



Dietary hazelnut skin prevents lipid oxidation in lamb enriched in omega-3 polyunsaturated fatty acids

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ABSTRACT

The aim of this study was to investigate the effect of partially replacing maize with hazelnut skin and linseed, alone or in combination, on the oxidative stability of lamb. Forty lambs were randomly assigned to 4 treatments and fed: a conventional cereal-based diet (C), or the same diet with 15 % of hazelnut skin (HS), or 8 % of extruded linseed (LS), or 7.5 % of hazelnut skin plus 4 % of linseed (H + L) as partial replacement of maize. After 60 days of feeding trial, lambs were slaughtered, and hydrophilic antioxidant capacity and fat-soluble vitamins content were evaluated in fresh meat. Colour stability, lipid and protein oxidation were assessed during 7 days of refrigerated storage. Dietary combination of hazelnut skin and linseed increased the content of tocopherols ($P < 0.001$) and of n-3 polyunsaturated fatty acids (PUFA n-3; $P < 0.001$), while reducing lipid oxidation (TBARS value) during 7 days of storage ($P < 0.001$). Feeding lamb with hazelnut skin and/or linseed did not affect meat hydrophilic antioxidant capacity, colour stability, nor the formation of hydroperoxides, thiols, or carbonyls ($P > 0.05$). These results suggest that vitamin E provided by hazelnut skin contributed to delay lipid oxidation in meat, despite the higher concentration of n-3 PUFA.

1. Introduction

The growing demand for healthy animal products implies the reduction of saturated fatty acids (SFA) and the increase in polyunsaturated ones (PUFA; Ederer et al., 2023). In the last decades, a trend towards a reduction in the consumption of red meat rich in SFA, such as lamb, in favour of white meat rich in PUFA has been observed (Rabadán et al., 2020). It has been shown that a greater content of PUFA in meat and milk is related to a lower incidence of cardiovascular diseases and greater anti-inflammatory activity (Givens, 2010). Particularly there is an increasing recognition of the health benefits of n-3 FA because these are essential for humans (Calder, 2017). In this context, the amount and composition of FA in the tissues of lambs are crucial elements for evaluating the quality of meat in terms of nutrition and health proprieties (Ebrahimi et al., 2017; Urrutia et al., 2016). To this purpose, strategies including incorporation in animals' diet of PUFA sources, such as linseed, have been studied to increase the meat concentration of n-3 PUFA, which are well known for their beneficial effects on human health

(Nguyen et al., 2018). For instance, Andrés et al. (2014) observed a higher concentration of n-3 PUFA in muscle from lambs fed 8.5 % linseed oil. Also Berthelot et al. (2010) obtained similar results, by supplementing lamb diets with 10 % extruded linseed. However, PUFA are highly susceptible to oxidative deterioration (Domínguez et al., 2019), so meat shelf-life can be worsened by discoloration and off-flavour development if an increase of PUFA is not adequately balanced by a concurrent increase of meat antioxidant capacity (Contini et al., 2014). A worsening of oxidative status of meat could also affect the organoleptic properties and colour of the meat with potential negative effects on consumer acceptance, caused by the browning of the meat and the development of off-flavours. Indeed, oxidative stability is a balance between antioxidants and pro-oxidant molecules (e.g., PUFA, heme iron, copper) and animal diet contributes enormously to regulating this balance (Luciano et al., 2013). Indeed, the dietary intake of antioxidants in livestock farming is considered to be more effective in preventing the oxidation of meat lipids than their post-mortem addition to meat products as preservatives (Govaris et al., 2004). Antioxidant

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molecules that have been used in ruminant diets to prevent oxidation in meat are naturally present in various feeds, such as pasture (Hajji et al., 2016; Santé-Lhoutellier et al., 2008), plant extracts (Cunha et al., 2018; Jerónimo et al., 2012), and agro-industrial by-products (Gravador et al., 2015; Natalello et al., 2020). The latter are sources of diverse antioxidants, such as polyphenols and fat-soluble vitamins (Vasta & Luciano, 2011). Furthermore, agro-industrial by-products are an underutilized feed resource which may also increase the sustainability of livestock production systems and reduce their environmental impact in a circular economy approach, while reducing feed costs (Salami et al., 2019).

In recent years, research has focused on recycling by-products from nuts industry as partial or total substitutes for traditional feedstuffs. Particularly, the hazelnut skin (*Corylus avellana* L.) has been gaining interest as feed ingredient due to its great availability and its interesting chemical composition (Musati et al., 2023). Hazelnut accounted for 11 % of world-wide dried fruit production, with a total of 585 thousand tons of fruits in 2022/2023 (International Nut Council, 2023). Although hazelnut skin represents only 2 % of total fruit weight, a huge amount of skin is produced by confectionary industries (Charron, 2019). Hazelnut skin is generated during peeling process by roasting, therefore it has a low moisture content making it easy to store and transport (Caccamo et al., 2019; Renna et al., 2020). Due to its high fibre content, ruminants are able to exploit this energy source without negative effects on animal metabolism and performance (Priolo et al., 2021; Renna et al., 2020). Moreover, hazelnut skin is characterized by high level of phenolic compounds, such as tannins, noteworthy for their ability to modulate rumen biohydrogenation of dietary PUFA (Musati, Hervás, et al., 2024), accumulating health promoting FA in muscles, such as PUFA or vaccenic and ruminic acids (Frutos et al., 2020). In a previous experiment, hazelnut skin was successfully employed in lamb diet at a concentration of 15 % as a partial replacement for maize (Priolo et al., 2021). We observed higher concentration of vaccenic acid and PUFA in meat without negative effects on growth performance in lambs (Priolo et al., 2021). Furthermore, meat from lambs given 15 % hazelnut skin showed an improved oxidative stability over 7 days storage by delaying lipid oxidation (Menci et al., 2023). This effect might be due to the antioxidant molecules (tocopherols and phenolic compounds) contained in hazelnut by-product. However, the replacement of maize with only hazelnut skin did not increase the meat content of n-3 PUFA (Priolo et al., 2021), desirable for their health benefits in humans. Therefore, the antioxidants provided by hazelnut skin (Menci et al., 2023) may be fully exploited with a feed source rich in n-3 PUFA.

Recently, Musati, Frutos, et al. (2024) explored the effects of partially replacing maize and soybean meal in lambs' diet either with linseed, or with hazelnut skin, or with a combination thereof in order to evaluate the potential benefits of integrating their distinct effects. Combining linseed and hazelnut skin resulted in meat with improved fatty acid composition, with greater contents of n-3 PUFA, ruminic and vaccenic acids. However, the study of Musati, Frutos, et al. (2024) did not investigate the oxidative stability of meat that, considering the lipid profile, could be more susceptible to oxidation. Indeed, taking into account the ability of linseed to increase the concentration of highly oxidizable PUFA and the high content of antioxidants in hazelnut skin, we hypothesized that the combination of linseed and hazelnut skin might be a strategy to improve the fatty acid composition of meat without compromising its oxidative stability. In a preceding study (Luciano et al., 2013) a similar approach consisted in combining linseed and olive cake in a diet for lambs, which resulted in meat with a high content of PUFA, but with an extended oxidative stability compared to a conventional cereal-based diet or to the a diet supplemented with linseed alone. To test this hypothesis, the meat samples collected from Musati, Frutos, et al. (2024) were used in the present study to investigate the effect of the dietary inclusion of hazelnut skin and extruded linseed, alone or in combination, on lamb oxidative stability. For this purpose, the meat content of fat-soluble vitamins and the antioxidant capacity of the hydrophilic fraction were determined, as well as the colour stability

and oxidation of lipids and proteins over 7 days of storage.

