Assessment of macroalgae and macroalgal extracts as a source of minerals in need of fine-tuning in multiple livestock production systems

Sabela Al-Soufi [1], Javier García [2], Antonio Muíños [3], Víctor Pereira [1], Verónica Piñeiro [4], Marta Miranda [5], Marco García-Vaquero [6] and Marta López-Alonso [1*]

[1] Departamento de Patoloxía Animal, Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela, 27002 Lugo, Spain; sabelaalsoufi.novo@usc.es (S.A.-S.); victor.pereira@usc.es (V. Pe.); marta.lopez.alonso@usc.es (M.L.-A.)

[2] Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, 28040 Madrid, Spain; javier.garcia@upm.es (J.G.)

[3] Porto Muíños S.L., 15185 Cerceda, Spain; antonio@portomuinos.com (A.M.)

[4] Unidade de Análise instrumental. Area de Infraestructuras de Investigación, Campus Terra, Universidade de Santiago de Compostela, 27002 Lugo, Spain; veronica.pineiro@usc.es (V. Pi.)

[5] Departamento de Anatomía, Produción Animal e Ciencias Clínicas Veterinarias, Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela, 27002 Lugo, Spain; marta.miranda@usc.es (M. M.)

[6] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland; marco.garciavaquero@ucd.ie (M.G.-V.)

[*] Correspondence: marco.garciavaquero@ucd.ie

Abstract: This study evaluates the levels of macrominerals (Ca, Mg, P, K, Na, S), essential trace elements (Co, Cr, Cu, Fe, I, Mn, Mo, Ni, Se, Zn) and potentially toxic trace elements (Cd, Hg, Pb, As (inorganic and organic)) in seven species of macroalgae and in their extracts. The potential maximum levels of inclusion of macroalgal biomass and extracts in feed were assessed in multiple livestock (swine, ruminant, poultry, leporine and pisciculture). Overall, macroalgae contained high levels of I, reaching its highest levels in *S. latissima* (4131 mg/kg DM) and *L. ochroleuca* (2780 mg/kg DM). Arsenic concentrations ranged from 4.10 mg/kg DM in *Ulva* spp. to 68.9 mg/kg DM in *S. latissima.* Arsenic was mainly present as arsenosugars, of relatively low toxicity. Extracts had higher macrominerals and I levels, and lower essential and toxic trace elements levels compared to the biomass. Macroalgal biomass and extracts can be added to feed at 1-5% to fulfill the physiological needs of multiple livestock, being I contents the main factor limiting highest inclusion rates. Inclusion of *S. latissima* and *L. ochroleuca* should be limited to 0.72 and 0.66%, respectively. Maximum level of inclusion of different macroalgal products, as dried biomass or extracts, must be finely tuned. Low levels of inclusion of macroalgae and/or extracts in feed can be considered as an efficient and natural strategy to fulfill the macrominerals and iodine needs of multiple livestock.

Keywords: essential trace and toxic elements, feed, seaweed, iodine, inorganic arsenic, organic arsenic

Abbreviations: Ca: calcium; Mg: magnesium; P: phosphorus; K: potassium; Na: sodium; S: sulphur; Co: cobalt; Cu: copper; Fe: iron; Mn: manganese; Mo: molybdenum; Se: selenium; Zn: zinc; I: iodine; Cd: cadmium; Pb: lead; Hg: mercury; As: arsenic; iAs: inorganic arsenic; DMA: dimethylarsinic acid; MMA: monomethylarsonic acid; AsB: arsenobetaine; ICP-MS: inductively coupled plasma mass spectrometry; HPLC: high performance liquid chromatography system; LD: limit of detection; CRM: certified reference material; NRC: National Research Council.

1. Introduction

Macroalgae are able to produce unique compounds to ensure the survival of the biomass in the extreme and changeable aquatic environments in which they grow (Biris-Dorhoi et al., 2020; Garcia-Vaquero et al., 2021). There are thousands of species of macroalgae capable of producing a wide array of compounds with multiple biological properties that can be used as pharmaceuticals, cosmetics, biofertilizers, biofuels, human food and animal feed (Evans and Critchley, 2014). Macroalgae, as a source of food and feed, offer advantages over traditional crops in terms of sustainability, as their production does not require freshwater, arable land or fertilizers (Mahadevan, 2015).

In animal feed, the inclusion of macroalgae has been studied for its prebiotic, antioxidant or immunomodulatory effects (De Jesus Raposo et al., 2016). Macroalgae are sources of dietary fibre, high-quality protein, vitamins, polyunsaturated fatty acids and polyphenolic compounds (Mahadevan, 2015; Øverland et al., 2019). Moreover, this biomass can provide greater amounts of minerals, with ash levels ranging from 8 to 40% of dry matter (DM), compared to terrestrial plants (5-10%) (Feedipedia, 2024). Thus, macroalgae could be exploited in animal feed production as an alternative and sustainable source of minerals to meet the animals' recommended daily intakes (Cabrita et al., 2016; Costa et al., 2021). However, the composition of the macroalgal biomass varies greatly depending on the class (i.e. green, red or brown macroalgae), species, geographical localization or environmental conditions (Biris-Dorhoi et al., 2020). Moreover, recent trends in the macroalgal processing sector include the extraction of compounds by innovative technologies, resulting in extracts of variable chemical nature that can be added to feed. Thus, the conditions used during the generation of these extracts represents a new source of variation in the composition of macroalgal-derived products worthy of further exploration (Garcia-Vaquero et al., 2021).

Macroalgae have been previously described as a source of macrominerals (calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na) and sulphur (S)), and trace minerals (cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn)) (Cabrita et al., 2016; Circuncisão et al., 2018). Some brown macroalgae, particularly from the order Laminariales, contain higher amounts of iodine (I), reaching up to 12.500 mg/kg DM (Garcia-Vaquero et al.,2021), compared to terrestrial plants (Circuncisão et al., 2018). In fact, macroalgae have been previously explored as feed supplements of I in multiple livestock (López-Alonso et al., 2016; Øverland et al., 2019; Rey-Crespo et al., 2014). However, it is crucial to ensure that the levels of I do not surpass the maximum levels permitted for this mineral in animal feed (EC 1334/2003). Thus, processing the biomass to lower the I content of the resulting macroalgal products (i.e. extracts) may be a desirable outcome if the contents of this mineral are a limiting factor restricting the inclusion of macroalgae in animal feed.

Macroalgae can accumulate undesirable elements, mainly arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), depending on the uptake capacity of each species, the environmental conditions (i.e. salinity, temperature, pH) and the level of pollution of the surrounding environment (Costa et al., 2021). Thereby, the high As concentrations found in some macroalgal species (up to 600 mg/kg DM; Garcia-Vaquero et al., 2021) can be a challenging factor for an increased utilization of macroalgae. Previous research demonstrated that the majority of the As in macroalgae is usually present in its low-toxic organic forms (Díaz et al., 2012; Wolle et al., 2021), which do not pose a significant risk in animal nutrition (Biancarosa et al., 2018). However, measures must be taken when using macroalgae in feed, ensuring that the levels of total and inorganic As (iAs) do not surpass the maximum limits established in animal feed (EC 32/2002, EC 1275/2013).

The use of macroalgae in animal feed has been previously explored; however, there are knowledge gaps related to the variable mineral composition of the biomass and their extracts. In fact, there are limited reports on the mineral contents of macroalgal products (biomass and/or extracts) used as feed supplements in recent studies. Moreover, the accumulation of potentially toxic elements, such as iAs, is mentioned by these researchers as a common concern when using macroalgal products in feed. However, most studies report only the levels of total As due to the complex analytical procedures needed for the speciation of As into organic and inorganic forms.

