. This explains at least part of the reason for the small differences in chemical composition between the silage and silage residue products. Furthermore, the sodium content was increased in the silage residue product. Sodium carbonate was added to establish alkaline extraction conditions, although the electrolyte balance was levelled between diets by adding potassium. Inclusion of either of the seaweed products significantly enhanced the feed intake of both treatment groups without beneficial effect on body weight gain, consequently showing an increased FCR. Birds fed the silage residue diet performed better than birds fed the silage diet based on the lower FCR and higher final body weight, which was likely caused by the low digestibility of the seaweed products. Literature on brown seaweed fed to poultry at nutritionally significant levels (>50 g/kg) is scarce. In one study, 20–60 g/kg fresh, boiled and autoclaved brown seaweed *Sargassum dentifolium* was added to finisher diets for broilers (El-deek et al., 2011). These authors did not observe differences in chemical composition or metabolizable energy of the seaweed products due to boiling for 20 min or autoclaving at 121 °C for 20 min. Additionally, no differences in the analysed metabolizable energy of the experimental diets, due to concentration or pre-treatment of the seaweed products added to the diets, were observed. Despite the lack of differences, birds fed the seaweed diets performed worse based on body weight, body weight gain, feed

intake and FCR with increasing concentrations of seaweed products added and with increasing severity of treatment of the seaweed product. The authors did not provide a clear explanation of these results, although they did not measure digestibility of the seaweed products. Furthermore, the differences in behaviour of the chyme in the intestinal tract of the birds, due to the inclusion of the differently treated seaweed products, were not analysed.

The digestibility of crude fibre was close to zero in the current study, both in the basal diet and the seaweed diets. This might be related to the other dietary ingredients being highly soluble, which might enhance shorter retention times of the digesta in the GIT. The negative apparent total tract digestibility values of for example certain inorganic components of the silage and silage residue products, indicated an interaction effect of the seaweeds with the basal diet.

Some differences were observed between the silage and silage residue products used in the *in vitro* and *in vivo* study. Compared to the silage product, the residue product used in the *in vitro* study showed a higher OM and a lower N content, as well as a lower OM and N digestibility. The opposite was observed for the products used in our *in vivo* study: compared to the silage product, in the residue product a lower OM and higher N content were observed, without differences in OM and N digestibility. The alkaline extraction process applied to the residue product used in the *in vivo* experiment, might have extracted different nutrients compared to non-alkaline conditions, leading to differences in chemical composition. Furthermore, large intra-species variations in chemical composition may explain part of the observed differences, since the products used in the *in vitro* and *in vivo* study originated from different batches. Intraspecies differences are ascribed to, amongst others, season of harvest, geographical characteristics and environmental factors (Schienen et al., 2015; Boderskov et al., 2016; Sharma et al., 2018). Additionally, when comparing *in vitro* and *in vivo* digestibility, it should be taken into account that the *in vitro* digestibility values reflect the solubilization of nutrients. Since not all soluble material is digestible, the *in vitro* analyses might overestimate digestibility. Furthermore, the seaweed products within the diets fed to the broilers might have interacted with the diet. For example, the viscosity of the chyme in the intestinal tract of the broilers might be altered by viscous substances (polysaccharides) in the seaweed products, which may influence the digestibility of the diets as a whole (Holdt and Kraan, 2011; Burg et al., 2012; Matthiesen et al., 2021). This means that the digestibility of the seaweed products in the *in vivo* experiment, as calculated by difference in digestibility compared to the basal diet, does not only reflect the digestibility of the seaweed products but also their impact on the digestibility of the basal diet.

## 5. Conclusion

The results of this study demonstrate that washing reduces the ash content of seaweed products, but simultaneously may reduce the nutritional value of the seaweed. Ensiling, as well as an extraction process as part of a biorefinery approach also reduces the nutritional value of seaweed.

From this study we conclude that the process of washing or ensiling alone does not make seaweed suitable for inclusion in broiler diets. Additional steps to be taken in order to create suitable feed ingredients out of seaweed products include a further reduction of the ash content, and an increase in digestibility. The latter might be achieved by different methods, like enzymatic hydrolysis, using suitable enzymes for seaweed species, related to the different chemical composition compared to land based plants. It is important to also gain more understanding of the behaviour of seaweed products in *in vitro* digestibility analyses as well as their behaviour in the gastro intestinal tract of broilers. This may allow to evaluate more precisely the nutritional value of the seaweed products. With this, a better understanding of the interaction between the seaweed products and the basal diets, including the consequences for the birds, their performance, and their health can be obtained.

## Author statement

Lotte Stokvis: Methodology, Formal analysis, Validation, Writing-original draft, Writing-review & editing, Visualization

Marinus van Krimpen: Conceptualization, Methodology, Formal analysis, Writing-original draft

Paul Bikker: Conceptualization, Methodology, Validation, Writing-original draft, Writing-review & editing, Supervision, Funding acquisition

René Kwakkel: Writing-review & editing, Validation

## Declaration of Competing Interest

The authors declare no conflict of interest.

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