2. Materials and methods

2.1. Animals and experimental diets

The experimental procedures were approved by the "Organismo Preposto al Benessere degli Animali (OPBA)" of the University of Catania (protocol No. 82427) and carried out according to the EU welfare guidelines (Directive 2010/63/EU; CR 1099/2009). The feeding trial is detailed in Musati, Frutos, et al. (2024).

The animals were raised in the pilot farm of the University of Catania. The trial involved 40 Valle del Belice × Comisana male lambs (60-days-old; initial body weight 17.3 kg ± 3.17) selected by the same local commercial farm. Lambs were transported in the experimental university facilities, weighed, and randomly assigned to 4 experimental treatments and individually fed. A complete description of the trial is detailed in Musati, Frutos, et al. (2024). Briefly, after 5 days of adaptation period, each group was fed ad libitum with one of the following concentrate-based diet for 60 days: a conventional maize-barley based concentrate diet for growing lambs (C); C diet with 15 % of hazelnut skin as partial replacement of maize (HS); C diet with 8 % extruded linseed as partial replacement of maize and soybean meal (LS); C diet with 7.5 % of hazelnut skin and 4 % of extruded linseed as partial replacement of maize (H + L). Ingredients and chemical composition of diets are reported in Table 1.

2.2. Feedstuff analyses

The proximate composition of each experimental feedstuffs is detailed in Musati, Frutos, et al. (2024), in which DM, ash and crude protein were analysed following ISO protocols (ISO 6496:1999, ISO

Table 1
Chemical composition (g/kg DM) of the experimental diets.

| | Experimental diets ^a | | | |
|---|---------------------------------|------|------|-------|
| | C | HS | LS | H + L |
| <i>Ingredients (g/kg DM)</i> | | | | |
| Maize | 260 | 110 | 210 | 145 |
| Barley | 260 | 260 | 260 | 260 |
| Alfalfa hay | 200 | 200 | 200 | 200 |
| Soybean meal | 160 | 160 | 130 | 160 |
| Wheat bran | 70 | 70 | 70 | 70 |
| Molasses | 30 | 30 | 30 | 30 |
| Mineral mix | 20 | 20 | 20 | 20 |
| Hazelnut skin | – | 150 | – | 75 |
| Extruded linseed | – | – | 80 | 40 |
| <i>Chemical composition (g/kg DM)</i> | | | | |
| Ash | 7.83 | 6.88 | 6.79 | 7.10 |
| CP | 166 | 167 | 164 | 178 |
| aNDF | 225 | 264 | 235 | 261 |
| ADF | 113 | 135 | 118 | 135 |
| Total polyphenols ^b | 5.17 | 24.6 | 7.16 | 12.4 |
| Total tannins ^b | 1.70 | 15.9 | 3.63 | 6.62 |
| Total tocopherols, mg/kg DM | 22.1 | 101 | 46.2 | 70.6 |
| α-tocopherol, % of total tocopherols | 29.1 | 42.8 | 30.0 | 34.9 |
| γ-tocopherol, % of total tocopherols | 70.9 | 57.2 | 70.0 | 65.1 |
| Total FA | 23.6 | 44.6 | 52.8 | 52.0 |
| <i>Individual fatty acid (% of total fatty acids)</i> | | | | |
| 16:0 | 19.2 | 11.8 | 12.1 | 13.6 |
| 18:0 | 3.27 | 2.2 | 3.24 | 4.86 |
| c9 18:1 | 22.0 | 54.3 | 30.6 | 36.5 |
| c9c12 18:2 | 51.3 | 28.8 | 32.7 | 27.8 |
| c9c12c15 18:3 | 2.95 | 1.54 | 19.8 | 15.1 |

Abbreviations: ADF: acid detergent fibre; aNDF: neutral detergent fibre; CP: crude protein; DM: dry matter; FA: fatty acids; OM: organic matter.

^a C: Control diet; HS: Hazelnut skin diet; LS: Linseed diet; H + L: Hazelnut skin + Linseed diet.

^b Expressed as g tannic acid equivalents/kg DM.

5984:2022, and ISO 5983-2:2009, respectively), while the fibre fraction (aNDF and ADF) was sequentially determined using an Ankom²⁰⁰⁰ fibre analyser (Ankom Technology Corp., Macedon, NY, USA). Total polyphenols and total tannins were determined in experimental diets following the Folin-Ciocalteu method developed by Makkar et al. (1993), using tannic acid (Sigma–Aldrich) as a reference standard, and minor adaptations described in Luciano et al. (2019). Feed FA were extracted and converted to FA methyl esters (FAME) using chloroform and 2 % (v/v) sulfuric acid in methanol and tridecanoic acid (Sigma–Aldrich) as internal standard. The FAME were separated and quantified using a gas chromatograph (GC) Thermo Finnigan Trace GC equipped with a flame ionization detector (FID; ThermoQuest, Milan, Italy) and 100 m high-polar fused silica capillary column (0.25 mm i.d.; 0.25 µm film thickness; SP-2560 fused silica, Supelco, Bellefonte, PA, USA). The helium was used as gas carrier. The FAME were identified comparing the retention time with commercial standard mixture of FAME (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmö, Sweden).

Tocopherols content of feedstuffs were quantified according to the extraction method developed by Rufino-Moya et al. (2020). An aliquot of 200 mg of feedstuffs were grounded and 300 µL methanol: acetone: petroleum ether (1:1:1, v/v/v) were added. Samples were centrifuged and the supernatant was collected, this operation was repeated three times. The supernatants were dried under N₂ and dissolved in 1 mL methanol and then filtered using 0.22 µm PTFE filters. Fat-soluble compounds were detected using a Nexera UHPLC (Shimadzu Corp., Japan) equipped with a C18 phase column (Zorbax ODS, Supelco, Bellefonte, PA; 25 cm × 4.6 mm; particle size: 5 µm) set as detailed by Natalello et al. (2022). Calibration curves were created with pure standards for quantifying the analytes (Merck Life Science s.r.l., Milano, Italy).

2.3. Slaughter procedure and meat sampling and analyses

After 60 days, animals were weighed and taken to a local slaughterhouse (80 km from the university experimental farm) where they were immediately slaughtered by stunning and exsanguination. Each carcass was weighed and the *longissimus thoracis and lumborum* (LTL) muscle was excised from both sides. The LTL muscle was removed, vacuum packed, and aged at 4 °C. After 24 h, muscle pH was recorded on the right side inserting the pH meter into a small incision (HI110; Hanna Instruments, Padova, Italy). The pH meter was calibrated using commercial standard solutions at the same temperature of the muscle (4 °C). The right LTL was subsequently vacuum-packed and stored at –80 °C until further analyses on FA profile, vitamins, and hydrophilic antioxidant capacity. The shelf-life study was performed on the left side (colour, lipid, and protein stability) as described below in paragraph 2.3.4.

