Animal Feed Science and Technology 332 (2026) 116607

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Replacing soybean meal and maize with white lupin (*Lupinus albus* L.) seeds alters growth performance, rumen fermentation, fatty acid metabolism, and meat quality in growing lambs

Guido Mangione [a], Martino Musati [a,*], Antonino Bertino [a], Manuel Scerra [b], Fabrizio Mangano [a], Marco Sebastiano Bella [a], Alessandro Priolo [a], Alfio Spina [c], Luisa Biondi [a], Massimiliano Lanza [a,1], Antonio Natalello [a]

[a] *Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Via Santa Sofia 100, Catania 95123, Italy*
[b] *Dipartimento di Agraria, Produzioni Animali, University of Reggio Calabria, Via dell'Università, 25, Reggio Calabria 89124, Italy*
[c] *Council for Agricultural Research and Economics (CREA), Research Centre for Cereal and Industrial Crops, Corso Savoia, 190, Acireale 95024, Italy*

ARTICLE INFO

Keywords:
Protein source
Lamb growth
Meat quality
Oxidative stability
Lupinus albus

ABSTRACT

This study evaluated the impact of replacing soybean meal and part of maize with white lupin (*Lupinus albus* L.) seeds in growing lamb diet on animal performance, rumen fermentation, fatty acid (FA) metabolism, and meat quality. Twenty male lambs were assigned to a control diet (CON) or to an experimental diet (LUP) in which white lupin seeds were included at 350 g/kg dry matter (DM) of the total diet. In the LUP diet, soybean meal was completely replaced and 30 % of maize was substituted with lupin seeds. Diets were formulated to be similar in crude protein content and metabolizable energy. Over 56 days, lambs fed the white lupin-based diet exhibited significantly lower feed intake, final body weight, and average daily gain compared to control, likely due to reduced palatability and the presence of antinutritional factors. Moreover, white lupin-based diet altered ruminal fermentation by increasing acetate and butyrate proportions, and shifted FA biohydrogenation pathways, favoring the production of C18:1 *t*11 and C18:2 *c*9, *t*11 beneficial to human health. Meat from white lupin-fed lambs had a higher n-3 PUFA content and a reduced n-6/n-3 ratio, with no detrimental effects on meat oxidative stability or color during storage. These findings suggest that fully replacing soybean meal and 30 % of maize with white lupin seeds could beneficially influence meat lipid quality, though adjustments to diet formulation are necessary to mitigate negative effects on feed intake and growth.

1. Introduction

The increasing global demand for animal-derived foods has placed growing pressure on livestock systems to adopt more sustainable and locally sourced feed strategies. Particularly, worldwide meat production has more than quadrupled since 1961, driven by both rapid population growth and rising per capita income leading to increased meat consumption (Food and Agriculture Organization of

* Corresponding author.
E-mail address: martino.musati@unict.it (M. Musati).
[1] Deceased 30 April 2025.

https://doi.org/10.1016/j.anifeedsci.2025.116607
Received 28 July 2025; Received in revised form 12 December 2025; Accepted 13 December 2025
Available online 13 December 2025

the United Nations, 2024). As the world heads toward a population of 10 billion by 2050, the demand for animal products will rise significantly, requiring more livestock and posing a major challenge, especially in securing adequate protein-rich feed sources (Food and Agriculture Organization of the United Nations, 2024). The European Union is largely self-sufficient in terms of meat production, accounting for over 96 % of its consumption (Eurostat, 2024). However, in the EU the livestock sector remains heavily dependent on imported protein-rich feedstuffs such as soybean and soybean meal, sourced mainly from South and North America (Šufliarský et al., 2024). This dependency poses several challenges, including vulnerability to global market fluctuations, increased feed costs, and significant environmental impacts such as deforestation and greenhouse gas emissions due to feed production and transportation (Parrini et al., 2023; da Silva et al., 2023). To mitigate these challenges, a potential solution could be to use locally available alternative feeds. Therefore, in the last decades, a wide range of alternative protein sources, primarily plant-based and often produced on a smaller scale, have been explored to enhance sustainability and cost-efficiency (Boudalia et al., 2024). Although many of these alternatives are produced in lower quantities compared to soybean meal, their local availability can provide farmers and feed manufacturers with more flexibility and potential cost savings opportunities (Fadel et al., 2017). Among these alternative feeds, white lupin (*Lupinus albus* L.), belonging to the *Fabaceae* family, has gained attention due to its high protein content, low starch levels, health-promoting fatty acid composition, and adaptability to marginal soils, especially varieties cultivated in Mediterranean climates (Chiofalo et al., 2012; Morsy et al., 2024). Total lupin seeds production reached more than 1.9 billion tons in 2023, with Australia as the largest producer (72.8 %) followed by Europe (19.4 %), where annually around 500,000 tons of lupin-derived products are consumed, including lupin bran, flour, milk, and tofu, used in various foods for humans or as an ingredient in animal diet, while the green plant is used as fertilizer as green manure (Caramona et al., 2024).

Lupin seeds have been successfully included in ruminant diets and have shown promise as a replacement of soybean meal without negative effects on dairy cow productivity (Joch and Kudrna, 2020) or young bull meat quality (Vicenti et al., 2009) due to their

Table 1
Ingredients and chemical composition of the lupin seeds and experimental diets.

