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Enhancing meat quality of weaned piglets with the dietary incorporation of *Ulva lactuca* and carbohydrases supplementation

José M. Pestana [a,b,1], Cristina M. Alfaia [a,b,1], David Miguel Ribeiro [c], Mónica M. Costa [a,b], Daniela F.P. Carvalho [c], Cátia F. Martins [a,b,c], Victor M.D. Alves [a,b], José P.C. Lemos [a,b], Miguel Mourato [c], Inês Delgado [d], Sandra Gueifão [d], Inês Coelho [d], André M. Almeida [c], João P.B. Freire [c], José A.M. Prates [a,b,*]

[a] *CIISA - Centre for Interdisciplinary Research in Animal Health, Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal*
[b] *Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), Faculdade de Medicina Veterinária, Universidade de Lisboa, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal*
[c] *LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[d] *INSA - Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisboa, Portugal*

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ABSTRACT

The impact of the dietary incorporation of 7% *Ulva lactuca*, a green seaweed, on the quality and nutritional value of piglet's meat was assessed. *U. lactuca* is rich in nutrients and bioactive compounds but its cell wall is composed of complex polysaccharides that reduce their bioavailability. Therefore, the effect of supplementing piglet diets with exogenous carbohydrases was also assessed here. A total of 40 male weaned piglets were divided into four dietary groups, each with 10 piglets: control (wheat, maize and soybean meal-based diet), UL (7% *U. lactuca* replacing the control diet), UL + R (UL and 0.005% Rovabio®), and UL + E (UL and 0.01% ulvan lyase). The piglets were fed the diets for 2 weeks. The results showed that incorporating *U. lactuca* in piglet diets did not influence most of the meat quality traits ($P > 0.05$). However, the incorporation of *U. lactuca* with the commercial carbohydrase (UL + R) increased the amount of the docosahexaenoic acid (DHA; 22:6*n*-3) in their meat ($P = 0.011$) compared with the control, by 54%. In addition, meat from piglets fed seaweed diets showed a nearly two-fold increase in iodine contents ($P < 0.001$). Meat tenderness, juiciness and overall acceptability of piglets fed the control diet and the UL diet were lower than those fed the diets containing seaweed and carbohydrases ($P < 0.001$). Overall, the findings indicate that 7% *U. lactuca* in the diets of weaned piglets had no major detrimental effects on meat quality and their carbohydrase supplementation has the potential to improve meat sensory traits.

1. Introduction

Conventional animal production is being pressured by increasing demand for meat, such as pork, to increase production whilst lowering environmental impact (Parlasca & Qaim, 2022). Commonly used feedstuffs in swine diets, such as soybean meal and cereal grains, have a significant environmental impact, with high input of production factors such as land and water. Additionally, the main cost of pig production is feeding, which is particularly important during the critical post-weaning period when piglets are introduced to solid feed, that requires high-quality ingredients (Mu, Pi, Zhang, & Zhu, 2022). Furthermore, this transition period is also characterized by increased vulnerability to pathogens and inflammatory stimuli, which can negatively affect piglet growth and health (Lallès & Montoya, 2012; Udit, Blake, & Chiu, 2022). Antibiotics and zinc oxide were previously used to mitigate these negative effects, however, their use has been banned in the EU for this purpose due to public health concerns. As an alternative, supplementing piglet diets with bioactive ingredients, like the prebiotic polysaccharides

* Corresponding author at: CIISA - Centre for Interdisciplinary Research in Animal Health, Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal.
E-mail address: japrates@fmv.ulisboa.pt (J.A.M. Prates).
[1] Authors with equal contribution.

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of seaweeds (Nguyen et al., 2023; Vigors, O'Doherty, Rattigan, & Sweeney, 2021), may reduce weaning-related stress and improve both piglet growth and meat quality (Lebret & Candek-Potokar, 2022).

There is a growing interest in using seaweeds as feed ingredients in livestock, particularly in swine, to enhance growth performance and health (Corino, Modina, Giancamillo, Chiapparini, & Rossi, 2019; Maghin, Ratti, & Corino, 2014; Ribeiro et al., 2021; Ribeiro et al., 2022. Seaweeds are multicellular, marine organisms, with a diverse nutritional composition. *Ulva lactuca*, a green seaweed, is widespread across a wide range of countries and environmental conditions, given its adaptability to different factors, including sunlight exposure and water salinity (Mantri, Kazi, Balar, Gupta, & Gajaria, 2020). *Ulva* sp. has a valuable nutritional composition with low to high crude protein contents (5–42% on a dry matter - DM basis) and low crude fat content (below 7% of DM basis) (Costa, Cardoso, Afonso, Bandarra, & Prates, 2021). *U. lactuca* is also rich in other nutrients and bioactive compounds, such as polysaccharides, *n*-3 polyunsaturated fatty acids (PUFA), iodine and other elements (Corino et al., 2019; Eismann, Reis, da Silva, & Cavalcanti, 2020; Taboada, Millán, & Míguez, 2010).

U. lactuca cell wall is made up of four polysaccharide families in *Ulva* sp.: two major ones, the water-soluble ulvan and insoluble cellulose, and two minor ones, a peculiar alkali-soluble linear xyloglucan and a glucuronan (Lahaye & Robic, 2007; Robic, Bertrand, Sassi, Lerat, & Lahaye, 2009), which are indigestible by the endogenous enzymes of monogastrics (Ribeiro et al., 2021). The supplementation of piglet diets with carbohydrases, such as the commercially available mix Rovabio® Excel AP, whose activities include cellulases, can help improve the bioavailability of the nutrients in *U. lactuca* (Pestana et al., 2020), by breaking down the cell wall during digestion. Costa et al. (2022) recently demonstrated the ability of a single recombinant ulvan lyase to partially disrupt *U. lactuca* cell walls in vitro, making its nutrients more accessible. This could, for instance, maximize the deposition of beneficial *n*-3 PUFA, which are present in relatively lower amounts in seaweed. Therefore, feeding piglet diets with *U. lactuca* and carbohydrases, it may be possible to improve the nutritional value and meat quality. This has the potential to increase the value of traditional roasted piglet meat products, such as *Leitão da Bairrada* in Portugal, *Porcheddu* in Italy or *Cochinillo Asado* in Spain (Santos Silva & Nunes, 2013), premium meat sectors of growing importance in Mediterranean Europe.

