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Running head: Dietary natural extract affects rabbit meat.

Effects of dietary levels of brown seaweeds and plant polyphenols on growth and meat quality parameters in growing rabbit.[1,2]

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ABSTRACT

Growth performances, carcass characteristics and meat quality parameters from growing rabbit fed with two levels of dietary brown seaweed (*Laminaria spp*) and plant polyphenols were investigated. One hundred and forty-four New Zealand White rabbits were allotted into three dietary treatments containing 0 (C), 0.3% (T1), and 0.6% (T2) of brown seaweed and plant polyphenols mixture for 42 days. Growth performances and carcass weight were improved in T1 group. Vitamin A and E content in *Longissimus thoracis and lumborum* (LTL) and *Semimembranosus* (SM) muscle were enhanced in the treated groups. In the SM muscle, the oxidative stability was improved in rabbit fed with both dosages of dietary supplement, and the cholesterol content tended to be lower in T1 than in T2 and C groups. The LTL and SM muscle sensory characteristics were improved. In conclusion, dietary integration with a low dosage of brown seaweed and plant polyphenols is a valid strategy for enhance growth performance and produce healthier rabbit meat.

Keywords: brown seaweed, growth performances, plant polyphenols, meat quality, rabbit.

1. Introduction

Animal welfare and farm sustainability are key factors in animal production systems (Dawkins, 2016). Consumers increasingly demand products of animal origin that come from production chains certified for animal welfare. Production institutions also restrict antibiotic use to prevent antibiotic resistance (Roca et al., 2015). Therefore, sustainable nutritional strategies able to support animal health and enhance product quality are required.

In recent years, herbs and spices containing polyphenols have been investigated as feed supplements to improve rabbit health and meat quality parameters, due to their effects on the digestive function and growth performance and their antioxidant and antimicrobial properties (Dalle Zotte, Celia & Szendrő, 2016). In this context, seaweeds are also potentially important in animal nutrition due to their high content of bioactive molecules (Makkar et al., 2016). In particular, brown seaweed has been of interest as a functional dietary ingredient, due to its various health benefits related to its sulfated polysaccharides, phlorotannin, diterpenes, minerals and vitamins content (Maghin, Ratti & Corino, 2014).

Rabbit meat is particularly appreciated by consumers due to its healthy properties (Wang, Su, Elzo, Jia, Chen, & Lai, 2016). Compared to other meats, rabbit has low fat and cholesterol content and high levels of protein with essential amino acids, and with a high digestibility value (Dalle Zotte, 2002). The high degree of unsaturation of fatty acids makes this meat particularly susceptible to oxidative processes during storage, with negative effects on sensory parameters and nutritional value (Dal Bosco et al., 2014). Previous studies have reported that in rabbit meat lipid oxidation can be prevented using vitamin E or natural extract supplements, which are good sources of dietary antioxidants (Corino, Pastorelli, Pantaleo, Oriani, & Salvatori, 1999; Dal Bosco et al., 2014; Dalle Zotte et al., 2016; Vizzarri, Palazzo, D'Alessandro, & Casamassima, 2017).

There is a growing interest in the use of natural supplements in rabbit nutrition to enhance productive performance, thus improving rabbit health and meat quality parameters (Hassan,

77 Mahrose, & Basyony, 2016). Dalle Zotte et al., (2016) reported that several herbs and spices
78 containing polyphenols have shown positive effects such as being growth promoters, antimicrobials
79 and antioxidants in rabbit species. Makkar et al. (2016) reported that dietary supplementation with
80 brown seaweed in rabbit has different effects: *Laminaria spp.* improved blood lipid profiles, but the
81 use of *Ascophillum nodosum* should be avoided because it had a toxic effect. No previous studies
82 have reported the effects of dietary brown seaweed in association with plant polyphenols in growing
83 rabbit and on growth performances and meat quality parameters. Thus, the aim of the study was to
84 investigate the effects of a dietary brown seaweeds and plant polyphenols mixture on productive
85 performance, carcass characteristics, and meat quality parameters in growing rabbits.
86

87 **2. Material and Methods**

88 ***2.1. Animal and experimental treatments***

89 Rabbits were handled following the guidelines for animal experiments, indicated in the EU
90 Directive 2010/63/EU and national guidelines for the care and use of animals were followed and all
91 experimental procedures involving animals were approved by the National Agricultural and Food
92 Centre ethical committee (No. NPPC 18-10-2016).

93 A total of 144 New Zealand White rabbits, half males and half females, were housed at the
94 National Agricultural and Food Centre, Nitra (Slovak Republic). At weaning, the 35-day-old rabbits
95 were randomly allotted into three experimental groups balanced for sex (48 rabbits per treatment).

96 Rabbits were housed in cages (2 female and 2 male rabbits/cage) and the trial lasted 42 days.
97 The cages were equipped with a hopper for feed and an automatic nipple drinking system. The
98 lighting cycle throughout the trial was 16h of light and 8h of dark. Heating and forced ventilation
99 systems allowed the building temperature to be maintained within 18 ± 4°C. The relative humidity
100 was about 70 ± 5%.

101 Rabbits were fed a control diet (C) or T1 and T2 diets, which were supplemented with 0.3%
102 and 0.6% of feed additive consisting of prebiotic polysaccharides from brown seaweeds (*Laminaria*

103 *Digitate and Hyperborea,* ratio 1:1) plus phenolic acid, hydroxycinnamic acids, tannins, and
104 flavonoids from plant extracts.
105 The diets included no anticoccidials, antibiotics or other medications. The supplement was
106 included in the basal mashed diet. All the experimental diets were pelleted. The two dosages of the
107 natural extract were chosen after an *in vitro* evaluation of the minimal inhibitory concentration
108 (MIC) against *Clostridium spp., Staphylococcus spp.,* and *Escherichia coli spp.* (Tosi, personal
109 communication). The ingredients and chemical composition of the experimental diets are reported
110 in Table 1.
111 The chemical composition of the diets and feed supplement were in accordance with the
112 methods of the Association of Analytical Chemists (AOAC, 2002). The quantitative analysis of the
113 phenolic compounds of the supplement was performed using HPLC-UV-DAD, according to Russo
114 et al. (2017). The quantification of beta-carotene of the feed supplement was performed in
115 accordance with the method proposed by Rakusa, Srecnik, & Roskar (2017). The chemical
116 composition, phenolic composition and carotenoid content of the feed supplement is reported in
117 Table 2.
118 Throughout the study feed was available *ad libitum* and animals were monitored daily to
119 assess their health conditions. They were weighed at the beginning (0 day), at 21 days and at the
120 end of the experimental trial (42 days). The daily feed intake was calculated from the amounts of
121 feed offered and refused weekly. These data were used to calculate the average daily gain (ADG),
122 average daily feed intake (ADFI) and feed conversion ratio (FCR).
123 At 77 days old, all rabbits were weighted and after 6 h fasting, 12 animals per group (1 male
124 rabbit/cage) were randomly selected and slaughtered at the research center slaughterhouse. Rabbits
125 were subjected to electrical stunning and sacrificed by bleeding according to the guidelines
126 established by the European Community (1099/2009/EC) for the protection of animals during
127 slaughter. Carcasses were chilled for 24 h at +4°C and then dissected, according to the
128 recommendations of the WRSA (Blasco & Ouhayoun, 1996), discarding the skin, the distal part of