Furthermore, the levels of inclusion of macroalgal products in the feed of multiple livestock production systems considering the current European Union (EU) regulations is worth further exploration to ensure compliance. Therefore, this study provides detailed information on the feasibility and safety of using macroalgae and their extracts as feed ingredients, establishing guidelines that could support their incorporation as an alternative source of minerals in animal nutrition.

The aim of this study was to analyze the mineral contents (essential macro and microminerals and potentially toxic elements, including iAs) of seven macroalgal species, including brown (*Fucus vesiculosus, Himanthalia elongata, Undaria pinnatifida, Laminaria ochroleuca, Saccharina latissima*), green (*Ulva* spp.) and red macroalgae (*Mastocarpus stellatus*) from the Atlantic coast of Galicia (NW Spain), as well as their extracts. The maximum concentrations of essential and toxic elements allowed in animal feed in the EU were used to assess the maximum levels of inclusion of both macroalgae and macroalgal extracts in the feed of multiple livestock production systems (swine, ruminant, poultry, leporine and pisciculture).

2. Material and methods

2.1. Macroalgal biomass and extracts

Seven macroalgal species were selected for this study, including brown (*Fucus vesiculosus, Himanthalia elongata, Undaria pinnatifida, Laminaria ochroleuca* and *Saccharina latissima*), green (*Ulva* spp.) and red (*Mastocarpus stellatus*) macroalgae. All samples were harvested from the Atlantic coasts of Galicia (NW Spain) by Porto-Muíños S.L. (Cerceda, A Coruña, Spain). *S. latissima* was cultivated in the same geographical region, as harvesting wild populations of this species is not permitted in the area. These species were selected for their potential in animal nutrition according to different criteria (for more details see (Al-Soufi et al., 2023)). Samples were washed with fresh water to remove external epitopes, oven-dried (< 40 °C, 4-5 days), ground and milled (Komodin K-160 P, Lleal, Granollers, Spain) to 1 mm particle size.

Macroalgal extracts were generated in this study from biomass discards produced during their industrial processing. Aqueous extracts were produced using a pressure reactor (Parr Instrument Company, IL, USA) operated in non-isothermal mode (160 °C, 110 psi), using water as solvent at a macroalgae:solvent ratio of 1:30 w/v. After the extraction process, the supernatants were collected and spray-dried (Büchi B-290, Flawil, Switzerland). Spray-drying was performed at 115 ºC inlet temperature, 4 mL/min feed solution flow rate, 1050 L/h atomization air flow rate and 4.1 bar pressure (Al-Soufi et al., 2023). The samples were then vacuum-packed and preserved at -20 °C for further analyses.

DM of biomass and extracts was determined by oven-drying the samples (105 °C, 16 h) until achieving constant weigh, following the official methods of analysis as described by AOAC.942.05 (Horwitz, 2010).

2.2. Sample preparation and trace element analyses

Trace element analyses were performed from macroalgal biomass and their freeze-dried extracts. Each sample was analysed in triplicate and an analytical quality control was performed throughout the process.

2.2.1. Acid digestion

0.3 g of each sample were mixed with 3 mL of concentrated 69% nitric acid (TMA, Hiperpure, PanReac, Barcelona, Spain) and 1 mL of ultrapure water. The mixtures were then digested in a microwave-assisted digestion system (Ethos Plus, Milestone, Sorisole, Italy) at 260 °C and 40 bar for 1 h (de Oliveira Filho et al., 2022; Garcia-Vaquero et al., 2021). All digested samples were transferred to polypropylene tubes and diluted to 50 mL with ultrapure water.

2.2.2. Alkaline extraction

Iodine was analysed following the European Standard guidelines for inductively coupled plasma mass spectrometry or ICP-MS (EN 15111:2007). Briefly, 0.1 g of samples were mixed with 5 mL of ultrapure water and 1 mL of tetramethylammonium hydroxide solution (TMAH, Sigma

Aldrich, Merck, Darmstadt, Germany). The mixtures were placed in a pre-heated oven (90 °C, 3 h). All the samples were cooled to room temperature, their volume adjusted to 25 mL with ultrapure water, and centrifuged (4,000 rpm, 15 min). Supernatants were filtered (0.45 µm) before the determination of I by ICP-MS within a day.

2.2.3. ICP-MS

The concentrations of macrominerals (Ca, Mg, P, K, Na and S) and microminerals (Co, Cr, Cu, Fe, I, Mn, Mo, Ni, Se and Zn) and of potentially toxic elements (Cd, Hg, Pb and As,) were determined by ICP-MS 7900 (Agilent, USA) following the conditions as described by de Oliveira Filho *et al.* (de Oliveira Filho et al., 2022).

The results of the control of analytical quality of this study are summarised in Table 1. Blank samples were included in all batches of samples. The limits of detection (LD) were calculated as three times the standard deviation (SD) of the blanks. Certified reference samples were analysed following the same procedures, and their recoveries were consistent with the certified levels of the 2 certified reference materials (CRMs). The 2 CRMs used were (1) apple leaves (NIST-1515), supplied by National Institute of Standards & Technology, Gaithersburg, USA; and (2) seaweed (ERM-CD200) supplied by Institute for Reference Materials and Measurements, Geel, Belgium. In the case of minerals not certified by these CRMs (Na, and I), spiked samples were used at concentrations producing intensity values up to 10 times the expected values, yielding recoveries of 86-93%.

Table 1. Limits of detection (LD) (µg/kg) of each element, certified levels (mg/kg) and % of recovery of the certified reference materials.

		Apple leaves (NIST 1515)		**Seaweed (ERM-CD200)**	
Minerals	LD	Certified levels (mg/kg)	Recovery (%)	Certified levels (mg/kg)	Recovery (%)
Ca	9.56	15250	109	-	-
Mg	0.31	2710	94	-	-
P	7.72	1593	113	-	-
K	7.37	16080	106	-	-

S	6.13	1800	125	-	-
Co	0.005	0.09	99	-	-
Cr	0.03	0.3	87	-	-
Cu	0.013	5.69	93	1.71	111
Fe	0.19	82.7	91	-	-
Mn	0.02	54.1	88	-	-
Mo	0.006	0.095	95	-	-
Ni	0.014	0.936	92	-	-
Se	0.005	-	-	0.088	124
Zn	0.18	12.45	88	25.3	123
Cd	0.005	0.0132	96	0.95	96
Hg	0.013	0.0432	102	0.0186	69
Pb	0.002	0.470	75	0.51	73
As	0.006	-	-	55	104

2.3. Arsenic speciation

For As speciation, the samples were digested using the microwave-assisted hot block method (Foster et al., 2007). Briefly, 0.5 g samples were mixed with 10 mL of 1% HNO_3 in polytetrafluoroethylene tubes (Milestone, Sorisole, Italy). The tubes were capped and placed in a pre-heated digestion block system (95 °C, 90 min). The samples were then cooled to room temperature, centrifuged (3,500 rpm, 10 min) and the supernatants filtered (0.45 µm). One g of the filtered supernatants was diluted with the mobile phase (10 mM ammonium phosphate, pH 8.25), achieving sample pHs ranging between 6 and 8.5. Sample solutions were then placed into polypropylene vials. The As speciation was performed based on the method described by Juskelis *et al.* (Juskelis et al., 2013) using high performance liquid chromatography system/inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The samples were analysed using an Agilent 7900 equipment (USA) connected to a HPLC (Agilent 1260, USA) with an anion-exchange column (Hamilton PRPX100).

The different arsenic fractions were determined after calibrating the equipment with the standard solutions for each arsenic species (Juskelis et al., 2013). Total inorganic As (iAs) was calculated as the sum of As (III) and As (V), and organic As was determined as the sum of dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenosugars and arsenobetaine (AsB). Due to the absence of calibration standards, arsenosugars were quantified by applying a compound independent calibration method according to the calibration curves of the nearest eluting standard species (Marschner et al., 2018; Morales-Rodríguez et al., 2022). The other As species were validated by the inclusion of blank solutions for the determination of LD and replicates of the CRM rice flour (SRM 1568b), provided by the National Institute of Standards & Technology, Gaithersburg, USA. The results of this quality control for As speciation are provided in Table 2.