The objective of this study was to determine the effect of dietary incorporation of 7% *U. lactuca*, with or without carbohydrases supplementation (commercial enzyme mixture or a recombinant ulvan lyase), on quality traits (pH, colour, lipid oxidation and sensory properties) and nutritional value (fatty acid composition, elemental profile and pigments) of meat from weaned piglets.

2. Materials and methods

2.1. Animals and experimental diets

The experimental protocol received approval from the School of Agriculture (ISA) of the University of Lisbon Ethics Commission and was authorized by the National Veterinary Authority (ref. 0421/000/000/2020–021337), following the European Union legislation (2010/63/EU Directive). The animal trial was carried out at the Animal Production Section of ISA (Lisbon, Portugal). A total of forty weaned male piglets (Large White × Duroc) with an initial body weight of 8.56 ± 0.85 kg (mean ± SD), were randomly assigned to one of the four experimental groups ($n = 10$): control (wheat, maize and soybean meal-based diet), UL (7% *U. lactuca* powder replacing control), UL + R (UL + 0.005% Rovabio® Excel AP from Adisseo (Antony, France)) and UL + E (UL + 0.01% of the recombinant ulvan lyase described by Costa, Pio, et al., 2022). The commercial enzyme mix (Rovabio®) included the following enzymatic activities: xylanase, β-glucanase, cellulase, pectinase and protease (Adisseo, 2023). The recombinant enzyme ulvan lyase has ulvan as its major substrate, which is a major component of the *U. lactuca* cell wall, along with cellulose (Costa, Pio, et al., 2022). Both enzymatic supplements were added to their respective diet during ingredient mixing, after being mixed in with an excipient (maize). The diets were kept at room temperature during the trial. The seaweed was wild-caught, bought to Aleor (Lézardrieux, France) and used as supplied (powder, < 250 μm). The piglets were housed individually in metabolic cages with free access to water, in the context of a nutritional trial. No salt was added to seaweed diets to prevent feed refusals or gastrointestinal issues. The experimental diets were formulated to be isocaloric and isonitrogenous (Table 1).

2.2. Chemical analysis of feeds

U. lactuca powder and diets, after ground to pass through a 1 mm sieve, were analysed for dry matter (103 °C oven drying), crude protein (Kjeldahl method), crude fat (acid hydrolysis followed by petroleum ether extraction) and ash (incineration at 500 °C) according to Association of Official Analytical Chemists International (2000). The neutral detergent fibre was determined using the Van Soest method (Van Soest, Robertson, & Lewis, 1991).

The fatty acid composition of *U. lactuca* and diets was analysed by one-step extraction and acid transesterification according to Sukhija and Palmquist (1988), with slight modifications. Succinctly, 1 mL of nonadecanoic acid (19:0) methyl ester in *n*-hexane, as the internal standard, was added to samples (around 0.1 g) followed by the addition of 1 mL of toluene and 3 mL of methanolic HCL 1.25 M. Then, fatty acid methyl esters (FAME) were injected into a Supelcowax® 10 capillary column (30 m × 0.20 mm i.d., 0.20 μm film thickness; Supelco, Bellefonte, PA, USA), incorporated in a gas chromatograph (HP7890A Hewlett-Packard, Avondale, PA) with flame ionization detector (GC-FID). Gas chromatographic conditions were: split/splitless injection system (1 μL), helium as carrier gas at a flow rate of 1.3 mL/min and the injector and detector temperatures were set at 250 and 280 °C, respectively (Alfaia et al., 2021). The results were expressed as a percentage of total fatty acids.

The diterpene profile (vitamin E homologues: tocopherols and tocotrienols) and β-carotene content in *U. lactuca* and diets (0.1 g each) were assessed, in duplicate, by a saponification reaction in a water bath at 80 °C for 15 min followed by single extraction with *n*-hexane, and

Table 1
Ingredients and feed supplements of dietary treatments (% as fed basis).

Ingredient	Dietary treatments[1]			
	Control	UL	UL + R	UL + E
Wheat	43.7	40.7	40.695	40.69
Maize	15.0	14.0	14.0	14.0
Soybean meal 44	25.0	23.3	23.3	23.3
Sweet wheat powder	10.0	9.3	9.3	9.3
Sunflower oil	3.00	2.9	2.9	2.9
Ulva lactuca powder	–	7.0	7.0	7.0
L-Lysine	0.500	0.470	0.470	0.470
DL-Methionine	0.100	0.090	0.090	0.090
L-Threonine	0.100	0.090	0.090	0.090
Calcium carbonate	0.500	0.470	0.470	0.470
Dicalcium phosphate	1.300	1.210	1.210	1.210
Sodium chloride	0.300	0.000	0.000	0.000
Vitamin-mineral premix[2]	0.500	0.470	0.470	0.470
Rovabio® Excel AP	–	0	0.005	0
Recombinant ulvan lyase	–	0	0	0.010

[1] Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase).

[2] Vitamin-mineral premix, VitaTec®, provided by Tecadi, Santarém Portugal. Per 1 kg of premix: Vitamin A – 3,000,000 UI, Vitamin D3–500,000 UI, Vitamin E – 10,000 mg, Vitamin B1–500 mg, Vitamin B2–1000 mg, Vitamin B6–500 mg, Vitamin B12–5 mg, Vitamin H2–18,75 mg, Vitamin K3–500 mg, Vitamin B5–3750 mg, Vitamin B3–6250 mg, Vitamin B9–62.5 mg, Choline chloride – 50,000 mg, Cu – 38,750 mg, Zn – 27,500 mg, Mn – 12,500 mg, I – 200 mg, Se – 50 mg, Fe – 25,000 mg, butyl-hydroxytoluene – 50 mg.

then, analysed through HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) using a Zorbax RX-Sil normal-phase silica column (250 mm × 4.6 mm i.d., 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA) and two detectors, a photodiode array detector and fluorescence detector coupled in series (Prates, Quaresma, Bessa, Fontes, & Alfaia, 2006). For fluorescence tocopherols and tocotrienols detection, the excitation wavelength was set at 295 nm with emission at 325 nm. For β-carotene detection, the UV spectrum was recorded at 450 nm. Vitamin E homologues and β-carotene contents were calculated using standard curves of peak area versus concentration.