129 the limbs, genitals, bladder, and gastrointestinal tract, and carcass measurements and meat quality
130 analyses were conducted. The *Longissimus thoracis and lumborum* (LTL) muscle and thighs (n =
131 12) were removed from each carcass. Samples were vacuum packed and stored at -20°C until lab
132 analyses. The *Semimembranosus* (SM) and the LTL muscles (n = 12) were subjected to lab analyses
133 to investigate their meat quality parameters.
134
135 ***2.2 Physical parameters***
136 The pH and color parameters were measured 24 h after slaughter. The pH was performed
137 using a pH meter (HI98191 microcomputer; Hanna Instruments, Vila do Conde, Portugal). The pH
138 probe was calibrated using standard buffers of pH 4.0 and 7.0 and the maintenance of calibration
139 was monitored between samples.
140 The International Commission on Illumination's (CIE) lightness (L*), redness (a*), and
141 yellowness (b*) values were measured for the samples using a CR-300 Chroma Meter (Minolta
142 Camera, Co., Osaka, Japan). The instrument was calibrated on the Commission Internationale
143 d'Eclairage (CIE) LAB color space system using a white calibration plate (Calibration Plate CR-
144 A43; Minolta Camera Co.). The colorimeter had an 8-mm measuring area and was illuminated with
145 a pulsed Xenon arc lamp (illuminat C) at a viewing angle of 0°. Reflectance measurements were
146 obtained at a viewing angle of 0° and the spectral component was included. The color variables
147 were measured at three different points on the central part of the samples. Moreover, total colour
148 differences (TCD) were calculated using the following equation: $\Delta E^*=(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$.
149
150 ***2.3 Chemical parameters***
151 Dry matter (DM), protein, ether extract (EE), and ash contents of the LTL and SM muscles
152 were determined according to AOAC (2002) methods. Dry matter was determined by the oven
153 drying method at 105°C until constant weight (method 950.46), protein by the Kjeldahl method
154 (method 990.03) using a 6.25 factor to convert the nitrogen content into total protein, the ether

155 extract by Soxhlet extraction (method 920.39), and ash by using a muffle furnace for 12 h at 550°C
156 (method 920.153).
157 The cholesterol content of the LTL and SM muscles was determined in accordance with the
158 procedure of Du and Ahn (2002). Lipids were extracted from 1.5 g of minced meat homogenate
159 with 33% KOH (ratio of 94:6), using Ultra-Turrax T18 Homogenizer (IKA, Cincinnati, USA) and
160 keeping in ice to avoid oxidation processes. Cholesterol was extracted with 5 ml of hexane, and 1 µl
161 was injected into the gas chromatograph. The cholesterol was identified based on the retention time
162 of the standard (Sigma Aldrich, St. Louis, USA), and quantified with the Chrom Card Data System
163 (version 1.17) software by comparing the peak area with the reference standard curve. All samples
164 were analyzed in triplicate.
165
166 ***2.4 Oxidative stability***
167 Meat oxidative stability was measured by evaluating the thiobarbituric acid-reactive
168 substances (TBARS) content of 4°C chilling SM meat samples at 0 h, and then at 24 h and 72 h, in
169 accordance with Meineri, Cornale, Tassone, and Peiretti (2010). The implemented method was as
170 follows: 500 mg of meat was homogenized with 10 mL of distilled water using a Homogenizer
171 Ultra Turrax T25 (IKA, Cincinnati, USA), and 2.5 mL of 25% trichloroacetic acid was added to the
172 homogenized sample, cooled at 4°C for 15 min, and then centrifuged at 4000 g at 4°C for 5 min.
173 The supernatant was filtered through Whatman 52 filter paper, and an aliquot of 3.5 mL was added
174 to 1.5 mL of 0.67% thiobarbituric acid and incubated at 70°C for 30 min.
175 Immediately after cooling, the absorbance of the sample was read in a spectrophotometer at
176 532 nm and compared to a standard curve of malondialdehyde (MDA; Sigma Aldrich, St. Louis,
177 USA). All analyses were performed in duplicate and the results were expressed as mg of MDA per
178 kg of meat.
179
180 ***2.5 Vitamin E and A content***

Alpha-tocopherol and retinol were determined in both muscles using a procedure modified from Zaspel and Csallany (1983). The muscles were analyzed using an HPLC system (Kontron Instruments, Milan, Italy) consisting of an autosampler (HPLC autosampler 360, Kontron Instruments, Milan, Italy) with a loop of 20 μL, a high-pressure pump and a C18 column 5 μm, 150 mm x 4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and methanol (75: 25 v/v) and a flow rate of 1 mL per min was used. The alpha-tocopherol and retinol were identified using a fluorimeter detector and comparing the samples' retention time with the pure standards (97%) purchased from Sigma Aldrich (St. Louis, USA). The quantification was carried out using the Geminyx system (version 1.91) by comparing the area sample peak with that of the reference standards curve.

2.6 Sensory evaluation

The LTL muscle and thigh preparation for sensory analysis was conducted after thawing for 24h at 4°C. Both samples were then prepared as single pieces in an uncovered stainless-steel dish in a conventional oven (REX, Italy) at 180°C. A thermocouple (Pentronic AB, Gunnebobruk, Sweden) was inserted into the center of each piece of meat to register the core temperature. The samples were removed from the oven at 75-80°C to allow for post-heating rise. After cooling the entire LTL muscle and thigh were cut into 1.5cm thick slices (Electrolux 50, 220-24, kW0.2). The slices were warmed to 60°C before the evaluation.

A trained sensory panel, consisting of eight members familiar with descriptive analysis procedures (EN ISO 13299, 2010), was established. All assessments were carried out in a sensory laboratory equipped according to EN ISO 8598, (1989) recommendations. The list of descriptors, definitions, and standards are reported in Palazzo, Vizzarri, Nardoia, Ratti, Pastorelli, and Casamassima (2015). The sensory profile was assessed according to EN ISO 13299 (2010) and the panel evaluated the two samples (thigh and LTL) on different days in triplicate. Within each session the design was balanced for order and carry-over effects (MacFie, Bratchell, Greenhoff, & Vallis,

1989). During training and sampling, the panelists had access to unlimited water and unsalted crackers. They were requested to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense).

2.7 Statistical analysis

Data on productive performance and slaughter parameters, were analyzed using one-way analysis of variance (ANOVA), with diet as fixed effect and cage as random effect (SPSS/PC Statistics 25.0 SPSS Inc., IBM). Meat physical and chemical parameters were processed with a one-way ANOVA, with diet as fixed effect. A repeated measure ANOVA with diet, storage time and their interaction as fixed effects, was used to analyzed TBARS data. Means were compared according to the Duncan's test. The sensory data were submitted to ANOVA with samples, judges, replicates, and their interactions as effects (EN ISO 13299, 2010). The significance of these effects was tested with F tests. Post-hoc pairwise contrasts were evaluated by Duncan's test. The cage was considered as the experimental unit for growth performance and the rabbit for the meat quality parameters. Data are reported as mean ± SEM. Differences among treatments were considered significant at $P < .05$.

3. Results and Discussion

3.1 Productive performance and carcass characteristics

The data of the productive performance of growing rabbits are reported in Table 3. The live weight was improved at 21 days ($P < .05$) and tended to be higher at 42 days ($P = .06$) in rabbit fed a lower dosage of the dietary supplement (T1 group) than the other two groups. Therefore, the ADG (0-42 d) tended to be higher ($P = .06$) in the T1 group. The feed conversion ratio was also improved ($P < .01$) in rabbits fed with a diet containing 0.3% of the natural extract mixture. The ADFI was lower in T2 than in C groups ($P < .05$) in the first period of the trial (0-21 d). Considering the ADFI in the first period of the trial, it is possible that the high dosage of bioactive compounds of the

supplement negatively affected diet palatability and consequently growth as previously observed in rat fed high dosage of ellagic acid (Cerdá, Cerón, Tomás-Barberán & Espín 2003).

The slaughter weight (2.71 ± 0.067 kg C *vs* 2.91± 0.035 kg T1 *vs* 2.80 ± 0.052 kg T2; P = .013) and carcass weight (1.61 ± 0.041 kg C *vs* 1.75 ± 0.026 kg T1 *vs* 1.66 ± 0.029 kg T2; P = .012) of the sampled rabbits were higher in the T1 group than in the others. The dressing percentage was not affected by the dietary treatments (59.4 ± 0.434 % C *vs* 59.6 ± 0.327 % T1 *vs* 59.7 ± 0.446 % T2; P = .929).

