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Feeding hazelnut skin to lambs delays lipid oxidation in meat

Ruggero Menci [a], Luisa Biondi [a], Antonio Natalello [a,*], Massimiliano Lanza [a], Alessandro Priolo [a], Bernardo Valenti [b], Antonino Bertino [a], Manuel Scerra [c], Giuseppe Luciano [a]

[a] Department Di3A, University of Catania, via Valdisavoia 5, 95123 Catania, Italy
[b] Department DSA3, University of Perugia, Borgo XX Giugno 74, 06123 Perugia, Italy
[c] Dipartimento di Agraria, Produzioni Animali, University of Reggio Calabria, Via dell'Università, 25, 89124 Reggio Calabria, Italy

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ABSTRACT

This study aimed to investigate the effect of dietary hazelnut skin (HNS), a by-product of confectionery industry, on the oxidative stability of lamb meat. Twenty-two finishing lambs were randomly assigned to 2 groups and fed ad libitum for 56 days on different concentrate-based diets: one control and one experimental, in which 150 g/kg of corn was replaced by HNS. After slaughter, the fat-soluble vitamins content and hydrophilic antioxidant capacity were assessed in fresh meat, as well as color, lipid and protein stability over 7 days of shelf-life trial. Dietary HNS increased ($P < 0.001$) the tocopherols content of meat and reduced ($P < 0.001$) lipid oxidation during 7 days of refrigerated storage. Meat from lambs fed HNS showed different ($P < 0.05$) instrumental color parameters. No diet effect ($P > 0.05$) was observed on the development of metmyoglobin, hydroperoxides, thiol groups, and carbonyl groups. Feeding HNS to lambs improves the oxidative stability of raw meat by delaying lipid oxidation thanks to the antioxidant molecules (tocopherols and phenolic compounds) contained in this by-product.

1. Introduction

The use of agro-industrial by-products in animal nutrition is one of the strategies that could help improve the sustainability of the livestock industry. As feed production is responsible for most of pollutant emissions of livestock sector (Gerber et al., 2013), replacing conventional feed crops with industrial by-products has the potential to reduce the environmental impact of animal feeding in a circular economy approach (Ponnampalam & Holman, 2023).

With a world production of about 1 million metric tons of in-shell fruits (FAO, 2021), hazelnut (*Corylus avellana* L.) is one of the most important nut fruits. Hazelnut skin (HNS) is a by-product of confectionery industry and consists of the perisperm of hazelnuts removed by roasting. Despite only accounting for 2% of in-shell weight, HNS is produced in large quantities by confectionery companies and its disposal can pose an economic and environmental problem (Charron, 2019). Hazelnut skin is low in moisture and high in fiber, crude fat, and C18:1 *c*9 (Caccamo et al., 2019), making it an attractive feed. Its low moisture content, for example, makes HNS easy to transport, store, and administer, which is usually the main issue with agro-industrial by-products (Salami et al., 2019). In recent years, research interest in the use of HNS in livestock nutrition has increased, and it was fed to ewes (288 g/d; Campione et al., 2020), dairy cows (1 kg/d; Renna et al., 2020), and rabbits (2.25 g/d; Candellone, Peiretti, Binello, Bergagna, & Meineri, 2019) with promising results. In addition to causing no adverse effects on animal metabolism and performance, dietary HNS has been found to improve product quality and animal welfare. In particular, feeding HNS to ewes and dairy cows increased the proportion of potentially beneficial fatty acids (FA), such as C18:1 *t*11 and C18:2 *c*9*t*11, in milk and cheese (Campione et al., 2020; Marino et al., 2021; Renna et al., 2020). Moreover, rabbits fed HNS showed lower oxidative stress markers in serum (Candellone et al., 2019). These effects are likely due to the bioactive compounds contained in HNS, which is an interesting source of phenolic compounds, condensed tannins (Del Rio, Calani, Dall'Asta, & Brighenti, 2011), and tocopherols (Renna et al., 2020). Indeed, phenolic compounds are able to modulate rumen biohydrogenation and thus modify the FA profile of ruminant products (Frutos et al., 2020), while tocopherols are the most important antioxidants in animal nutrition, involved in the oxidative balance of tissues (Ponnampalam et al., 2014).

In a recent experiment, we replaced 150 g/kg DM of corn with the same amount of HNS in the diet of finishing lambs to investigate the

* Corresponding author.
E-mail address: antonio.natalello@unict.it (A. Natalello).

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effects on growth performance and FA metabolism (Priolo et al., 2021). After 56 days of feeding, we observed no detrimental effect on average daily gain (ADG) and final bodyweight, and an increase in poly-unsaturated fatty acids (PUFA) and C18:1 *t*11 in meat. Considering the bioactive compounds contained in HNS, we hypothesized that feeding this by-product to lambs could enhance the oxidative stability of meat and improve its shelf-life despite the increase of PUFA, which are particularly prone to oxidation. The oxidative processes occurring in fresh meat at the expense of pigments, lipids, and proteins lead to a well-known deterioration in the organoleptic qualities of the product that negatively affect consumer acceptance. The dietary supplementation of by-products that contain antioxidant molecules is a strategy proved to delay the oxidative processes in fresh meat (Chikwanha et al., 2019; Inserra et al., 2014; Natalello et al., 2020).