Total pigments were assessed as previously described by Pestana et al. (2020). Pigments were extracted from *U. lactuca* and diets (0.1 g) with 5 mL of acetone and homogenized with a T25 UltraTurrax (IKA, Königswinter, Germany). Afterwards, samples were centrifuged at 2500 rpm for 10 min. for separation of the supernatant. The measurement of chlorophyll *a* and *b* and total carotenoids was done at 662, 645 and 470 nm, respectively, using a UV–VIS spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, Little Chalfont, UK). All analyses were carried out in dim light because pigments are very photosensitive. The pigment content was calculated using the following equations: chlorophyll a = 11.75 A66–2.350 A645; chlorophyll b = 18.61 A645–3.960 A662 and total carotenoids = 1000 (A470–2.270 Ca - 81.4 Cb)/227 (Dere, Güneş, & Sivaci, 1998).

The mineral profile was determined as suggested by Ribeiro et al. (2020). Briefly, the *U. lactuca* and the experimental diets were digested, in triplicate, from 0.3 g of sample with concentrated nitric and hydrochloric acids before the addition of hydrogen peroxide. Subsequently, samples were analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Thermo Scientific, Waltham, MA, USA). Iodine and bromine concentrations were determined, in triplicate, as described by Delgado et al. (2019). After alkaline extraction of *U. lactuca* seaweed and diets (around 0.2 g) with tetramethylammonium hydroxide (TMAH) solution at 25% (*v*/*v*) and ultra-pure water (Milli-Q Element system, Millipore Corporation, Saint-Quentin, France) in a Heating Graphite Block System (DigiPREP MS, SCP Science, Quebec, Canada), at 90 °C during 3 h, the samples were centrifuged, filtered with 0.45 μm pore size hydrophilic filters (Merck, Darmstadt, Germany) and analysed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) (Thermo X series II, Thermo Fisher Scientific, Waltham, MA, USA). The chemical composition of *U. lactuca* and experimental diets is depicted in Table 2.

Table 2
Chemical composition of experimental diets.

	Macroalga	Dietary treatments[1]			
Item	*U. lactuca*	Control	UL	UL + R	UL + E
Proximate composition (% as dry matter)					
Dry matter	88.7	89.4	89.3	89.2	89.3
Crude protein	28.2	18.0	18.3	18.7	18.6
Crude fat	2.9	5.6	5.5	5.7	5.3
Ash	31.7	5.9	7.2	7.5	7.3
Neutral detergent fibre	27.1	15.4	19.9	20.0	19.9
Fatty acid profile (% total fatty acids)					
14:0	0.608	0.384	0.434	0.387	0.409
16:0	33.6	10.8	12.0	11.5	11.5
16:1*c*9	4.17	0.163	0.270	0.273	0.289
17:0	0.580	0.088	0.077	0.081	0.076
17:1*c*9	0.958	0.049	0.052	0.052	0.047
18:0	1.26	3.62	3.52	3.50	3.52
18:1*c*9	14.9	26.0	25.0	25.8	25.4
18:2*n*-6	6.14	55.2	52.7	52.8	53.1
18:3*n*-3	17.3	1.32	1.91	1.72	1.72
18:4*n*-3	12.8	0.000	0.619	0.599	0.578
20:0	0.344	0.320	0.336	0.329	0.323
22:0	1.54	0.638	0.637	0.645	0.627
Diterpene profile (μg/g of wet weight)					
α-Tocopherol	79.3	52.4	46.8	47.3	51.0
β-Tocopherol	n.d.	1.01	0.992	0.839	0.849
γ-Tocopherol+β-tocotrienol	n.d.	2.39	2.38	1.93	1.94
δ-Tocopherol	n.d.	0.522	0.508	0.457	0.487
γ-Tocotrienol	n.d.	1.63	1.68	1.34	1.42
Pigments (μg/g of wet weight)[2]					
β-Carotene	170	0.441	14.5	16.7	15.2
Chlorophyll *a*	2311	3.86	239	238	247
Chlorophyll *b*	1666	5.80	166	167	170
Total carotenoids	510	1.91	43	43	45
Element profile, mg/kg dry matter					
Bromine	694	11.7	77.3	86.3	81.8
Calcium	6202	14,661	14,966	15,017	14,011
Copper	3.73	256	240	258	227
Iodine	45.1	1.45	5.88	7.12	5.66
Iron	537	265	258	294	253
Magnesium	25,889	1456	4186	3980	4018
Manganese	39.0	142	129	145	145
Phosphorous	2786	8722	8876	9340	8638
Potassium	38,822	10,967	14,206	13,946	14,431
Sodium	52,133	4082	7486	7212	7618
Sulphur	49,265	2998	8906	8323	8720
Zinc	8.96	257	261	256	281

[1] Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase), n.d., not detected.
[2] Chlorophylls and total carotenoids were obtained using the formulas reported by Dere et al. (1998).

2.3. Growth performance of piglets

The growth performance of the piglets was evaluated by recording their weight at the beginning (28-day old) and end of each week over a two-week period. The daily feed refusals were also recorded to monitor the feed intake of the piglets. After the two-week period, the piglets were humanely slaughtered using electrical stunning and exsanguination. The *longissimus lumborum* (LL) muscle was then harvested from each carcass, with the right side being used for meat quality and sensory analysis, and the left side being minced, vacuum packed and stored at −20 °C for biochemical analysis.

2.4. Determination of meat quality traits and sensory analysis

2.4.1. Measurement of meat pH and colour parameters

The pH of LL muscle was measured 24 h *post-mortem,* in triplicate, using a pH meter (Hanna Instruments, Woonsocket, RI, USA) with a glass penetrating electrode equipped with temperature compensation after calibration with pH buffers at ambient temperature. Meat colour parameters, including the International Commission on Illumination (CIE) system colour profile of lightness (L^*), redness (a^*), and yellowness (b^*), were assessed 24 h *post-mortem* after 1 h of refrigeration at 4 °C. The measurements were taken, after 1-h air exposure to allow blooming, on three spots of the cut surface of the LL muscle using a colourimeter (Minolta CR-300, Konica Minolta, Tokyo, Japan) with illuminant D65, 2° viewing angle geometry, 11-mm-diametre aperture, 8-mm-diametre measurement area and a C light source.

