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Marine macroalgae in rabbit feed – Effects on meat quality

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ABSTRACT

The addition of macroalgae to livestock diets has demonstrated to enhance the quality of meat by improving the muscle stability, antioxidant capacity and fatty acid profile. However, information regarding rabbit meat is scarce. This study evaluated the effect of adding 1.025% of different macroalgae, dehydrated and as extracts (*Saccharina latissima, Himanthalia elongata* and *Ulva* spp.) to the diet of growing rabbits. Dietary supplementation with the *Ulva* spp. extract increased the fat content (0.96% vs 0.33% in control group) and the proportion of monounsaturated fatty acids (by 22%; $P \leq 0.022$), but did not affect the moisture, protein or ash contents or the physicochemical properties of the rabbit *longissiumus lumborum* muscle. The antioxidant status of the meat was adequate and was not affected by the dietary supplements. The sensorial properties of the meat were also not affected, and dietary supplementation with both *S. latissima* and *H. elongata* actually enhanced the flavour and juiciness of the meat ($P \leq 0.01$). Altogether, the study findings indicate that the addition of these sustainable ingredients to rabbit feed did not negatively affect meat quality, and some of them may potentially improve specific characteristics, which could make this meat more attractive to consumers.

1. Introduction

Rabbit meat production is an important industry in Europe, particularly in Spain, Italy and France (Cullere & Dalle Zotte, 2018). The meat is valued for its good quality (high quality protein and low-fat content) and its nutritional properties (Dalle Zotte, 2002). Nevertheless, the industry is facing some production-related difficulties (increased mortalities due to digestive diseases) and a reduction in sales of rabbit meat, mainly due to the lack of tradition of consumption and to the taste (Buitrago-Vera, Escribá-Pérez, Baviera-Puig, & Montero-Vicente, 2016), as well as to the lack of availability of processed products suited to more modern types of consumption (at a competitive price) and the increasing perception of rabbits as pets (Al-Soufi, García, Muíños, & López-Alonso, 2022; Cullere & Dalle Zotte, 2018).

Marine macroalgae have gained increasing interest as a sustainable ingredient in animal feed (Costa, Cardoso, Afonso, Bandarra, & Prates, 2021), as their unique composition has great potential to improve gut health, mainly due to the potential prebiotic effect of some of their polysaccharides or their influence on the immune response (Al-Soufi et al., 2022; Evans & Critchley, 2014), which have already proved beneficial in piglets (Heim, Sweeney, O'Shea, Doyle, & O'Doherty, 2014; Walsh, Sweeney, O'Shea, Doyle, & O'Doherty, 2013). Processing macroalgae extracts is very promising as it enables concentration of some polysaccharides of interest, such as laminarin, fucoidan and ulvans, which potentially act as prebiotics, as they can be fermented by gut microbiota (Al-Soufi et al., 2023), or immune stimulators (Bussy et al.,

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2019). Moreover, when added to animal feed, macroalgae might enhance meat quality, by modulating the fatty acid composition (Moroney et al., 2015; Ribeiro et al., 2021) and by improving the antioxidant (Michalak et al., 2022) and sensory properties (Rossi et al., 2020).

The inclusion of macroalgae in rabbit feed is not usual and the effects are still not fully understood. However, some studies have already demonstrated some benefits. Some macroalgae species (e.g. *Saccharina latissima* and *Himanthalia elongata*) have been demonstrated to be easily fermentable and/or increase the butyrate concentration by the caecal microbiota of rabbits (Al-Soufi et al., 2023), although they had no effect on mortality (Al-Soufi et al., 2024). Regarding meat quality, a recent study in Italy reported a lower cholesterol content and an improvement in the oxidative stability and the sensory quality of the meat when *Laminaria* spp. and plant polyphenols were added to the rabbit diets at doses of 0.3 and 0.6% (Rossi et al., 2020). Nevertheless, the potential effects of macroalgae will depend on various factors such as the species, the type of processing of macroalgae and the amount added. In all cases, it is also important to demonstrate that the inclusion of the macroalgae in the diet does not have a negative effect on meat quality or in its sensory properties, so that the product will be acceptable to consumers.

This research aimed to evaluate the effects on rabbit meat quality (proximate composition, physico-chemical and antioxidant properties, fatty acid profile and sensory properties) of the addition of different marine macroalgae products *(Saccharina latissima, Himanthalia elongata* and *Ulva* spp.*)* to the rabbit diet, in the context of valorizing discards generated by the macroalgae industry in Galicia (north-western Spain) in a circular-economy approach. Demonstration of positive effects on rabbit meat quality would enable this strategy to be used as a marketing tool to attract customers searching for healthy, sustainable meat products.

2. Material and methods

2.1. Experimental trial

All of the experimental procedures were in accordance with the Spanish guidelines for the care and use of animals in research (Spanish Royal Degree 53/2013; BOE, 2013). The procedures were approved by the Bioethics Committee of the Universidad Politécnica de Madrid, Spain (protocol code 2021–002, approved 24 February 2021).