In the light of the above, the aim of the present study was to determine the antioxidant capacity and the color, lipid and protein stability of the meat obtained from the experiment of Priolo et al. (2021), in order to investigate the effect of dietary HNS on the oxidative stability of lamb meat.

2. Material and methods

2.1. Animals and diets

The experiment took place in the experimental farm of the University of Catania (37°24′35.3" N, 15°03′34.9″ E). Twenty-two 2-months-old male lambs (*Valle del Belice* breed) were individually penned indoor and randomly assigned to 2 groups ($n = 11$), balanced for bodyweight (15.3 kg, SD 1.79). The control group received a conventional concentrate-based pelleted diet composed of corn (260 g/kg DM), barley (260), soybean meal (160), alfalfa hay (200), wheat bran (70), molasses (30), and vitamin-mineral mix (20). The other group (HNS) received a similar pelleted diet in which part (58%) of corn was replaced with 150 g/kg DM of hazelnut skin (Dalma Mangimi S.p.a.; via Sperina Alta 18, Marene, Cuneo, Italy). Hazelnut skin was included in the pellet as it was obtained by the confectionary company, with no further treatment. The chemical composition of HNS and experimental diets is shown in Table 1. During a 5-days adaptation period, weaning concentrate was gradually replaced with the experimental diets. Then, the lambs were fed ad libitum with the experimental diets for 56 days, and orts were weighed daily to measure voluntary intake. On the last day of the feeding trial, all the lambs were weighed, transported to a commercial slaughterhouse (45 min transfer), and immediately slaughtered by stunning and exsanguination. All the experimental procedures were conducted in accordance with the European Union welfare guidelines (Directive 2010/63/EU; Council Regulation 1099/2009) and approved by "Organismo Preposto al Benessere degli Animali (OP BA)" of the University of Catania (protocol nr. 15,295). A detailed description of the experiment was reported by Priolo et al. (2021).

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

Item[1]	Hazelnut skin	Diet[2]	
		CON	HNS
DM, g/kg as fed	904	893	891
Crude fat	303	25.3	60.2
Crude protein	102	194	222
NDF	355	214	253
ADF	268	110	144
Lignin	146	26.8	49.2
Ash	24.8	62.8	67.2
Phenols (TA eq)	133	3.2	22.4
Tannins (TA eq)	78.3	1.5	16.0
α-tocopherol, mg/kg DM	161	6.67	29.2
γ-tocopherol, mg/kg DM	217	10.5	41.8
δ-tocopherol, mg/kg DM	22.4	2.56	6.08
Metabolizable energy, Mcal/kg DM	3.15	2.33	2.47

[1] ADF, acid detergent fiber; DM, dry matter; NDF, neutral detergent fiber; TA eq, tannic acid equivalents.
[2] CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin.

2.2. Feedstuff sampling and analyses

During the feeding trial, subsamples of the experimental feedstuffs were collected weekly and vacuum-stored at −30 °C. The subsamples were then ground and pooled to obtain representative samples of each feedstuff. The feedstuff samples were analyzed for fiber fractions (Van Soest, Robertson, & Lewis, 1991), crude protein, crude fat, and ash (methods 984.13, 920.39, and 942.05, respectively; AOAC, 1995). Total phenolic compounds and total tannins were determined following the Folin-Ciocalteau method, as described by Luciano et al. (2019).

Tocopherols were extracted according to Rufino-Moya, Joy, Lobón, Bertolín, and Blanco (2020). Briefly, 200 mg of feedstuff was mixed with 3 mL of methanol:acetone:petroleum ether (1:1:1, v:v:v) with BHT (0.1 g/L), and vortexed 1 min. Then, samples were centrifugated at 1000 × g for 5 min, and the supernatants were collected. This procedure was performed for a total of 3 times. The residues after evaporation under N_2 were dissolved in methanol (1 mL) and filtered with 0.22 μm PTFE filters. A Nexera UHPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a C18 phase column (Zorbax ODS, Supelco, Bellefonte, PA; length: 25 cm; internal diameter: 4.6 mm; particle size: 5 μm) was used to quantify the analytes. Settings and temperatures were the same described by Natalello et al. (2022). Tocopherols were detected by fluorescence (RF-20AXS, Shimadzu) at the excitation wavelength of 295 nm and emission wavelength of 330 nm. The comparison with the retention time of pure standards (Merck Life Science s.r.l., Milano, Italy) was used for analytes identification. For each analyte, external calibration curves were created with pure standards.

2.3. Meat sampling and analyses

After 24 h of refrigerated storage (0–4 °C), carcasses were weighed and the *longissimus thoracis and lumborum* muscle (LTL) was excised from both sides. Muscle pH was measured on the right LTL using a HI110 pH-meter (Hanna Instruments, Padova, Italy) fitted with temperature probe and calibrated using 2 buffer solutions at pH 4 and 7, kept at 0–4 °C. The right LTL was then vacuum-packed and stored at −80 °C pending analyses of fatty acid composition, fat-soluble vitamins, and hydrophilic antioxidant capacity. The left LTL was used for the shelf-life trial and the consecutive analyses of lipid and protein oxidations.