The data is thus in agreement with previous studies of post-weaning piglets that reported an improvement in ADG due to dietary supplementation with *Laminaria* spp. extract (Gahan, Lynch, Callan, O'Sullivan, & O'Doherty, 2009; McDonnell, Figat, & O'Doherty, 2010). An enhancement of growth performance was also observed in broilers with a dietary supplementation of *Ascophillum nodosum* meal (Evans & Critchley, 2014). Brown seaweed contains a high amount of water-soluble polysaccharides such as laminarins, fucoidans, and alginates. These constituents have been shown to have prebiotic effects and to reduce pathogenic microorganisms in the gastrointestinal tract in both *in vitro* and *in vivo* studies (Chen et al., 2018; Sweeney et al., 2011). *In vitro* prebiotic activity of fucoidans and alginates from brown seaweed has been found with an increase in the growth rate of *Lactobacillus spp.* (Okolie, Rajendran, Udenigwe, Aryee, & Mason, 2017). In addition, Lynch, Sweeney, Callan, O'Sullivan, and O'Doherty (2010) reported that laminarins have antibacterial properties, stimulate *Bifidobacteria*, and increase the production of short chain fatty acid (SFA) in the gut.

Studies have reported conflicting data on plant polyphenols supplementation and the enhancement of growth performance. Zhao, Xu, Du, Li, and Zhang (2005) found an improvement in feed intake and growth performance in growing rabbits fed traditional Chinese herbs, which contain polyphenols. In another study, dietary supplementation with microalgae spirulina and thyme was not found to affect rabbit performance (Dalle Zotte, Sartori, Bohatir, Remignon, & Ricci,

2013). Palazzo et al. (2015) also reported no differences in ADG and the final weight of rabbit fed *Lippia citriodora* extract.

In the present study, the active principles of brown seaweed and plant polyphenols had a positive effect on antioxidant status, as shown by the TBARS values and the vitamin E content of the muscles, and probably on gut bacteria populations, due to more efficient feed utilization and consequently an improvement in growth performance.

Indeed, positive effects on digestibility of nitrogen (N), gross energy (GE), fibers (NDF), and ash have been reported in post-weaning weaned piglets (O'Doherty, Dillon, Figat, Callan, & Sweeney, 2010; O'Shea, McAlpine, Sweeney Varley & O'Doherty, 2014).

3.2 Meat quality parameters of LTL and SM muscles

The data on the physical and chemical parameters of the LTL and SM muscles are reported in Table 4 and 5, respectively. The pH values were affected by dietary treatments in both LTL and SM muscles ($P < .05$), but the ranges fall within the values reported in previous studies (Maj, Bieniek, & Łapa, 2008; Carrilho, López, & Campo, 2009).

No difference ($P > .05$) was observed for the color indexes in either muscle in terms of dietary treatments, in agreement with the data reported for the LTL and SM muscles (Daszkiewicz, Gugolek, Janiszewski, Kubiak, & Czoik, 2012; Tůmová, Bízková, Skřivanová, Chodová, Martinec, & Volek, 2014). The TCD values resulted 1.41 and 1.33 for T1 and T2 treatment respectively, indicating small difference in perceivable colour (TCD < 1.5) (Adekunte, Tiwari, Cullen, Scannell, & O'donnell, 2010).

The chemical composition of the LTL muscle was not affected by the dietary treatment, except that the ash content was lower ($P < .001$) in muscles from rabbit in the T1 group than those in the C and T2 groups. These data are in line with the results of previous studies on growing rabbit (Dal Bosco, Castellini, Bianchi, & Mugnai, 2004; Daszkiewicz et al., 2012).

283 In the SM muscle, the cholesterol content tended to be lower (P = .052) in rabbit fed with
284 the low dosage of dietary supplement (T1) than in those in the T2 and C groups. A previous study
285 showed that dietary *Laminaria* spp. (1 g/d for 14 days) lowered cholesterol and triglycerides in
286 rabbits with experimental hyperlipoproteinemia (Tang & Shen, 1989). In addition, Vizzarri et al.
287 (2017) reported a lower cholesterol content in rabbit LTL muscle due to dietary supplementation
288 with plant polyphenols, which can modulate the activity of enzyme 5-hydroxy-3-methylglutaryl-
289 coenzyme A (HMG-CoA) reductase, which regulates the metabolic pathway for cholesterol
290 synthesis (Kowalska & Bielanski, 2009).
291 In other animal species such as pigs, donkeys, horses and lambs dietary supplementation
292 with natural extracts containing plant polyphenols was found to slightly affect the pH, color indexes
293 and muscle chemical composition (Rossi, Pastorelli, Cannata, Tavaniello, Maiorano, & Corino,
294 2013; Rossi et al., 2017a; Valenti et al., 2019).
295
296 ***3.3 Vitamin content of LTL and SM muscles***
297 Figure 1 (A, B) shows the vitamin A and E content of LTL and SM muscles in terms of the
298 dietary treatments. The vitamin A content was higher (P < .001) in the LTL muscle of rabbit fed
299 brown seaweed and plant polyphenols (T1 and T2 groups) than in the control. In the SM muscle a
300 higher content (P < .001) of vitamin A was observed in the T1 group than in the C and T2 groups.
301 Vitamin E content was higher in the LTL muscles of rabbit in the T1 and T2 groups than in the
302 control group. In the SM muscle a higher content (P < .001) of vitamin E was observed in the T1
303 group than in the C and T2 groups.
304 higher content (P < .001) of vitamin E was observed in the T1 group than in the C and T2
305 groups. The higher content of vitamin A in muscles from rabbit of T1 and T2 groups than control
306 should be related to the carotene content and the several antioxidant compounds from the dietary
307 supplement. The carotenoids are present in *Laminaria spp.* in amount variable from 468 to1065
308 mg/kg DM as reported by Jacobsen, Sorensen Holdt, Akoh, & Hermund (2019).

309 The dietary supplement contained several polyphenol compounds such as neochlorogenic
310 acid, syringic acid and ellagic acids that possess a high antioxidant activity. As observed in rat the
311 dietary hydroxycinnamic acid derivatives protecting vitamin E from oxidation in all tissues (Frank,
312 Kamal-Eldin, Razdan, Lundh, & Vessby, 2003). Moreover, it is reported by Kumar, Prahalathan, &
313 Raja (2012) that dietary supplementation with syringic acid in hypertensive rat positively affect
314 vitamin E and C serum and tissue, reducing oxidative stress.
315 A previous study reported that *Ascophyllum nodosum* extract increases serum vitamin A in
316 lamb and liver vitamin E in beef (Allen et al., 2001). Other studies of pigs reported that dietary
317 supplementation with brown seaweed (*Ascophyllum* spp.*; Laminaria* spp.) increased the antioxidant
318 status of piglets, measured as plasma superoxide dismutase, catalase, and muscle TBARS (Wang et
319 al., 2016; Moroney, O'Grady, Robertson, Stanton, O' Doherty, & Kerry, 2015). The present data
320 agree with Palazzo et al. (2015), who reported an increase in vitamin E content in the LTL muscle
321 of New Zealand white rabbit fed a high dosage of *Lippia Citriodora* extract. Palazzo, Schiavitto,
322 Cinone, and Vizzarri (2019) recently reported an increase in fat-soluble vitamins (vitamin A and
323 vitamin E) in LTL muscles of rabbit fed natural extract containing the hydroxycinnamic ester
324 derivative widely distributed in plants, called verbascoside, with a high antioxidant activity (Gil,
325 Enache, & Oliveira-Brett, 2013).
326 Thus, the higher muscle vitamin A and E content is related to the content of phenols,
327 carotenoid, tannins and phlorotannins and polysaccharides in brown seaweed as fucoidans,
328 laminarans and vitamins (Jacobsen et al., 2019) and to the antioxidant activity of plant polyphenols
329 that preserve vitamin E oxidation through several well-known mechanisms (Cimmino et al., 2018).
330
331 ***3.4 Oxidative stability of SM muscle***
332 The oxidative stability of the SM muscle in terms of the dietary treatments and time of
333 storage is reported in Figure 2. We analyzed the SM muscle to verify the antioxidant activity of the
334 natural mixture on a muscle with a higher fat content than the LTL muscle and a different oxidative