Overall, in this trial, experimental diets, each including one of the four macroalgae products, were tested relative to a control diet without macroalgae. The control diet was formulated considering the nutritional standards for growing rabbits (De Blas and Mateos, 2020; Table 1). The macroalgae products were provided by Porto-Muíños S.L. (Cerceda, A Coruña, Spain) and selected according to the better results obtained from their in vitro characterization (Al-Soufi et al., 2023). Four macroalgae products were prepared: dehydrated *Saccharina latissima* (DSL diet), aqueous extract of hydrolysed *S. latissima* (ESL diet), aqueous extract of *Himanthalia elongata* (EHE diet) and aqueous extract of hydrolysed *Ulva* spp. (EU diet). Dehydrated *S. latissima* was obtained by drying the sample at low temperature (<40 °C) for 4–5 days before grinding it in a micro-grinding mill (Komodin K-160 P, Lleal, Granollers, Spain) that rendered powder samples. In order to obtain the aqueous extract of *H. elongata*, the whole macroalgae was mixed with water (1:30 m:v) and subjected to autohydrolysis in a Parr pressure reactor operating in a non-isothermal mode up to 160 °C and 110 psi. The supernatant was then collected and dried with a spray dryer (Büchi B-290, Flawil, Switzerland) equipped with a standard cyclone (1.5 mm nozzle). The operating settings were 115 °C inlet temperature, 4 mL/min (pump at 15%) feed solution flow rate and 1050 L/h atomization air flow rate and 4.1 bar pressure. Finally, the aqueous extract of the enzymatically hydrolysed macroalgae (*S. latissima* and *Ulva* spp.) was obtained. Samples were mixed with water (1:10 ratio) containing the enzyme Celluclast (Novozymes) at 4%, and the mixture was incubated at 50 °C for 6 h.

Table 1
Formulation and chemical composition of the control diet. For each experimental diet (DSL, ESL, EHE, EU), the corresponding macroalgae product was additionally added to the control diet at a dose of 1.025% (*w*/w).

Ingredient	%
Alfalfa meal	20.0
Wheat bran 15% CP	19.9
Orange pulp	15.0
Sunflower meal extracted	11.8
Oat hulls	10.0
Corn gluten feed	8.00
Barley	4.54
Beet molasses	3.00
Soybean meal 47% CP	2.81
Rice hulls	2.17
Corn DDGS	1.04
Soybean oil	0.50
Sodium chloride	0.39
Vitamin and mineral premix[1]	0.30
Diclazuril 0.5%	0.20
L-Lysine sulphate	0.16
L-Threonine	0.09
Amino acid composition	%
Lysine	0.65
Methionine	0.23
Met + Cys	0.47
Threonine	0.59
Arginine	0.83
Nutritional value[2]	
Digestible energy, kcal/kg	2237
Digestible protein, %	10.3

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissimi;* EHE: aqueous extract of *Himanthalia elongate;* EU: aqueous extract of hydrolysed *Ulva* spp. w/w: wet weight.

[1] The vitamin and mineral premix provided the following substances, per kg of complete diet: Vit A: 10,000 IU; Vit D_3: 1000 IU; Vit E: 40 mg; Vit K_3: 2 mg; Vit B_1: 2 mg; Vit B_2: 6 mg; Vit B_6: 3.9 mg; Vit B_{12}: 15 μg; calcium pantothenate: 16.30 mg; niacin: 40 mg; biotin: 100 μg; folic acid: 1 mg; betaine hydrochloride: 131.6 mg; Mn: 10 mg; I: 1 mg; Fe: 50 mg; Cu: 8 mg; Zn: 50 mg; Co: 0.2 mg; Se: 0.2 mg.[2] Values obtained by Alfonzo et al. (2023).

The enzyme was inactivated raising the temperature to 90 °C for 15 min. The supernatant was then dried and ground as commented before (Al-Soufi et al., 2023). The basal growing diet was supplemented with the macroalgae products at a dose of 1.025% (*w*/w;) (Table 1). Antibiotics were not added to any of the diets throughout the trial. The chemical composition and in vitro faecal digestibility of the four macroalgae products (Table 2) and the experimental diets (Table 3) were determined as previously described (Al-Soufi et al., 2023). The fatty acid (FA) profile of all experimental diets was determined as described in Section 2.4 and is shown in Table 4.

This study was conducted within a broader experimental trial (TIRAC project) whose aim was to evaluate the benefits of four marine macroalgae products on the gut health of post-weaning rabbits. As part of this research, a total of 40 mixed-sex crossbred (New Zealand White X Californian) weaned rabbits were randomly assigned to the groups fed the different experimental diets or the control diet without macroalgae. Each group was composed of eight rabbits, which were fed ad libitum with the corresponding diets between weaning (age 30 days, mean weight 701 ± 80 g) and slaughter (age 63 days; mean weight 2154 ± 162 g). The rabbits were housed individually in flat desk cages and water was provided ad libitum.

2.2. Sample collection

On reaching 63 days of age, eight rabbits from each group were

Table 2
Chemical composition of macroalgae products (% DM).

	Dehydrated	Aqueous extracts		
Chemical composition	*S. latissima* (DSL)	*S. latissima* (ESL)	*H. elongata* (EHE)	*Ulva* spp. (EU)
Moisture	6.60	4.91	7.68	19.6
On DM basis				
Ash	37.8	54.9	50.3	38.6
Total dietary fibre	33.2	4.02	20.5	37.4
Neutral detergent fibre	11.8	0.00	1.58	BDL
Acid detergent fibre	9.07	0.00	2.74	BDL
Acid detergent lignin	0.64	0.00	1.34	BDL
Soluble fibre	21.4	4.02	18.9	37.4
Crude protein	19.9	15.9	6.31	6.33
CP-TDF	5.15	BDL	2.54	1.46
CP-NDF	5.79	BDL	BDL	BDL
Ether extract	3.81	3.64	3.80	10.8
In vitro faecal digestibility, %				
ivDMd	68.4	99.9	98.4	100
ivOMd	59.1	99.8	97.3	99.7
ivCPd	67.0	100	94.2	100

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissimi;* EHE: aqueous extract of *Himanthalia elongate;* EU: aqueous extract of hydrolysed *Ulva* spp. DM: dry matter. ivDMd: in vitro dry matter digestibility; ivOMd: in vitro organic matter digestibility; ivCPd: in vitro crude protein digestibility. BDL: below detection level.