2.3.1. Fatty acids and fat-soluble vitamins

The intramuscular fat was extracted from 10 g of homogenized meat samples using 2:1 (v:v) chloroform:methanol (Folch, Lees, & Sloane Stanley, 1957). The FA contained in intramuscular fat were trans-esterified with methanolic CH_3ONa (Christie, 1982). The FA profile of meat was determined by gas chromatographic analysis using a Thermo Finnigan Trace GC equipped with a flame ionization detector (ThermoQuest, Milan, Italy) and a high-polar fused silica capillary column (length 100 m; internal diameter: 0.25 mm; film thickness 0.25 μm; SP-2560 fused silica, Supelco, Bellafonte, PA), set as reported by Priolo et al. (2021).

Tocopherols, retinol, and cholesterol in meat were quantified following the method of Bertolín, Joy, Rufino-Moya, Lobón, and Blanco (2018), as modified by Menci et al. (2022). In brief, 2.5 g of meat was saponified overnight with 200 mg of L-ascorbic acid and 7.5 mL of KOH (10% in 1:1 ethanol:water) in an incubator shaker. Lipids were extracted two times by reaction with 5 mL of 9:1 hexane:ethyl acetate (with 25 mg/L of BHT) and subsequent centrifugation at 2000 ×*g* for 5 min at 10 °C. After evaporation of the supernatant under N_2 flow, the pellet was dissolved in 1 mL of methanol (HPLC grade) and filtered through PTFE syringe filters (0.2 μm/13 mm). Tocopherols were quantified by UHPLC as described above (Section 2.2), whereas cholesterol and retinol were

detected using a photodiode array detector (SPD-M40, Shimadzu) at the absorbance wavelength of 220 nm and 325 nm, respectively.

2.3.2. Antioxidant capacity

Three different assays were performed to assess the hydrophilic antioxidant capacity in terms of radical scavenging activity and reducing power, as described by Luciano et al. (2017). First, 1 g of minced lean meat was homogenized for 1 min at 9000 rpm (Ultra Turrax T-18 Homogenizer) with 10 mL of distilled water in a test tube kept in an ice-water bath. The sample was centrifuged at 2500 ×*g* for 20 min at 4 °C, and the so obtained meat aqueous extract (MAE) was filtered (Whatman 541 filter paper) and collected into different aliquots for the following antioxidant assays, as described by Natalello et al. (2022).

For the Trolox equivalent antioxidant capacity (TEAC) assay (Re et al., 1999), 20 μL of MAE was mixed with 2 mL of $ABTS^{\bullet+}$ solution (Aouadi et al., 2014) and the absorbance at 734 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy) after 60 min incubation at 30 °C. To account for the spontaneous discoloration, a blank was prepared using distilled water instead of MAE. Results were obtained by comparison to a 5-points calibration curve (0–400 μg/mL) of Trolox standard (238,813; Merck Life Science s.r.l., Milano, Italy) in phosphate buffer saline (pH 7.4).

For the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996), 50 μL of MAE was mixed with 150 μL of distilled water and 1.5 mL of a 10:1:1 (v:v:v) solution of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (2,4,6-tripyridyl-*s*-triazine in 40 mM HCl), and 20 mM aqueous ferric chloride. The absorbance at 593 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy) after 60 min incubation at 37 °C. Results were obtained by comparison to a 7-points calibration curve (0–280 μg/mL) of $FeSO_4 \times 7H_2O$.

For the Folin-Ciocalteau assay (Makkar, Blümmel, Borowy, & Becker, 1993), 500 μL of MAE was mixed with 500 μL of distilled water, 500 μL of 1 N Folin-Ciocalteau reagent, and 2.5 mL of 20% sodium carbonate. After 40 min incubation in the dark at room temperature, the sample was centrifuged at 2500 × *g* for 10 min at 4 °C and the absorbance was read at 725 nm (UV-1601; Shimadzu Corporation, Milan, Italy). Results were obtained by comparison to a 7-points calibration curve (0–100 μg/mL) of aqueous tannic acid.

2.3.3. Shelf-life trial and color measurement

The shelf-life trial was performed as described by Valenti et al. (2019), with some changes as follows. Three slices (thickness 2 cm) were cut from the 9th to the 13th rib of each left LTL. The slices were placed in polystyrene trays, over-wrapped with 3 layers of domestic plastic wrap, and stored in the dark at 4 °C for 0 d (2 h for blooming), 4 d, or 7 d. After storage, the color parameters L^* (lightness), a^* (redness), b^* (yellowness), C^* (saturation), and hue angle were measured in the CIE L*a*b* space (Minolta CM 2022, d/8° geometry, Minolta Co. Ltd. Osaka, Japan; mode SCE; illuminant A; standard observer 10°) with 3 measurements on non-overlapping areas. The reflectance spectrum from 400 nm to 700 nm was recorded for the calculation of metmyoglobin, expressed as a percentage of the total forms of myoglobin (Krzywicki, 1979). After color analysis, the meat slices were aliquoted into different subsamples for the analyses of lipid oxidation and protein oxidation, and stored at −80 °C.