Table 2. Limits of detection (µg/L) of each element, certified levels (mg/kg) and % recovery of the certified reference material for arsenic speciation.

		Rice flour (SRM 1568b)	
	LD	Certified levels (mg/kg)	Recovery (%)
DMA	0.196	0.180	118
MMA	0.0262	0.0116	108
iAs	0.3823	0.092	101

LD: limits of detection; DMA: dimethylarsinic acid; MMA: monomethylarsonic acid; iAs: As (III) + As (V).

2.4. Calculation of maximum level of inclusion of macroalgae and extracts in animal feed

The maximum level of inclusion of macroalgal biomass and their extracts were calculated for each essential and potentially toxic trace element in porcine, broilers, laying hens, ruminants, dairy cows, rabbits and fish. These calculations were based on: (1) the total concentration allowed by current EU legislation in complete feed (Regulation EC 1334/2003 and EU 1039/2018 – were used in the case of Co, Cu, Fe, Mn, Se and Zn; and Regulation EC 1459/2005 – was used in the case of I; Directive EC 32/2002 and Regulation EC 1275/2013 – were the references used in the case of As, Cd, Hg, Pb). (2) The basal mineral concentration of standard diets from different livestock species, without additional mineral supplements (Van Paemel et al., 2010).

Since there is no legislation regarding maximum levels of macrominerals in animal feed, the maximum mineral tolerance of each species (National Research Council, 2005) were used in the calculations as described in Eq. 1.

$$[M]diet \times (100 - I\ algae) + [M]algae \times I\ algae = [M]total \times 100 \qquad \text{Eq.1}$$

[M]diet: Basal mineral concentration in the standard diet (expressed in mg/kg DM).

[M]algae: Mineral concentration in the macroalgal product (expressed in mg/kg DM).

[M]total: Maximum concentration of mineral permitted in the prevailing legislation (expressed in mg/kg DM; converted from complete feed considering 88% DM).

I algae: Maximum level of inclusion of macroalgae in the diet (expressed in %).

3. Results

3.1. Mineral composition of dried macroalgal biomass

The concentrations of macrominerals in selected dried macroalgal species in this study are presented in Table 3. Ca concentrations were quite similar in most macroalgal species (12.3-31.1 g/kg macroalgae on dry matter basis (DM)), while P was the least abundant macromineral in all macroalgal species (3.41-13.5 mg/kg DM). The concentrations of Mg in *Ulva* spp. (54.04 g/kg DM) and S in *Ulva* spp. and *M. stellatus* of 121.8 and 134.0 g/kg DM, respectively; were remarkably high compared to the rest of the species analyzed. The levels of other macrominerals showed remarkable variations between the species analyzed. Thereby, K was present at very high levels in most of the brown macroalgae (47.32-160.2 g/kg DM) compared to red and green macroalgae (12.3-31.1 g/kg DM). In the case of Na, this macromineral was present at levels ranging between 28.6 and 67.2 g/kg DM in red and brown macroalgae, being the highest values the ones of *H. elongata* and the lowest levels described in green macroalgae.

Table 3. Average concentrations (expressed as mean ± standard deviation, n=3) of macrominerals (g/kg DM) in selected macroalgal species from the Atlantic coast of Galicia (NW Spain).

Species	**Ca**	**Mg**	**P**	**K**	**Na**	**S**
F. vesiculosus	23.6±3.7	12.5±4.5	13.5±1.58	47.3±0.9	28.3±7.6	49.4±11.7
H. elongata	17.2±2.9	11.1±3.9	10.5±6.4	148.9±3.4	67.2±3.4	25.6±6.6
U. pinnatifida	17.2±2.0	8.89±1.96	8.71±3.27	126.5±50.5	59.8±10.0	15.2±2.5
L. ochroleuca	23.7±0.9	10.5±2.2	5.16±1.81	160.2±7.8	54.4±21.0	21.3±0.3
S. latissima	21.4±8.4	7.27±3.29	4.39±2.58	144.6±3.4	54.0±3.4	12.6±4.7
Ulva spp.	12.3±3.1	54.0±11.0	3.41±0.84	12.3±2.1	16.4±0.5	121.8±20.0
M. stellatus	31.1±9.4	14.5±0.3	3.85±0.47	31.1±3.1	66.1±20.7	134.0±4.1

Essential trace element concentrations were very variable (up 2-fold differences) in the macroalgal species studied (see Table 4). *F. vesiculosus* was remarkably rich in most elements, being one order of magnitude higher than those in other macroalgal species. In the case of Cr, Fe and Ni, *F. vesiculosus* had levels comparable only to those present in *M. stellatus*. All macroalgal species studied contained exceptionally high amounts of iodine compared to other minerals, with *Ulva* spp. containing the lowest amount of I (92.8 mg/kg DM), and *S. latissima* the highest levels of I (4131 mg/kg DM) followed by *L. ochroleuca* (2780 mg/kg DM).

Table 4. Average concentrations (expressed as mean ± standard deviation, n=3) of essential trace elements (mg/kg DM) in selected macroalgal species from the Atlantic coast of Galicia (NW Spain).

Species	**Co**	**Cr**	**Cu**	**Fe**	**I**	**Mn**	**Mo**	**Ni**	**Se**	**Zn**
F. vesiculosus	1.85±1.07	15.7±8.4	5.83±0.43	1233±107	396±64	597±74	1.070±0.281	8.45±2.80	0.296±0.016	92.4±70.7
H. elongata	1.41±0.06	2.01±0.47	2.06±0.16	57±8	134±7	53.8±12.4	0.280±0.010	3.25±2.80	0.096±0.032	53.2±3.0
U. pinnatifida	0.353±0.029	1.53±1.32	0.84±0.10	97±24	284±27	10.3±2.2	0.232±0.026	0.957±0.42	0.143±0.041	23.2±6.61
L. ochroleuca	0.256±0.018	1.62±0.78	1.43±0.24	122±38	2780±216	10.8±3.8	0.224±0.031	0.881±0.334	0.183±0.021	25.0±2.4
S. latissima	0.196±0.064	4.12±1.80	1.18±0.29	446±174	4131±202	14.1±6.21	0.320±0.087	1.47±0.342	0.149±0.043	32.4±8.6
Ulva spp.	0.283±0.042	4.29±2.94	2.65±0.39	460±155	92.8±4.42	37.7±10.1	0.257±0.038	5.01±0.17	0.144±0.034	10.4±0.7
M. stellatus	0.582±0.070	12.5±6.23	3.86±1.90	1403±103	204±118	45.9±17.9	0.567±0.058	9.88±2.99	0.172±0.038	50.9±28.9

Regarding potentially toxic trace elements, the macroalgae evaluated in this study accumulated lower levels of Cd (ranging from 0.095 to 1.18 mg/kg DM), Hg (0.004-0.039 mg/kg DM) and Pb (0.267-1.48 mg/kg DM) (see Table 5) compared to the aforementioned levels of macrominerals and essential trace elements. By contrast, As was the only toxic element detected at similar levels of magnitude to those of macrominerals and essential trace elements. Brown macroalgal samples, had the highest contents of As (46.3-68.9 mg/kg DM), while the lowest levels of this toxic element were detected in *Ulva* spp. (4.10 mg/kg DM).

Table 5. Average concentrations (expressed as mean ± standard deviation, n=3) of potentially toxic trace elements (mg/kg DM) in selected macroalgal species from the Atlantic coast of Galicia (NW Spain).