2.4.2. Cooking treatment, cooking loss and shear force determination

Meat samples were defrosted overnight at 4 °C and cooked in a water bath set at 80 °C until they reached an internal temperature of 78 °C, as monitored by a Lufft C120 (Munich, Germany) thermocouple. Cooking loss was calculated by weighing the meat samples before and after cooking.

Meat shear force was determined by cutting longitudinal sections towards the fibres with a 1 cm^2 cross-section and using a Warner-

Bratzler blade coupled to a texture analyser (TA-XT Plus texture analyser, Stable Micro Systems, Surrey, UK), as described by Silva et al. (2015). The mean of four replicates was recorded for meat shear force.

2.4.3. Sensory analysis by a trained panel

Sensory analysis of the meat samples was conducted by a trained sensory panel of 12 panellists, who were selected to form a homogeneous group (age, income and cultural background) and intensively trained on this meat type (Faculty of Veterinary Medicine, Lisbon, Portugal). The muscle samples were trimmed of external connective tissue, cut into cubes, and cooked in a water bath, as described earlier for cooking loss. The samples were then distributed to five panels, with 8 random samples *per* session. The panellists evaluated the attributes of tenderness, juiciness, flavour, and overall acceptability using a numerical scale, with scores ranging from 1 (low/negative) to 8 (high/positive). For off-flavour, the scale ranged from 0 (absence) to 8 (maximum).

2.5. Determination of total pigments, diterpene profile and lipid oxidation

The methodology used for the analysis of chlorophylls *a* and *b* and total carotenoid contents in LL muscle was the one described by Tolpeznikaite et al. (2021), with minor modifications. Pigments were extracted from 2.5 g of fresh muscle samples using acetone as an extraction solvent. Pigment contents were measured as described for seaweed and diets using the formulas reported by Dere et al. (1998).

The determination of total cholesterol, β-carotene and tocopherols in meat samples, in duplicate, was performed according to Prates et al. (2006). Concisely, cholesterol and terpenoids were extracted, in duplicate, from 0.750 g of fresh muscle samples by direct saponification, using single *n*-hexane extraction and subsequently HPLC analysis, using UV–visible photodiode array detector for total cholesterol and β-carotene detection ($\lambda = 202$ nm and $\lambda = 450$ nm, respectively) linked to a fluorescence detector for tocopherols and tocotrienols detection (excitation $\lambda = 295$ nm and emission $\lambda = 325$ nm), according to the procedures described for seaweed and diets.

Lipid oxidation of meat was assessed by thiobarbituric acid reactive substances (TBARS) at days 0 and 8, kept at 4 °C, as previously described by Grau, Guardiola, Boatella, Barroeta, and Codony (2000). TBARS values were calculated, in duplicate, and expressed as mg of malondialdehyde/kg of meat.

2.6. Determination of intramuscular fat content and fatty acid composition

The extraction of intramuscular fat (IMF) content from lyophilized LL samples (0.250 g) was done using the Folch, Lees, and Stanley (1957) extraction method with a modification by Carlson (1985), using a dichloromethane-methanol (2:1, *v*/v) solution. IMF was determined gravimetrically by evaporating the solvent and weighing the remaining residue. Fatty acids were converted to fatty acid methyl esters (FAME) through sequential alkaline and acid transesterification, as described by Pestana et al. (2020). FAME were separated in Supelcowax 10 capillary column using GC-FID, in accordance in accordance with procedures detailed for *U. lactuca* and diets (Alfaia et al., 2021; Prates, Prates, & Bessa, 2009; Siciliano et al., 2013). The identification of FAME was achieved by comparison of retention times with a standard (37 Component FAME mixture from Supelco Inc. Bellefonte, PA, USA). Nonadecanoic acid (19:0) was used as the internal standard and the amount of fatty acids were expressed as mg/100 g of meat, using the lipid conversion factor of 0.91 (Weirauch, Posati, Anderson, & Exler, 1977).

2.7. Determination of element profile

The element profile in LL muscle was evaluated as mentioned above for *U. lactuca* and the experimental diets (see Section 2.1). Briefly, the minerals were extracted from 0.3 g of freeze-dried muscle, digested and analysed with ICP-AES, except iodine and bromine. The analysis of iodine and bromine was performed, in triplicate, from 0.6 g of freeze-dried LL muscle by ICP-MS after extraction at 90 °C for 3 h following the same procedure as *U. lactuca* and diet samples.

2.8. Statistical analysis

Data analysis was performed by ANOVA using the GLM procedure of SAS software version 9.4; SAS Institute Inc., Cary, NC, USA), except TBARS which were analysed with the MIXED procedure of SAS to consider the repeated measures in time and, thus, dependent observations. The experimental unit was the piglet. For the analysis of growth performance and meat colour, sensorial analysis, total pigments and diterpenes, fatty acid and elements, the dietary treatment was considered as a fixed factor and the animal as a random term in the model. The analysis of the sensory panel also included session as a co-factor. The same model was considered for the analysis of cooking loss and texture data. The cooking batch was not included in the model, since there was only one cooking batch, which contained all samples randomly distributed. For TBARS analysis, both dietary treatment and storage time were fixed factors, the animal was a random term, and the interaction between dietary treatment and storage time was included in the model. Least-square means were compared using the PDIFF option, adjusted for the Tukey-Kramer method. All statistical tests were considered significant at $P < 0.05$.

3. Results

3.1. Experimental diets, feed intake and piglet's growth performance

Table 2 shows the different chemical compositions of dietary treatments. Notably, the levels of total carotenoids and chlorophylls in seaweed diets were 23- to 50-fold higher than those with the control, whereas an increase of 7- and 4.3-fold of bromine and iodine, respectively, was detected in the diets with *U. lactuca*.

Experimental diets had no effect on body weight ($p > 0.05$). Piglets had, on average, 9.6 kg and 14.8 kg of initial and final weight, respectively. The average daily gain (376 ± 4.13 g), average daily feed intake (572 ± 15.0 g) and feed conversion ratio (1.6 ± 0.05) were also unaffected by diets (see Table S1 at Supplementary Materials).