	Lupin seeds	Experimental diet[a]	
		CON	LUP
Ingredient, g/kg DM			
Maize		430	300
Soybean meal		220	0
Molasses		30	30
Vitamins and minerals mix[b]		20	20
Hay		300	300
Lupin seeds		0	350
Chemical composition, g/kg DM			
DM, g/kg as fed	919	907	919
Ash	31.3	79.8	96.3
Crude protein	341	193	201
Ether extract	71.5	19.5	42.1
aNDF[c]	240	218	244
ADF[c]	229	154	179
ADL[c]	188	135	126
Total polyphenols[d]	7.96	5.96	5.96
Total tannins[d]	n.d.	1.72	0.34
α-tocopherol, μg/g DM	n.d.	8.67	6.88
γ-tocopherol, μg/g DM	160.8	16.6	67.5
Protein fractions[e], g/kg DM			
A1	3.92	3.39	5.64
B1	19.9	7.17	4.83
B2	7.53	3.49	2.37
B3	1.69	4.67	5.99
C	1.04	0.59	1.29
Metabolizable Energy, Mcal/kg DM	5.20	4.60	4.61
Individual fatty acid, g/kg DM			
C16:0	6.07	6.99	5.25
C18:0	1.65	2.33	1.39
C18:1 *c*9	31.6	7.55	16.1
C18:1 *c*11	1.81	0.52	1.20
C18:2 *c*9, *c*12	15.3	13.5	13.7
C18:3 *c*9, *c*12, *c*15	5.72	1.91	3.70

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b] Containing: 40 % calcium carbonate, 15 % sodium bicarbonate, 15 % monocalcium phosphate, 12.5 Vitamin mix, 10 % salt, and 7.5 % magnesium oxide.
[c]aNDF: amylase-treated Neutral Detergent Fiber; ADF: acid detergent fiber, ADL: acid detergent lignin.
[d]Expressed as g tannic acid equivalents/kg DM
[e] Protein fractions: A1 = nonprotein nitrogen; B1 = buffer-soluble true protein; B2 = buffer-insoluble protein–neutral detergent soluble protein; B3 = neutral detergent insoluble protein–acid detergent insoluble protein; C = acid detergent insoluble protein (Licitra et al., 1996). n.d. = not detected.

protein being rapidly degradable in the rumen (Froidmont and Bartiaux-Thill, 2004; Kung et al., 1991). Compared to soybean meal, lupin seeds showed a lower protein content with a higher rumen degradability, but higher fibre and lipid content (Froidmont and Bartiaux-Thill, 2003). However, their nutritional value can be limited by the presence of quinolizidine alkaloids, such as lupanine 11, 12-dehydrolupanine and sparteine, and other phytochemicals which may reduce feed palatability and intake, and interfere with nutrient digestibility (Bernhoft, 2010; Khan et al., 2015). Consequently, the appropriateness of this legume in ruminant diet largely depends on its alkaloid content, which can be minimized by selecting low-alkaloid varieties, the so-called sweet, that can be used as feed (Dronne et al., 2003; Gresta et al., 2023).

Despite an increasing body of research, studies specifically focusing on the implications of including white lupin in lamb diets and its impact on rumen fermentation, meat quality and oxidative stability, remain limited. In this context, the present study investigated the effects of total replacement of soybean meal and partial maize with 350 g/kg of sweet white lupin (*Lupinus albus* L.) seeds in the diet of growing lambs. We hypothesized that replacing soybean meal and part of the maize with sweet white lupin seeds would maintain lamb growth performance while positively affecting rumen fermentation, muscle fatty acid composition, and meat oxidative stability during refrigerated storage.

2. Materials and methods

2.1. Experimental design, animals and diets

The experimental trial was conducted at the experimental facilities of the University of Catania (37°24′35.3″N 15°03′34.9″E). All the experimental procedures with animals were conducted following the European Union legislation (CD 2010/63/EU guidelines) and according to the protocol evaluated by the "Organismo Preposto al Benessere degli Animali (OPBA)" of the University of Catania (protocol No. 169878). Twenty Valle del Belice × Pinzirita 40-day-old male lambs (initial body weight 12.8 ± SD 2.33 kg) were selected from a local dairy sheep farm. The lambs were moved from the native farm to the experimental university facilities, allocated indoors in individual pens (1.5 m^2 each) with straw litter, and assigned to two experimental dietary treatments, balanced for the initial body weight. The control group (CON; 10 lambs) was fed with a conventional concentrate-based diet. The other group (LUP; 10 lambs) received a diet in which white lupin seeds were included at 350 g/kg dry matter (DM) of the total diet, completely replacing soybean meal and substituting 30 % of maize. Lupin seeds were included at a level of 35 % of the total dietary DM, with the primary aim of fully replacing soybean meal. As a consequence of their chemical composition, this inclusion also entailed a partial reduction of maize in the ratio. Diets were formulated to be similar in crude protein content and metabolizable energy. White lupin seeds (*Lupinus albus* L., cv. Tennis), sweet variety with low quinolizidine alkaloids content, were supplied by a local company (Canicattini Bagni, Siracusa, Italy). The diet formulation and chemical composition of lupin seeds and experimental diets are shown in Table 1. The lambs were gradually adapted over 5 days to the two experimental diets by progressively replacing the weaning diet with the experimental feeds. Following this adaptation phase, all animals were individually offered their respective diets *ad libitum* for 56 days, pelletized to prevent feed selection. Fresh drinking water was available throughout the entire experiment. For each animal, offered and refused diet was recorded every day to calculate the dry matter intake (DMI). All lambs were weighed weekly throughout the experimental trial (Spider 3, Mettler Toledo, Columbus, OH, USA) to determine their average daily gain (ADG).