Table 3
Chemical composition of experimental diets (% DM).

Chemical composition	Control	DSL	ESL	EHE	EU
Ash	8.38	8.53	8.64	8.81	8.79
Crude protein	17.0	16.9	17.0	16.4	16.6
Ether extract	3.74	3.31	3.46	3.48	3.10
Neutral detergent fibre	44.5	43.4	42.6	45.2	45.2
Acid detergent fibre	20.1	20.0	19.7	20.5	20.4
Acid detergent lignin	3.63	3.73	3.80	4.10	4.39
In vitro faecal digestibility, %					
ivDMd	65.0	65.9	66.2	65.5	66.5
ivCPd	89.1	88.6	89.2	91.0	91.0

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissimi;* EHE: aqueous extract of *Himanthalia elongate;* EU: aqueous extract of hydrolysed *Ulva* spp. DM: dry matter.

slaughtered by head concussion and subsequent bleeding. The whole carcass of each animal was collected after removing the skin, the distal parts of the limbs and tail, the gastrointestinal tract and the urogenital tract, as recommended by the World Rabbit Science Association (Blasco & Ouhayoun, 1993). The carcasses were immediately refrigerated at 4 °C and chilled for 24 h before analysis). Samples from the *longissimus lumborum* (LL) muscle from each animal were used in the analytical procedures.

2.3. Proximate composition, physicochemical and antioxidant analysis of meat

The chemical composition of LL muscle (moisture, protein, ash and fat contents) and the physicochemical parameters (pH and the *L**lightness, *a**redness, *b**yellowness, chroma, Hue angle and ΔE colour parameters) were determined following the procedures described by Lorenzo, Munekata, Pateiro, Campagnol, and Domínguez (2016). Meat colour was measured on the LL surface after 60 min of blooming using a portable colorimeter (Konica Minolta CM-600 d, Osaka, Japan) with an illuminant D65, 0° viewing angle geometry and 8 mm of aperture size.

The antioxidant capacity of the meat (LL) was determined by the 2,2-diphenyl −1-picrilhihidrazil (DPPH) radical scavenging activity, as mg trolox/g sample, according to de Carvalho et al. (2020); and lipid oxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) assay (as mg MDA/kg sample), following Tarladgis, Watts, Younathan, and Dugan (1960).

Table 4
Fatty acid composition of complete experimental diets (mg/100 g feed).

Fatty acid	Experimental diets				
	Control	DSL	ESL	EHE	EU
C6:0	1.35	1.40	1.24	1.31	1.38
C8:0	1.48	1.41	1.38	1.52	1.45
C10:0	0.664	0.651	0.673	0.669	0.659
C11:0	0.244	0.252	0.235	0.249	0.238
C12:0	1.84	1.93	1.71	1.88	1.97
C14:0	5.08	5.58	5.12	5.46	4.86
C15:0	2.02	2.22	1.85	2.16	2.97
C16:0	368	353	387	374	361
C16:1*n*-7	4.48	4.98	4.03	4.25	4.67
C17:0	1.80	1.68	1.87	1.74	1.91
C18:0	48.3	45.6	47.1	50.3	46.9
9 t-C18:1	0.667	0.663	0.672	0.661	0.670
11 t-C18:1	2.41	2.48	2.35	2.44	2.39
C18:1*n*-9	446	432	454	441	460
C18:1*n*-7	26.9	27.3	25.1	26.3	25.8
C18:2*n*-6	994	962	984	979	998
C18:3*n*-3	110	119	103	105	116
9c,11 t-C18:2 (CLA)	0.625	1.09	0.640	0.795	0.629
C20:0	9.67	9.51	9.79	9.83	9.37
C20:1*n*-9	10.4	10.9	11.2	9.76	10.1
C20:2*n*-6	1.94	1.85	1.98	1.79	1.87
C21:0	1.22	1.15	1.24	1.31	1.29
C20:3*n*-6	1.35	1.31	1.43	1.29	1.37
C20:4*n*-6	0.293	0.741	0.315	0.678	0.292
C20:3*n*-3	0.417	0.436	0.393	0.427	0.432
C20:5*n*-3 (EPA)	1.21	1.51	1.11	1.39	1.29
C22:0	9.51	9.92	9.31	8.84	9.72
C22:1*n*-9	4.56	4.22	4.31	4.72	4.87
C22:5*n*-3 (DPA)	18.2	19.6	17.4	18.8	19.3
C23:0	3.41	3.32	3.58	3.14	3.47
C24:0	16.7	15.2	17.6	16.1	15.9
C24:1*n*-9	2.83	2.96	2.64	3.02	2.76
SFA	472	452	489	478	463
MUFA	498	485	504	492	511
PUFA	1128	1110	1113	1112	1141
n-3	130.2	140	122	126	137
n-6	998	965	987	982	1001
Total	2099	2045	2104	2079	2113

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissima;* EHE: aqueous extract of *Himanthalia elongata;* EU: aqueous extract of hydrolysed *Ulva* spp.