3.2. Meat quality traits and sensory analysis

Table 3 presents the effect of the experimental diets on meat colour, pH and sensory analysis. The results show that the dietary treatments had varying impacts on meat quality. There were no significant differences in pH, colour parameters, cooking loss and shear force between the four experimental groups ($P > 0.05$). However, most sensorial attributes showed significant differences between experimental diets. The UL + R and UL + E groups showed higher tenderness and juiciness compared to the control and UL groups ($P < 0.001$). There was no significant difference in the flavour or off-flavour between groups ($P > 0.05$). The overall acceptability was also significantly different, with the UL + R and UL + E groups having higher acceptability compared to the control and UL groups ($P < 0.001$).

3.3. Total pigments and diterpene profile and meat oxidative stability

The effect of experimental diets on pigments and vitamin E homologues is shown in Table 4. Data showed no significant influence of dietary treatments on chlorophylls *a* and *b* and total carotenoids ($P > 0.05$). β-carotene was not detected in LL muscle. Furthermore, only α-tocopherol, among vitamin E homologues, was detected even though with no statistically significant impact ($P > 0.05$) due to the experimental diets.

Table 3
Effect of experimental diets on pH 24 h, colour parameters (L^*, a^*, b^*) cooking loss, shear force and sensorial analysis of *longissimus lumborum* of piglets.

	Dietary treatments[1]					*p*-Value	
Item	Control	UL	UL + R	UL + E	SEM[2]	Session[3]	Diet
pH 24 h	5.38	5.49	5.48	5.51	0.038	–	0.099
Colour parameters							
Lightness (L^*)	50.8	50.1	49.7	49.0	0.571	–	0.213
Redness (a^*)	7.76	7.92	7.39	7.71	0.257	–	0.529
Yellowness (b^*)	1.42	1.00	0.987	0.986	0.2461	–	0.517
Cooking loss (%)	36.4	35.9	35.4	35.2	0.572	–	0.418
Shear force (N)	28.4	26.5	25.7	28.0	1.078	–	0.284
Sensorial attributes							
Tenderness	5.24^b	5.14^b	5.88^a	6.24^a	0.140	0.164	<0.0001
Juiciness	5.17^b	5.19^b	5.75^a	5.95^a	0.139	0.001	<0.0001
Flavour	5.45	5.43	5.53	5.54	0.125	0.071	0.892
Off-flavour	0.587	0.771	0.623	0.884	0.1019	0.545	0.140
Overall acceptability	5.07^b	5.14^b	5.63^a	5.76^a	0.129	0.691	<0.0001

1 Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL+R (UL with 0.005% of Rovabio® Excel AP) and UL+E (UL with 0.01% recombinant ulvan lyase).
2 SEM, standard error of the mean.
3 The effect of the session was added as a block to analyse sensorial panel data.
a,b Different superscript letters within a row are significantly different ($P < 0.05$).
The scores for sensory attributes (except for off-flavour) range from 1 (low/negative) to 8 (high/positive) and for off-flavour, the scale ranges from 0 (absence) to 8 (maximum).

Table 4
Effect of experimental diets on total pigments and α-tocopherol contents (μg/100 g of wet weight) in *longissimus lumborum* of piglets.

	Dietary treatments[1]					
Item	Control	UL	UL + R	UL + E	SEM[2]	*p*-Value
Total pigments						
Chlorophyll *a*	17.2	14.7	21.0	27.0	3.81	0.094
Chlorophyll *b*	27.3	23.2	35.7	37.1	5.34	0.100
Total carotenoids	4.99	7.22	5.55	7.26	0.916	0.144
Diterpene profile						
α-Tocopherol	1.34	1.34	1.21	1.16	0.104	0.533

1 Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase).
2 SEM, standard error of the mean.

Fig. 1 displays the effect of the experimental diets on meat lipid oxidation. There was a significant effect on the interaction of diet and time ($P = 0.045$), although oxidation was mostly increased by UL + E with time ($P < 0.05$).

3.4. Intramuscular fat, total cholesterol and fatty acid composition

Table 5 summarizes the effect of the experimental diets on intramuscular fat, total cholesterol and fatty acid composition of LL muscle. Intramuscular fat and total cholesterol were unaffected by dietary treatment ($P > 0.05$). Minor saturated fatty acids (SFA), including 15:0 ($P = 0.001$) and 17:0 ($P = 0.003$), decreased with seaweed diets compared to the control. Regarding PUFA, the content of 22:6*n*-3 was significantly higher ($P = 0.011$) with UL + R diet, when compared with the control. The experimental diets had no effect ($P > 0.05$) on the sums of fatty acids, notwithstanding *n*-6/*n*-3 ratio was significantly lower ($P = 0.001$) in the meat of piglets fed with the seaweed diets relative to the control.

3.5. Element profile

The element profile of LL muscle is presented in Table 6. The elements most abundant in LL muscle were potassium, phosphorous and sulphur. Data show that the dietary treatments have influenced the level of some elements in the meat of piglets. In fact, calcium ($P = 0.007$), sodium ($P = 0.013$) and manganese ($P = 0.001$) were lower with UL + E diet compared to the other treatments, but without significant ($P > 0.050$) differences between UL + E diet and control for sodium concentration. In contrast, bromine ($P < 0.001$) and iodine ($P < 0.001$) increased in the meat of piglets fed with the seaweed diets compared with the control.

4. Discussion

Although brown seaweeds (e.g. *Laminaria* spp. and *Ascophylum nodosum*) have already been used for supplementing piglet diets (Corino et al., 2019; Ribeiro et al., 2021), to the best of our knowledge, the inclusion of *U. lactuca* as a feed ingredient in piglet diets, has not been addressed so far. This is the first study to evaluate the incorporation of whole biomass *U. lactuca* at the ingredient level (7%) and the effects of carbohydrases supplementation on growth performance and meat quality traits of weaned piglets. There were no detrimental effects on growth performance by feeding piglets up to 7% *U. lactuca,* regardless of enzymatic supplementation. Michalak, Chojnacka, and Korniewicz (2015) have reported no negative effect on growth performance and feed intake of growing pigs (initial live weight: 20.9 kg) fed a green seaweed, *Enteromorpha* sp., for 87 days with up to 4%. In the present study, no significant impact on the growth performance of piglets was found, revealing that 7% of dietary incorporation of *U. lactuca* did not compromise the productive parameters. However, we must point out that these results were obtained with piglets housed in metabolic cages, in the framework of a nutritional trial, and do not reflect standard production conditions, which requires further research.