2.2. Slaughter procedure and sample collection

After 56 days of trial, the experimental diets and water were removed 3 h before slaughter. All lambs were weighed, transported to a commercial abattoir (30 min travel from the University facilities) and immediately slaughtered by stunning and exsanguination according to the European regulation (C.R. 1099/2009).

For each lamb, the whole ruminal and abomasal contents were sampled within 15 min from slaughter, manually homogenized, and pH was immediately measured for both fluids using a pH-meter (HI-110; Hanna Instruments, Padova, Italy) calibrated using 2 buffer solutions at pH 4 and 7. Afterward, approximately 100 mL of each unfiltered ruminal sample was directly frozen using dry ice, later lyophilized using a freeze-drying system (LyoQuest Mod. −55 PLUS, © Syntegon Telstar, SLU - Barcelona, Spain), and stored at –80 °C for further FA profile analyses. Another aliquot of rumen fluid was strained through four layers of cheesecloth and centrifuged at 3122 ×*g* for 5 min (MPW-54; MPW Med. Instruments, Warsaw, Poland). Afterward five mL of supernatant was transferred to test tubes containing 5 mL of 0.2 *N* HCl for ammonia analysis. Furthermore, an additional 0.8 mL of the centrifuged rumen sample was added to 0.5 mL of a deproteinizing solution (2 % methaphosphoric and 0.4 % crotonic acids, w/v, in 0.5 *N* HCl) to be analyzed for volatile FA (VFA). Both ruminal samples for ammonia and VFA determination were stored at –80 °C until analyses.

After the evisceration, the carcasses were weighed in order to obtain hot carcass weight and cooled at 4 °C for 24 h and weighed again to obtain cold carcass weight. Subsequently, the entire *longissimus thoracis et lumborum muscle* (LTL) was excised from both sides. The pH of the muscle was measured on the right LTL using a HI-110 pH meter (Hanna Instruments, Padova, Italy) equipped with a temperature probe and calibrated with two standard solutions at pH 4 and 7, both maintained at 4 °C. The right LTL was vacuum-sealed and stored at −80 °C for later analysis of FA composition, fat-soluble vitamins, and hydrophilic antioxidant capacity. The left LTL was designated for shelf-life study and subsequent evaluations of lipid oxidation.

2.3. Feedstuff analyses

Feedstuff samples were collected at the beginning, middle, and end of the experimental trial, and vacuum packaged and then stored

at −20°C. Equal portions of the subsamples collected during the trial for each diet were ground and homogenized to prepare representative samples of each feedstuff for proximate analyses. Particularly, amylase-treated neutral detergent fiber (aNDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991), and the results were expressed with residual ash. Dry matter, crude protein, crude fat (ether extract) and ash were determined using AOAC (Association of Official Analytical Chemists), 1995 official methods (930.15, 984.13, 920.39 and 942.05, respectively). Protein fractions were analyzed following the method detailed by Licitra et al. (1996), while metabolizable energy was estimated based on Cannas et al. (2004).

According to the method developed by Makkar et al. (1993) the total phenolic compounds were analyzed in the extract using Folin-Ciocalteu reagent (1 *N*) and sodium carbonate 20 % (w/v) with a spectrophotometer (model UV-1601; Shimadzu Corporation, Milan, Italy). Whereas non-tannin phenolics were determined by precipitating tannins from the extract with insoluble polyvinylpyrrolidone. Total tannins concentration was calculated as the difference between total phenols and total non-tannin phenols. Phenolic compounds and tannins were quantified using tannic acid solutions (TA; Sigma–Aldrich) as a reference standard, to prepare calibration curves, ranging from 0 to 100 μg/mL, and the results were expressed as mg TA equivalents/g DM.

Tocopherols of feedstuffs were extracted according to the method developed by Rufino-Moya et al. (2020). In brief, 200 mg of feedstuff were mixed with 3 mL of methanol:acetone:petroleum ether (1:1:1, v:v:v) with 0.01 % (w/v) butylated hydroxytoluene (BHT) and vortexed for 1 min. Samples were centrifuged at 1000 × *g* for 5 min, and the supernatants were collected, this operation was repeated three times. Afterwards, the supernatants were dried under N_2, dissolved in 1 mL methanol, and then filtered through 0.22 μm PTFE filters. The fat-soluble compounds were quantified 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 according to Natalello et al. (2022). Briefly, a 10 μL sample was injected into the system, using methanol as the isocratic mobile phase at a flow rate of 1.3 mL/min. The autosampler (SIL-40C XS, Shimadzu) and column oven (CTO-40C, Shimadzu) were maintained at 25 °C and 40 °C, respectively. Tocopherols were measured with a spectrofluorometric detector (RF-20AXS, Shimadzu) set to an excitation wavelength of 295 nm and an emission wavelength of 330 nm. External calibration curves were prepared using pure standards (Merck Life Science s.r.l., Milano, Italy) for each analyte quantification.

Feedstuff FA were extracted using chloroform and converted to FA methyl esters (FAME) with 2 % (v/v) sulfuric acid in methanol, using tridecanoic acid (C13:0; Sigma-Aldrich) as an internal standard (Shingfield et al., 2003). Gas chromatographic analysis was performed as later detailed for meat FA analysis.