2.3.4. Products of lipid oxidation

Lipid oxidation was assessed by measuring the production of hydroperoxides and thiobarbituric acid reactive substances (TBARS) in meat samples after the shelf-life trial (Section 2.3.3). Hydroperoxides were determined following the procedure described by Maqsood, Benjakul, and Balange (2012), modified as follows. One g of meat in 15 mL 2:1 chloroform:methanol was homogenized for 60 s (30 s, pause, 30 s) and filtered (Whatman No. 1 filter paper). Then, 7 mL of the filtrate was mixed with 2 mL of 0.5% NaCl, vortexed, and centrifuged at 2800 ×*g* for 5 min at 4 °C. After removing the supernatant, 750 μL of the underlying phase was 1:1 diluted with chloroform, and mixed with 1 mL of 2:1 chloroform:methanol, 12.5 μL of ammonium thiocyanate, and 12.5 μL of iron chloride. The sample was incubated in the dark at room temperature for 20 min, and the absorbance at 500 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy). Results were compared to a 7-points calibration curve (0–3 ppm) of cumene hydroperoxide in 2:1 chloroform:methanol.

The TBARS determination followed the procedure described by Natalello et al. (2020): 2.5 g of meat were homogenized (Ultra Turrax T-18 Homogenizer) with 12.5 mL of distilled water, keeping the sample in an ice-water bath. After adding 12.5 mL of 10% trichloroacetic acid, the sample was filtered (Whatman No. 1 filter paper). Four mL of the filtrate was mixed with 1 mL of 0.06 M aqueous thiobarbituric acid, and the tube was incubated in a water bath at 80 °C for 90 min. The absorbance at 532 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy) and compared to a 5-points calibration curve (0–200 nmol/L) of TEP standard (1,1,3,3,-tetraethoxypropane) in distilled water.

2.3.5. Isolation of myofibrillar proteins

Myofibrillar proteins (MP) were isolated from meat samples after the shelf-life trial (Section 2.3.3) following the method described by Park, Xiong, and Alderton (2007), with modifications. Briefly, 3 g of finely chopped lean meat was mixed with 15 mL of isolation buffer (10 mM sodium phosphate, 0.1 M NaCl, 2 mM $MgCl_2$, and 1 mM EDTA; pH 7.0), and homogenized at 13,500 rpm (Ultra Turrax T-18 Homogenizer). The homogenization lasted 60 s (30 s, pause, 30 s) and during this time the sample was always kept in an ice-water bath. The homogenate was centrifuged at 2600 ×*g* for 15 min at 4 °C, and the supernatant was discarded. The pellet was mixed with 10 mM isolation buffer, and homogenized and centrifuged as above; this step was performed twice. After discarding the supernatant, the pellet is suspended in 10 mL NaCl (0.1 M), vortexed, and centrifuged as above; this step was performed twice. The pellet was suspended in 10 mL NaCl (0.1 M), homogenized for 30 s, collected in two tubes (2 aliquots of 5 mL), and centrifuged as above. Last, the supernatant was discarded and the MP pellet was stored at −80 °C.

2.3.6. Protein thiol groups

Protein thiol groups were assessed in MP basing on the method described by Jongberg, Skov, Tørngren, Skibsted, and Lund (2011), modified as follows. The MP pellet (Section 2.3.5) was mixed with 12.5 mL of SDS buffer and centrifuged at 1000 ×*g* for 10 min at 4 °C. Two mL of sample was incubated in a water bath for 1 h at 80 °C. Then, 0.5 mL of sample were transferred in a cuvette and mixed with 2 mL of 0.1 M tris (hydroxymethyl)aminomethane (TRIS) buffer (pH 8.0). The absorbance at 412 nm was read (UV-1601; Shimadzu Corporation, Milan, Italy) immediately (T0) and after exactly 30 min of reaction in the dark with 0.5 mL of freshly made Ellman reagent (T1). The sample absorbance was compared to a 5% SDS buffer blank:

$$A_{412} = A_{412}\mathrm{T1}(Sample) - A_{412}\mathrm{T0}(Sample) - A_{412}\mathrm{T1}(Blank) + A_{412}\mathrm{T0}(Blank)$$

The thiol concentration was calculated based on a 6-points L-cysteine standard curve in 5% SDS buffer (from 0 to 1 mM).

Thiols concentration was expressed as μmol per g of protein. After the incubation step, 100 μL of each sample was appropriately diluted with 5% SDS buffer, and the absorbance at 280 nm was measured. After correction against the value of 5% SDS buffer blank, results were compared to a 6-points bovine serum albumin curve in 5% SDS buffer (from 0 to 2 g/L).