2.3.1. Fatty acids profile

For the FA profile of wet meat, the analyses and instrument parameters are described above in paragraph 2.2. For the quantification of FAME, the nonadecanoic acid was used as internal standard. In the previous study (Musati, Frutos, et al., 2024) we reported the FA profile as g/100 g of total FA according to the purpose of the research. In the present investigation, the main classes of FA (SFA, MUFA, PUFA, n-3 PUFA, and n-6 PUFA) are expressed as mg/100 g meat, as the quantity of fatty acid is considered a more relevant information to investigate the oxidative stability of meat. Moreover, the susceptibility of FA to lipid oxidation was estimated calculating the total amount of highly peroxidizable (HP) PUFA (Natalello et al., 2020), for more details see Priolo et al. (2021).

2.3.2. Hydrophilic antioxidant capacity of meat

The hydrophilic antioxidant capacity was determined with five different assays: Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), Folin-Ciocalteu assay, ferrous ion

chelating activity (FICA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. For TEAC, FRAP, Folin-Ciocalteu, and FICA assays samples were prepared as follows. Two grams of meat was homogenized for 1 min (Ultra Turrax T-18; Ika, Germany) in ice-water bath with 18 mL of distilled water. Samples were centrifugated (2500 ×g for 20 min at 4 °C) and the supernatant filtered (Whatman 1 filter paper). The filtered meat aqueous extract (MAE) was divided into different aliquots for subsequent analyses.

The TEAC assay was performed following protocol of Re et al. (1999). Briefly, 20 µL of MAE was mixed with 2 mL of ABTS^{•+} solution (Aouadi et al., 2014). Absorbance at 734 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy) after an incubation of 1 h at 30 °C. A blank prepared with a solution of phosphate buffer saline (PBS) instead of MAE was used for measuring the spontaneous discoloration. Results were obtained by comparison to a 6-points calibration curve (0–400 µg/mL; $y = 141 + 4.45x$; $r^2 = 0.999$) of Trolox standard (CAS: 53188-07-1; Merck Life Science s.r.l., Milano, Italy) in PBS (pH 7.4).

For the FRAP assay (Benzie & Strain, 1996), 50 µL of MAE was mixed with 150 µL of water and 1.5 mL of a 10:1:1 (v:v:v) solution [300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (2,4,6-Tri(2-pyridyl)-s-triazine in 40 mM HCl), and 20 mM aqueous ferric chloride]. After 1 h at 37 °C of incubation, absorbance at 593 nm was measured (UV-1601 Shimadzu). Results were obtained by comparison to a 7-points calibration curve (0–280 µg/mL; $y = 2.29 + 0.007x$; $r^2 = 0.999$) of FeSO₄ × 7H₂O.

The Folin-Ciocalteu assay was performed following the protocol of Makkar et al. (1993). An aliquot of 0.5 mL of MAE was mixed with 0.5 mL of distilled water, 0.5 mL of 1 N Folin-Ciocalteu reagent, and 2.5 mL of 20 % sodium carbonate. Samples were incubated 40 min at room temperature, and then centrifugated at 2500 ×g for 10 min at 4 °C. Absorbance was read at 725 nm (UV-1601 Shimadzu). Results were obtained by comparison to a 6-points calibration curve (0–50 µg/mL; $y = 0.0241x - 0.0078$; $r^2 = 0.999$) of aqueous tannic acid.

For the FICA assay (Yen & Wu, 1999), a sample of 0.5 mL of MAE was mixed with 0.1 mL of Fe(II) chloride (2 mmol/L in distilled water), 0.2 mL of ferrozine solution (5 mmol/L in distilled water), 4.2 mL of distilled water, and incubated in darkness for 60 min. Results of the absorbance were recorded at 562 nm (UV-1601 Shimadzu) and then compared to a 6-points calibration curve (0–60 µg/mL; $y = 1.45x + 0.169$; $r^2 = 0.996$) of EDTA.

For the DPPH assay, the method proposed by Yen and Wu (1999) was followed with some adaptations. Briefly, 2 g of LTL was homogenized with 18 mL of 0.05 M PBS for 1 min (Ultra Turrax T-18 Homogenizer) in ice-water bath. Two mL of 10 % C₂HCl₃O₂ was added to the mixture for precipitating proteins. The samples were centrifugated (2500 ×g for 20 min at 4 °C) and the supernatant filtered (Whatman No. 1). Subsequently, 0.6 mL of filtrate, 2.4 mL water and 3 mL of 0.2 mM DPPH were mixed in methanol. Results of the absorbance were measured at 517 nm (UV-1601 Shimadzu) and then compared to a 6-points calibration curve (0–90.9 µg/mL; $y = 0.775x - 2.13$; $r^2 = 0.997$) of Trolox standard buffer solution (0.1 mg/mL).

2.3.3. Lipophilic vitamins in meat

The concentration of fat-soluble vitamins (α- and γ-tocopherols and retinol) and cholesterol in LTL were determined according to Bertolín et al. (2018) with adaptations as detailed in Menci et al. (2023). A sample of 2.5 g was mixed with 0.2 g ascorbic acid and 7.5 mL of 10 % potassium hydroxide in ethanol:water and incubated in orbital shaker overnight. For extracting lipids, 5 mL of 9:1 hexane:ethyl acetate with 25 mg/L of BHT was added. Then samples were centrifugated and supernatant was collected. This operation was repeated two times. Supernatants were dried under N₂ and residues were dissolved with methanol and filtered (0.22 µm PTFE filters). Quantification of tocopherols, retinol, and cholesterol were performed using UHPLC analysis. Instrument, setting, and procedure are described above in paragraph 2.2.

2.3.4. Oxidative meat stability

Oxidative stability in fresh meat over aerobic refrigerated storage was determined following Menci et al. (2023). Meat samples from the left LTL were cut in 3 slices (2 cm of thickness). The slices were stored at 4 °C in the dark in polystyrene trays, covered with a double layers of food stretch film, for 0 (2 h of blooming), 4, and 7 days, simulating the storage conditions of a consumer in a domestic or commercial refrigerated environment. Each slide was used to monitor the colour stability and oxidation status of lipids and proteins, as described in the following chapters.

2.3.4.1. Colour measurement. At each day of storage, colour of meat samples was measured with Minolta CM 2022 (Minolta Co., Japan Minolta CM 2022; d/8° geometry, mode SCE; illuminant A; standard observer 10°). The mean values of three measurements were taken from different areas of meat, according to the colour descriptor L^* , a^* , b^* , C^* , and h_{ab} recorded in the CIELAB colour space. The metmyoglobin percentage formation was measured according to Krzywicki (1979) considering the reflectance spectrum from 400 nm to 700 nm. The metmyoglobin accumulation was also estimated with the ratio between scattering coefficient at 572 and 525 nm (KS572/KS525; Stewart et al., 1965). Subsequently, slices of meat were frozen (−80 °C) until oxidation analyses.

2.3.4.2. Lipid and protein oxidation. Lipid oxidation was evaluated measuring the primary (hydroperoxides) and secondary (2-thio-barbituric acid reactive substances; TBARS) products in meat during 7 days of refrigerated storage (4 °C). The hydroperoxide and TBARS analyses followed the original procedure described in Maqsood et al. (2012) and Siu and Draper (1978), respectively, but with some adaptations as detailed in Menci et al. (2023) and Natalello et al. (2020), respectively. Concerning protein oxidation, primary (thiols) and secondary (carbonyls) products of protein oxidation were analysed in myofibrillar protein (MP) fraction, isolated following the method described in Park et al. (2007). The analysis of thiol and carbonyl content in MP is detailed in Menci et al. (2023).