2.4. Rumen and abomasum analyses

Ammonia concentration in ruminal fluid samples was quantified by spectrophotometric analysis (model UV-1601; Shimadzu Corporation, Milan, Italy) employing the salicylate method, following the procedure outlined by Reardon et al. (1966). The VFA concentrations in ruminal fluid were determined using a gas chromatograph (ThermoQuest, Milan, Italy) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm; Supelco, Bellefonte, PA, USA) as indicated by Priolo et al. (2021). In short, rumen liquors were centrifuged at 16000 × *g* for 15 min at 4 °C, and the resulting supernatant was transferred to a GC vial. One μL was injected under isothermal conditions at 140 °C, with a split ratio of 1:100. Helium served as the carrier gas at a constant flow rate of 2 mL/min. The injector and detector temperatures were maintained at 250 °C. Crotonic acid ($C_4H_6O_2$) was used as the internal standard. Individual VFA were identified by comparing their retention times to those of a standard VFA mixture (Volatile Free Acid Mix, CRM46975, Sigma-Aldrich, USA). The FA from rumen and abomasal contents were directly transesterified to FA methyl esters (FAME) using a two-step process involving basic followed by acid catalysis, with nonadecanoic acid (C19:0) as the internal standard, according to the method described by Natalello et al. (2019). Gas chromatography conditions for FAME analysis are described in the section on meat FA determination.

2.5. Meat FA analysis

The LTL muscle samples were deprived of visible fat, finely minced, and 10 g homogenized with a solution of chloroform and methanol (2:1, v/v). Total intramuscular fat (IMF) was gravimetrically measured following solvents evaporation using a rotary evaporator system (Rotavapor R-114, Büchi, Flawil, Switzerland). The IMF was extracted using a hexane and 2-propanol mixture (4:1, v/v), and 50 mg of the isolated lipids were methylated with 1 mL of 0.5 *N* sodium methoxide in methanol and 2 mL of hexane containing nonadecanoic acid (C19:0) as an internal standard (Valenti et al., 2018). All the FAME were separated and quantified using a Thermo Finnigan Trace GC equipped with a flame ionization detector (FID; ThermoQuest, Milan, Italy) and 100-m high-polar fused silica capillary column (100 m × 0.25 mm i.d.; film thickness 0.25 μm; SP – 2560 fused silica, Supelco, Bellafonte, PA). Total FAME were quantified using a temperature gradient program with a split ratio of 1:80, employing helium as gas carrier at a constant flow rate of 1 mL/min, as detailed by Priolo et al. (2021). The C18:1 *t*10 and C18:1 *t*11 isomers were further separated in a dedicated analysis under isothermal conditions at 165°C. The FAME peaks were identified by comparing their retention times with those of a commercial FAME standard mixture (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmö, Sweden).

2.6. Antioxidant capacity of meat

Five different assays were performed to estimate the hydrophilic antioxidant capacity in meat, such as 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. The aqueous extract preparation and analytical procedures were based on the methods of

Benzie and Strain (1996) for the FRAP assay, Makkar et al. (1993) for the Folin–Ciocalteu method, Re et al. (1999) for the TEAC assay, and Yen and Wu (1999) for the FICA and DPPH assays, with slight modifications as described by Musati et al. (2025). In brief, the muscle samples were trimmed of visible fat and finely minced with a knife. For the TEAC, FRAP, Folin-Ciocalteu, and FICA assays, 2 g of meat was homogenized for 1 min (Ultra Turrax T-18; Ika, Germany) with 18 mL of distilled water in a test tube constantly kept in an ice-water bath. Subsequently, the samples were centrifuged at 2500 × *g* for 20 min at 4°C and the supernatant filtered (Whatman 1 filter paper). The so-obtained meat aqueous extract (MAE) was then collected into different aliquots for the following antioxidant analyses.

For TEAC assay, 20 μL of MAE was mixed with 2 mL of ABTS•+ solution (Aouadi et al., 2014) and the absorbance read at 734 nm (UV- 1601; Shimadzu Corporation, Milan, Italy) after 60 min incubation at 30°C according to Re et al. (1999). To measure the spontaneous discoloration, a blank containing phosphate-buffered saline (PBS) in place of MAE was prepared. Results were determined by referencing a 5-points calibration curve (0–400 μg/mL) prepared with Trolox standard (CAS 53188–07–1; Merck Life Science s.r.l., Milano, Italy) in PBS (pH 7.4).

The FRAP assay was performed according to Benzie and Strain (1996) with minor adaptations. Briefly, a 50 μL aliquot of the filtered MAE sample was mixed with 150 μL of distilled water and 1.5 mL of a reagent solution (10:1:1, v:v:v) composed of 300 m*M* acetate buffer (pH 3.6), 10 m*M* TPTZ (2,4,6-tripyridyl-s-triazine in 40 m*M* HCl), and 20 m*M* aqueous ferric chloride. After incubation at 37 °C for 60 min, absorbance was recorded at 593 nm using a UV-1601 spectrophotometer (Shimadzu, Milan, Italy). Quantification was performed by reference to a 7-point calibration curve (0–280 μg/mL) prepared with aqueous ferrous sulfate heptahydrate ($FeSO_4 \times 7H_2O$).

The Folin–Ciocalteu assay was conducted according to the method of Makkar et al. (1993). Briefly, 0.5 mL of MAE was combined with 0.5 mL distilled water, 0.5 mL of 1 *N* Folin–Ciocalteu reagent, and 2.5 mL of 20 % (w/v) sodium carbonate. Following 40 min of incubation at room temperature in the dark, samples were centrifuged at 2500 × *g* for 10 min at 4 °C. Absorbance was measured at 725 nm using a UV-1601 spectrophotometer (Shimadzu, Milan, Italy). Quantification was based on a six-point calibration curve (0–100 μg/mL) prepared with aqueous tannic acid.