2.4. Fatty acid profile of meat

For the fatty acid analysis of LL muscle (10 g of wet muscle) and the complete diets, fat extraction (using methanol, chloroform, and water) and fatty acid transesterification (first a base-catalysed transesterification with sodium methoxide in methanol followed by an acid-catalysed transesterification with methanolic sulfuric acid was used) were carried out according to the protocol described by Domínguez, Crecente, Borrajo, Agregán, and Lorenzo (2015). Fatty acids methyl esters (FAMEs) were separated and quantified by gas chromatography-FID (Agilent Technologies, Santa Clara, CA, USA), following the chromatographic conditions described by Domínguez et al. (2015). One μL in split mode (50:1) was injected. For the separation of fatty acid, a DB-23 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies) was used. Individual fatty acids were identified by comparing their retention times with those of authenticated standards (FAME Mix-37 components-; docosapentaenoic acid (C22:5n-3; DPA); trans-11 vaccenic acid (11 t-C18:1; TVA); cis-vaccenic acid (18:1n-7, CVA, Supelco, Madrid, Spain) and conjugated linoleic acid

(9c,11 t-C18:2, CLA, Matreya, State College, PA, USA). As internal standard C19 was used. The results were expressed as mg/100 g of muscle tissue.

2.5. Sensory analysis of meat

The sensory quality of the meat was evaluated in the sensorial analysis laboratory of the Meat Technology Center of Galicia, which is equipped with individual cabinets under white light, according to UNE-EN ISO 8589:2010 regulation (International Organization of Standardization, 2014). Quantitative-descriptive analysis (QDA) was conducted to determine the sensorial profile of the five treatments evaluated (Cittadini, et al. 2022), following the ISO 13299:2017 regulations (International Organization for Standardization, 2017). The evaluation panel was composed by seven assessors (four women and three men, aged between 32 and 49 years) selected from the Meat Technology Center of Galicia Staff (Ourense, Spain), who were previously trained following the UNE-EN ISO 8586:2014 methodology (International Organization for Standardization, 2014) with the attributes and scale used during three sessions.

Steaks (of thickness 2 cm) were cut from the LL muscle and cooked in a convection oven at 180 °C until an internal temperature of 80 °C was reached. The tasters were provided water and unsalted bread at the beginning of session and between samples, to cleanse their palates and remove residual flavours. Samples were individually labelled with a randomized 3-digit number and served together on white dishes. The tasting order was designed and indicated to the panellists to avoid first sample and carry-over effects (Macfie, Bratchell, & Greenhoff, 1989). Tasters evaluated five attributes (odour, flavour, tenderness, juiciness and fibrousness) on a structured scale ranging from 0 (absence or minimal intensity of the attribute) to 10 (presence or maximal intensity). The scores obtained in the descriptive analysis were used to determine the sensory profile for each treatment.

2.6. Statistical analysis

The effect of the macroalgae treatments on the proximate composition, physicochemical parameters, antioxidant properties and fatty acid profiles was evaluated using a two-way ANOVA, with sex included as a main factor. Neither sex nor the interaction between sex and treatment were significant in the analysis; therefore, the effect of the treatment was evaluated by a post-hoc Tukey test. The effect on the sensorial properties was examined by a two-way ANOVA, including panellists as a fixed factor and session as random term, and considering the interaction between panellists × treatment. A significance level of $\alpha = 0.05$ was established. The IBM SPSS statistical software (version 27.0) was used for all statistical tests.

3. Results and discussion

3.1. Proximate composition, physicochemical and antioxidant properties of meat

Inclusion of the macroalgae products in the diet did not affect the final body weight or dressing out percentage, which were on average 2162 g and 60.2%, respectively (Table 5). These data are consistent with the lack of effect of the inclusion of low levels of macroalgae on growth rate in rabbits obtained in a parallel experiment (Al-Soufi et al., 2024).

The results of the proximate composition and the physicochemical and antioxidant properties of meat are presented, for each treatment, in Table 6. The average moisture (76.36%), protein (21.81%) and ash (1.23%) contents for the five treatments were consistent with the reference values reported by Hernández and Dalle Zotte (2020) for this meat cut (75% moisture, 22% protein, 1% ash). Regarding the lipid content, the values obtained were much lower (average 0.53%) than reference values for the loin, 1.4% (and 38% coefficient of variation; Combes (2004)) and 2 ± 1.5% (mean ± standard deviation; Hernández and Dalle Zotte (2020). This discrepancy can be explained by the age of slaughter. In this study rabbits were slaughtered at a younger age (63 days) than in the countries of origin of most of the reference data (France and Italy; age around 70 and 80 days). The fat content of the *longissimus* muscle increases gradually from weaning to 20 weeks (Cavani et al., 2000; Gondret, Mourot, Lebas, & Bonneau, 1998), and the main enzyme involved in the novo synthesis of fatty acids (acetyl-CoA carboxylase) remains low up to 9 weeks of age (Gondret, Mourot, & Bonneau, 1997).

Table 5
Effect of dietary inclusion of macroalgae products on dressing percentage at age 63 days.

	Control	DSL	ESL	EHE	EU	SEM	*P*-value
Body weight, g	2141	2257	2167	2138	2109	63.1	0.53
Hot carcass weight, g	1302	1357	1307	1277	1268	41.6	0.65
Dressing out percentage, %	60.8	60.1	60.3	59.7	60.1	0.51	0.65

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissima;* EHE: aqueous extract of *Himanthalia elongata;* EU: aqueous extract of hydrolysed *Ulva* spp. $n = 8$ per treatment.