2.4. Statistical analyses

Statistical analyses were performed with SPSS V. 27 (SPSS Inc., Chicago). Data on animal performance, FA profile, tocopherols, and antioxidant capacity of meat were analysed using the GLM to test the dietary treatment effect and considering the individual lambs as the experimental units. A mixed model for testing the effect of the dietary treatment, time of storage, and their interaction as fixed factors was applied to parameters of colour and lipid and protein oxidation, using individual lambs as random factor. Significance differences were declared at $P < 0.05$.

3. Results

3.1. Feed composition and in vivo performance

The chemical composition of the experimental diets is included in Table 1. The partial replacement of maize with hazelnut skin and extruded linseed generated some slight changes in the chemical composition of the diets. In particular, diets containing hazelnut skin apported fibre, with higher values of aNDF and ADF in HS and H + L compared to C and LS diets. Hazelnut skin was characterized by a high polyphenols and tannins content, therefore HS and H + L diets had a greater quantity of these compounds compared to C and LS. Regarding fat-soluble vitamins, HS and H + L diets had a greater quantity of tocopherols compared to C and LS diets (101 and 70.6 mg/kg DM vs 22.1 and 46.2 mg/kg DM, respectively) and γ -tocopherol was the main vitamin E isomer. However, γ -tocopherol represented approximately 70

% of the vitamin E isomers in the C and LS diets, while hazelnut skin diets provided a higher proportion of α -tocopherol, between 48.8 and 34.9 %, respectively for HS and H + L. Concerning FA content, LS and H + L diet had more than double content of total FA compared to C (5.28 and 5.20 vs. 2.36 mg/g DM, respectively). Diets with linseed were characterized by a content 5.1-fold for H + L and 6.7-fold higher for LS of α -linolenic acid (c9c12c15 18:3) compared to C (15.1 and 19.8 vs. 2.95 %, respectively). While oleic acid (c9 18:1) was higher in diets with hazelnut skin increasing by +65 % in H + L and +147 % in HS compared to C diet.

As shown in Table 2, the dietary treatments did not affect the animal performance, expressed in final body weight, carcass weight, average daily gain, feed conversion ratio, and DM intake ($P > 0.05$). Considering the different content of total polyphenols and tocopherol in the diet, the intake of total polyphenols, total tannins, and vitamin E increased in the order: $C \leq LS < H + L < HS$ ($P < 0.001$).

3.2. Fatty acids, fat-soluble vitamins, and antioxidant capacity of meat

The dietary treatments did not influence the pH of meat neither the intramuscular fat content ($P > 0.05$; Table 3). Feeding hazelnut skin and/or extruded linseed affected the quantity of saturated and polyunsaturated FA ($P = 0.043$ and 0.003 , respectively). Concerning SFA, H + L had higher quantity compared to HS samples, while PUFA content was higher in H + L than C and HS. Also, n-6 PUFA content in H + L meat was greater than LS ($P = 0.014$). Contrary, n-3 PUFA reached higher value in H + L and LS meat compared to C and HS ($P < 0.001$). Consequently, diets containing linseed (LS and H + L) significantly reduced the n-6/n-3 ratio compared to HS and C ($P < 0.001$). Meat from LS and H + L lambs had higher quantity of HP-PUFA (highly peroxidizable PUFA, with at least three double bonds) compared to C and HS meat ($P < 0.001$). Concerning fat-soluble vitamins, feeding lambs with hazelnut skin increased the vitamin E content in meat, indeed HS and H + L meat had greater amount of total tocopherols than that of C and LS ($P < 0.001$). In particular, α -tocopherol was the main isomer in all treatments, but its proportion was greater in C treatment than in the others ($P < 0.001$), conversely γ -tocopherol proportion was higher in lamb meat of HS, LS, and H + L meat ($P < 0.001$). Considering the effect of dietary treatment on concentration of HP-PUFA and vitamin E, lambs fed LS had the highest HP-PUFA/vitamin E ratio, HS had the lowest, while the C and H + L had intermediate values ($P < 0.001$). The dietary treatment did not influence retinol and cholesterol concentration in meat ($P > 0.05$). Also, the antioxidant assays of hydrophilic fraction of meat, such as TEAC, FRAP, Folin-Ciocalteu, FICA, and DPPH, were not affected by the dietary treatment ($P > 0.05$).

3.3. Meat oxidative stability

Table 4 reports the effects of dietary treatment, time of storage, and their interaction on meat discolouration, lipid and protein oxidation over 7 days of refrigerated storage. The diet did not influence meat colour ($P > 0.05$) and all meat colour parameters were affected by time of storage ($P \leq 0.001$). Particularly, L^* reached maximum value on day 4 of storage and minimum on day 0 and 7 ($P = 0.001$). While, a^* , b^* , C^* , and KS572/KS525 decreased over time of storage, while hue angle (h_{ab}), and percentages of metmyoglobin increased, indicating meat browning. Only exception, the interaction diet \times time was significant for L^* and Hue angle ($P = 0.003$ and 0.012 , respectively). Fig. 1 shows that the highest brightness value was reached on day 4 and the lowest on day 0, both in the HS treatment. Regarding h_{ab} , values increased over time for all groups, but with different trends as shown in Fig. 2. The hydroperoxide content was not influenced by diet ($P > 0.05$) but tended to reach higher values on day 4 of storage ($P = 0.082$), while TBARS values increased over storage time (Fig. 3; $P < 0.001$). Moreover, the dietary treatment affected secondary products of lipid oxidation, with an average higher TBARS value in C meat, followed by LS and then H + L

Table 2
Effect of dietary treatment on animal performance and intakes.

| | Experimental diets ¹ | | | | SED ² | P-value |
|--------------------------|---------------------------------|-------------------|-------------------|-------------------|------------------|---------|
| | C | HS | LS | H + L | | |
| Final BW (kg) | 30.9 | 28.5 | 31.4 | 29.9 | 2.13 | 0.555 |
| Carcass weight (kg) | 14.1 | 13.1 | 14.4 | 14.3 | 1.19 | 0.691 |
| ADG (g/d) | 227 | 193 | 237 | 214 | 24.8 | 0.323 |
| FCR | 4.30 | 5.17 | 4.07 | 5.03 | 0.531 | 0.118 |
| DMI (g/d) | 831 | 862 | 852 | 893 | 77.4 | 0.870 |
| <i>Intakes</i> | | | | | | |
| Polyphenols (g TA eq/d) | 4.29 ^c | 21.2 ^a | 6.10 ^c | 11.1 ^b | 1.10 | <0.001 |
| Tannins (g TA eq/d) | 1.41 ^c | 13.7 ^a | 3.09 ^c | 5.91 ^b | 0.656 | <0.001 |
| Total tocopherols (mg/d) | 18.3 ^a | 87.5 ^a | 39.3 ^c | 63.1 ^b | 5.38 | <0.001 |
| α-tocopherol (mg/d) | 5.33 ^d | 37.5 ^a | 11.8 ^c | 22.0 ^b | 2.04 | <0.001 |
| γ-tocopherol (mg/d) | 13.0 ^d | 50.1 ^a | 27.5 ^c | 41.0 ^b | 3.37 | <0.001 |

Abbreviations: ADG: average daily gain; BW: body weight; DMI: dry matter intake; FCR: feed conversion ratio; TA eq: tannic acid equivalents.