The FICA assay was performed as described by Yen and Wu (1999). Briefly, 0.5 mL of MAE was mixed with 0.1 mL of 2 mM $FeCl_2$, 0.2 mL of 5 m*M* ferrozine solution, and 4.2 mL of distilled water. The mixture was incubated in the dark for 60 min, after which absorbance was measured at 562 nm using a UV-1601 spectrophotometer (Shimadzu, Milan, Italy). Chelating activity was quantified using a 6-point calibration curve of EDTA (0–60 μg/mL).

The DPPH radical scavenging activity was assessed following the method of Yen and Wu (1999), with minor modifications. In brief, 2 g of *longissimus thoracis et lumborum* (LTL) muscle was homogenized with 18 mL of 0.05 *M* PBS for 1 min using an Ultra-Turrax T-18 homogenizer (Ika, Germany) in an ice-water bath. Proteins were precipitated by adding 2 mL of 10 % trichloroacetic acid ($C_2HCl_3O_2$), followed by centrifugation at 2500 × *g* for 20 min at 4 °C, and the supernatant was filtered (Whatman 1 filter paper). Then, 0.6 mL of the filtrate was mixed with 2.4 mL of distilled water and 3 mL of 0.2 m*M* DPPH in methanol. After incubation, absorbance was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Milan, Italy). Results were quantified by comparison to a 6-point calibration curve of Trolox (0–90.9 μg/mL) prepared in 0.1 mg/mL buffer solution.

2.7. Fat-soluble vitamins and cholesterol in meat

The concentrations of tocopherols (α and γ), retinol, and cholesterol in LTL muscle were quantified according to the protocol of Bertolín et al. (2018), with modifications outlined by Menci et al. (2023). Briefly, 2.5 g of minced meat was mixed with 0.2 g of L-ascorbic acid and 7.5 mL of 10 % potassium hydroxide prepared in a 1:1 ethanol:water solution. The mixture was saponified overnight in an orbital shaker (KS 4000 i control, Ika, Germany). Lipid extraction was performed twice using 5 mL of a 9:1 (v/v) hexane:ethyl acetate solution containing 25 mg/L BHT, followed by centrifugation at 2000 × *g* for 5 min at 10 °C. The combined supernatants were evaporated under N_2 flow, and the residue was reconstituted in 1 mL of HPLC-grade methanol and filtered through a 0.2 μm PTFE syringe filter. Tocopherols were quantified by UHPLC as described in Section 2.4, while retinol and cholesterol were detected using a photodiode array detector (SPD-M40, Shimadzu) at 325 nm and 220 nm respectively.

2.8. Shelf-life study and color measurement

The shelf-life study on fresh meat was performed by cutting three 2-cm-thick slices from the left of each LTL muscle with a knife, then placed in polystyrene trays wrapped with food stretch film, and stored in the dark at 4°C for 0 (2 h of blooming), 4, and 7 days to mimic domestic storage conditions. Meat color was measured at each day of storage using a portable Minolta spectrophotometer (CM-2022, Minolta Co. Ltd., Japan; SCE mode; illuminant A; 10° standard observer) to detect the color descriptors L^* (lightness), a^* (redness), b^* (yellowness), C^* (chroma), and hue angle. For each slice, 3 measurements on non-overlapping areas of the surface were taken. The reflectance spectra (400–700 nm) were recorded to quantify metmyoglobin (MMb) formation (Krzywicki, 1979). Afterwards, each slice of meat was vacuum-packed and frozen until the upcoming lipid oxidation analysis.

2.9. Lipid oxidation

Lipid oxidation in meat samples was evaluated at the end of the storage period by quantifying both primary (hydroperoxides) and secondary (thiobarbituric acid reactive substances, TBARS) oxidation products. The hydroperoxide content was determined following the method of Maqsood et al. (2012), with modifications described by Menci et al. (2023). Briefly, 1 g of meat was homogenized with

15 mL of chloroform:methanol (2:1, v/v) for 60 s (30 s intervals with a pause in between.) and filtered through filter paper (Whatman No. 1). A 7 mL aliquot 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 upper layer, 750 μL of the lower phase was diluted (1:1) with chloroform and reacted with 1 mL of chloroform:methanol (2:1), 12.5 μL of ammonium thiocyanate, and 12.5 μL of ferric chloride. After 20 min incubation at room temperature in the dark, absorbance was measured at 500 nm (UV-1601; Shimadzu Corporation, Milan, Italy). Quantification was achieved using a seven-point calibration curve (0–3 ppm) of cumene hydroperoxide in 2:1 chloroform:methanol.

The TBARS analysis was performed according to Siu and Draper (1978) with slight adjustments (Natalello et al., 2020). A sample of 2.5 g of meat was homogenized with 12.5 mL of distilled water in an ice-water bath (Ultra-Turrax T-18 homogenizer; Ika, Germany). After the addition of 12.5 mL of 10 % trichloroacetic acid (w/v), the mixture was filtered (Whatman No. 1). An aliquot of 4 mL of the filtrate was mixed with 1 mL of 0.06 M thiobarbituric acid and incubated in a water bath at 80 °C for 90 min. Absorbance was recorded at 532 nm (UV-1601; Shimadzu Corporation, Milan, Italy), and values were calculated against a 5-point calibration curve (0–200 nmol/L) of tetraethoxypropane (TEP) in distilled water. Results were expressed as mg malondialdehyde (MDA)/kg of meat.