There were no significant differences between experimental groups in the moisture, protein or ash contents of the LL muscle ($P \geq 0.2$). By contrast, regarding the lipids, the LL fat content was higher ($P < 0.05$) in rabbits fed the diet containing the *Ulva* spp. extract (0.96%) than in those fed the diets containing the other two macroalgae extracts, and in the control group (0.33%). In all cases, the lipid content was below the lower limit of the reference range for this cut of meat. The differences did not seem to be related to the dietary fat level. The relatively low fat content of the experimental diets (on average, 3.5% of dry matter content (DM)) is within the range (2.5–3.5% DM) at which minor or no changes are observed in the intramuscular fat (Dalle Zotte, 2000). In fact, dietary and LL fat contents were not linearly correlated ($r = -0.25$; $P = 0.12$; $n = 40$). In pigs, low protein (or lysine) diets enhanced fat accumulation in muscle without affecting subcutaneous fat (Madeira et al., 2013, 2017). This may partly explain the relatively high fat content in the group fed the diet containing *Ulva* spp., as in a parallel experiment rabbits fed this diet showed the lowest digestible [protein/energy] ratio (Alfonzo et al., 2023), and a lower feed intake than control diet (unpublished results), which led to the *Ulva* group to the lowest digestible protein intake.

Regarding the physicochemical properties, neither the colour parameters nor the pH differed in relation to the experimental diets ($P \geq 0.2$). In all groups, the colour parameters indicated that the meat was slightly lighter (average $L^* = 62.64$), less red (average $a^* = -1.74$) and more yellowish (average $b^* = 10.31$) than previously reported for LL muscle of rabbits at the same age of slaughter (Pla, 2008). Redness is associated with oxidation of the meat (Trombetti et al., 2022), and in this case the lower values of a^* can therefore be explained by a very low degree of lipid oxidation, as explained below. According to Adekunte, Tiwari, Cullen, Scannell, and O'Donnell (2010), colour differences can be considered noticeable when ΔE^* values are higher than 3. In our study, *Ulva* group presented the highest ΔE^* values (3.39), whereas the other treatments showed ΔE^* values from 1.93 to 2.92 (Table 6).

Regarding the antioxidant properties of meat, neither the free radical scavenging activity of meat (DPPH) nor the degree of lipid oxidation (TBARS) differed in relation to the experimental diets ($P \geq 0.1$). The TBARS values were remarkably low in all treatments (mean TBARS = 0.02 mg MDA/kg), indicating very low lipid oxidation. As already explained, the lipid content of the LL muscle was very low, which could partly explain the very low degree of lipid oxidation. These values are within the range reported by some authors (Lo Fiego et al., 2004; Mattioli et al., 2019, 2016), but lower than those reported by other authors (Dabbou et al., 2017; Liu et al., 2009) for this type of meat. The level and

Table 6
Effect of dietary inclusion of macroalgae products on proximate composition, physicochemical and antioxidant properties of rabbit *longissimus lumborum* muscle.

Parameter	Control	DSL	ESL	EHE	EU	SEM	*P*-value
Proximate composition							
Moisture (%)	76.6	76.5	76.3	76.4	75.9	0.099	0.21
Lipids (%)	0.33^{a}	0.55^{ab}	0.44^{a}	0.36^{a}	0.96^{b}	0.059	0.002
Protein (%)	21.8	21.5	21.9	22.0	21.8	0.083	0.42
Ash (%)	1.22	1.23	1.20	1.23	1.27	0.014	0.65
Physicochemical properties							
*L** (lightness)	61.3	63.0	62.4	62.9	63.5	0.305	0.17
*a** (redness)	−1.80	−1.51	−1.86	−1.93	−1.61	0.108	0.74
*b** (yellowness)	9.90	10.5	9.96	10.34	10.8	0.163	0.32
Chroma	10.2	10.6	10.1	10.5	10.9	0.155	0.59
Hue Angle	−80.2	−81.5	−79.3	−79.4	−57.9	4.18	0.62
ΔE	–	2.92	1.93	2.51	3.39	0.330	0.33
pH	5.72	5.71	5.67	5.73	5.74	0.052	0.75
Antioxidant properties							
TBARs (mg MDA/kg)	0.00891	0.0162	0.0448	0.0239	0.0295	0.006	0.37
DPPH (μg trolox/g)	115	118	108	131	108	3.17	0.13

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissima;* EHE: aqueous extract of *Himanthalia elongata;* EU: aqueous extract of hydrolysed *Ulva* spp. n = 8 per treatment. TBARS: degree of lipid oxidation; DPPH: free radical scavenging activity of meat. $^{a\text{-}b}$ for each parameter (row) different letters indicate that the mean values were different ($P < 0.05$; Tukey test).

type of fat, the type of sample (refrigerated vs. frozen) and the storage time may all influence these values.

Marine macroalgae have been shown to have both positive and negative effects on the antioxidant capacity of meat. On the one hand, they are known for their high contents of polyphenols and other antioxidant compounds (Garcia-Vaquero et al., 2021; Morais et al., 2020),

Table 7
Fatty acid (FA) composition of *longissimus lumborum* muscle (mg/100 g of muscle) in relation to each experimental diet.