2.3.7. Protein carbonyl groups

Protein carbonyl groups were assessed in MP based on the method described by Jongberg et al. (2011), modified as follows. The MP pellet (Section 2.3.5) was mixed with 25 mL of carbonate buffer (1 M NaCl, 50 mM Na_2CO_3, 50 mM $NaHCO_3$; pH 9.6), and centrifuged at 1500 ×*g* for

15 min. Then, 2 mL of supernatant was placed in a microtube and centrifuged at 20,000 ×*g* for 1 h. Two 0.5 mL aliquots of supernatant were placed in two different microtubes and added with 0.5 mL of DNPH (10 mM 2,4-dinitrophenylhydrazine in 2 M HCl) or 0.5 mL of 2 M HCl (blank sample), respectively. All samples were incubated in a water bath at 37 °C for 1 h, vortexing every 10 min. The samples were then added with 325 μL of 50% TCA, vortexed, placed in ice for 10 min, and centrifuged at 16,500 ×*g* for 10 min. The protein pellet was washed with 1 mL of ethanol-ethyl acetate in HCl, let to react for 10 min, and centrifuged at 16,500 ×*g* for 10 min; this step was performed three times. Then, the pellet was dried under N_2 flow and dissolved in 1 mL of 6 M guanidine hydrochloride (in 20 mM potassium dihydrogen phosphate; pH 2.3). The samples were incubated at 37 °C for 30 min, and the absorbance (A) at 280 nm and 370 nm was measured (UV-1601; Shimadzu Corporation, Milan, Italy). The carbonyl concentration was expressed as μmol per g of protein, according to the following formula:

$$Carbonyl\ concentration = \frac{A_{370}}{22,000\ (A_{280} - A_{370} \times 0.43)} \times 10^6$$

where 22,000 is $\varepsilon_{hydrazone,370}$, and 0.43 is the ratio between $\varepsilon_{hydrazone,280}$ and $\varepsilon_{hydrazone,370}$. The blank sample value was subtracted from the corresponding sample value.

2.4. Statistical analysis

Statistical analysis was performed using the software IBM SPSS version 26 (SPSS Inc., Chicago, Illinois, USA). The individual animal was the statistical unit. A one-way ANOVA was applied to the data of intake, growth performance, carcass weights, FA profile, fat-soluble vitamins, and cholesterol, with the diet as fixed factor. A mixed ANOVA for repeated measures was applied to the data of color, and lipid and protein oxidations, with diet, storage time, and their interaction as fixed factors, and the individual animal as random factor. Differences were considered significant when $P \leq 0.050$ and the Tukey post hoc test was performed for multiple comparisons.

3. Results and discussion

3.1. In vivo performance

The HNS diet was formulated with the aim of reducing the reliance on conventional feed while still meeting the dietary needs of lambs. In particular, we decided to replace corn because it is a feed crop with high economic and resource costs in the Mediterranean area, and plays a key role in the feed-food-fuel competition. The level of HNS dietary supplementation (i.e., 150 g/kg DM, equal to 58% of corn) was chosen considering the nutritional limitation posed by its high fat and tannin content. For instance, Niderkorn et al. (2020) observed that 20 g/kg of condensed tannins from HNS lowered the in vitro rumen fermentability of diet, reducing gas production and protein degradability. This is due to the antimicrobial activity and the protein-binding ability of tannins, which make them able to affect rumen digestion (Vasta et al., 2019). However, the HNS diet of the present study was formulated to keep the tannin level below the 2% dry matter intake (DMI) threshold (Table 1), which is generally considered harmless to lambs (Vasta et al., 2019). Indeed, the data on growth performance reported by Priolo et al. (2021) confirms the suitability of the HNS diet for lamb feeding, as no detrimental effect was observed on DMI, ADG, final bodyweight, and carcass weight. The daily phenols and tannins intakes of HNS lambs were 7 and 11 times higher ($P < 0.001$) than that of CON lambs, respectively (Table 2). Also, the α-tocopherol and γ-tocopherol intakes were 4 times higher ($P < 0.001$) in the HNS group than in the CON group (Table 2). These results were predictable considering the high phenols, tannins, and tocopherols contents of HNS (Table 1).

Table 2
Intakes of the experimental lambs.

Item[1]	Diet[2]		SEM[3]	*P*-value
	CON	HNS		
DMI, g/d	836	851	29.8	0.803
Phenols, g TA eq/d	2.6	19.1	1.87	<0.001
Tannins, g TA eq/d	1.2	13.6	1.40	<0.001
α-tocopherol, mg/d	5.6	24.9	2.22	<0.001
γ-tocopherol, mg/d	8.8	35.6	3.09	<0.001
δ-tocopherol, mg/d	2.14	5.17	0.364	<0.001

[1] ADG, average daily gain; DMI, dry matter intake; TA eq, tannic acid equivalents.
[2] CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin.
[3] SEM, standard error of the mean.