335 metabolism (Gondret et al., 2004). The oxidative stability of the SM muscle was affected ($P < .001$)
336 by dietary treatments and storage time. A significant interaction between storage time and dietary
337 treatment was also observed ($P < .001$). The oxidative stability at both sampling times was higher in
338 groups fed the brown seaweed and polyphenols mixture than in the control. In agreement with the
339 present data, Moroney, O' Grady, O'Doherty, and Kerry (2012) reported a high oxidative stability in
340 the *Longissimus dorsi* muscle of pigs fed with seaweed extract (*Laminaria digitata*). In beef fed
341 *Ascophyllum nodosum* extract a high muscle oxidative stability was also observed (Allen et al.,
342 2001).
343 A high oxidative stability in the LTL muscles of animals fed plant polyphenols was also
344 reported in pigs, *Equidae,* goats and rabbit (Cimmino et al., 2018; Rossi et al., 2017a; Rossi, Stella,
345 Ratti, Maghin, Tirloni, & Corino, 2017b; Palazzo et al., 2015; Palazzo et al., 2019). Usually natural
346 antioxidant can reduce lipid oxidation, enhancing meat shelf-life, since they are able to block the
347 oxidative chain propagation reactions. Polyphenols have a high antioxidant activity, through several
348 mechanisms: as a scavenger of free radicals (Zheng et al., 2009), as transition metal chelators
349 (Andjelković et al., 2006) and as quencher of free singlet oxygen (Mukai, Nagai, & Ohara 2003).
350
351 ***3.5 Sensory profile***
352 The F values for the aroma, taste, flavor and texture parameters of the LTL sensory profile are
353 reported in Table 6. The results indicate that dietary supplementation with brown seaweed and plant
354 polyphenols affected the aroma and flavor of the LTL muscle ($P <. 05$) The F values for replicates
355 and interactions were not affected ($P > .05$) in all the descriptors, while judges presented differences
356 ($P < .01$) for aroma and flavor. Differences between judges are common in sensory evaluations, due
357 to the different use of the scale (Lea, Naes, & Rodbotten, 1997). No interaction between panelists ×
358 replicates and samples × replicates were observed.
359 In Table 7, the F values for aroma, taste, flavor, and texture parameters of the thigh sensory
360 profile are reported. The data showed that dietary supplementation with brown seaweed and plant

polyphenols affected the aroma and texture of the thigh ($P <$. 05). The F values for replicates were not affected ($P > .05$) for all the descriptors, while judges presented differences ($P < .05$) for aroma, flavor and texture. No interaction between judges × replicates and samples × replicates were observed in LTL, while in thigh, the interaction judges × samples was significant ($P < .05$). It is probably due to the type of samples analyzed. In fact, the thigh is more variable from a chemical point of view than LTL muscle that is more homogeneous, where we did not observe any statistical interaction. These results did not influence the overall sensory evaluation, in fact no differences were reported for the same attributed perceived as flavour. The F values for aroma, taste, flavor, and texture parameters in both the LTL muscle and the thigh highlight the excellent reproducibility of the scores given by the panelists and the homogeneity of samples during replicates.

The least squares mean of the different attributes for the LTL muscle and thigh are reported Table 8 and 9, respectively. The data shows that taste and texture parameters were comparable in all the experimental groups. In the LTL muscle, a difference ($P < .05$) for aroma (rabbit, liver and rancid) and rabbit flavor were observed. The intensity of rabbit aroma and flavour was higher ($P <$ .05) in the T1 and T2 groups than in the control, and the same result was observed for liver aroma. The rancid aroma was higher ($P < .05$) in the T2 group than in the T1 and control groups.

In the thigh, dietary treatments affected ($P < .05$) the aroma (rabbit, liver, and metallic) and the texture parameters. The rabbit aroma and flavor were higher ($P < .01$) in the T1 and T2 groups than in the controls and the same result was observed for liver aroma. The rancid aroma was higher ($P < .05$) in the T2 group than in T1 and the control groups. In terms of texture data, higher scores for tenderness and juiciness were observed in thighs from animals fed the natural supplement (T1 and T2) than in the control, while a lower score was given for stringiness ($P < .05$) in T1 and T2 than in the control. As previously observed in rabbit fed natural antioxidants, the high values for tenderness may be a result of the protection against the oxidation process (Palazzo et al., 2015). In our previous study of *Equidae*, dietary supplementation with an extract containing plant polyphenols enhanced meat texture parameters (Rossi et al., 2017a). This parameter is important for

consumers' eating habits and is closely linked with consumer expectations of rabbit meat quality. The results of the present study indicate that the mean scores for each descriptor could be assumed to be satisfactory for the sensory profile of rabbit meat. The sensory evaluation showed that dietary supplementation with natural extract mixture containing brown seaweeds and plant polyphenols affects aroma in the LTL muscle and the aroma and texture in the thigh.

4. Conclusion

Dietary supplementation with the low dosage of brown seaweeds and plant polyphenols mixture can be considered a useful nutritional strategy in rabbit meat production, since an improvement of feed conversion ratio and muscles vitamin A and E content was achieved. The higher muscle vitamin content enhances both nutritional quality and oxidative stability. Sensory parameters related to aroma, flavour and texture are positively affected by dietary treatment. In the present experimental condition, the low dosage of the natural extract mixture seems to be useful for enhancing rabbit meat production. The higher dosage of supplement does not produce any adverse effects on rabbit performance or meat quality parameters and should be utilized in a more stressful breeding condition.

Declaration of Competing Interests

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

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References

411 Adekunte, A. O., Tiwari, B. K., Cullen, P. J., Scannell, A. G. M., & O'donnell, C. P. (2010). Effect
412 of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food*
413 *Chemistry*, 122, 500-507.
414 Allen, V. G., Pond, K. R., Saker, K. E., Fontenot, J. P., Bagley, C.P., Ivy, R. L., Evans R. R.,
415 Schmidt, R.E., Fike, J. H., Zhang, X., Ayad, J. Y., Brown, C. P., Miller, M. F.,
416 Montgomery, J. L., Mahan, J., Wester, D. B., & Melton, C. (2001). Tasco: Influence of a
417 brown seaweed on antioxidants in forages and livestock—A review. *Journal of Animal*
418 *Science*, 79, E21–E3.
419 Andjelković, M., Van Camp, J., De Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., &
420 Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl
421 groups. *Food Chemistry* 98, 23–31.
422 AOAC. (2002). Official methods of analysis. 17th edition. *Journal - Association of Official*
423 *Analytical Chemists*, Arlington, VA.
424 Blasco, A., & Ouhayoun, J. (1996). Harmonization of criteria and terminology in rabbit meat
425 research. Revised proposal. *World Rabbit Science,* 4, 93–99.
426 Carrilho, M. C., López, M., & Campo, M. M. (2009). Effect of the fattening diet on the
427 development of the fatty acid profile in rabbits from weaning. *Meat Science*, 83(1), 88–95.
428 Cerdá, B., Cerón, J. J., Tomás-Barberán, F. A., & Espín, J. C. (2003). Repeated oral administration
429 of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days is not toxic.
430 *Journal of Agricultural and Food Chemistry* 51(11), 3493-3501.
431 Chen, X., Sun, Y., Hu, L., Liu, S., Yu, H., Xing, R., Li, R., Wang X., & Li, P. (2018). In vitro
432 prebiotic effects of seaweed polysaccharides. *Journal of Oceanology and Limnology* 36(3),
433 926–932.
434 Cimmino, R., Barone, C. M. A., Claps, S., Varricchio, E., Rufrano, D., Caroprese, M., Albenzio,
435 M., De Palo, P., Campanile, G., & Neglia, G. (2018). Effects of dietary supplementation

436 with polyphenols on meat quality in Saanen goat kids. *BMC Veterinary Research*, 14(1),
437 181.
438 Corino, C., Pastorelli, G., Pantaleo, L., Oriani, G., & Salvatori, G. (1999). Improvement of color
439 and lipid stability of rabbit meat by dietary supplementation with vitamin E. *Meat Science*,
440 52, 285–289.
441 Dal Bosco, A., Castellini, C., Bianchi, L., & Mugnai C. (2004). Effect of dietary α-linolenic acid
442 and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit
443 meat. *Meat Science*, 66, 407–413.
444 Dal Bosco, A., Mugnai, C., Roscini, V., Mattioli, S., Ruggeri, S., & Castellini, C. (2014). Effect of
445 dietary alfalfa on the fatty acid composition and indexes of lipid metabolism of rabbit meat.
446 *Meat Science*, 96, 606–609.
447 Dalle Zotte, A. (2002). Perception of rabbit meat quality and major factors influencing the rabbit
448 carcass and meat quality. *Livestock Production Science*, 75(1), 11–32.
449 Dalle Zotte, A., Sartori, A., Bohatir, P., Remignon, H., & Ricci R. (2013). Effect of dietary
450 supplementation of Spirulina (Arthrospira platensis) and Thyme (Thymus vulgaris) on
451 growth performance, apparent digestibility and health status of companion dwarf rabbits.
452 *Livestock Science*, 152, 182–191.
453 Dalle Zotte, A., Celia, C., & Szendrő, Zs. (2016). Herbs and spices inclusion as feedstuff for
454 additive in growin grabbit diets and as additive in rabbit meat: A review. *Livestock Science*,
455 189, 82–90.
456 Daszkiewicz, T., Gugolek, A., Janiszewski, P., Kubiak, D., & Czoik, M. (2012). The effect of
457 intensive and extensive production systems on carcass quality in New Zealand White
458 rabbits. *World Rabbit Science*, 20, 25–32.
459 Dawkins, M. S. (2016). Animal welfare and efficient farming: is conflict inevitable?. *Animal*
460 *Production Science*, 57, 201–208.