Species	**Cd**	**Hg**	**Pb**	**As**
F. vesiculosus	0.693±0.151	0.012±0.004	1.480±0.628	54.6±10.6
H. elongata	0.383±0.017	0.004±0.001	0.267±0.039	46.3±2.69
U. pinnatifida	0.388±0.055	0.012±0.007	0.541±0.310	48.1±4.49
L. ochroleuca	0.225±0.113	0.010±0.003	0.347±0.087	56.0±2.0
S. latissima	0.264±0.095	0.019±0.005	0.452±0.085	68.9±13.0
Ulva spp.	0.095±0.002	0.007±0.000	0.624±0.059	4.10±0.37
M. stellatus	1.184±0.531	0.039±0.025	0.447±0.116	14.1±0.35

The speciation of As into its inorganic and organic forms was performed in all the macroalgal samples as summarized in Table 6. This analysis aimed to elucidate the most common As species and potential risks associated to the total As concentrations described previously. Total recoveries ranged between 60 and 90% of total As. iAs occurred as As (III) and As (V), and the concentrations of these species in all macroalgae were below 1 mg/kg DM. Most As was present in an organic form, being the arsenosugars the most relevant fraction of those studied. DMA, MMA and AsB concentrations were, by comparison, the least common As species in all samples.

Table 6. Average concentrations (expressed as mean ± standard deviation, n=3) of As speciation (mg/kg DM) in selected macroalgal species from the Atlantic coast of Galicia (NW Spain).

Abbreviations within the table are as follows DMA (dimethylarsinic acid), MMA (monomethylarsonic acid), AsB (arsenobetaine) and LD (limit of detection).

Species	**As III**	**As V**	**As-sugars**	**DMA**	**MMA**	**AsB**
F. vesiculosus	0.189±0.167	0.089±0.100	26.11±11.5	0.801±0.158	0.538±0.483	0.119±0.124
H. elongata	0.337±0.337	0.093±0.087	16.79±5.11	0.577±0.046	0.127±0.012	0.061±0.004
U. pinnatifida	0.149±0.243	0.001±0.001	27.58±2.76	1.088±0.001	0.124±0.095	0.077±0.147
L. ochroleuca	0.362±0.296	0.126±0.101	31.81±6.24	0.779±0.054	0.158±0.065	0.049±0.040
S. latissima	0.193±0.280	0.406±0.051	49.87±11.66	0.938±0.389	0.342±0.203	0.188±0.067
Ulva spp.	0.004±0.004	0.182±0.018	1.39±0.17	0.478±0.638	0.737±0.720	0.113±0.052
M. stellatus	<LD	0.150±0.107	7.60±0.36	0.318±0.186	0.349±0.007	0.010±0.013

3.2. Variation of mineral concentrations in macroalgal extracts

The variation in mineral concentrations between extracts with respect to the intact biomass are represented in Figure 1. There were high variations in the levels of the majority of macrominerals (except Ca and P) between the extracts and the biomass. Overall, the levels of macrominerals in the extracts were higher, ranging from to 6-162%, compared to the ones detected in the intact macroalgae, except in the case of *S. latissima* and *M. stellatus*. The concentration of K in the extracts of *U. pinnatifida* (331.9 g/kg DM) and *L. ochroleuca* (313.4 g/kg DM), and the concentration of most minerals in the extract from *Ulva* spp., were higher compared to those of the dried biomass. The concentrations of essential trace elements were generally lower (by 20-98%) in the extracts compared to those of the biomass, with a few exceptions (i.e. Cr in *H. elongata*). The concentration of I was maintained or increased greatly in the extracts of most species compared to those of the intact macroalgal biomass, especially in the case of *L. ochroleuca* and *M. stellatus* that showed increases of 110 and 197%, respectively. By contrast, the extraction process had a positive effect on the content of potentially toxic trace elements, as the levels of most of these elements were greatly reduced by 20-97% compared to those of the biomass. In the case of As, the extraction had variable effects depending on the species, being at slightly lower (20-25%) or sometimes higher (7-40%) concentrations in the extracts compared to those described in the dried biomass. In turn, iAs was significantly lower (up to 100%) in the extracts of most macroalgal species, being only slightly higher in *H. elongata* and *M. stellatus*, compared to those of the original macroalgae. Extracts of *Ulva* spp. had levels of iAs 200% higher than those of the original biomass. Nonetheless, iAs was present at concentrations below 0.6 mg/kg DM in all extracts studied.

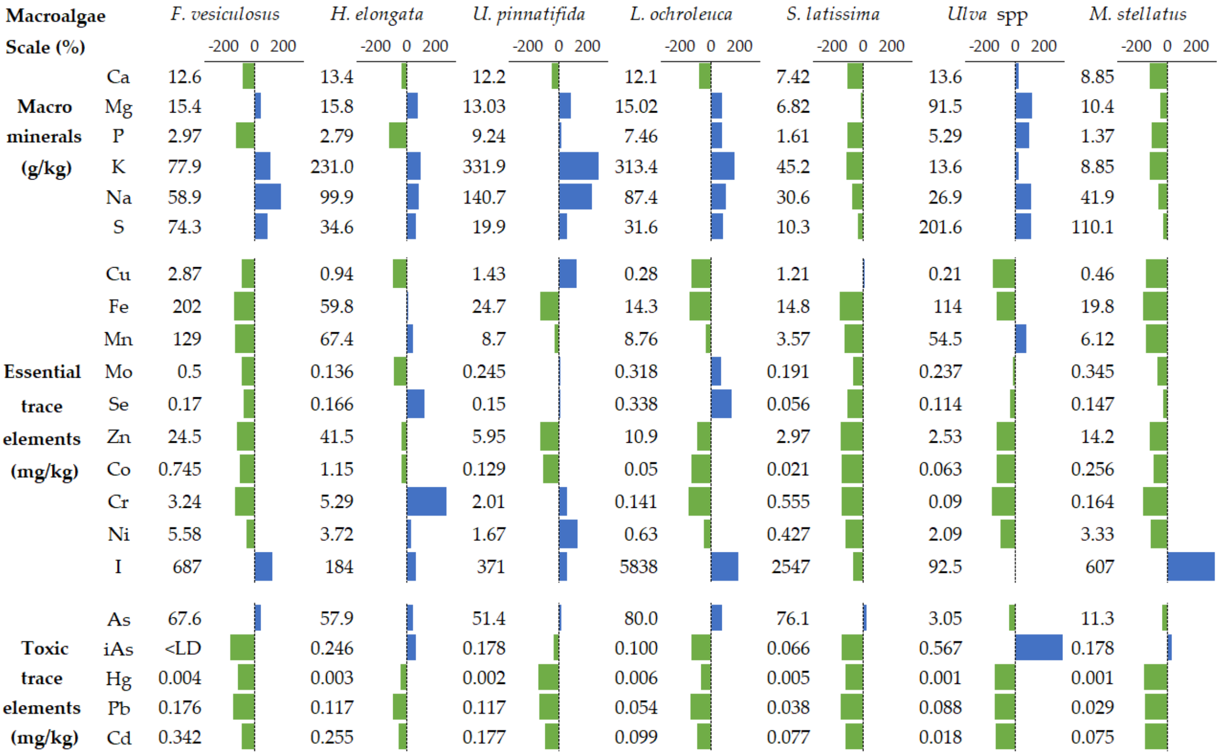

Figure 1. Variation of the mineral concentrations in macroalgal extracts represented as percentage of increase (blue) or decrease (green) relative to those the dried macroalgae (200% scale range).

3.3. Limits of inclusion of macroalgal biomass and extracts in animal feed

According to our results, I was the main limiting factor when including macroalgal products (biomass and extracts) in animal feed. The limits of inclusion calculated for macroalgal biomass and extracts are presented in Table 7. These limits were calculated considering the amount of I already provided by feed materials in standard diets (without mineral supplementation; data taken from Van Paemel *et al*. (Van Paemel et al., 2010)) and the maximum I concentration allowed in complete feed (Regulation EC 1459/2005). The levels of inclusion varied between the different livestock species/categories, as the maximum I concentration in feed allowed by legislation varies between livestock. Thereby, maximum I concentration varied from

5 mg/kg, allowed in the complete feed of laying hens and dairy cows, to 20 mg/kg allowed in complete feed of fish. The background iodine in standard diets is relatively low and similar between all livestock species studied, except in the case of fish diets.