^{a,b,c,d} Within a row, different superscripts indicate significant differences ($P < 0.05$).

¹ C: Control diet; HS: Hazelnut skin diet; LS: Linseed diet; H + L: Hazelnut skin + Linseed diet.

² SED: standard error of the difference.

Table 3
Effect of dietary treatment on the pH, intramuscular fat, vitamins, cholesterol, and antioxidant capacity of meat.

| | Experimental diets ¹ | | | | SED ² | P-value |
|-------------------------------------|---------------------------------|-------------------|--------------------|-------------------|------------------|---------|
| | C | HS | LS | H + L | | |
| pH | 5.55 | 5.51 | 5.52 | 5.54 | 0.082 | 0.943 |
| Intramuscular fat (g/100 g meat) | 2.40 | 1.91 | 2.41 | 2.42 | 0.251 | 0.124 |
| <i>Fatty acid (mg/100 g meat)</i> | | | | | | |
| Saturated | 679 ^{ab} | 511 ^b | 667 ^{ab} | 783 ^a | 93.9 | 0.043 |
| Monounsaturated | 797 | 599 | 796 | 873 | 110.25 | 0.087 |
| Polyunsaturated | 142 ^b | 146 ^b | 159 ^{ab} | 192 ^a | 13.7 | 0.003 |
| n-6 PUFA | 122 ^{ab} | 127 ^{ab} | 105 ^b | 138 ^a | 9.53 | 0.014 |
| n-3 PUFA | 14.8 ^b | 14.1 ^b | 47.3 ^a | 45.2 ^a | 4.29 | <0.001 |
| HP-PUFA | 22.9 ^b | 21.6 ^b | 52.8 ^a | 51.7 ^a | 4.86 | <0.001 |
| n-6/n-3 Ratio | 8.30 ^a | 9.21 ^a | 2.24 ^b | 3.14 ^b | 0.454 | < 0.001 |
| Cholesterol (g/kg) | 0.633 | 0.668 | 0.666 | 0.631 | 0.0415 | 0.700 |
| <i>Fat-soluble vitamins (mg/kg)</i> | | | | | | |
| Total tocopherols | 0.746 ^b | 1.76 ^a | 0.830 ^b | 1.34 ^a | 0.185 | <0.001 |
| α-tocopherol (%) | 89.7 ^a | 81.3 ^b | 78.4 ^b | 77.7 ^b | 2.35 | <0.001 |
| γ-tocopherol (%) | 10.3 ^b | 18.7 ^a | 21.6 ^a | 22.4 ^a | 2.35 | <0.001 |
| Retinol | 0.067 | 0.057 | 0.065 | 0.055 | 0.006 | 0.143 |
| HP-PUFA/vitamin E ³ | 2.50 ^b | 2.11 ^c | 2.80 ^a | 2.58 ^b | 0.072 | <0.001 |
| <i>Antioxidant capacity (mg/g)</i> | | | | | | |
| TEAC (Trolox eq) | 64.1 | 58.1 | 67.9 | 62.9 | 4.30 | 0.162 |
| FRAP (Fe2+ eq) | 20.7 | 19.8 | 22.0 | 20.3 | 0.99 | 0.178 |
| Folin-Ciocalteu (TA eq) | 0.295 | 0.286 | 0.307 | 0.290 | 0.0090 | 0.111 |
| FICA (EDTA eq) | 0.767 | 0.795 | 0.751 | 0.767 | 0.0319 | 0.574 |
| DPPH (Trolox eq) | 0.707 | 0.661 | 0.751 | 0.734 | 0.0402 | 0.105 |

Abbreviations: DPPH: 1,1-diphenyl-2-picrylhydrazyl; EDTA: ethylenediaminetetraacetic acid; FICA: ferrous ion chelating activity; FRAP: ferric reducing antioxidant power; HP-PUFA: highly peroxidizable polyunsaturated fatty acid; PUFA: polyunsaturated fatty acid; TA eq: tannic acid equivalents; TEAC: Trolox equivalent antioxidant capacity.

^{a,b,c} Within a row, different superscripts indicate significant differences ($P < 0.05$).

¹ C: Control diet; HS: Hazelnut skin diet; LS: linseed diet; H + L: Hazelnut skin + Linseed diet.

² SED: standard error of the difference.

³ Calculated as the ratio between HP-PUFA and vitamin E (total tocopherols), both expressed in mg/g meat. Original value obtained was not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

and HS ($P = 0.001$). A similar trend was shown by interaction diet \times time in TBARS, with notable differences only starting from day 4 of storage ($P < 0.001$). In particular, the concentration of TBARS values increased over time and the C and LS treatments on day 4 showed values comparable to those of H + L and HS on day 7 (Fig. 3). The differences between groups were significant on day 4 and 7 of storage. In particular, after 4 days the C and LS treatments showed higher TBARS values compared to HS and H + L ($P < 0.001$), while after 7 days the TBARS content was significantly higher in C and LS compared to HS ($P < 0.001$). Concerning protein oxidation, primary (thiols) and secondary (carbonyl) products were not affected by diet nor time of storage (Table 4; $P > 0.05$).

4. Discussion

The intramuscular fat content of LTL was similar between dietary treatments; however, meat lipid composition was affected by diet. In particular, PUFA and HP-PUFA content was increased when lambs received the combination of hazelnut skin and linseed compared to the control. However, animal products rich in unsaturated FA may be more susceptible to oxidation processes, as these FA, being more prone to oxidation, act as pro-oxidant factors (Bekhit et al., 2013). Furthermore, oxidation processes are favoured as the degree of lipid unsaturation increases (Johnson & Decker, 2015). The present study investigated the concentration (mg/100 g of meat) of the main FA class, particularly unsaturated FA, which are those that are most subject to the oxidative

Table 4

Effect of dietary treatment on colour stability and lipid and protein oxidation in lamb over 7 days of refrigerated storage.