461 Du, M., & Ahn, D. U. (2002). Simultaneous analysis of tocopherols, cholesterol, and phytosterols
462 using gas chromatography. *Journal of Food Science*, 67(5), 1696–1700.
463 European Community. (2010). Council Directive 2010/63/EU of 22 September 2010 on the
464 protection of animals used for scientific purposes. – [accessed May 23, 2019].
465 https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF
466 European Community. (2009). Council Directive 1099/2009/EC of of 24 September 2009 on the
467 protection of animals at the time of killing. - [accessed May 23, 2019]. https://eur-
468 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:303:0001:0030:EN:PDF.
469 Evans, F. D., & Critchley, A. T. (2014). Seaweeds for animal production use. *Journal of Applied*
470 *Phycology*, 26, 891–899.
471 Frank, J., Kamal-Eldin, A., Razdan, A., Lundh, T., & Vessby B. (2003). The dietary
472 hydroxycinnamate caffeic acid and its conjugate chlorogenic acid increase vitamin E and
473 cholesterol concentrations in sprague−dawley rats. Journal of Agricultural and Food
474 Chemistry 519, 2526-2531.
475 Gahan, D. A., Lynch, M. B., Callan, J. J., O'Sullivan, J. T., & O'Doherty, J. V. (2009).
476 Performance of weanling piglets offered low-, medium-or high-lactose diets supplemented
477 with a seaweed extract from *Laminaria* spp. *Animal*, 3, 24–31.
478 Gil, E. S., Enache, T. A., & Oliveira-Brett A. M. (2013). Redox Behaviour of Verbascoside and
479 Rosmarinic Acid. *Combinatorial Chemistry & High Throughput Screening*, 16(2), 92–97.
480 Gondret, F., Damon, M., Jadhao, S., Houdebine, L. M., Herpin, P., & Hocquette, J. F. (2004). Age-
481 related changes in glucose utilization and fatty acid oxidation. *Journal of Muscle Research*
482 *and Cell Motility*, 25, 405–410.
483 Hassan, F. A., Mahrose, K. M., & Basyony, M. M. (2016). Effects of grape seed extract as a natural
484 antioxidant on growth performance, carcass characteristics and antioxidant status of rabbits
485 during heat stress. *Archives of Animal Nutrition*, 70, 141–154.

ISO 8598. (1989). Sensory analysis: General guidance for design of test rooms, International Organization for Standardization, Geneva, Switzerland.

ISO 13299. (2010). International Organization for Standardization: Sensory analysis-Methodology-General guidance to establish a sensory profile, Geneva, Switzerland.

Jacobsen, C., Sorensen A. M., Holdt, S. L., Akoh, C. C., & Hermund, D. B. (2019). Source, Extraction, Characterization, and Applications of Novel Antioxidants from Seaweed. *Annual Review of Food Science and Technology,* 10, 541–68.

Kowalska, D. & Bielański, P. (2009). Meat quality of rabbits fed a diet supplemented with fish oil and antioxidant. *Animal Science Papers and Reports*, 27, 139–148.

Kumar, S., Prahalathan, P., & Raja B. (2012). Syringic acid ameliorates l-NAME-induced hypertension by reducing oxidative stress Na*unyn-Schmiedeberg's Archives of Pharmacology*, 385, 1175–1184.

Lea, P., Naes, T., & Rodbotten, M. (1997). Analysis of variance for sensory data. New York: Chirchester John Wilej & Sons Ltd.

Lynch, M. B., Sweeney, T., Callan, J. J., O'Sullivan, J. T., & O'Doherty, J. V. (2010). The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *Journal Science of Food and Agriculture*, 90, 430–437.

MacFie, H. J., Bratchell, N., Greenhoff, K., & Vallis, L. V. (1989). Designs to Balance the Effect of Order of Presentation and First-Order Carry-Over Effect in Halls Tests. *Journal of Sensors and Sensor Systems*, 4, 129–148.

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, 212, 1–17.

510 Maghin, F., Ratti, S., & Corino, C. (2014). Biological functions and health promoting effects of
511 brown seaweeds in swine nutrition. *Journal of Dairy Veterinary & Animal Research,* 1(1),
512 00005.
513 Maj, D., Bieniek, J., & Łapa, P. (2008). Meat quality of New Zealand White and Californian rabbits
514 and their crosses. *Medycyna Weterynaryjna*, 64, 351–353.
515 McDonnell, P., Figat, S., & O'Doherty, J.V. (2010). The effect of dietary laminarin and fucoidan in
516 the diet of the weanling piglet on performance, selectedfaecal microbial populations and
517 volatile fatty acid concentrations. *Animal,* 4, 579–585.
518 Meineri, G., Cornale, P., Tassone, S., & Peiretti, P. G. (2010). Effects of Chia (Salvia hispanica L.)
519 seed supplementation on rabbit meat quality, oxidative stability and sensory traits. *Italian*
520 *Journal of Animal Science*, 9, 45–49.
521 Moroney, N. C., O' Grady, M. N., O'Doherty, J. V., & Kerry, J. P. (2012). Addition of seaweed
522 (*Laminaria digitata*) extracts containing laminarin and fucoidan to porcine diets: Influence
523 on the quality and shelf-life of fresh pork. *Meat Science*, 92(4), 423–429.
524 Moroney, N. C., O'Grady, M. N., Robertson, R. C., Stanton, C., O' Doherty, J. V., & Kerry, J. P.
525 (2015). Influence of level and duration of feeding polysaccharide (laminarin and fucoidan)
526 extracts from brown seaweed (*Laminaria digitata*) on quality indices of fresh pork. *Meat*
527 *Science*, 99, 132–141.
528 Mukai, K., Nagai, S., & Ohara, K. (2005). Kinetic study of the quenching reaction of singlet oxygen
529 by tea catechins in ethanol solution. *Free Radical Biology and Medicine* 39, 752–761.
530 O'Doherty, J. V., Dillon, S., Figat, S., Callan, J. J., & Sweeney, T. (2010). The effects of lactose
531 inclusion and seaweed extract derived from Laminaria spp. on performance, digestibility of
532 diet components and microbial populations in newly weaned pigs. *Animal Feed Science and*
533 *Technology*, 157, 173–180.