Most macroalgal species could be included in animal feed at rates ranging between 1 and 3% (*F. vesiculosus, H. elongata, U. pinnatifida* and *M. stellatus*) and 5% (*Ulva* spp.) when used as dried biomass for laying hens and dairy cattle. These levels of inclusion increased by 4 times when considering their use in fish diets and 2 times in other livestock species studied. Overall, as seen in Table 7, the limits of inclusion of macroalgal extracts were lower than those of the original biomass in all cases, except for *Ulva* spp. The rates of inclusion for *L. ochroleuca* and *S. latissima*, were lower compared to the other macroalgal species studied due to their iodine contents. *L. ochroleuca* could be added at a rate between 0.16 and 0.66% when used as dried biomass, and between 0.076-0.32% when using their extracts. Regarding *S. latissima*, the dried macroalgae could be added at a rate ranging between 0.11 and 0.45%, while the amount of extracts was limited to 0.18-0.72%.

Table 7. Maximum levels of inclusion in the diet (expressed in %) of the macroalgal biomass and extracts from selected macroalgal species in multiple livestock species.

Livestock species		**Porcine**	**Broilers**	**Laying hens**	**Ruminants**	**Dairy cows**	**Rabbits**	**Fish**
I concentration standard diet (mg/kg)		0.177	0.103	0.073	0.491	0.538	0.252	1.61
Maximum I regulatory limits (mg/kg)[1]		10	10	5	10	5	10	20
F. vesiculosus	Biomass	2.48	2.50	1.25	2.41	1.13	2.47	4.67
	Extract	1.43	1.44	0.72	1.39	0.65	1.42	2.69
H. elongata	Biomass	7.32	7.37	3.67	7.10	3.33	7.27	13.9
	Extract	5.35	5.39	2.68	5.19	2.44	5.31	10.1
U. pinnatifida	Biomass	3.46	3.48	1.73	3.35	1.57	3.43	6.51
	Extract	2.65	2.67	1.33	2.57	1.21	2.63	4.98
L. ochroleuca	Biomass	0.35	0.36	0.18	0.34	0.16	0.35	0.66
	Extract	0.17	0.17	0.084	0.16	0.076	0.17	0.32
S. latissima	Biomass	0.24	0.24	0.12	0.23	0.11	0.24	0.45

	Extract	0.39	0.39	0.19	0.37	0.18	0.38	0.72
Ulva spp.[2]	Biomass	10.6	10.7	5.31	10.3	4.84	10.5	20.2
	Extract	10.6	10.7	5.33	10.3	4.85	10.6	20.2
M. stellatus [2]	Biomass	4.83	4.87	2.42	4.68	2.19	4.80	9.11
	Extract	1.62	1.63	0.81	1.57	0.74	1.61	3.04

[1] Regulation EC 1459/2005. [2] For *Ulva* spp. and *M. stellatus* the recommended inclusion rates according to the S content should be used.

Apart from I, most essential trace elements were found in macroalgae at concentrations one order of magnitude lower than the limits established by legislation (Table 8) for the different livestock species/categories. However, some macroalgal products exceeded these limits for some minerals. Thereby, the limits of Fe (450-750 mg/kg) were exceeded by *F. vesiculosus* and *M. stellatus* biomass; those of Mn (100-150 mg/kg) were surpassed by dried *F. vesiculosus*; and in the case of Co (1 mg/kg) this limit was exceeded by dried *F. vesiculosus*, and extracts from *H. elongata*. Nevertheless, for all these macroalgal products, the iodine content restricted the amount of macroalgae that could be included in the diet, and therefore, the limits of inclusion of the other essential trace elements (> 20%; data not shown) would always be fulfilled by small % of inclusion of macroalgal products in feed.

Table 8. Maximum levels of essential trace elements allowed in complete feed (mg/kg complete feed, moisture: 12%)[1].

Maximum level	**Porcine**	**Poultry**	**Bovine**	**Ovine**	**Caprine**	**Rabbits**	**Fish**
Co	1	1	1	1	1	1	1
Cu	25 (100-150)[2]	25	30 (15)[3]	15	35	25	25
Fe	750	450	450	500	750	750	750
Mn	150	150	150	150	150	150	100
Se	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zn	150	120	120	120	120	150	150

[1] Regulation EC 1334/2003 and EU 1039/2018. [2] In pigs and sows: 25 mg/kg; in sucking and weaned piglets up to 8 weeks after weaning: 150 mg/kg; from 5-8 weeks after weaning: 100 mg/kg. [3] Bovine species before the start of rumination: 15 mg/kg; other bovines: 30 mg/kg.

When considering the concentrations of potentially toxic elements in the macroalgae evaluated in our study, Cd, Pb and Hg were in all cases below the maximum levels allowed for feed materials (1, 5 and 0.1 mg/kg feed with a moisture content of 12 %; Directive EC 32/ 2002 and Regulation EC 1275/2013) (Table 9). By contrast, total As concentrations were above the maximum allowed concentration for seaweed meal and feed materials derived from seaweed (40 mg/kg relative to a feed with a moisture content of 12 %, equivalent to 45.5 mg/kg DM) in all brown macroalgal species, even though iAs was in all cases lower than 2 mg/kg feed. Nonetheless, the maximum levels of inclusion of macroalgae in complete feed (maximum total As concentration of 10 mg/kg feed) were in all cases higher (> 12%) than those estimated for I.

Table 9. Maximum allowed levels of potentially toxic trace elements in complete feed (mg/kg complete feed, moisture:12%)[1].

Mineral	**Cd**	**Hg**	**Pb**	**As**
Feed materials derived from seaweed	1	0.1	10	40[2]
Complete feed	0.5	0.1	5	10

[1] Directive EC 32/ 2002 and Regulation EC 1275/2013. [2] Upon request by the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg. This analysis is of particular importance for the macroalgal species *Hizikia fusiforme.*

No legal limits have been established yet in the EU regarding macrominerals in animal feed. The maximum mineral tolerances (Table 10) established by the National Research Council (National Research Council, 2005) were used to estimate the maximum amounts of macroalgae that can be included in the diets. Livestock species generally tolerate macromineral concentrations in the diet that are higher than those of the present study. Only two exceptions were appreciated, the case of Mg for *Ulva* spp., and S for both *Ulva* spp. and *M. stellatus*. Mg was mostly limiting in the case of porcine diets, and S was mainly limiting for ruminants. Therefore, in these livestock, the levels of inclusion in feed of both macroalgal species would have to be adjusted. Out of these two minerals, S was the main limiting for both macroalgal species, as it limited the amounts of macroalgae that could be included in livestock's diets: 1.67% inclusion of *Ulva* spp. biomass, and 1% of its extract; and 1.5% of dried *M. stellatus*, and 1.84% for its extract (considering the amount of S already present in ruminants' diets). These inclusion rates would be similar in other livestock species, for which the maximum recommended levels of S are just slightly higher (4 g/kg in swine and poultry) than those described in ruminants.

Table 10. Maximum mineral tolerance (% in complete feed) for macrominerals in different livestock species (NRC, 2005).