Fatty acid	Control	DSL	ESL	EHE	EU	SEM	*P*-value
C6:0	0.369^{a}	0.545^{ab}	0.475^{ab}	0.423^{ab}	0.639^{b}	0.030	0.031
C8:0	0.280	0.371	0.330	0.304	0.430	0.021	0.15
C:10	0.770^{a}	1.40^{ab}	1.10^{ab}	1.15^{ab}	2.44^{b}	0.170	0.017
C12:0	1.032^{a}	1.67^{ab}	1.44^{ab}	1.59^{ab}	3.19^{b}	0.207	0.007
C13:0	0.136^{a}	0.152^{ab}	0.145^{ab}	0.143^{a}	0.207^{b}	0.008	0.017
C14:0	14.8^{a}	20.3^{ab}	19.3^{ab}	17.6^{a}	28.4^{b}	1.23	0.004
C14:1*n*-5	0.932^{a}	1.97^{ab}	2.06^{ab}	1.44^{ab}	3.09^{b}	0.230	0.032
C15:0	4.15	4.51	4.50	4.36	5.86	0.204	0.056
C16:0	239^{a}	276^{ab}	284^{ab}	259^{a}	369^{b}	13.1	0.013
C16:1*n*-7	19.6^{a}	33.3^{ab}	36.2^{ab}	27.4^{a}	50.0^{b}	2.79	0.005
C17:0	5.09	5.30	5.19	5.05	6.77	0.242	0.12
C18:0	54.0	58.8	56.6	54.8	72.5	2.29	0.060
9 t-C18:1	2.26	2.24	2.39	2.23	2.34	0.070	0.94
11 t-C18:1	185^{a}	208^{ab}	224^{ab}	197^{a}	289^{b}	11.0	0.017
C18:1*n*-9	163^{a}	212^{ab}	229^{ab}	201^{ab}	296^{b}	12.4	0.009
C18:1*n*-7	12.7	13.6	15.1	12.5	17.2	0.623	0.083
C18:2*n*-6	206^{a}	224^{ab}	224^{ab}	219^{ab}	289^{b}	9.67	0.045
C18:3*n*-6	0.949	1.06	1.11	1.10	1.38	0.053	0.11
C18:3*n*-3	13.5^{a}	15.8^{ab}	15.2^{ab}	14.6^{a}	22.8^{b}	0.963	0.012
9c,11 t-C18:2 (CLA)	1.12	1.45	1.34	1.19	1.67	0.068	0.072
C20:0	0.845^{a}	1.01^{ab}	0.944^{ab}	0.900^{a}	1.35^{b}	0.052	0.012
C20:1*n*-9	2.09	2.34	2.50	2.04	3.30	0.162	0.088
C20:2*n*-6	3.89	4.13	4.12	3.74	4.38	0.146	0.71
C21:0	1.26	1.28	1.28	1.15	1.14	0.039	0.65
C20:3*n*-6	4.69	4.48	4.76	4.44	4.67	0.126	0.92
C20:4*n*-6	41.0	36.8	40.6	39.2	34.3	1.08	0.25
C20:3*n*-3	0.492	0.467	0.462	0.418	0.553	0.030	0.72
C20:5*n*-3 (EPA)	1.59	1.53	1.55	1.52	1.45	0.042	0.87
C22:0	0.474	0.518	0.491	0.509	0.627	0.024	0.28
C22:1*n*-9	0.237	0.262	0.278	0.228	0.287	0.017	0.79
C22:2*n*-6	1.13	1.09	1.58	1.28	1.25	0.085	0.41
C24:0	0.413	0.509	0.426	0.323	0.659	0.067	0.61
C22:5*n*-3 (DPA)	7.27	6.55	7.19	6.64	6.55	0.164	0.44
C22:6*n*-3 (DHA)	1.75	1.56	1.82	1.62	1.59	0.047	0.34
C24:1*n*-9	0.336	0.324	0.375	0.335	0.410	0.020	0.66
SFA	324^{a}	374^{ab}	377^{ab}	348^{a}	494^{b}	17.3	0.01
MUFA	387^{a}	475^{ab}	513^{ab}	445^{a}	663^{b}	26.3	0.008
PUFA	281	298	302	293	369	11.3	0.098
n-3	22.9	24.6	23.9	22.7	31.9	1.11	0.041
n-6	258	272	276	269	337	10.2	0.11
Total	992^{a}	1146^{ab}	1191^{ab}	1086^{a}	1527^{b}	53.8	0.015

DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissima;* EHE: aqueous extract of *Himanthalia elongata;* EU: aqueous extract of hydrolysed *Ulva* spp. SFAs: Saturated fatty acids; MUFAs: Monounsaturated fatty acids; PUFAs: Polyunsaturated fatty acids; *n*-3: Omega-3; *n*-6: Omega-6. $^{a\text{-}b}$ for each parameter (row) different letters indicate that the mean values were different ($P < 0.05$; Tukey test). SEM: standard error of the mean.

which could reduce the lipid oxidation of meat when included in animal feed (for a review, see – Michalak et al. (2022)). The amounts of antioxidant compounds in macroalgae vary widely depending on the species, time of harvesting, temperature and stressors, and the antioxidant contents must be determined if this effect is to be pursued (Michalak et al., 2022). On the other hand, both macroalgae and rabbit meat are rich in PUFAs (Biris-Dorhoi et al., 2020), leading to a higher degree of oxidation, and consequently to a reduced shelf-life and to a possible rancid flavour of some meat cuts (Dalle Zotte, 2002; Moroney et al., 2015; Nutautaitė, Racevičiūtė-Stupelienė, Bliznikas, & Vilienė, 2023). Achieving an equilibrium between the antioxidant contents and the healthy fatty acid profile of rabbit meat is therefore important.