534 Okolie, C. L., Rajendran, S. R. C. K., Udenigwe, C. C., Aryee, A. N. A., & Mason, B. (2017).
535 Prospects of brown seaweed polysaccharides (BSP) as prebiotics and potential
536 immunomodulators. *Journal of Food Biochemestry*, 41, e12392.
537 O'Shea, C. J., McAlpine, P., Sweeney T., Varley P. F., & O'Doherty, J. V. (2014). Effect of the
538 interaction of seaweed extracts containing laminarin and fucoidan with zinc oxide on the
539 growth performance, digestibility and faecal characteristics of growing piglets. *British*
540 *Journal of Nutrition*, 111, 798–807.
541 Palazzo, M., Vizzarri, F., Nardoia, M., Ratti, S., Pastorelli, G., & Casamassima, D. (2015). Dietary
542 Lippia citriodora extract in rabbit feeding: effects on quality of carcass and meat. *Archives*
543 *Animal Breeding*, 58, 355–364.
544 Rakusa, Z. T., Srecnik, E., & Roskar, R. (2017). Novel HPLC-UV method for simultaneous
545 determination of fat-soluble vitamins and coenzyme Q10 in medicines and supplements.
546 *Acta Chimica Slovenica*, 64, 523-529.
547 Palazzo, M., Schiavitto, M., Cinone, M., & Vizzarri, F. (2019). Rabbit metabolic response and
548 selected meat quality traits: Evaluation of dietary PLX® 23 and LycoBeads® feed
549 supplement. *Journal of Animal Physiology and Animal Nutrition*, 103, 383–394.
550 Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Cohen, J., Findlay, D.,
551 Gyssens, I., Heure, O. E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liébana, E., López-
552 Cerero, L., MacGowan, A., Martins, M., Rodríguez-Baño, J., Rolain, J. M., Segovia, C.,
553 Sigauque, B., Taconelli, E., Wellington, E., & Vila, J. (2015). The global threat of
554 antimicrobial resistance: science for intervention. *New Microbes New Infect*, 6, 22–9.
555 Rossi, R., Pastorelli, G., Cannata, S., Tavaniello, S., Maiorano, G., & Corino, C. (2013). Effect of
556 long term dietary supplementation with plant extract on carcass characteristics meat quality
557 and oxidative stability in pork. *Meat Science*, 95(3), 542–548.
558 Rossi, R., Ratti, S., Pastorelli, G., Maghin, F., Martemucci, G., Casamassima, D., D'Alessandro, A.
559 G. & Corino, C. (2017a). Effect of dietary plant extract on meat quality and sensory

560 parameters of meat from Equidae. *Journal of the Science of Food and Agricolture,* 97(14),
561 4690–4696.
562 Rossi, R., Stella, S., Ratti, S., Maghin, F., Tirloni, E., & Corino, C. (2017b). Effects of antioxidant
563 mixtures in the diet of finishing pigs on the oxidative status and shelf life of Longissimus
564 dorsi muscle packaged under modified atmosphere. *Journal of Animal Science*, 95(11),
565 4986–4997.
566 Russo, R., Pucci, L., Giorgetti, L., Árvay, J., Vizzarri, F., Longo, V., & Pozzo, L. (2017).
567 Polyphenolic characterisation of plant mixture (Lisosan® Reduction) and its
568 hypocholesterolaemic effect in high fat diet-fed mice. *Natural Product Research*.
569 Sweeney, T., Dillon, S., Fanning, J., Egan, J., O'Shea, C. J., Figat, S., Gutierrez, J. J M. Mannion C,
570 Leonard, F., & O'Doherty, J. V. (2011). Evaluation of seaweed-derived polysaccharides on
571 indices of gastrointestinal fermentation and selected populations of microbiota in newly
572 weaned pigs challenged with Salmonella Typhimurium. *Animal Feed Science and*
573 *Technology*, 165, 85–94.
574 Tang, Z. L., & Shen, S. F. (1989). A study of *Laminaria digitata* powder on experimental
575 hyperlipoproteinemia and its hemorrheology. *Chinese journal of modern developments in*
576 *traditional medicine*, 9(4), 223–5.
577 Tůmová, E., Bízková, Z., Skřivanová, V., Chodová, D., Martinec, M., & Volek, Z. (2014).
578 Comparisons of carcass and meat quality among rabbit breeds of different sizes, and hybrid
579 rabbits. *Livestock Science,* 165, 8–14.
580 Valenti, B., Natalello, A., Vasta, V., Campidonico, L., Roscini, V., Mattioli, S., Pauselli, M., Priolo,
581 A., Lanza, M & Luciano, G. (2019). Effect of different dietary tannin extracts on lamb
582 growth performances and meat oxidative stability: comparison between mimosa, chestnut
583 and tara. *Animal*, 13(2), 435–443.
584 Vizzarri, F., Palazzo, M., D'Alessandro, A. G., & Casamassima, D. (2017). Productive performance
585 and meat quality traits in growing 1 rabbit following the dietary supplementation of *Lippia*

citriodora, Raphanus sativus and *Solanum lycopersicum* extracts. *Livestock Science,* 200, 53–59.

Wang, J., Su, Y., Elzo, M. A., Jia, X., Chen, S., & Lai, S. (2016). Comparison of carcass and meat quality traits among three rabbit breeds. *Korean Journal of Food Science of Animal Resources*, 36, 84–89.

Zaspel, B. J., & Csallany, A. S. (1983). Determination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. *Analitycal Biochemestry*, 1,146–150.

Zhao, H. Q., Xu, H. J., Du, W. B., Li, L., & Zhang, D. F. (2005). Research and applications of Chinese herbal additives, *Chinese Journal Rabbit Farming*, 1, 31–33.

Zheng, G., Xu, L., Wua, P., Xie, H., Jiang, Y., Chen, F., & Wie, X. (2009). Polyphenols from longan seeds and their radical-scavenging activity. *Food Chemistry,* 116, 433–436.

612 **Table 1.** Ingredients and chemical composition of experimental diets (g/kg)

	Experimental diet[1]		
Ingredients	CON	T1	T2
Maize	282	279	276
Alfalfa hay	305	305	305
Sunflower meal	135	135	135
Palm seed oil	8	8	8
Soybean oil	7	7	7
Wheat	80	80	80
Cane molasses	20	20	20
Carob bean meal	90	90	90
Oat	53	53	53
Calcium carbonate	7	7	7
Sodium Chloride	3	3	3
Dicalcium phosphate	2	2	2
DL-Methionine (99%)	2.5	2.5	2.5
L-Lysine HCl (78.5%)	1.6	1.6	1.6
Choline (75%)	1.4	1.4	1.4
Vitamin and mineral premix*	2.5	2.5	2.5
Dietary supplement	0	3	6
Chemical composition, [2]			
Crude protein	184	183.6	183.5
Ether extract	35.7	35.5	35.5
Crude fibre	187	186.8	187
Ash	86	85.7	85.8
Nitrogen free extract	507	507.1	506.9
NDF	302.1	301.5	301.7
ADF	195.8	195.4	195.3

ADL	39.9	39.5	39.5

[1] CON= control group; T1= group supplemented with 0.3% of brown seaweed and plant polyphenols ; T2= group supplemented with 0.6% of brown seaweed and plant polyphenols ; [*]Supplied per kg diet: 13,500 I.U. vitamin A (trans-retinyl acetate); 800 I.U. vitamin D3 (cholecalciferol); 35 mg vitamin E (α-tocopherol min 91%), 35 mg copper (cupric sulphate pentahydrate);

[2] analyses determined in triplicate.

641 **Table 2.** Chemical composition and polyphenols content of the dietary supplement.

Item	% on dry matter
Dry matter	93.58 ± 5.05
Crude Protein	7.21 ± 0.99
Ether extract	0.32 ± 0.01
Crude fibre	11.20 ± 1.02
Carbohydrates	49.64 ± 3.18
Ash	32.68 ± 1.38
Compounds:[1]	mg/kg dry weight
β-Carotene	402 ± 30.89
Phenolic Acid:	
Dihydroxybenzoic acid	≤ LOD*
Syringic acid	1059.79 ± 62.82
Hydroxycinnamic acids:	
Neochlorogenic acid	7979.23 ± 468.11
Rosmarinic acid	126.54 ± 8.67
Trans sinapic acid	105.54 ± 8.09
Chlorogenic acid	21.45 ± 3.65
Tannins:	
Ellagic acid	2440.88 ± 148.29
Rutin	272.37 ± 20.82
Flavonoids:	
Myricetin	53.88 ± 5.68
Kaempferol	≤ LOD

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643 * Limit of detection;

644 [1] value are expressed as means (n= 4) ± standard deviation.