Maximum level (%)	**Porcine**	**Broilers**	**Laying hens**	**Ruminants**	**Rabbits**	**Fish**
Ca	1	1.5	5	1.5	2	0.9
Mg	0.3	0.5	0.75	0.6	-	-
P	1.5	1	0.8	1	1	-
K	3	3	3	3	3	3
Na	0.1	0.15	-	3	-	-
S	0.4	0.4	-	0.3	-	-

4. Discussion

4.1. Mineral composition of dried macroalgal biomass

Only a broad comparison of our macromineral results can be made with previous data, as previously published findings often refer to an entire genus (e.g. *Laminaria* or *Fucus*) or to macroalgal species similar to those of this study, but not identical (i.e. *L. ochroleuca* was selected while most reports focus on *Laminaria digitata*). Moreover, as mentioned previously, the levels of minerals in macroalgae can be influenced by the season of collection and geographical location of the biomass used in each study (Garcia-Vaquero et al., 2021).

Overall, the macromineral composition reported in this study was similar to that of previous reports (see Supplementary Table 1). The levels of K, Na, Mg, Ca and S accounted for more than 97% of total mineral content of macroalgae (Biris-Dorhoi et al., 2020; Cabrita et al., 2016; Circuncisão et al., 2018), while the levels of P were lower compared to those of the other macrominerals. Previous studies reported differences in mineral content between brown, red and green macroalgae. The contents of Na and K in brown macroalgae were described as higher compared to any other macroalgal types; red macroalgae contained remarkably higher Ca levels compared to those of brown and green macroalgae; and green macroalgae was described as a biomass rich in Mg (Circuncisão et al., 2018). Sulphur is a main component of sulphated polysaccharides, such as fucoidans from brown macroalgae, ulvans in green macroalgae and galactans in red macroalgae (Rupérez, 2002), fact that could possibly explain the relatively high concentrations of this element in *F. vesiculosus, Ulva* spp. and *M. stellatus* compared to other species (Circuncisão et al., 2018).

When comparing the macromineral levels of the macroalgae studied with other commonly used ingredients in animal nutrition (INRA feed tables), the concentrations of most macrominerals were at least one order of magnitude higher in macroalgae than in most cereals and legumes. For instance, Ca was present at low concentrations in most cereals and legumes (0.4-5.5 g/kg DM), while the values of this micromineral reached levels ranging from 12.3 to 31.1 g Ca/kg DM in macroalgae. Calcium concentrations in macroalgae were comparable to other ingredients of animal origin, such as those of fish meal and processed animal proteins (20.9-48.6 g/kg DM). Likewise, in the case of P, this element was present at similar levels in macroalgae (3.41-13.5 g/kg DM) and in most processed animal proteins (6.4-44.4 g/kg DM), but at higher levels than those reported in most cereals and legumes (2.4-6.3 g/kg DM). Macroalgae had much higher concentrations of Mg, K, Na and S than any other animal feed ingredient, since those were found at very low levels in all cereals (Mg: 1-2; K: 2-5.5; Na: 0.04-0.35; S: 0.5-1.8 g/kg DM), legumes (Mg: 1.3-4; K: 8.2-20.2; Na: 0-0.95; S: 1.6-3.6 g/kg DM) and animal-based ingredients (Mg: 0.7-9.1; K: 1.4-11.9; Na: 0.99-11.7; S: 1.2-18.2 g/kg DM). Indeed, complete feed for livestock is usually supplemented with mineral premixes which include essential macrominerals (National Research Council, 2005) to counteract the low levels of most of these elements in commonly used feed ingredients (Bhanderi et al., 2016). Thus, macroalgae are a source of these minerals, and their inclusion at the doses recommended in this study in different livestock's feed would help to provide the majority of the macromineral requirements needed by the animals. This would help to overcome the economic challenge of the soaring prices of macrominerals in modern animal production systems due to a growing global demand and limited availability of traditional mineral natural resources (Yıldız, 2022).

Regarding essential trace elements composition, the findings of this study were in agreement with those published in recent literature (Supplementary Table 2). However, the levels of Fe and Mn reported in this study were relatively higher than those reported elsewhere for *F. vesiculosus*, and *M. stellatus*; while the concentrations of Se were lower in *S. latissima* or *Ulva* spp. compared to previous reports. The I contents in this study were within the range of those previously reported; although the levels of I reported in some species, such as *L. ochroleuca* and *S. latissima*, varied widely across studies.

Unlike macrominerals, most essential trace elements were present in macroalgae within the same concentration ranges as those described for the main ingredients used in livestock nutrition (INRA feed tables) including cereals (Zn: 15-34; Cu: 3-13; Se: 0.08-0.5; Co: 0.03-0.3; Mo: 0.2-1 mg/kg DM) and legumes (Zn: 29-52; Cu: 3-61; Se: 0.01-0.8; Co: 0.01-0.4; Mo: 0.2-4 mg/kg DM). However, in some cases their concentrations were lower compared to those of animal-based ingredients (Zn: 24-138; Cu: 10-40; Se: 0.4-2; Co: 0.1-3; Mo: 0.1-1 mg/kg DM), with I being the most remarkable exception, as it exceeded the content of any other feed ingredient (range 0.01-3 mg I/kg DM) by several orders of magnitude, especially in *L. ochroleuca* and *S. latissima*. The concentrations of Fe were high in *F. vesiculosus* (1233 mg/kg DM) and *M. stellatus* (1403 mg/kg DM) compared to most cereals and legumes (40-422 mg/kg DM), and they were similar to those of animal-based ingredients (59-5131 mg/kg DM). The concentration of Mn was higher in *F. vesiculosus* (597 mg/kg DM) than in most feed ingredients (8-65 mg/kg DM in cereals and legumes, 3-258 mg/kg DM in animal-based ingredients).

These findings support the idea that macroalgae, particularly *F. vesiculosus, M. stellatus, L. ochroleuca* and *S. latissima*, can be potentially used as sources of Fe, Mn and I (Lozano Muñoz and Díaz, 2020). Furthermore, these macroalgal species could be included in animal feed to meet the mineral requirements of livestock (Cabrita et al., 2016; Costa et al., 2021; Øverland et al., 2019), while enriching animal-derived products with some essential minerals (Circuncisão et al., 2018; Morais et al., 2020). In fact, some macroalgae have been used historically to prevent goitre, and their addition to animal feed, as an alternative to iodized salts, has been explored as a mechanism to prevent iodine deficiency in many European countries (Circuncisão et al., 2018; Rey-Crespo et al., 2014).

The levels of toxic trace elements reported in this study were also in agreement with previous findings (Supplementary Table 3), although there are relatively few studies using the macroalgal species selected in this study. As mentioned previously, the absorption of toxic substances by macroalgae is dependent on many environmental factors, as well as on the polysaccharide composition of each macroalgae, as these polymers have variable affinity for different toxic trace elements (Circuncisão et al., 2018). Overall, the data reported in the literature on the toxic trace elements of macroalgae is extremely variable, with levels similar to those described in other terrestrial feedstuffs from relatively unpolluted regions (López-Alonso, 2012). However, total As residues in macroalgal species, particularly in brown algae, are up to some orders of magnitude higher compared to those in most terrestrial feedstuffs/feed ingredients (Van Paemel et al., 2010).

The results for As speciation in this study showed good recoveries of total As, similarly to previous studies analysing macroalgae (Wolle et al., 2021). Contents below 1 mg/kg DM of iAs were reported in this study for all macroalgae, similarly to previous reports (Rose et al., 2007; Wolle et al., 2021). The high proportion of organic forms, like arsenosugars, has been previously reported in several macroalgal species (Biancarosa et al., 2018; Díaz et al., 2012; Wolle et al., 2021). Arsenosugars have not been defined yet as non-toxic, therefore, their level of consumption has to be considered as a potential health risk (Camurati and Salomone, 2020). Other As species considered as having low levels of toxicity, i.e. dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), or with no risk of toxicity, i.e. arsenobetaine (AsB), were present at negligible concentrations. Similar patterns of As speciation have been reported in most macroalgal species (Camurati and Salomone, 2020; Rose et al., 2007), indicating the capacity of macroalgae to metabolize the toxic iAs species (As III and As V) present in seawater to less toxic forms, such as DMA, MMA and arsenosugars. However, other macroalgae (e.g. brown macroalgal species from the Sargassaceae family or Hijiki) do not have this capacity; and therefore, most As of this biomass is composed of iAs, which constitutes a risk for consumers (Rose et al., 2007). Our results confirm that in these particular macroalgal species, As would not pose a risk either to animals or to consumers of animal-derived products.