3.2. Fatty acid profile of meat

The fatty acid profile of LL muscle is shown, for each experimental group, in Table 7. MUFAs were predominant in all groups (on average 496 mg/100 g muscle), followed by SFAs (on average 383 mg/100 g muscle) and PUFAs (on average 309 mg/100 g muscle). These values differ from reference values reported for this type of meat (Combes, 2004; Dalle Zotte, 2002; D'Arco et al., 2012). Although rabbit meat is characterised by a healthy FA profile relative to other meat types, which generally has lower SFA and cholesterol contents and higher amounts of PUFAs and balanced n-3 and n-6 FA, it appears that the highest proportion of FA is constituted by the SFAs (38–40%), followed by MUFAs (22–32%) or PUFAs (24–37%), depending on the cut of meat. Therefore, the change in the proportions of FAs (MUFAs > SFAs > PUFAs) is a positive effect because MUFAs are considered beneficial for human health, in comparison with SFAs, which in this case were much lower than the usual values. Regarding the individual FAs, the most abundant in all treatments were palmitic (C16:0), trans-vaccenic (11 t-C18:1), oleic (C18:1n-9), and linoleic (C18:2n-6) acids. These results are consistent with values previously reported for this meat, with palmitic, oleic, and linoleic acids also constituting the most abundant FAs (Dalle Zotte, 2002).

Regarding the experimental diets, rabbits fed the diet containing the *Ulva* spp. extract showed a significant different composition of fatty acids. Both MUFA and SFA contents in EU meat (663 and 494 mg/100 g muscle, respectively) were significantly higher (1.7 and 1.5-fold increase) than in the control group (387 and 324 mg/100 g muscle, respectively). Both DSL and ESL groups showed higher values of these FA than the control group, although not significant; and PUFA content was also higher in EU group (369 mg/100 g muscle) than in the control group (281 mg/100 g muscle), although not significant. This effect might be associated with the higher LL fat content in this group, since total FA content was significantly higher in EU group (1527 mg/100 g muscle) than in control group (992 mg/100 g muscle). As described before, these effects might come from the lower protein digestibility of this group. In fact, in pigs fed low protein (or lysine) diets, that enhanced fat accumulation in muscle, the MUFA and SFA contents also increased and the PUFA content decreased (Madeira et al., 2013, 2017). Anyway, both the total fat content and the FA profile of pork meat are very different from those of the rabbit meat, and therefore the results must be compared with caution.

The contents of some other individual FAs, such as myristic (C14:0), palmitic (C16:0), trans-vaccenic (11 t-C18:1), oleic (C18:1*n*-9) and linoleic (C18:2*n*-6) acids, were also higher ($P < 0.05$) in the meat from rabbits fed the diet supplemented with *Ulva* spp. Moreover, both odd-chain fatty acids (C15:0 and C17:0) showed a tendency to increase in EU group compared to the control group.

These findings differ from those of other studies, although to the best of our knowledge, there are no comparable studies in which the same macroalgae and dose were tested in rabbits. For example, the inclusion of laminarin and fucoidan extracts derived from *Laminaria digitata* (brown macroalgae) in pig diets at doses of 450–900 mg/kg feed (Moroney et al., 2015) decreased the SFA content of the LL muscle. Similar results were observed after inclusion of *Cladophora glomerata* (freshwater green macroalgae) at doses of 4 and 8% (*w*/w) in diets fed to weaned rabbits (Nutautaitė et al., 2023), which enhanced the meat profile by decreasing SFAs and MUFAs and increasing the proportion of PUFAs. In another study conducted in rabbits and in which the microalgae *Spirulina platensis* was added to their diets at rates of 50–150 g/kg feed, the meat (LL muscle) also had lower SFA and MUFA contents and a higher PUFA content (Peiretti & Meineri, 2011). Comparison of these results must take into account that the possible effect of macroalgae on the FA profile of meat will vary depending on numerous factors. For example, *Spirulina platensis is* a microalga with a very different composition from that of marine macroalgae. On the other hand, the dose included in animal feed also determines the possible effects, and these studies in rabbits included a higher proportion of algae (4–8% for *Cladophora glomerata* and 5–15% for *Spirulina platensis*) than those used in the present research.

Despite these differences, some marine macroalgae appear to modify the FA profile of meat when included in animal feed. In order to understand why these effects occur, it is important to consider that the lipid content of most marine macroalgae is <5% DM (Biris-Dorhoi et al., 2020; Costa et al., 2021), and that they are generally included in animal feed at low doses (< 2%), as in the present study. Indeed, some researchers have observed that the direct contribution of FAs from macroalgae to diet would not explain the effect on the meat FA profile (Moroney et al., 2015). In the present study, the dose of macroalgae included was very low (1.025%) and the crude fat content of the diet was relatively low. Moreover, three of the macroalgae products used were aqueous extracts, in which lipids are expected to be almost absent (except for the unexpected high fat content of the *Ulva* spp. extract), as they were processed with the objective of enhancing digestibility and concentrating beneficial fermentable polysaccharides. In fact, the FA profile of all experimental diets was very similar (Table 4), which means that the increase of FA in EU group cannot come from a direct contribution in the diet. Therefore, the explanation might be that macroalgae alter the FA profile of meat by interacting with gut microbiota and/or with fat metabolism. Some macroalgae-derived polysaccharides have been demonstrated to act as prebiotics and therefore alter the microbiome, with the consequent increase in short-chain fatty acids (SCFAs). These SCFAs act as substrates for the endogenous synthesis of FAs, which explains their effect on the FA profile of meat (Moroney et al., 2015; Ribeiro et al., 2021). This influence might be reflected in odd-chain fatty acids, which are synthesized by the intestinal microbiota through the elongation of propionate and valerate, minor volatile fatty acids derived from microbial fermentation in rabbits, and which are used in ruminants as biomarkers of ruminal function (Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006). In this case, the inclusion of *Ulva* spp., rich in soluble fibre (Al-Soufi et al., 2023), showed a tendency to increase the proportion of odd fatty acids in LL, what might be an indication of this type of effect on rabbit microbiota. Similarly, the inclusion of sugar beet pulp (soluble fibre) increased both odd and branched-chain fatty acids in rabbit LL (Papadomichelakis, Karagiannidou, Anastasopoulos, & Fegeros, 2010) and milk (Delgado et al., 2018).