2.10. Calculations and statistical analysis

The Biohydrogenation (BH) estimates (%) of C18:1 *c*9 (oleic acid – OA), C18:2 *c*9, *c*12 (linoleic acid – LA) and C18:3 *c*9, *c*12, *c*15 (alpha linolenic acid – α-LnA) in both rumen and abomasal fluid were calculated as described by Oliveira et al. (2016) using the following formula:

$$BH_{FAx} = \frac{FAx_{diet} - FAx_{rumen\ or\ abomasum}}{FAx_{diet}} \times 100$$

The BH completeness (%) was determined based on the method described by Alves et al. (2017), considering the maximum C18:0 in rumen and abomasal fluid, assuming a complete BH of the C18 FA from diet, as detailed below:

$$BH\ completeness(\%) = \frac{C18:0_{rumen\ or\ abomasum}}{\begin{array}{c}C18:0_{diet} + (C18:1\ c9_{diet^-} - C18:1\ c9_{rumen\ or\ abomasum}) + \\ (C18:2\ c9,c12_{diet^-} - C18:2\ c9,c12_{rumen\ or\ abomasum}) + \\ (C18:3\ c9,c12,c15_{diet^-} - C18:3\ c9,c12,c15_{rumen\ or\ abomasum})\end{array}} \times 100$$

The atherogenicity index (AI), which reflects the balance between FA that promote or prevent plaque formation, indicating their impact on atherosclerosis was calculated as described by Ulbricht and Southgate (1991), using the following formulas:

$$AI = \frac{C12:0 + (4x\ C14:0) + C16:0}{\Sigma MUFA + \Sigma n-6PUFA + \Sigma n-3PUFA}$$

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

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Animal performance				
DMI[c], g/d	963	333	85.5	< 0.001
Initial BW[c], kg	13.1	12.4	0.59	0.533
Final BW[c], kg	27.1	14.4	1.84	< 0.001
Hot carcass, kg	12.0	6.07	0.89	< 0.001
Cold carcass, kg	11.8	6.05	8.95	< 0.001
ADG[c], g/d	248	37.6	31.3	< 0.001
FCR[c]	3.89	8.86	6.81	0.193
Intakes				
Crude protein intake, g/d	180	67.0	10.2	< 0.001
aNDF[c] intake, g/d	204	81.3	12.2	< 0.001
Total polyphenols intake[d]	55.7	19.8	3.08	< 0.001
Total tannins intake[d]	16.1	1.13	0.53	< 0.001
α-tocopherol intake, μg/d	81.1	22.9	3.89	< 0.001
γ-tocopherol intake, μg/d	155	225	28.9	< 0.001
Total FA[c] intake, g/d	30.7	13.8	2.92	< 0.001
C16:0, intake, g/d	6.54	1.75	3.04	< 0.001
C18:0, intake, g/d	2.18	0.46	0.91	< 0.001
C18:1 *c*9, intake, g/d	7.07	5.36	7.16	0.114
C18:2 *c*9, *c*12, intake, g/d	12.6	4.59	7.07	< 0.001
C18:3 *c*9, *c*12, *c*15, intake, g/d	1.79	1.23	1.66	< 0.05

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c] DMI = dry matter intake; BW = body weight; ADG = average daily gain; FCR = feed conversion ratio, calculated as DMI/ADG; FA= fatty acids; aNDF: amylase-treated Neutral Detergent Fiber.
[d]Expressed as g tannic acid equivalents/d

The statistical analysis was performed with the JMP 18 software (SAS Institute Inc., 2024), using the GLM to test the dietary treatment effect on animal performance, FA profile, tocopherols, and antioxidant capacity of meat, considering the individual lambs as the experimental units. A mixed model was used to assess the impact of dietary treatment, storage time, and their interaction (fixed effects) on color and lipid oxidation parameters, with individual lambs treated as a random effect. Statistical significance was declared when $P \leq 0.05$.

3. Results

3.1. Animal performance, intakes and fermentation parameters

As shown in Table 2, dietary treatment significantly affected almost all animal performance and feed intake parameters, despite the initial BW being comparable (P > 0.05). Particularly, lambs fed LUP diet showed lower DMI (P < 0.001), final BW (P < 0.001), carcass weights (P < 0.001), and ADG (P < 0.001) compared to those fed CON diet. Moreover, the intakes of CP, aNDF, total phenols, total tannins and tocopherols were greater for lambs in the CON treatment compared to the LUP treatment (P < 0.001) as well as the daily intake of total FA and most individual fatty acids (P < 0.05). The only exceptions were C18:1 *c*9 and C18:1 *c*11 which were similar between the two treatments (P > 0.05).

Table 3 shows the impacts of dietary treatments on ruminal fermentation parameters. Notably, the ruminal pH had higher values (P < 0.001) in LUP than CON treatment, while total VFA concentration tended to be higher (P = 0.064) in CON treatment compared to LUP. Conversely, the ammonia level was not affected by dietary treatments. However, LUP increased the molar proportions of acetate and butyrate (P < 0.001 and P < 0.05, respectively) while decreased the proportion of propionate and valerate (P < 0.001). Consequently, acetate:propionate ratio was higher (P < 0.001) in the ruminal fluid from LUP lambs.