4.2. Variation of mineral concentrations in the macroalgal extracts

In recent years, extracts from macroalgae have been incorporated in animal feed due to their prebiotic content or potential immunomodulatory properties. Our findings indicate that macroalgal extracts can provide valuable contents of macrominerals, especially I, which at the same time constitute a limitation for the inclusion of high amounts of extracts in animal feed. It is worth noting that the results of this study refer to extracts generated using a specific processing technology. However, variations in the extraction technology used (conventional versus innovative technologies) and even processing conditions used with the same technology (i.e. temperature, solvents, pH) have a significant influence on the chemical composition of the extracts achieved from the initial biomass (Kadam et al., 2013), and thus, the levels of minerals in extracts may vary widely depending on the processing conditions of the biomass. Further research will be needed exploring the effect of these technologies and other processing conditions on the mineral levels of the resulting macroalgal products.

4.3. Limits of inclusion of macroalgal biomass and extracts in animal feed

In order to guarantee safety to animals, consumers and the environment, the EU has established maximum permissible levels of essential trace and potentially toxic elements in complete feed. The maximum I concentrations allowed in animal feed were exceeded by up two-fold in all the macroalgal species studied. Thus, the maximum level of inclusion of different macroalgal products, as dried biomass or extracts, must be finely tuned.

The ranges of inclusion of macroalgae calculated in this study are in agreement with current inclusion rates of dried macroalgae in animal studies, since the beneficial effects of the addition of macroalgae are generally appreciated at levels of inclusion of less than 2% macroalgae in feed (Evans and Critchley, 2014). The limits of inclusion of extracts recommended in this study are lower than those of their original biomass (except in the case of *Ulva* spp.), supporting also the addition of less than 1 % of extract in animal studies. *L. ochroleuca* and *S. latissima* deserve special attention due to their extremely levels of I, and therefore, lower amounts of these macroalgae and their extracts can be included in animal feed compared to the other macroalgal species studied.

It is important to note that all inclusion rates were calculated from the values obtained for the macroalgae under study, which, as already discussed, can vary widely depending on multiple factors. Therefore, these values only provide an idea of the range of inclusion of some species and highlight the importance of analysing macroalgae before their inclusion in animal feed.

5. Conclusions

This study provides a detailed mineral profiling (essential macro and microminerals and potentially toxic elements) of multiple macroalgal species growing on the Atlantic coast of Galicia (NW Spain) and their extracts. Additionally, the study demonstrates the suitability of macroalgal biomass and their derived extracts for their inclusion in animal feed as source of minerals to meet the physiological requirements of multiple livestock. In comparison with other feed ingredients, macroalgae had high levels of most macrominerals (Ca, Na, K, Mg, S) and in some species, essential trace elements, mainly I, as well as Fe and Mn. Although macroalgae had high levels of As compared to other feed ingredients, this element was mainly present in its organic form, mostly as arsenosugars, of relatively low toxicity, while the inorganic and toxic As form was comparatively present at really low levels in all samples. Therefore, the inclusion of these macroalgal species and extracts will not constitute a risk when used at the doses currently used in animal feed. Overall, the generation of aqueous extracts increased the concentrations of all macrominerals and I, and reduced those of essential and potentially toxic trace elements compared to the original biomass. Calculation of the limits of inclusion in feed according to the EU maximum regulatory concentrations in animal nutrition demonstrated that I was the main element limiting the inclusion of macroalgal biomass and extracts in all animal species studied. Most of these products can be used in feed at the current levels of inclusion reported in the literature, ranging from 1 to 5%, without negative effects. The recommended levels of inclusion in feed were lower in the case of *L. ochroleuca* and *S. latissima* compared to the other species studies due to their exceptionally high levels of I. Thus, the inclusion of these species in animal feed should be finely measured.

Overall, this study demonstrates that macroalgal biomass and their extracts are an excellent source of macrominerals, mainly I, and that their inclusion in feed can be considered as good quality source of minerals in animal feed. This strategy could help to overcome the challenge of the soaring prices of these minerals used as feed supplements in multiple livestock production systems. Future research should explore in detail the economic and sustainability implications of this shift in mineral supplementation if implemented at larger scale globally for all livestock's feed, aiming to confirm the potential benefits of the inclusion of macroalgae compared to the traditional sources of minerals.

Ethics approval: Not applicable**.**

Competing interest: There are no conflicts to declare.

Acknowledgements: Authors would like to thank the use of RIAIDT-USC analytical facilities.

Authors contribution: Conceptualization: J.G., M.L.-A., M.G.-V. and A.M.; methodology: M.M., V.Pi., V.Pe.; investigation: S.A.-S., M.L.-A., M.M., V.Pi. and V.Pe.; resources: E.C., A.M., M.L.-A. and J.G.; data curation: S.A.-S. and M.L.-A.; writing—original draft preparation: S.A.-S. and M.L.-A.; writing—review and editing: S.A.-S., M.L.-A., M.M. and M.G.-V.; supervision: M.L.-A. and M.M.; project administration: E.C., A.M., M.L.-A. and J.G.; funding acquisition: E.C., A.M., M.L.-A. and J.G. All authors read and approved the final manuscript.

Funding: This research was carried out within the innovation project TIRAC, co-financed by 80% by the European Agricultural Fund for Rural Development (EAFRD) of the European Union and by 20% by the Ministry of Agriculture, Fisheries and Food, within the framework of the National Rural Development Program 2014–2020. The General Directorate for Rural Development, Innovation and Agrifood Training (DGDRIFA) is the authority in charge of applying this aid. Budget: EUR 492,580.38. Total grant: EUR 485,043.58. Funding number: 2020-PN216 (O00000226e2000044671). Sabela Al-Soufi, Víctor Pereira, Marta Miranda and Marta López-Alonso belong to "Grupo de Potencial Crecimiento" financed by GAIN (Axencia Galega de Innovación) (grant number ED431B 2023/008).

Availability of data and materials: Data will be made available on reasonable request.

References

Almela, C., Algora, S., Benito, V., Clemente, M.J., Devesa, V., Súñer, M.A., Vélez, D., Montoro, R., 2002. Heavy metal, total arsenic, and inorganic arsenic contents of algae food products. J Agric Food Chem 50, 918–923. https://doi.org/10.1021/jf0110250

Almela, C., Jesús Clemente, M., Vélez, D., Montoro, R., 2006. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. Food and Chemical Toxicology 44, 1901–1908. https://doi.org/10.1016/j.fct.2006.06.011

Al-Soufi, S., Nicodemus, N., Carro, M.D., López-Alonso, M., Miranda, M., Muíños, A., Cegarra, E., Vázquez-Belda, B., Domínguez, H., Torres, M.D., Flórez-Fernández, N., García, J., 2023. Marine Macroalgae in Rabbit Nutrition: In Vitro Digestibility, Caecal Fermentability, and Microbial Inhibitory Activity of Seven Macroalgae Species from Galicia (NW Spain). Agriculture 13. https://doi.org/10.3390/agriculture13101995

Bhanderi, B.M., Goswami, A., Garg, M.R., Samanta, S., 2016. Study on minerals status of dairy cows and their supplementation through area specific mineral mixture in the state of Jharkhand. J Anim Sci Technol 58. https://doi.org/10.1186/s40781-016-0124-2