3.2. Meat oxidative stability

To the best of our knowledge, this study makes part of the first experiment aimed at assessing the effect of dietary HNS on meat quality. Meat oxidative stability depends on the delicate balance between pro-oxidant molecules, such as PUFA, and antioxidant molecules, such as α-tocopherol (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Table 3 shows the main FA classes, fat-soluble antioxidant vitamins, and hydrophilic antioxidant capacity of meat from experimental lambs. Dietary HNS increased ($P < 0.05$) the PUFA content in meat, especially *n*-6 series. This was combined with a modification of meat FA profile, including higher proportions of C18:2 *c*9*c*12 and C18:1 *t*11, due to the specific effects of HNS tannins on rumen biohydrogenation and rumen microbiota (Daghio et al., 2021; Priolo et al., 2021). Despite PUFA being more sensitive to oxidation, no difference ($P > 0.05$) in meat peroxidability between HNS and CON groups was observed, according to the calculations based on FA profile (Table 3). Concerning antioxidant vitamins, the HNS meat had more than twice the content of α-tocopherol and γ-tocopherol than the control meat ($P < 0.001$). Similarly, Renna et al. (2020) and Marino et al. (2021) observed higher content of tocopherols in cow milk and ewe cheese when they fed animals 1 kg/d and 288 g/d of HNS, respectively. The transfer of tocopherols from diet to tissues is a well-known phenomenon (Bellés, del Mar Campo, Roncalés, & Beltrán, 2019), and the effect we observed was likely due to the higher vitamin E content of HNS. In addition, the HNS diet contained a greater amount of crude fat than the CON diet, which can further contribute to the absorption of tocopherols at intestinal level (Lodge, Hall, Jeanes, & Proteggente, 2004). Furthermore, the presence of phenolic compounds in HNS could have indirectly preserved vitamin E during digestion thanks to their antioxidant activity, allowing for a higher quota of tocopherols to be absorbed by the animal (Soldado, Bessa, & Jerónimo, 2021). Dietary phenolic compounds are also suggested to directly contribute to the antioxidant capacity of meat through the intestinal absorption of their metabolites. For example, Luciano et al. (2011) observed a higher antioxidant activity in meat, assessed by TEAC and FRAP assays, after feeding lambs 40 g/kg DM of tannins from quebracho (*Schinopsis lorentzii* Engl.). As these assays are performed on aqueous meat extracts, they exclude the activity of fat-soluble antioxidant vitamins and target hydrophilic antioxidants, such as phenolic compounds, vitamin C, and many others. Despite the higher intake of phenolic compounds by HNS lambs, in the present study the diet had no effect ($P > 0.05$) on meat antioxidant capacity assessed by TEAC, FRAP, and Folin-Ciocalteau assays (Table 3). Also, the result of the Folin-Ciocalteau assay suggests that there is no direct transfer of phenolic compounds from diet to muscle. However, our results are not surprising considering that the mechanism of action of phenolic compounds is still unclear and that the wide variety of plant sources makes studies difficult to compare (Soldado et al., 2021). For example, in agreement with our results, Jerónimo et al. (2020) observed no effect on the FRAP and TEAC values of meat after feeding lambs up to 22 g/kg DM of phenolic compounds (mainly tannins) from *Cistus ladanifer* L.

Table 3
Effect of the diet on pH, intramuscular fat, fatty acids, vitamins, cholesterol, and antioxidant capacity of lamb.

Item[1]	Diet[2]		SEM[3]	*P*-value
	CON	HNS		
pH	5.780	5.752	0.0209	0.514
Intramuscular fat, g/kg	20.28	21.14	0.985	0.675
Fatty acid, g/kg				
SFA	6.08	6.37	0.378	0.708
MUFA	6.58	7.01	0.428	0.622
PUFA	1.275	1.451	0.0416	0.031
PUFA *n*-6	1.118	1.301	0.0388	0.014
PUFA *n*-3	0.1233	0.1055	0.0050	0.074
HP-PUFA	0.405	0.369	0.0139	0.195
Peroxidability index[4], %	15.38	14.93	0.950	0.817
Cholesterol, g/kg	0.721	0.740	0.0144	0.510
Fat-soluble antioxidant vitamin				
α-tocopherol, mg/kg	0.587	1.292	0.0865	<0.001
γ-tocopherol, mg/kg	0.148	0.470	0.0367	<0.001
Retinol, μg/kg	32.0	31.6	1.32	0.894
Antioxidant capacity, mg/g				
TEAC (Trolox eq)	61.9	53.0	4.08	0.286
FRAP (Fe^{2+} eq)	19.10	19.28	0.875	0.919
Folin-Ciocalteau (TA eq)	0.594	0.601	0.0121	0.783

[1] eq, equivalents; FRAP, ferric reducing antioxidant power; HP-PUFA, highly peroxidizable polyunsaturated fatty acids (sum of PUFA with three or more unsaturated bonds); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TA, tannic acid; TEAC, Trolox equivalent antioxidant capacity.
[2] CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin.
[3] SEM, standard error of the mean.
[4] Calculated as: %dienoic×1 + %trienoic×2 + %tetraenoic×3 + %pentaenoic×4 + %hexaenoic×5 (Scislowski, Bauchart, Gruffat, Laplaud, & Durand, 2005).