645

646 **Table 3.** Productive performances of growing rabbits fed control diet and diets supplemented with
647 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

Item	Dietary treatment				
	C	**T1**	**T2**	**SEM**	***P-value***
Live weight, g					
0d	830.2	846.0	789.4	21.02	0.161
21d	1860.9 [b]	1996.3 [a]	1825.3 [b]	44.15	0.024
42d	2655.9	2834.8	2725.2	52.40	0.066
ADG†, g/d					
0d-21d	49.1	54.8	49.3	1.84	0.062
21d-42d	37.9	39.9	42.9	2.19	0.284
0d-42d	43.5	47.4	46.1	1.15	0.067
ADFI*, g/d					
0d-21d	154.9[a]	142.0 [ab]	136.8 [b]	5.07	0.046
21d-42d	188.8	175.6	192.6	8.99	0.382
0d-42d	171.8	158.8	164.7	6.54	0.379
FC‡, kg/kg					
0d-21d	3.20	2.59	2.89	0.17	0.057
21d-42d	5.03 [a]	4.41 [b]	4.58 [ab]	0.18	0.049
0d-42d	3.94 [a]	3.35 [b]	3.60 [ab]	0.11	0.003

648

649 Data are reported as mean ± pooled SEM n=12 (cages with 4 rabbits per cage)

650 C= Control; T1 = dietary supplementation of 0.3% of polyphenols and seaweeds mixture and T2 =
651 dietary supplementation of 0.6% of polyphenols and seaweeds

652 †ADG= average daily gain; *ADFI= feed intake; ‡FC = feed conversion ratio;

653 [a, b] values in the same row are different at $P < 0.05$.

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Table 4. Physical parameters of *Longissimus thoracis and lumborum* and *Semimembranosus muscle* of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

	Dietary treatment				
Item	**C**	**T1**	**T2**	**SEM**	***P-value***
Longissimus thoracis and lumborum					
pH, 24 h	5.86 [b]	5.92 [a]	5.86 [b]	0.010	0.020
Color indexes:					
L*	55.91	55.38	57.41	0.460	0.180
a*	4.01	3.90	3.96	0.252	0.944
b*	11.89	11.71	11.58	0.212	0.845
Semimembranosus muscle					
pH, 24 h	5.75 [a]	5.75 [a]	5.84 [b]	0.015	0.021
Color indexes:					
L*	64.24	63.44	64.31	3.391	0.994
a*	5.46	6.25	6.31	0.523	0.776
b*	3.97	4.23	2.95	0.392	0.394

n=12; data are reported as mean ± pooled SEM;

[a, b] values in the same row are different at $P < 0.05$.

669 **Table 5.** Chemical composition of *Longissimus thoracis and lumborum* and *Semimembranosus*
670 muscle of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and
671 plant polyphenols mixture (T1 and T2 respectively).

672

	Dietary treatment				
Item	**C**	**T1**	**T2**	**SEM**	***P-value***
Longissimus thoracic and lumborum					
Moisture, %	72.82	73.02	73.41	0.182	0.412
Crude protein, %	24.50	23.76	24.40	0.176	0.210
Ether extract, %	1.17	0.90	0.95	0.064	0.123
Ash, %	1.20^{A}	0.94^{B}	1.04^{B}	0.030	<0.001
Cholesterol, mg/100g	32.72	27.22	34.62	2.210	0.373
Semimembranosus muscle					
Moisture, %	73.79	73.67	73.67	0.269	0.533
Crude protein, %	22.62	22.82	22.81	0.176	0.803
Ether extract, %	1.52	1.91	1.52	0.015	0.981
Ash, %	1.21	1.19	1.15	0.030	0.329
Cholesterol, mg/100g	53.25	30.47	42.08	4.052	0.056

673 n=12; data are reported as mean ± pooled SEM;

674 $^{A, B}$ values in the same row are different at $P < 0.01$.

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682 **Table 6.** Sensory evaluation of *Longissumus thoracis and lumborum* muscle: F value and statistical
683 significance of treatments (n=3), judges (n = 8), replicates (n = 3) and their interaction for each
684 sensory descriptor.

	F value					
Descriptors	Samples	Judges	Replicates	SxJ[1]	SxR	JxR
Aroma						
Rabbit	10.75***	1.40	0.92	1.21	1.16	0.64
Liver	7.88**	3.65**	0.31	2.86	0.12	0.27
Rancid	5.97*	4.17**	1.05	1.65	1.83	1.20
Taste						
Sweet	1.25	1.80	0.90	1.08	1.66	0.57
Salty	1.55	1.30	1.88	0.50	1.11	0.94
Flavour						
Rabbit	11.56***	4.07**	0.34	2.43	1.51	0.85
Liver	2.77	2.39**	0.81	1.49	0.60	0.31
Rancid	1.74	4.26**	0.32	2.20	0.39	0.56
Texture						
Tender	1.57	1.37	0.50	0.81	0.78	1.11
Juicy	0.24	1.39	1.89	0.76	0.83	1.33
Stringy	2.80	1.66	1.12	0.87	2.00	1.20

685

686 [1] SxJ = Samples x Judges; SxR= Samples x Replicates; JxR= Judges x Replicates.

687 Significant: ***= 99,9%; ** = 99%; * = 95%; n.s. = no significant

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692 **Table 7.** Sensory evaluation of thigh: F value and statistical significance of treatments, judges (n =
693 8), replicates (n = 3) and their interaction for each sensory descriptor.

	F value					
Descriptors	Samples	Judges	Replicates	SxJ[1]	SxR	JxR
Aroma						
Rabbit	4.88*	1.51	1.54	0.60	0.67	1.13
Liver	3.92*	11.73***	1.38	5.31***	0.53	1.44
Metallic	3.61*	9.96***	0.20	4.90***	0.61	2.00
Taste						
Sweet	0.63	1.10	1.04	1.21	0.62	1.17
Salty	2.16	0.76	1.43	0.28	0.51	1.65
Flavour						
Rabbit	2.04	0.67	1.52	0.25	0.57	2.00
Liver	0.25	4.20	0.21	1.30	0.54	0.75
Metallic	0.29	4.26**	1.44	1.25	0.43	0.79
Texture						
Tender	3.30*	5.34	0.19	1.78	0.43	1.40
Juicy	8.70**	2.95*	1.30	0.84	0.69	0.97
Stringy	3.38*	4.26**	1.88	2.45*	0.97	1.09

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695 [1] SxJ = Samples x Judges; SxR= Samples x Replicates; JxR= Judges x Replicates.

696 Significant: ***= 99,9%; ** = 99%; * = 95%; n.s. = no significant

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701 **Table 8.** Mean values of sensory attributes of *Longissimus thoracis and lumborum* and
702 *Semimembranosus muscle* of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of
703 brown seaweed and plant polyphenols mixture (T1 and T2 respectively).
704

	Dietary treatment		
Descriptors	C	T1	T2
Aroma			
Rabbit	5.0 [a]	5.8 [b]	6.4 [b]
Liver	5.0 [a]	5.4 [b]	5.7 [b]
Rancid	5.0 [a]	5.1 [a]	5.5 [b]
Taste			
Sweet	5.0	4.4	5.0
Salty	5.0	5.4	5.4
Flavour			
Rabbit	5.0 [a]	5.8 [b]	6.1 [b]
Liver	5.0	5.4	5.6
Rancid	5.0	5.2	5.3
Texture			
Tender	5.0	5.3	5.5
Juicy	5.0	4.8	5.0
Stringy	5.0	5.6	5.7

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706 [a, b] means within rows with different superscript letters differ significantly for $P < 0.05$.
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712 Table 9. Mean values of sensory attributes of thight of rabbits fed control diet (C) or diet
713 supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2
714 respectively).
715

	Dietary treatment		
Descriptors	C	T1	T2
Aroma			
Rabbit	5.0 [a]	5.0 [a]	5.8 [b]
Liver	5.0 [b]	4.5 [a]	4.9 [b]
Metallic	5.0 [b]	4.6 [a]	5.0 [b]
Taste			
Sweet	5.0	5.1	5.3
Salty	5.0	5.4	5.4
Flavour			
Rabbit	5.0	5.4	5.5
Liver	5.0	4.8	4.8
Metallic	5.0	4.9	4.8
Texture			
Tender	5.0 [a]	5.3 [ab]	5.5 [b]
Juicy	5.0 [a]	5.8 [b]	5.8 [b]
Stringy	5.0 [b]	4.2 [a]	4.4 [ab]