Concerning meat quality traits, the effect of 7% *U. lactuca* in piglet diets, individually or combined with exogenous carbohydrases, did not change pH, colour, cooking loss and shear force. However, the inclusion of such high levels of seaweed supplemented with carbohydrases enhanced meat acceptability, which was positively scored (> 4.0) by an increase in tenderness and juiciness. The fact that we did not find shear force differences between treatments but found tenderness effects on the sensory panel might be related to the complexity of the sensorial

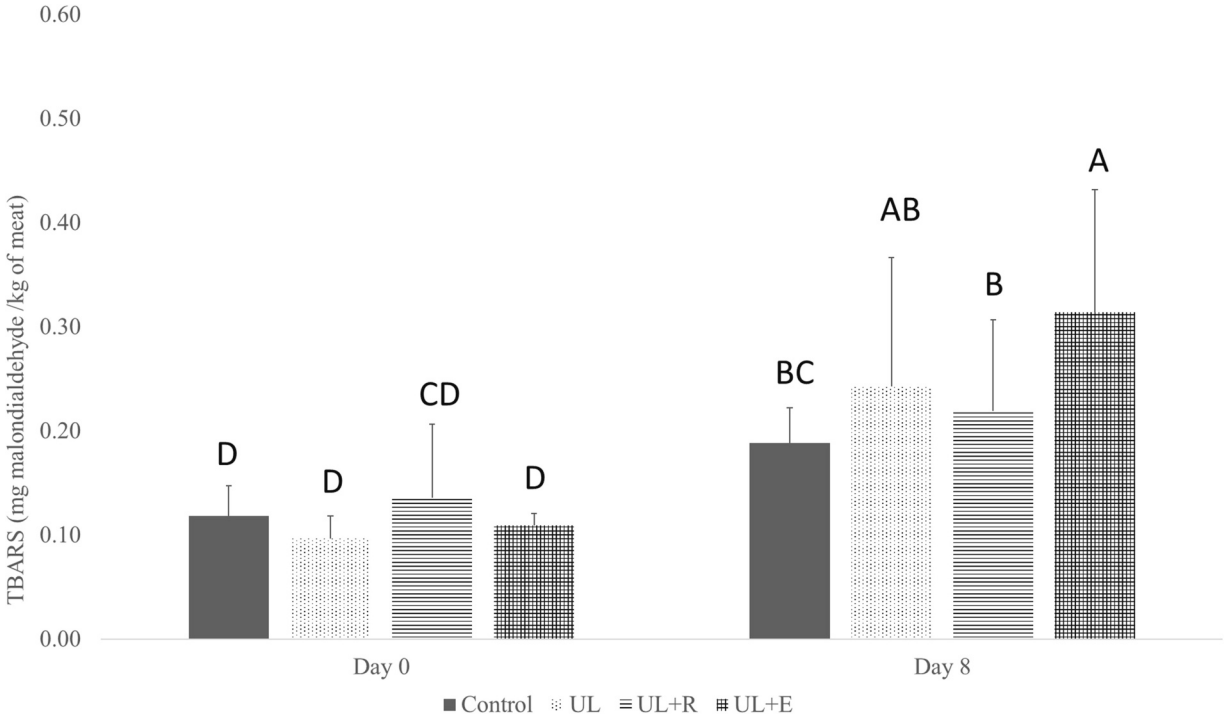

Fig. 1. TBARS levels (mean ± standard error) at 0 and after 8 days under refrigeration in *longissimus lumborum* muscle of weaned piglets fed with Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase). ^{A,B} Bars with different letters are significantly different ($P < 0.05$).

assessment, which is not as objective as the former analysis. Moreover, according to our trained sensory panel, *U. lactuca* had no negative effect on meat flavour (absence of fishy flavours), thus contributing to consumers' acceptance of meat.

Green seaweeds have high antioxidant metabolites and may be used to improve meat quality (Rajauria, Draper, McDonnell, & O'Doherty, 2016). The effect of dietary incorporation of 7% *U. lactuca* in piglet diets, with or without carbohydrases, on LL contents of pigments and vitamin E was also exploited. Unexpectedly, we found that feeding piglets with this level of *U. lactuca* did not change the contents of chlorophylls and total carotenoids, contrary to what we found in broilers fed with similar diets (Costa et al., 2022). In addition, the sum of carotenoids in the meat of piglets did not reflect the number of total carotenoids in seaweed diets, which was 23-fold higher than the control. This is coherent with our data on lipid oxidation, since the UL + E group significantly increased malonaldehyde compared with the remaining diets at day 8. The presumably increased accumulation of antioxidant pigments could have counterbalanced this effect. The different availability of the phenolic content, or other components of the seaweed with pro-oxidative or anti-oxidative capacities, can also be playing a part in generating these differences. The higher accumulation of PUFA has been linked to higher lipid oxidation in pig meat (Domínguez et al., 2019). Surprisingly, this PUFA increment was not seen in our study. The reasons for such a lack of PUFA accumulation require further research.

Regarding the fatty acid profile, the addition of 7% *U. lactuca* to piglet diets, with or without carbohydrases supplementation, had a minimal impact on the fatty acid profile of the piglet meat. The combination of seaweed and commercial carbohydrase significantly increased the accumulation of the docosahexaenoic acid (DHA, 22:6*n*-3) in piglet meat, but without modifications on the levels of alpha-linolenic acid (18:3*n*-3), which does not point out for a higher conversion rate of ALA to DHA through the action of elongases with this treatment than with control. Therefore, the reason for such DHA accumulation in piglet meat with this dietary treatment requires further research. According to the criteria of the Food Advisory Committee (1990), these meats are considered lean meats (fat content <5%). In the present study, intramuscular fat ranged from 1.3% to 1.37%, meeting that criterion.