Meat color is one of the major factors affecting consumer acceptance, and reflectance measurements are often used to assess color parameters. Interestingly, feeding lambs with HNS increased ($P < 0.05$) the L^*, a^*, b^*, and C^* values of meat (Table 4), and this could be related with the higher intake of phenolic compounds. Indeed, an effect on meat color, and particularly increased lightness, had already been observed in lambs fed tannin-containing by-products such as pomegranate pomace (Natalello et al., 2020) or carob pulp (Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000), but the reason for this is still unclear. Recently, Malva et al. (2023) found that feeding HSN changed the expression of 42 proteins in the muscle proteome of lambs, which might be responsible for different light absorption and thus different meat instrumental color. However, the variation in instrumental colorimetric parameters observed in our study was of such low magnitude that it is very unlikely to affect the visual appearance of meat. During storage, the oxidation of myoglobin, the main pigment of meat, entails the conversion of oxymyoglobin to metmyoglobin, which leads to the well-known meat browning (Faustman, Sun, Mancini, & Suman, 2010). Enhancing the antioxidant status of muscle by feeding antioxidant molecules, either fat-soluble or hydrophilic, is a proven strategy to delay meat discoloration (Luciano et al., 2011; Natalello et al., 2020; Ripoll, González-Calvo, Molino, Calvo, & Joy, 2013). However, dietary HNS had no effect on meat color development during 7 days of refrigerated storage. Indeed, L^*, b^*, and hue angle increased while a^* decreased over time ($P < 0.001$), following the typical meat discoloration pattern (Chikwanha et al., 2019; Luciano et al., 2019; Natalello et al., 2020), without any significant interaction with the diet (Table 4). Moreover, the percentage of metmyoglobin in meat was similar ($P > 0.05$) between the two experimental groups and increased ($P < 0.001$) over time regardless of diet (Table 4). These results show a lack of effectiveness of dietary HNS in delaying meat discoloration, despite the increase in vitamin E content.

Lipids and proteins are also subject to oxidation processes during meat storage, leading to profound changes in organoleptic qualities. Lipid oxidation is a chain reaction involving a free radical mechanism, which preferentially affects fatty acids with higher levels of unsaturation (Domínguez et al., 2019). Hydroperoxides and TBARS represent the primary and secondary products of lipid oxidation, respectively. In the present experiment, hydroperoxides increased in the first few days of refrigerated storage and then slowly decreased ($P = 0.009$; Table 5). At the same time, TBARS increased ($P < 0.001$) over the 7 days of shelf-life trial (Table 5). These trends reflect the pattern of lipid oxidation in which primary products are rapidly decomposed into secondary products, such as malondialdehyde, as already observed in fresh lamb meat

Table 4
Effect of diet on color stability in lamb over 7 days of refrigerated storage.

Item	Diet[1] (D)		Time (T), d			SEM[2]	*P*-value		
	CON	HNS	0	4	7		D	T	D × T
L^* (lightness)	45.2	46.9	45.7^{b}	44.9^{b}	47.5^{a}	0.265	0.007	<0.001	0.529
a^* (redness)	12.7	13.3	14.7^{a}	11.5^{c}	12.8^{b}	0.204	0.036	<0.001	0.606
b^* (yellowness)	11.5	12.2	11.6^{b}	10.9^{b}	13.1^{a}	0.177	0.024	<0.001	0.682
C* (saturation)	17.1	18.1	19.0^{a}	15.9^{b}	18.3^{a}	0.237	0.024	<0.001	0.615
Hue angle, deg	42.2	42.4	38.1^{c}	43.3^{b}	45.5^{a}	0.415	0.578	<0.001	0.887
630/580[3]	1.594	1.613	1.816^{a}	1.494^{b}	1.500^{b}	0.0202	0.359	<0.001	0.587
Metmyoglobin, %	47.24	47.76	42.21^{c}	48.81^{b}	51.49^{a}	0.563	0.537	<0.001	0.552

a, b, c For the Time factor, means within a row that do not share a superscript letter are statistically different.
[1] CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin.
[2] SEM, standard error of the mean.
[3] 630/580, ratio between the reflectance at 630 nm and 580 nm.

Table 5
Effect of diet on the products of lipid and protein oxidations in lamb over 7 days of refrigerated storage.

Item	Diet[1] (D)		Time (T), d			SEM[2]	*P*-value		
	CON	HNS	0	4	7		D	T	D × T
Lipid oxidation, mg/kg meat									
Hydroperoxides	15.96	16.14	14.16^{b}	18.85^{a}	15.14^{ab}	0.666	0.891	0.009	0.784
TBARS[3]	0.991	0.418	0.247^{c}	0.722^{b}	1.144^{a}	0.0759	<0.001	<0.001	<0.001
Myofibrillar protein oxidation, μmol/g protein									
Thiols	80.3	79.7	82.9	78.4	78.7	3.05	0.888	0.548	0.873
Carbonyls	3.50	4.01	4.01	3.72	3.50	0.190	0.259	0.456	0.539

a, b, c For the Time factor, means within a row that do not share a superscript letter are statistically different.
[1] CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin.
[2] SEM, standard error of the mean.
[3] TBARS, thiobarbituric acid reactive substances (expressed as mg of malondialdehyde per kg of meat).