716
717 [a, b] means within rows with different superscript letters differ significantly for $P < 0.05$.
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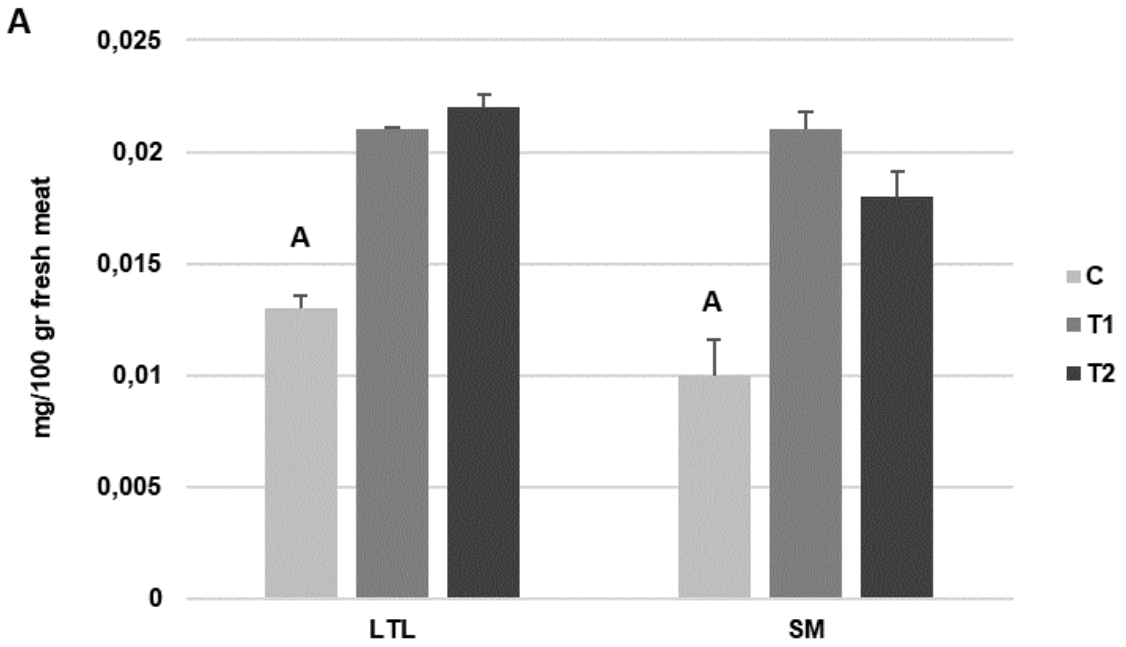
Figure 1. Vitamin A (A) and Vitamin E (B) content of *Longissimus thoracis and lumborum* (LTL) and *Semimembranosus* muscle (SM) of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively; n=12). Data are reported as mean ± SEM; $^{A, B, C}$ values with differ superscript letters are different at P < .001.



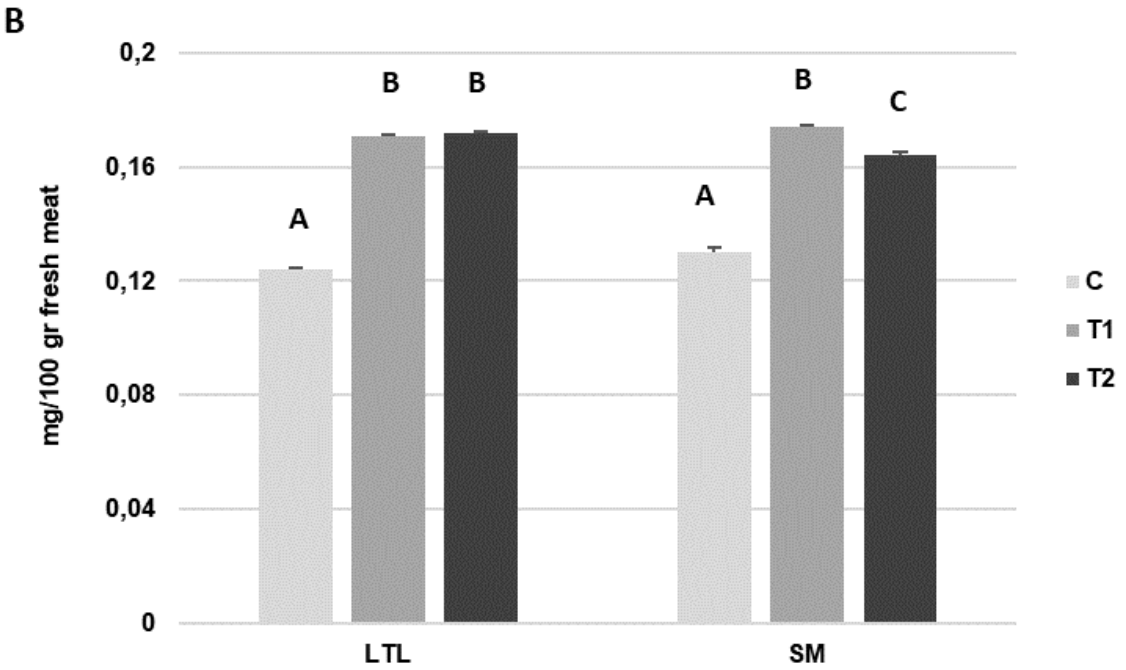
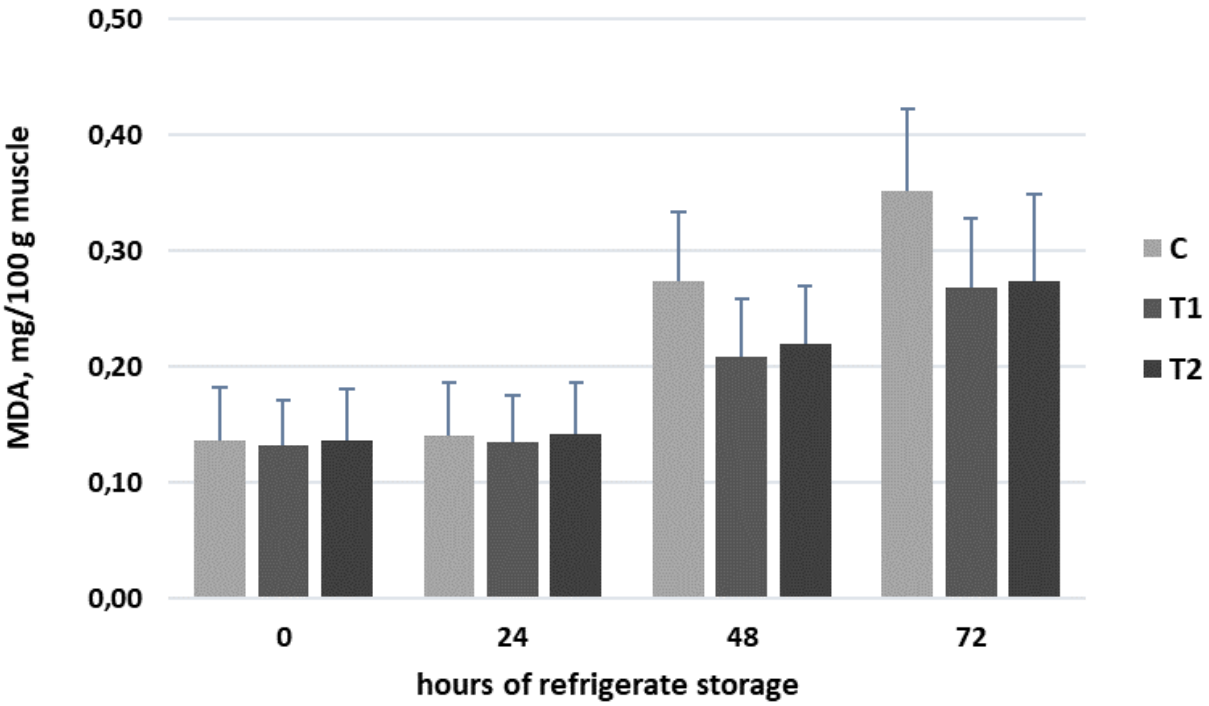


Figure 2. Oxidative stability of *Semimembranosus* muscle of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively) in relation to dietary treatments and time of refrigerated storage at 4°C. Results are expressed as mean values ± SEM (n=12). Time effect for $P < .001$, Treatment effect for $P < .001$, interaction between time x Treatment for $P < .001$



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