Although seaweeds are known to have low-fat contents when compared to other feed ingredients, in general, they can have a high proportion of *n*-3 PUFA in their fatty acid composition (Costa et al., 2021; Ribeiro et al., 2021), which has been reported to reach beyond 10% (Tabarsa, Rezaei, Ramezanpour, & Waaland, 2012; Coelho et al., 2020). The benefits of *n*-3 PUFA, such as reducing the risk of cardiovascular diseases, have been extensively demonstrated in animal and epidemiological studies (Jerez-Timaure, Sánchez-Hidalgo, Pulido, & Mendoza, 2021; Matarneh, England, Scheffler, & Gerrard, 2017). This has led several international organizations, such as the American Heart Association, the Food and Agriculture Organization of the United Nations, and the World Health Organization, to recommend a daily intake of at least 500 mg of eicosapentaenoic acid (EPA, 20:5*n*-3) and DHA (Aranceta & Pérez-Rodrigo, 2012; Molendi-Coste, Legry, & Leclerq, 2011). Despite such recommendations, most Western societies still have a daily consumption of *n*-3 long-chain PUFA that is below 500 mg/day (Martins et al., 2013). Only a small increase in DHA content was observed in piglet meat from piglets fed combined with the commercial carbohydrase (5.08 mg/100 g) relative to the control (3.29 mg/100 g). Although the muscle of the piglets supplemented with the commercial carbohydrase is far from providing the recommended daily intake of EPA + DHA, since the maximum content determined in this tissue was 10 mg EPA + DHA/100 g, around 50 times lower than the recommended one, the improvement is positive when consumption well below the recommendations, as is the case in Western societies. Moreover, it is worth noting that the higher levels of DHA in meat, around 5.08 mg/100 g muscle, is unlikely to affect its oxidative stability, as evidenced by both TBARS values and sensory panel evaluations. Although the extent of lipid oxidation did increase between day 0 and day 8, it remained below the critical value of 0.5 mg malondialdehyde/kg, which is typically considered detectable by consumers (Wood et al., 2008).

The inclusion of 7% *U. lactuca* in piglet diets affected the element levels in their LL muscle. Diets containing seaweed led to a higher

Table 5
Effect of experimental diets on intramuscular fat (g/100 g of dry matter), total cholesterol (mg/100 g fresh muscle) and fatty acid (FA) composition (mg/100 g of dry matter) in *longissimus lumborum* muscle of piglets.

Item	Dietary treatments[1]				SEM[2]	*p*-Value
	Control	UL	UL + R	UL + E		
Intramuscular fat (g/100 g of dry matter)	1.37	1.30	1.36	1.32	0.090	0.938
Total cholesterol (mg/100 g of wet weight)	78.1	76.3	76.8	78.0	0.020	0.925
Fatty acid profile (mg/100 g of dry matter)						
Lauric acid (12:0)	1.25	1.32	0.924	1.29	0.115	0.073
Myristic acid (14:0)	12.1	9.66	12.3	11.8	1.24	0.423
Myristoleic acid (14:1*c*9)	0.467	0.437	0.422	0.476	0.043	0.794
Pentadecanoic acid (15:0)	2.97^{a}	1.89^{b}	1.65^{b}	1.90^{b}	0.219	0.001
Palmitic acid (16:0)	290	283	306	281	21.2	0.838
cis-7 Hexadecenoic acid (16:1*c*7)	5.46	4.89	5.41	5.56	0.430	0.694
Palmitoleic acid (16:1*c*9)	34.6	23.6	33.7	32.5	3.83	0.171
Margaric acid (17:0)	9.28^{a}	6.79^{b}	5.79^{b}	6.55^{b}	0.638	0.003
cis-9 Margaric acid (17:1*c*9)	5.73	4.71	4.98	5.66	0.471	0.344
Stearic acid (18:0)	165	175	177	158	13.6	0.724
Oleic acid (18:1*c*9)	284	218	278	275	26.9	0.291
Vaccenic acid (18:1*c*11)	55.9	53.1	56.4	54.5	4.16	0.946
Linoleic acid (18:2*n*-6)	249	260	240	249	18.3	0.882
γ-Linolenic acid (18:3*n*-6)	0.872	1.05	0.947	0.977	0.062	0.253
Linolelaidic acid (18:2*t*9*t*12)	1.49	1.58	1.37	1.49	0.154	0.643
α-Linolenic acid (18:3*n*-3)	3.52	3.75	4.08	4.18	0.299	0.397
Arachidic acid (20:0)	1.85	1.73	1.85	1.62	0.163	0.704
Eicosenoic acid (20:1*c*11)	5.04	3.79	4.91	4.72	0.452	0.212
Eicosadienoic acid (20:2*n*-6)	8.43	7.52	7.31	8.18	0.667	0.595
γ-homolinolenic acid (20:3*n*-6)	6.32	6.75	5.56	5.63	0.644	0.515
Arachidonic acid (20:4*n*-6)	55.9	62.9	46.1	54.2	7.47	0.471
Eicosatrienoic acid (20:3*n*-3)	0.598	0.660	0.583	0.734	0.055	0.222
Eicosapentaenoic acid (20:5*n*-3)	2.10	2.93	1.56	2.60	0.449	0.165
Behenic acid (22:0)	0.539	0.668	0.631	0.697	0.070	0.419
Erucic acid (22:1*n*-9)	0.847	0.958	1.25	1.12	0.108	0.055
Docosapentaenoic acid (22:5*n*-3)	5.42	6.80	5.22	5.62	0.709	0.401
Docosahexaenoic acid (22:6*n*-3)	3.29^{b}	4.69^{ab}	5.08^{a}	4.51^{ab}	0.374	0.011
Other	30.8	31.9	25.7	26.0	3.92	0.584
Fatty acid partial sums						
SFA	483	480	506	462	36.1	0.861
MUFA	391	309	384	378	34.0	0.301
PUFA	337	358	318	331	27.2	0.767
n-3 PUFA	14.9	18.8	16.5	17.6	1.30	0.195
n-6 PUFA	320	338	300	312	25.9	0.770
Fatty acid ratios						
PUFA:SFA	0.709	0.778	0.654	0.714	0.058	0.521
n-6:*n*-3	21.6^{a}	18.2^{b}	18.1^{b}	17.5^{b}	0.740	0.001

1 Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase).
2 SEM, standard error of the mean.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
a,b Different superscript letters within a row are significantly different ($P < 0.05$).

Table 6
Element content (mg/100 g for macroelements and μg/100 g for microelements) in *longissimus lumborum* muscle of piglets fed the experimental diets.