(Luciano et al., 2013). However, dietary HNS influenced the lipid oxidation of meat with regard to the accumulation of secondary compounds. As shown in Fig. 1, the two groups had similar initial TBARS content, but the HNS meat showed lower ($P < 0.001$) values after 4 and 7 days of storage. In particular, the TBARS content in HNS meat slowly increased, reaching 0.59 mg/kg on day 7, whereas in CON meat it steadily increased, reaching 0.98 mg/kg on day 4, and 1.70 mg/kg on day 7 (Fig. 1). The TBARS content is important in fresh meat as, unlike hydroperoxides, secondary lipid oxidation compounds are responsible for off-flavors. For instance, 2 mg/kg of TBARS is the suggested threshold for rancidity perception and consumer acceptance in fresh meat (Berruga, Vergara, & Gallego, 2005; Campo et al., 2006). Although even the TBARS values of control meat did not reach this threshold after 7 days, it is important to note that dietary HNS showed the potential to delay the development of rancidity in fresh meat. This is likely due to better antioxidant power related to the higher α-tocopherol content in HNS meat. Indeed, differences in meat α-tocopherol content of the same order as those we observed can lead to significant differences in lipid oxidation, as Ripoll et al. (2013) demonstrated by feeding lambs different levels of dl-α-tocopheryl acetate. Despite lipid oxidation and myoglobin oxidation being suggested to be interconnected (Faustman et al., 2010), in the present study we found dietary HNS to delay the former and have no effect on the latter. However, this phenomenon is not new to meat shelf-life studies. For example, Inserra et al. (2014) found that dietary citrus pulp reduced TBARS production after 3 and 6 days of storage in lamb meat, while no effect on metmyoglobin development was observed. Also, Santé-Lhoutellier, Engel, and Gatellier (2008) observed that meat from pasture-raised lambs had higher α-tocopherol content and lower TBARS development than lamb fed a concentrate diet, but no difference in meat discoloration was found.

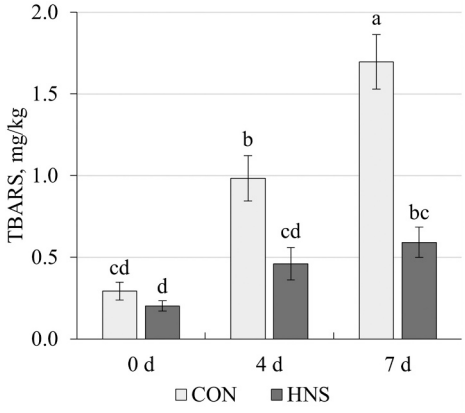

Fig. 1. Interaction between diet and storage time on TBARS (mg/kg) development in lamb.
CON, control diet; HNS, diet containing 150 g/kg DM of hazelnut skin; TBARS, thiobarbituric acid reactive substances (expressed as mg of malondialdehyde per kg of meat).
Error bars represent the standard error of the mean.
a, b, c, d, Bars that do not share a letter are statistically different at $P < 0.001$.

Protein oxidation in meat involves the chemical modification of amino acid side chains with loss of thiol groups and formation of carbonyl groups (Zhang, Xiao, & Ahn, 2013). This alteration changes the structure of proteins, thus affecting meat water-holding capacity and texture. As shown in Table 5, dietary HNS had no effect ($P > 0.05$) on myofibrillar proteins oxidation as assessed by thiol and carbonyl groups determinations. Supplementing animals' diet with antioxidants is suggested to protect meat proteins from oxidation, as observed in lambs fed dl-α-tocopheryl acetate (Ortuño, Serrano, & Bañón, 2015), citrus pulp (Gravador et al., 2014), or grape pomace (Chikwanha et al., 2019). However, there is still much to understand in the field of protein oxidation, and contrasting results have already been reported. For example, no diet effect on carbonyl development has been observed in meat from pasture-raised lambs (Santé-Lhoutellier et al., 2008), or lambs fed with phenol-rich carob pulp (Gravador et al., 2015) or anthocyanin-rich citrus extracts (Maggiolino et al., 2021). Interestingly, in the present study the storage time had no effect ($P > 0.05$) on myofibrillar protein oxidation (Table 5). Probably, the storage conditions we adopted were not particularly oxidizing to proteins. Indeed, we stored the meat in the dark, and light is known to induce the formation of free radicals (Zhang et al., 2013). In fact, in other studies where the shelf-life conditions adopted were similar to ours, a very slow protein oxidation has been reported (Gravador et al., 2014; Gravador et al., 2015; Maggiolino et al., 2021).

4. Conclusion

The results of the present study show that feeding lambs hazelnut skin, a by-product of confectionery industry, can contribute to improve the oxidative stability of raw meat by delaying lipid oxidation. This is most likely due to the antioxidant molecules contained in HNS, primarily tocopherols and secondarily phenolic compounds. However, we cannot assume that the same effect would also occur in animals reared in different farming systems. Nevertheless, we observed no effect on meat discoloration and protein oxidation during 7 days of shelf-life trial. Considering the high cost of synthetic vitamin E and the growing concern of consumers about chemical additives, HNS gains importance in livestock feeding as an agro-industrial by-product source of natural antioxidants.

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CRediT authorship contribution statement

Ruggero Menci: Investigation, Formal analysis, Visualization, Writing – original draft. **Luisa Biondi:** Writing – original draft. **Antonio Natalello:** Methodology, Investigation, Formal analysis, Writing – original draft. **Massimiliano Lanza:** Writing – original draft. **Alessandro Priolo:** Conceptualization, Project administration, Supervision, Resources. **Bernardo Valenti:** Methodology, Investigation, Writing – review & editing. **Antonino Bertino:** Investigation, Writing – review & editing. **Manuel Scerra:** Writing – original draft. **Giuseppe Luciano:** Funding acquisition, Methodology, Project administration, Writing – original draft.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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