3.2. Fatty acid profile of rumen and abomasum contents

The FA profile of rumen content is reported in Table 4. Several ruminal FA were affected by the dietary inclusion of LUP compared to the CON diet. Individual SFA, such as C12:0, C14:0, and C16:0 were found at lower concentrations in the LUP treatment compared to CON (P < 0.05). In contrast, LUP diet increased the level of C17:0 *anteiso* (P = 0.041). The MUFA contents were variably affected by the dietary treatments with higher C17:1 *c*10 (P < 0.001) and C16:1 *t*9 (P = 0.038) and lower C18:1 *c*11 (P = 0.001) in LUP compared to CON. Concerning BH intermediates, the ruminal digesta of the animals given LUP had a more than 2-fold higher proportion of VA than the CON animals (P = 0.008), whereas the C18:1 *t*10 concentration was higher in the CON group than in the LUP group (P = 0.005), resulting in a markedly reduced C18:1 *t*10/*t*11 ratio in LUP (P = 0.001). In addition, LUP-fed animals exhibited significantly greater levels of several long-chain saturated fatty acids, including C20:0, C22:0, C23:0, C24:0, and C24:1 *c*9 (P < 0.001). Within the PUFA, LUP markedly increased the concentration of α-LnA (P = 0.001), and rumenic acid (RA; P = 0.033), while reducing LA (P < 0.001) compared with CON diet. Moreover, the rumen concentration of C20:4 n-6 was higher (P < 0.001) in animals given LUP than CON animals. Finally, the sum of PUFA (P = 0.001), n-3 PUFA (P = 0.001), and n-6 PUFA (P < 0.001) were all affected by the dietary treatments, with significantly reduced levels of PUFA and n-6 and elevated levels of n-3 in LUP compared to CON.

The dietary inclusion of 35 % lupin seeds resulted in significant modifications in the FA profile of abomasal digesta (Table 5). The LUP diet significantly decreased (P ≤ 0.05) or showed a tendency to decrease (P < 0.1) the levels of FA containing 16 or fewer carbon atoms with the exception of C15:0 *iso* and C16:1 *t*9, which were lower in the abomasal content from LUP-fed lambs (P = 0.023 and P = 0.029, respectively). A marked reduction in C16:0 (P < 0.001) along with an increase in C16:1 *t*9 (P = 0.029) was observed with LUP than CON. The proportion of C17:1 *c*10 was lower in LUP compared to CON treatment (P = 0.037). Among long-chain saturated fatty acids, C18:0 (P = 0.012), C22:0 (P < 0.001), C23:0 (P = 0.042), and C24:0 (P < 0.001) were present in greater concentrations in

Table 3
Effect of dietary treatment on pH, ammonia and volatile fatty acids (VFA) of rumen fluid.

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Rumen pH	6.12	6.58	6.34	0.006
Ammonia, mg/L	129	162	34.81	0.513
Total VFA, mmol/L	290	225	22.69	0.064
Molar proportions, mmol/100 mmol VFA				
Acetate	74.4	81.8	1.19	< 0.001
Propionate	20.9	12.33	1.14	< 0.001
Isobutyrate	0.26	0.37	0.04	0.064
Butyrate	3.35	4.86	0.38	< 0.05
Isovalerate	0.27	0.36	0.06	0.259
Valerate	0.74	0.26	0.08	< 0.001
Caproate	0.11	0.03	0.03	0.115
Acetate:propionate ratio	3.65	7.06	0.51	< 0.001

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.

Table 4
Effect of dietary treatment on rumen digesta fatty acid composition (g/100 g of freeze-dried rumen content).

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Total FA (mg/100 g freeze-dried rumen content)	4157	3695	237	0.347
C12:0	0.56	0.32	0.049	0.011
C13:0	0.16	0.13	0.012	0.125
C14:0	1.75	1.27	0.115	0.034
C14:1 *t*9	0.51	0.58	0.099	0.763
C15:0 *iso*	0.67	1.02	0.130	0.188
C15:0 *anteiso*	0.27	0.10	0.063	0.180
C14:1 *c*9	1.33	1.04	0.107	0.188
C15:0	1.35	1.08	0.074	0.067
C15:1 *t*10	0.46	0.31	0.049	0.113
C16:0	17.8	14.7	0.454	< 0.001
C16:1 *t*9	0.21	0.50	0.072	0.038
C17:0 *iso*	0.17	0.26	0.034	0.161
C16:1 *c*9	0.17	0.23	0.021	0.219
C17:0 *anteiso*	0.35	0.54	0.046	0.041
C17:0	0.59	0.51	0.030	0.234
C17:1 *c*10	0.40	0.22	0.029	< 0.001
C18:0	30.4	34.7	1.616	0.188
C18:1 *t*6, *t*7, *t*8	0.65	0.87	0.090	0.240
C18:1 *t*9	0.39	0.49	0.043	0.253
C18:1 *t*10	3.68	1.08	0.500	0.005
C18:1 *t*11 (VA)	2.57	5.30	0.553	0.008
C18:1 *c*6	1.53	1.34	0.126	0.456
C18:1 *c*9 (OA)	7.19	9.82	0.689	0.052
C18:1 *c*11	1.34	0.70	0.108	0.001
C19:1 *t*7	0.67	0.38	0.077	0.061
C18:2 *c*9, *c*12 (LA)	8.98	2.70	0.927	< 0.001
C20:0	0.60	1.64	0.150	< 0.001
C20:1 *c*11	1.73	0.77	0.144	< 0.001
C18:3 *c*9, *c*12, *c*15 (α-LnA)	0.06	0.73	0.117	0.001
C18:2 *c*9, *t*11 (RA)	0.18	0.53	0.086	0.033
C18:2 *t*10, *c*12	0.19	0.09	0.045	0.284
C21:0	0.14	0.20	0.025	0.208
C22:0	0.40	1.64	0.161	< 0.001
C20:4 n-6	0.00	0.41	0.067	< 0.001
C23:0	0.01	0.20	0.035	0.002
C24:0	0.35	0.66	0.050	< 0.001
C24:1 *c*9	0.04	0.18	0.023	< 0.001
C22:4 n-6	0.04	0.08	0.019	0.236
Fatty acid groups (%)	55.60	59.02	1.881	0.382
SFA				
MUFA	22.88	23.80	1.338	0.743
PUFA	9.44	4.60	0.838	0.001
n-3	0.06	0.73	0.117	0.001
n-6	9.21	3.33	0.899	< 0.001
OBCFA	3.71	4.05	0.220	0.462
Ratio				
C18:1 *t*10/*t*11 ratio	1.56	0.21	0.240	0.001