3.3. Sensory analysis of meat

The results obtained for the sensory analysis of meat from each treatment are shown in Fig. 1. The odour, tenderness and fibrousness of the meat were not affected by the experimental diets ($P > 0.05$). The juiciness of meat was improved when rabbits were fed with *H. elongata* extract, relative to the meat from the control group (5.6 vs. 4.8; $P = 0.01$). The flavour of the meat was also affected by the experimental diet containing *S. latissima* extract (5.8 vs 5.2, $P = 0.008$). These preliminary results are very positive because it is important to verify that the addition of marine macroalgae to rabbit diets will not give the meat an unpleasant taste. The macroalgae-supplemented diets did not negatively

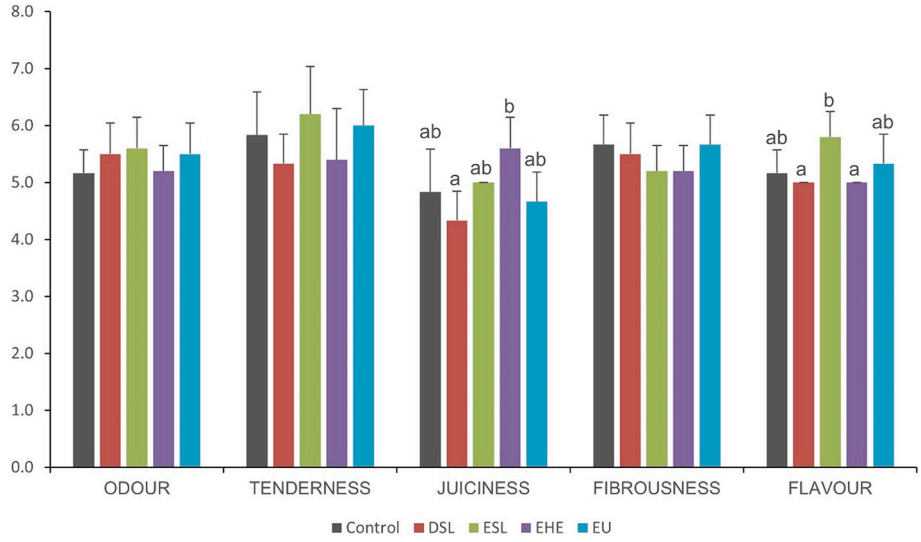

Fig. 1. Average scores (0–8 scale) for the sensorial properties of meat, for each treatment (DSL, ESL, EHE, EU). DSL: dehydrated *Saccharina latissimi;* ESL: aqueous extract of hydrolysed *S. latissima;* EHE: aqueous extract of *Himanthalia elongata;* EU: aqueous extract of hydrolysed *Ulva* spp.

affect the sensory attributes of meat, and therefore the acceptability of the products by consumers should not be affected. Moreover, some sensory attributes of this already highly appreciated meat were actually improved, as the panellists considered that the juiciness of meat was enhanced by the addition of *H. elongata* extract to the rabbit diet. In the case of the diet including the *S. latissima* extract, the flavour of the meat was considered more pleasant than the control meat.

Very few studies are available for comparison, but other authors also observed overall positive sensorial effects of including macroalgae in the diet of different species. The inclusion of laminarin and fucoidan extracts in a pig diet (Moroney et al., 2015) improved the visual sensory properties of meat and did not modify the sensory properties (appearance, texture, acceptability), and the researchers concluded that these extracts could be used without affecting consumer acceptance. In a previous study conducted with rabbits (Rossi et al., 2020), polysaccharides derived from *Laminaria* spp. were included in the diets at doses of 0.3 and 0.6% (*w*/w) and the meat obtained was enhanced in terms of texture and flavour attributes. Our results showing that both flavour and texture of rabbit meat were improved by the inclusion of brown macroalgae in the diet are consistent with these earlier findings.

4. Conclusions

In summary, the addition of different marine macroalgae products (*S. latissima, H. elongata* and *Ulva* spp.) to rabbit diets at a dose of 1.025% (w/w), positively modified the FA profile of meat, by increasing the proportion of omega-3 fatty acids, with the increase being significant in the case of the *Ulva* spp. extract. This change may deserve further research as it may be related to changes in SCFA production by microbiota. Regarding the sensory profile of meat, dietary supplementation with macroalgae did not negatively affect the sensorial properties of meat, and therefore they could be used in rabbit feed without affecting consumer acceptance of the product. Moreover, dietary supplementation with *S. latissima* and *H. elongata* enhanced the flavour and juiciness of the meat. Altogether, the study findings indicate that the addition of these sustainable ingredients to rabbit feed did not negatively impact the meat quality, and some of them may even improve specific characteristics, which could make the meat product more attractive to consumers.

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CRediT authorship contribution statement

Sabela Al-Soufi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Javier García:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Nuria Nicodemus:** Writing – review & editing, Investigation, Conceptualization. **Jose M. Lorenzo:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Eugenio Cegarra:** Resources, Project administration, Funding acquisition. **Antonio Muíños:** Resources, Project administration, Funding acquisition, Conceptualization. **Ana Paula Losada:** Investigation, Conceptualization. **Marta Miranda:** Writing – review & editing, Methodology, Investigation. **Marta López-Alonso:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Data availability

Data will be made available on request.

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