Biancarosa, I., Belghit, I., Bruckner, C.G., Liland, N.S., Waagbø, R., Amlund, H., Heesch, S., Lock, E.J., 2018. Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed. J Sci Food Agric 98, 2035–2042. https://doi.org/10.1002/jsfa.8798

Bikker, P., Stokvis, L., van Krimpen, M.M., van Wikselaar, P.G., Cone, J.W., 2020. Evaluation of seaweeds from marine waters in Northwestern Europe for application in animal nutrition. Anim Feed Sci Technol 263. https://doi.org/10.1016/j.anifeedsci.2020.114460

Biris-Dorhoi, E.S., Michiu, D., Pop, C.R., Rotar, A.M., Tofana, M., Pop, O.L., Socaci, S.A., Farcas, A.C., 2020. Macroalgae—A sustainable source of chemical compounds with biological activities. Nutrients 12, 1–23. https://doi.org/10.3390/nu12103085

Cabrita, A.R.J., Maia, M.R.G., Oliveira, H.M., Sousa-Pinto, I., Almeida, A.A., Pinto, E., Fonseca, A.J.M., 2016. Tracing seaweeds as mineral sources for farm-animals. J Appl Phycol 28, 3135–3150. https://doi.org/10.1007/s10811-016-0839-y

Camurati, J.R., Salomone, V.N., 2020. Arsenic in edible macroalgae: an integrated approach. J Toxicol Environ Health B Crit Rev 23, 1–12. https://doi.org/10.1080/10937404.2019.1672364

Circuncisão, A.R., Catarino, M.D., Cardoso, S.M., Silva, A.M.S., 2018. Minerals from macroalgae origin: Health benefits and risks for consumers. Mar Drugs. https://doi.org/10.3390/md16110400

Costa, M., Cardoso, C., Afonso, C., Bandarra, N.M., Prates, J.A.M., 2021. Current knowledge and future perspectives of the use of seaweeds for livestock production and meat quality: a systematic review. J Anim Physiol Anim Nutr (Berl) 105, 1075–1102. https://doi.org/10.1111/jpn.13509

De Jesus Raposo, M.F., De Morais, A.M.M.B., De Morais, R.M.S.C., 2016. Emergent sources of prebiotics: Seaweeds and microalgae. Mar Drugs 14, 1–27. https://doi.org/10.3390/md14020027

de Oliveira Filho, E.F., Miranda, M., Ferreiro, T., Herrero-Latorre, C., Castro Soares, P., López-Alonso, M., 2022. Concentrations of Essential Trace and Toxic Elements Associated with Production and Manufacturing Processes in Galician Cheese. Molecules 27. https://doi.org/10.3390/molecules27154938

Díaz, O., Tapia, Y., Muñoz, O., Montoro, R., Velez, D., Almela, C., 2012. Total and inorganic arsenic concentrations in different species of economically important algae harvested from coastal zones of Chile. Food and Chemical Toxicology 50, 744–749. https://doi.org/10.1016/j.fct.2011.11.024

Evans, F.D., Critchley, A.T., 2014. Seaweeds for animal production use. J Appl Phycol 26, 891–899. https://doi.org/10.1007/s10811-013-0162-9

Feedipedia. (2024). Feed categories. Forage plants. INRA, CIRAD and FAO https://www.feedipedia.org/content/feeds?category=15965. Accessed on 29th October 2024.

Foster, S., Maher, W., Krikowa, F., Apte, S., 2007. A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues. Talanta 71, 537–549. https://doi.org/10.1016/j.talanta.2006.04.027

Garcia-Vaquero, M., Rajauria, G., Miranda, M., Sweeney, T., Lopez-Alonso, M., O'doherty, J., 2021. Seasonal Variation of the Proximate Composition, Mineral Content, Fatty Acid Profiles and Other Phytochemical Constituents of Selected Brown Macroalgae. Mar Drugs 19. https://doi.org/10.3390/MD19040204

Horwitz, W., 2010. Agricultural Chemicals, Contaminants, Drugs, in: Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, MD, USA.

INRA-CIRAC-AFZ feed tables (2017-2021) [WWW Document], 2023. . https://www.feedtables.com .

Juskelis, R., Li, W., Nelson, J., Cappozzo, J.C., 2013. Arsenic speciation in rice cereals for infants. J Agric Food Chem 61, 10670–10676. https://doi.org/10.1021/jf401873z

Kadam, S.U., Tiwari, B.K., O'Donnell, C.P., 2013. Application of novel extraction technologies for bioactives from marine algae. J Agric Food Chem. https://doi.org/10.1021/jf400819p

López-Alonso, M., 2012. Animal feed contamination by toxic metals, in: Animal Feed Contamination: Effects on Livestock and Food Safety. Woodhead Publishing, pp. 183–204. https://doi.org/10.1533/9780857093615.2.183

López-Alonso, M., Rey-Crespo, F., Orjales, I., Rodríguez-Bermúdez, R., Miranda, M., 2016. Effects of different strategies of mineral supplementation (marine algae alone or combined with rumen boluses) in organic dairy systems. J Anim Physiol Anim Nutr (Berl) 100, 836–843. https://doi.org/10.1111/jpn.12449

Lozano Muñoz, I., Díaz, N.F., 2020. Minerals in edible seaweed: health benefits and food safety issues. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2020.1844637

Mahadevan, K., 2015. Seaweeds: A sustainable food source, in: Seaweed Sustainability: Food and Non-Food Applications. Elsevier Inc., pp. 347–364. https://doi.org/10.1016/B978-0-12-418697-2.00013-1

Marschner, K., Musil, S., Mikšík, I., Dědina, J., 2018. Investigation of hydride generation from arsenosugars - Is it feasible for speciation analysis? Anal Chim Acta 1008, 8–17. https://doi.org/10.1016/j.aca.2018.01.009

Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L., Bahcevandziev, K., 2020. Seaweed potential in the animal feed: A review. J Mar Sci Eng 8, 1–24. https://doi.org/10.3390/JMSE8080559

Morales-Rodríguez, A., Pérez-López, M., Puigpelat, E., Sahuquillo, À., Barrón, D., López-Sánchez, J.F., 2022. Arsenosugar extracted from algae: Isolation by anionic exchange solid-phase extraction. J Chromatogr A 1684. https://doi.org/10.1016/j.chroma.2022.463549

National Research Council, 2005. Mineral tolerance of animals, 2nd Revised Edition. ed. The National Academies Press, Washington DC.

Øverland, M., Mydland, L.T., Skrede, A., 2019. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. J Sci Food Agric 99, 13–24. https://doi.org/10.1002/jsfa.9143

Rey-Crespo, F., López-Alonso, M., Miranda, M., 2014. The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. Animal 8, 580–586. https://doi.org/10.1017/S1751731113002474

Romarís-Hortas, V., García-Sartal, C., Barciela-Alonso, M. del C., Domínguez-González, R., Moreda-Piñeiro, A., Bermejo-Barrera, P., 2011. Bioavailability study using an in-vitro method of iodine and bromine in edible seaweed. Food Chem 124, 1747–1752. https://doi.org/10.1016/j.foodchem.2010.07.117

Rose, M., Lewis, J., Langford, N., Baxter, M., Origgi, S., Barber, M., MacBain, H., Thomas, K., 2007. Arsenic in seaweed-Forms, concentration and dietary exposure. Food and Chemical Toxicology 45, 1263–1267. https://doi.org/10.1016/j.fct.2007.01.007

Van Paemel, M., Dierick, N., Janssens, G., Fievez, V., De Smet, S., 2010. Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. EFSA Supporting Publications 7, 1–1132. https://doi.org/10.2903/sp.efsa.2010.en-68

Wolle, M.M., Todorov, T.I., Conklin, S.D., 2021. Arsenic Species in Seaweeds Commercially Available in the United States. ACS Food Science and Technology 1, 511–523. https://doi.org/10.1021/acsfoodscitech.0c00120