| | Experimental diets (D) ¹ | | | | Time (T), d | | | SED ² | P-value | | |
|--|-------------------------------------|--------------------|--------------------|---------------------|--------------------|--------------------|--------------------|------------------|---------|--------|--------|
| | C | HS | LS | H + L | 0 | 4 | 7 | | D | T | D × T |
| Colour stability | | | | | | | | | | | |
| <i>L*</i> (lightness) | 45.7 | 44.8 | 44.9 | 44.3 | 44.4 ^y | 45.6 ^x | 44.7 ^{xy} | 1.17 | 0.691 | 0.001 | 0.003 |
| <i>a*</i> (redness) | 15.3 | 14.4 | 14.8 | 14.5 | 16.7 ^x | 15.0 ^y | 12.6 ^z | 0.401 | 0.106 | <0.001 | 0.477 |
| <i>b*</i> (yellowness) | 13.5 | 12.8 | 12.7 | 12.5 | 13.3 ^x | 13.2 ^x | 12.2 ^y | 0.490 | 0.235 | 0.001 | 0.272 |
| <i>C*</i> (saturation) | 20.5 | 19.3 | 19.5 | 19.2 | 21.4 ^x | 19.9 ^y | 17.6 ^z | 0.610 | 0.165 | <0.001 | 0.427 |
| <i>h_{ab}</i> (Hue angle; deg) | 41.5 | 41.7 | 40.7 | 40.9 | 38.2 ^z | 41.3 ^y | 44.1 ^x | 0.531 | 0.211 | <0.001 | 0.012 |
| KS572/KS525 ³ | 0.900 | 0.901 | 0.909 | 0.909 | 0.934 ^x | 0.904 ^y | 0.877 ^z | 0.0095 | 0.628 | <0.001 | 0.082 |
| Metmyoglobin (%) | 49.2 | 49.5 | 48.5 | 48.6 | 45.7 ^z | 48.8 ^y | 52.3 ^x | 0.825 | 0.562 | <0.001 | 0.052 |
| Lipid oxidation, mg/kg meat | | | | | | | | | | | |
| Hydroperoxide | 14.2 | 12.6 | 10.6 | 13.3 | 12.7 | 14.9 | 10.4 | 4.59 | 0.723 | 0.082 | 0.814 |
| TBARS ⁴ | 1.55 ^a | 0.632 ^c | 1.36 ^{ab} | 0.781 ^{bc} | 0.153 ^z | 1.10 ^y | 1.99 ^x | 0.239 | 0.001 | <0.001 | <0.001 |
| Protein oxidation, μmol/g protein | | | | | | | | | | | |
| Thiols | 55.2 | 61.5 | 49.0 | 51.9 | 57.6 | 54.6 | 51.0 | 8.25 | 0.449 | 0.082 | 0.918 |
| Carbonyls | 5.72 | 4.36 | 5.69 | 4.29 | 5.16 | 4.56 | 5.33 | 0.913 | 0.223 | 0.071 | 0.283 |

^{a,b,c} Within a row, different superscripts indicate significant differences between dietary treatments ($P < 0.05$).

^{x,y,z} Within a row, different superscripts indicate significant differences between storage time ($P < 0.05$).

¹ C: Control diet; HS: Hazelnut skin diet; LS: linseed diet; H + L: Hazelnut skin + Linseed diet.

² SED: standard error of the difference.

³ ratio between scattering coefficient at 572 nm and 525 nm.

⁴ TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).

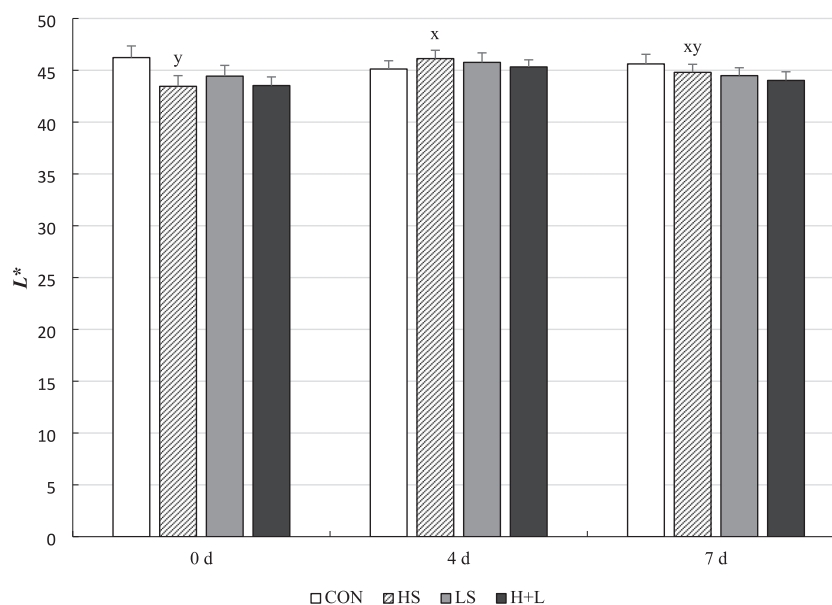


Fig. 1. Interaction between diet and storage time on *L** (lightness) in lamb meat.

C: control diet; HS: Hazelnut skin diet; H + L: Hazelnut skin + Linseed diet; LS: Linseed diet.

Error bars represent the standard error of the mean.

x, y Within each dietary treatment, different superscripts indicate differences between days of storage ($P \leq 0.003$).

deterioration (Bekhit et al., 2013). Moreover, HP-PUFA were estimated as the sum of FA with 3 or more double bonds, as reported in many meat shelf-life studies (e.g., Luciano et al., 2019; Natalello et al., 2020; Siphambili et al., 2020). In this study, HP-PUFA quantity was higher in meat from H + L and LS lambs. Thus, it might be expected that feeding linseed might result in a more pronounced lipid oxidation of meat by increasing pro-oxidant substrates.

However, according to Bekhit et al. (2013), the oxidative process in meat is a delicate balance between pro-oxidant (e.g., PUFA, heme iron, copper, etc.) and antioxidant (vitamins, glutathione, antioxidant enzymes, etc.) factors. Among the antioxidants, the vitamin E is considered one of the most powerful in meat (Bellés et al., 2019). In the present study, its higher concentration in HS and H + L meat was likely due to the greater amount of vitamin E (tocopherols) present in the hazelnut skin by-product. In fact, the HS and H + L diets resulted in a greater

ingestion of total tocopherols compared to LS and C. Similar results have also been previously highlighted by other studies in which hazelnut skin was administered to lambs (Menci et al., 2023), dairy sheep (Marino et al., 2021) and cows (Renna et al., 2020). Tocopherols are largely bioavailable and absorbable by the intestine with lipid micelles (Bellés et al., 2019), and in turn it has been demonstrated that lipids may favour the intestinal absorption of tocopherols (Lodge et al., 2004). Our experimental diets containing linseed and hazelnut skin had more than double of fat compared to C diet. However, although LS lambs ingested higher contents of vitamin E and fat compared to C lambs, the concentration of total tocopherols in meat was comparable between these two treatments. Therefore, a higher concentration of vitamin E in the LS lambs was expected in regard of C animals. The lack of difference in muscle vitamin E concentration between C and LS lambs is not easy to explain. A possible explanation could lie in the high concentration of

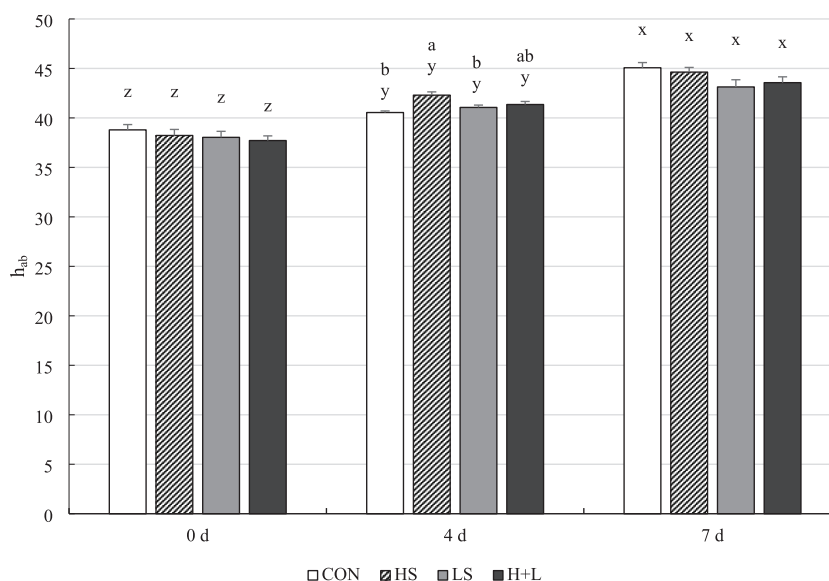


Fig. 2. Interaction between diet and storage time on h_{ab} (hue angle) in lamb meat.