Item	Dietary treatments[1]				SEM[2]	*p*-Value
	Control	UL	UL + R	UL + E		
Macroelements (mg/100 g of dry matter)						
Calcium	23.3^{a}	23.8^{a}	23.9^{a}	20.1^{b}	0.47	0.007
Magnesium	28.4	29.9	29.6	28.3	0.25	0.078
Potassium	423	436	434	417	3.5	0.175
Phosphorous	259	266	268	264	2.3	0.527
Sodium	128^{ab}	134^{a}	132^{a}	111^{b}	2.9	0.013
Sulphur	209	212	213	210	2.0	0.928
Total	1072	1101	1101	1050	8.3	0.072
Microelements (μg/100 g of dry matter)						
Bromine	104^{b}	237^{a}	232^{a}	229^{a}	9.5	<0.001
Copper	184	176	186	185	3.87	0.813
Iodine	1.27^{b}	2.00^{a}	1.84^{a}	1.90^{a}	0.053	<0.001
Iron	1644	1798	1637	1389	54.6	0.059
Manganese	69.3^{a}	69.3^{a}	69.3^{a}	51.7^{b}	2.02	0.001
Zinc	1593	1566	1579	1614	24.2	0.917
Total	3594	3848	3705	3471	67.7	0.243
Total macro and microelements (mg/100 g)	1075	1105	1104	1053	8.32	0.071

1 Control (wheat, maize and soybean diet); UL (7% *U. lactuca* replacing control), UL + R (UL with 0.005% of Rovabio® Excel AP) and UL + E (UL with 0.01% recombinant ulvan lyase).
2 SEM, standard error of the mean.
a,b Different superscript letters within a row are significantly different ($P < 0.05$).

accumulation of bromine and iodine in the LL muscle compared to the control group, though when supplemented with ulvan lyase, there was a decrease in calcium and manganese, and a tendency to reduce sodium levels. These effects are consistent with the amounts of bromine and iodine found in *U. lactuca* and align with experimental diet concentrations. Although iodine accumulation in meat can have either positive or toxic effects on metabolism (Gärtner, 2016), and bromine is toxic without proven benefits (Pearson & Ashmore, 2020), the changes in meat element composition are not expected to pose a hazard to human health. The maximum amount of iodine in meat (2.00 μg/100 g) was 75 times lower than the recommended daily intake (150 μg/day) for an adult consuming 100 g of meat per day (World Health Organization, 2007). Similarly, the accumulation of bromine in meat with macroalga treatments (average of 233 μg/100 g) was well below the maximum allowable limit (1000 μg/kg body weight/day), even with a 2-fold increase compared to control (Pearson & Ashmore, 2020). Nevertheless, we do point out that the Br/I ratio is increased by at least 1.4-fold from control to seaweed-fed piglet meat, which is important to consider, given that bromine is particularly detrimental to health when the intake of iodine is low (Sobolev et al., 2020). The small decrease in calcium (23.3 to 20.1 mg/100 g) and sodium (128 to 110 mg/100 g) in meat from piglets fed with *U. lactuca* supplemented with ulvan lyase relative to other diets is not expected to impact their essential roles in bone density (Li et al., 2018) and extracellular fluid regulation and cell membrane transport (Doyle & Glass, 2010). In addition, the reduction of sodium in the meat can have benefits for human health, since it contributes to reducing hypertension and cardiovascular diseases (Doyle & Glass, 2010; Li et al., 2018), although that is not likely to occur when feeding 7% *U. lactuca* supplemented with ulvan lyase to piglets due to a low magnitude and significance of the dietary effect. Other minerals were also diminished in piglet meat with this dietary treatment, which

included manganese and, numerically, iron. Several studies indicated feeding brown and green seaweeds to pigs reduced mineral composition in meat (Jerez-Timaure et al., 2021) and mineral digestibility (Michalak, Chojnacka, & Korniewicz, 2020). The reason why this happened in the current study is uncertain but could be due to the formation of insoluble complexes that prevent the absorption of these micronutrients and the consequent tissue deposition. However, it is beyond the scope of this paper to evaluate that. It is furthermore important to mention that we have not analysed other heavy metal contents in meat (e.g., arsenic and lead) or the health issues on both animals and consumers of long-term feeding of *U. lactuca* to pigs. The repercussions of incorporating such bioaccumulation of minerals as seaweed (Costa et al., 2021) in the diet of the growing-finishing stage warrants further research. Studying how heavy metals may be accumulated or excreted by the animals feeding on these seaweeds is also necessary in order to evaluate the safety of its use, for consumer health and environmental purposes, respectively. Finally, feeding this seaweed for longer periods of time, to growing and/or finishing pigs, could more accurately reflect the effects on pork quality.

5. Conclusions

The results showed that 7% *U. lactuca*, when combined with the commercial Rovabio® 94 Excel AP supplementation in piglet diets, has the potential to slightly improve the content of the beneficial DHA in meat. Moreover, seaweed diets supplemented with carbohydrases enhanced meat sensory traits, through an increase of meat acceptability, tenderness and juiciness, and iodine content. Overall, data reveal the potential impact of incorporating *U. lactuca* in piglet diets, especially when combined with carbohydrases. We also point out that due to the heterogeneity of the nutritional composition of the seaweed depending on harvesting season, production conditions and post-harvesting treatment, other results could be obtained with this seaweed. Therefore, additional research is necessary to determine the optimal incorporation level of *U. lactuca* and the potential benefits of enzyme supplementation in piglet diets. The digestibility of seaweed-containing diets, the impact on piglet metabolism and the role of carbohydrases in improving nutrient bioavailability also warrant further investigation. This will help to fully ascertain the benefits of using this seaweed as an alternative feed ingredient in swine nutrition.

CRediT authorship contribution statement

José M. Pestana: Methodology, Writing – original draft. **Cristina M. Alfaia:** Methodology, Writing – original draft. **David Miguel Ribeiro:** Methodology, Writing – original draft. **Mónica M. Costa:** Methodology, Writing – original draft. **Daniela F.P. Carvalho:** Methodology. **Cátia F. Martins:** Methodology. **Victor M.D. Alves:** Methodology. **José P.C. Lemos:** Methodology. **Miguel Mourato:** Methodology. **Inês Delgado:** Methodology. **Sandra Gueifão:** Methodology. **Inês Coelho:** Methodology. **André M. Almeida:** Writing – review & editing. **João P.B. Freire:** Conceptualization, Writing – review & editing. **José A.M. Prates:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2023.109306.

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