C: Control diet; HS: Hazelnut skin diet; H + L: Hazelnut skin + Linseed diet; LS: Linseed diet.

Error bars represent the standard error of the mean.

a, b Within each day of storage, different superscripts indicate differences between dietary treatments ($P \leq 0.05$).

x, y, z Within each dietary treatment, different superscripts indicate differences between days of storage ($P \leq 0.05$).

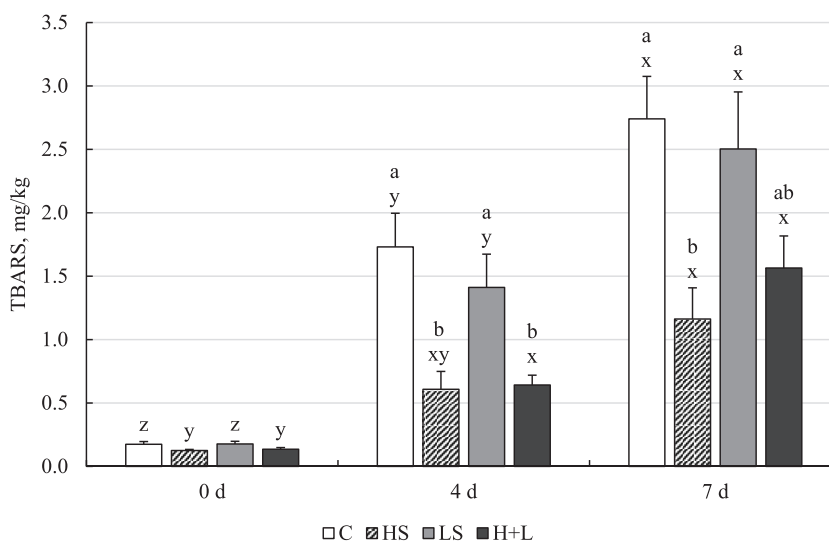


Fig. 3. Interaction between diet and storage time on TBARS (mg/kg) development in lambs.

C: Control diet; HS: Hazelnut skin diet; H + L: Hazelnut skin + Linseed diet; LS: Linseed diet; TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).

Error bars represent the standard error of the mean.

a, b Within each day of storage, different superscripts indicate differences between dietary treatments ($P \leq 0.05$).

x, y, z Within each dietary treatment, different superscripts indicate differences between days of storage ($P \leq 0.05$).

PUFA present in linseed. Indeed, PUFA can interfere with tocopherol bioavailability in different manners: *i*) PUFA inhibit the vitamin E absorption (McDowell, 2000), probably due to their competition for micelle incorporation in the intestine; *ii*) PUFA might be easily peroxidized in the rumen or intestine, leading to the generation of radical species. These radicals might be neutralized by vitamin E, resulting in an oxidated tocopheroxyl radical (Bellés et al., 2019). Further studies should be planned specifically on ruminants fed with diets rich in PUFA and vitamin E to better understand these mechanisms involved in absorption and storage in meat.

To assess the balance between pro-oxidant and antioxidant factors,

we calculated the HP-PUFA/vitamin E ratio, as reported in some studies (e.g., Luciano et al., 2019; Luciano, Moloney, et al., 2011; Luciano, Vasta, et al., 2011). A high HP-PUFA/vitamin E value indicates an imbalance towards pro-oxidant molecules, while low values suggest a greater proportion of antioxidants, thus increasing the resistance of meat to oxidation. In our study, the HP-PUFA/vitamin E ratio was significantly higher in LS meat than C and HS, with the H + L combination showing intermediate value. This finding may suggest that meat from the LS treatment would be more susceptible to lipid oxidation than meat from other treatments and, among these, HS would be the most resistant to the oxidative process.

Lipid oxidation is the main non-bacterial degradation process that deteriorates the physio-chemical quality of meat generating changes in colour, texture, odour, and flavour, which influence consumer acceptance (Huang & Ahn, 2019; Lorenzo & Gómez, 2012). The first oxidation products are hydroperoxides, which are responsible to exert adverse consequences on human digestion, with cytotoxic effects on cells (Angeli et al., 2011). Despite FA profile might explain most of the formation of hydroperoxides (Yi et al., 2013), in the present study we found no differences between dietary treatments. Surprisingly, hydroperoxide formation only showed a tendency to increase during day 4 of storage and decrease at day 7. This may be explained by the unstable nature of hydroperoxides. Indeed, the latter rapidly decompose into various secondary oxidation compounds including alcohols, ketones, aldehydes, and hydrocarbons (Rivas-Cañedo et al., 2009), probably preventing their accumulation in meat. Among the secondary products generated by the oxidation of lipids, malondialdehyde represents one of the main and, together with other secondary lipid oxidation products, is quantified using TBARS assay. In the present study, TBARS value increased during storage. On day 7 of refrigerated storage, C and LS treatments exceeded values of 2.5 mg MDA/kg meat, whereas HS and H + L treatments showed values below 1.6. According to Campo et al. (2006), the 2 mg MDA/kg meat is the limit of perception of the unpleasant rancidity off-flavour by consumer for fresh meat. Therefore, the higher values of TBARS in C and LS meat may negatively influence the flavour and the aroma perceived by the consumers. On the contrary, a diet containing hazelnut skin appeared to delay the lipid oxidation process, and therefore extending the shelf life of fresh meat. Indeed, feeding hazelnut skin (HS and H + L) delayed the secondary lipid oxidation compared to C. For the HS treatment, this result is consistent with the lower HP-PUFA/vitamin E ratio.

Unexpectedly, in the present study, the meat from LS lambs did not show significant differences for the TBARS values compared to meat from C lambs. Our findings contrast with the literature, which indicates that meat produced with PUFA-rich feeding (such as linseed supplementation) is generally more susceptible to oxidation, resulting in a greater accumulation of TBARS compared to a diet not enriched with PUFA (Andrés et al., 2014; Luciano et al., 2013). Moreover, in our study, meat from LS lambs had an unfavourable HP-PUFA/vitamin E ratio compared to C. The comparable development of secondary products of lipid oxidation (TBARS value) between C and LS treatments over the storage period implies that other factors – not measured in this study – may have contributed to the oxidative balance, such as essential amino acids, including histidine (Marino et al., 2019), notable to be the precursor of carnosine and anserine, which may improve lipid stability of lambs (Morrissey et al., 1998). Furthermore, n-3 PUFA of linseed might improve the immune response of lambs, reducing oxidative stress, and enhance the expression of enzymes, such as superoxide dismutase, which have antioxidant effects (Acuti et al., 2012; Hou et al., 2023). We therefore cannot rule out that changes in the metabolism of the lambs could have probably affected the oxidative status of the meat.

Contrary to expectations, on day 4 of refrigerated storage the meat deriving from H + L lambs had a lower TBARS value compared to C, although HP-PUFA/vitamin E ratio was comparable. This result suggests that not only tocopherols may have acted as antioxidants, but also other factors or molecules present in hazelnut skin by-product may have contributed to the improved antioxidant capacity of H + L. The evaluation of antioxidant capacity in food matrixes represents a complex issue, as each analytical method individually cannot fully represent the antioxidant reactions that occur. Indeed, structure, concentration, and reactivity to free radicals and pro-oxidants can influence the efficacy of the antioxidants (Rumpf et al., 2023). In the present study, the antioxidant capacity of hydrophilic fraction of fresh meat was assessed by five assays, none of them depicting any significant differences among dietary treatments. Concerning water-soluble molecules, dietary polyphenols are an important class of antioxidants. The literature reports conflicting information regarding the effects of polyphenols supplementation in

ruminants' diet on the antioxidant capacity of meat. For instance, quebracho tannin supplementation had positive effects on lamb meat, improving its antioxidant capacity (Luciano, Moloney, et al., 2011; Luciano, Vasta, et al., 2011) while dietary *Cistus ladanifer* L. tannins did not influence meat TEAC and FRAP values (Jerónimo et al., 2020). These contradictory effects may be explained by the chemical nature of polyphenols, that are a large and heterogeneous group of bioactive compounds (Valenti et al., 2019). Furthermore, due to their high molecular weight, the bioavailability of polyphenols is scarce, hence intestinal absorption is limited as well as the deposition in animal tissues, as demonstrated by Soldado et al. (2021) for condensed tannins.

However, dietary polyphenols can contribute to oxidative balance in other manners. In our study, although total polyphenols did not seem to show direct antioxidant effects in meat – as observed through the Folin-Ciocalteu antioxidant assay and other hydrophilic antioxidant assays – likely due to their low intestinal bioavailability (López-Andrés et al., 2013), these compounds may have preserved dietary vitamin E, making it more bioavailable to the animals in the gastro-intestinal tract. For instance, Lobón et al. (2017) investigated the effects on the meat quality of suckling lambs from mothers fed quebracho tannins. Despite a similar intake of vitamin E of lactating sheep, the diet containing quebracho tannins increased α -tocopherol concentration and reduced lipid oxidation in meat from suckling lambs. Therefore, the results obtained in the present study may suggest that the dietary inclusion of hazelnut skin improved the absorption of bioavailable antioxidants (mainly, vitamin E) by through increasing both the direct intake of tocopherols and their protection towards oxidation mediated by total polyphenols, such as tannins.

The protein oxidation involves the degradation of aminoacidic chains with the formation of carbonyl groups and the loss of thiols groups (Zhang et al., 2013) negatively influencing the water-holding capacity and texture of the meat (Lund et al., 2011). The manipulation of the animal diet might be a strategy to prevent protein oxidation, but literature reports conflicting results (Gravador et al., 2015; Holman et al., 2019; Menci et al., 2023; Ortuño et al., 2015; Santé-Lhoutellier et al., 2008). In the present study, the diet and time did not influence the concentration of thiols and carbonyls. We hypothesize that storage conditions (7 days, 4 °C) used in this study may have not exerted oxidative stresses enough to sufficiently stimulate the protein oxidation process. Indeed, conditions such as high levels of oxygen in modified atmosphere packaging and very long storage time were shown to be necessary for inducing protein oxidation (Zhang et al., 2013).

Another important factor that influences consumer acceptance is meat colour. In this study, the experimental diets did not affect the colour parameters and these results are in contrast with other studies who suggested that a diet rich in polyphenols may affect meat colour parameters (Menci et al., 2023; Natalello et al., 2020; Priolo et al., 2002). Nevertheless, the reasons for these results are still unclear. Concerning time of storage, it affected all the investigated parameters over the 7-day period. Colour descriptors followed a discoloration pattern typical of the browning of meat over time (Faustman et al., 2010). Indeed, redness (a^*) and saturation (C^*) decreased, while hue angle (h_{ab}) increased, as generally observed in other studies under similar experimental conditions (e.g., Luciano et al., 2019; Menci et al., 2023). Yellowness (b^*) has been reported to increase with advancing storage time following browning of meat (Menci et al., 2023; Natalello et al., 2020), but in the present experiment it decreased. However, although b^* decreased over time, h_{ab} (a combination of a^* and b^*) confirmed the typical increase during storage, indicating that the decrease observed in a^* was more pronounced than that of b^* , with that determining a colour change towards brown. A significant diet \times time interaction was observed for lightness. However, changes in lightness over time are often considered not strictly related to the browning of the meat. The latter is a natural process caused by the oxidation of oxy-myoglobin to metmyoglobin (Faustman et al., 2010). In accordance with this, the proportion of metmyoglobin increased and reached its

maximum on the day 7 of storage, and the KS572/KS525 decreased over time of storage accordingly. It was often reported that the reduction in meat discoloration is related to the oxidation state of lipids (Faustman et al., 2010). In fact, in our study, both the browning colour parameters and TBARS values increased over time. However, when the colour descriptors were referred to the dietary treatment, they did not follow the same pattern of TBARS values, being lower in hazelnut treatments (HS and H + L) compared to the control. This is in accordance with other studies which reported that in some cases the colour stability does not reflect the oxidative stability of the lipids referring to dietary treatments (Luciano et al., 2019; Menci et al., 2023).

5. Conclusions

The present study evidenced that a lamb diet containing 4 % extruded linseed and 7.5 % hazelnut skin, partially replacing maize, may be a useful strategy to exploit an agro-industrial by-product in a circular economy approach while improving the quality of meat.

Dietary supplementation with extruded linseed at 8 % enriched lamb with health-promoting n-3 PUFA. Contrary to expectations due to the well-known higher oxidative susceptibility of PUFA, linseed supplementation had no impact on the oxidative stability of the meat.

Conversely, the inclusion of hazelnut skin in the diet may be a strategy to delay lipid oxidation of the meat, probably thanks to the supply of antioxidant molecules, such as tocopherols, and to the action of polyphenols that may have preserved the tocopherols in the diet. However, the hazelnut skin alone did not contribute to improving fairly the fatty acid profile of the meat.

The combination of both linseed and hazelnut skin showed the positive sides of the individual by-products, improving the acid profile in terms of n-6/n-3 PUFA ratio and increasing the resistance of meat to lipid oxidation. To sum up, the combination of linseed and hazelnut skin appears to be a valid strategy to improve both fatty acid profile and oxidative stability of meat, and at the same time employ an agro-industrial by-product that lowers the costs of the diet in a circular economy approach.

CRedit authorship contribution statement

Martino Musati: Writing – original draft, Visualization, Formal analysis, Data curation. **Antonino Bertino:** Writing – review & editing, Formal analysis. **Marco Sebastiano Cannone:** Writing – review & editing, Formal analysis. **Fabrizio Mangano:** Writing – review & editing, Formal analysis. **Giuseppe Luciano:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Alessandro Priolo:** Writing – review & editing, Investigation, Conceptualization. **Marco Sebastiano Bella:** Writing – review & editing. **Luisa Biondi:** Writing – review & editing. **Manuel Scerra:** Writing – review & editing. **Guido Mangione:** Writing – review & editing, Formal analysis. **Antonio Natalello:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Consent form

All authors consent to the publication of this manuscript.

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Declaration of competing interest

The authors declared that they have no conflicts of interest in this work.

Data availability

Data will be made available on request.

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