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; OBCFA, odd and branched chain fatty acids; PUFA, polyunsaturated fatty acids.

the abomasum of animals given LUP diet. Similar to rumen content, the LUP abomasum content had a higher VA ($P = 0.045$) and lower C18:1 *t*10 ($P = 0.015$) proportion than the CON group. In addition, C18:1 *c*11 was lower in LUP than CON ($P = 0.001$). These changes led to a strongly reduced C18:1 *t*10/*t*11 ratio in LUP treatment ($P = 0.005$). Concerning the individual PUFA, the abomasum of animals given LUP diet showed lower ($P < 0.001$) LA and higher α-LnA ($P = 0.018$), C20:4 n-6 ($P < 0.001$), C22:4 n-6 ($P < 0.001$) compared to CON animals. The concentration of PUFA in the abomasum was significantly lower ($P < 0.001$) in lambs fed the LUP diet, whereas the concentration of SFA showed a tendency to increase ($P = 0.063$). Finally, LUP showed higher content of total n-3 PUFA ($P = 0.007$) and a lower n-6 content ($P < 0.001$), resulting in a drastic reduction in the n-6/n-3 ratio ($P < 0.001$) compared to CON.

The analysis of BH indices of OA, LA, and α-LnA and the BH completeness in rumen and abomasal fluid are reported in Table 6. The extent of BH of α-LnA was greater ($P < 0.001$) in the rumen content of CON lambs, while that of LA was higher ($P < 0.001$) in LUP. A similar trend was observed in the abomasum, where LUP enhanced the BH rate of OA ($P = 0.003$) and LA ($P < 0.001$). Moreover, the BH of α-LnA was also lower ($P = 0.028$) in LUP compared to CON group. The completeness of BH process was not influenced by the diet ($P > 0.05$).

Table 5
Effect of dietary treatment on abomasum digesta fatty acid composition (g/100 g of freeze-dried abomasum content).

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Total FA (mg/100 g freeze-dried abomasum content)	3784	4408	274	0.270
C10:0	0.01	0.02	0.006	0.583
C12:0	0.46	0.29	0.037	0.010
C13:0	0.14	0.10	0.011	0.026
C14:0	1.58	1.21	0.108	0.086
C14:1 *t*9	0.07	0.04	0.014	0.174
C15:0 *iso*	0.48	1.09	0.139	0.023
C15:0 *anteiso*	0.13	0.08	0.009	0.006
C14:1 *c*9	1.26	1.01	0.071	0.075
C15:0	1.19	1.11	0.078	0.623
C15:1 *t*10	0.39	0.24	0.057	0.196
C16:0	18.18	13.68	0.628	< 0.001
C16:1 *t*9	0.18	0.51	0.078	0.029
C17:0 *iso*	0.24	0.19	0.019	0.246
C16:1 *c*9	0.25	0.20	0.019	0.166
C17:0 *anteiso*	0.40	0.45	0.031	0.388
C17:0	0.60	0.60	0.035	0.946
C17:1 *t*10	0.01	0.04	0.013	0.276
C17:1 *c*10	0.23	0.14	0.022	0.037
C18:0	32.35	42.59	2.156	0.012
C18:1 *t*6, *t*7, *t*8	0.77	0.77	0.117	0.993
C18:1 *t*9	0.44	0.39	0.055	0.699
C18:1 *t*10	3.09	0.86	0.485	0.015
C18:1 *t*11 (VA)	2.19	3.89	0.433	0.045
C18:1 *c*6	1.49	1.34	0.163	0.664
C18:1 *c*9 (OA)	7.71	7.68	0.684	0.984
C18:1 *c*11	1.28	0.65	0.110	0.001
C19:1 *t*7	0.58	0.24	0.070	0.010
C18:2 *c*9, *c*12 (LA)	9.67	3.11	0.933	< 0.001
C20:0	0.67	2.07	0.201	< 0.001
C20:1 *c*11	1.72	0.80	0.142	< 0.001
C18:3 *c*9, *c*12, *c*15 (α-LnA)	0.11	0.62	0.113	0.018
C18:2 *c*9, *t*11 (RA)	0.03	0.09	0.020	0.128
C21:0	0.20	0.22	0.020	0.659
C22:0	0.42	1.97	0.200	< 0.001
C20:4 n-6	0.00	0.45	0.072	< 0.001
C23:0	0.20	0.30	0.023	0.042
C24:0	0.44	0.79	0.050	< 0.001
C24:1 *c*9	0.02	0.21	0.030	< 0.001
C22:4 n-6	0.05	0.13	0.014	< 0.001
C22:5 n-6	0.08	0.07	0.015	0.693
Fatty acid groups (%)	57.7	66.7	2.456	0.063
SFA[c]				
MUFA[c]	21.7	19.0	1.586	0.426
PUFA[c]	9.94	4.65	0.868	< 0.001
n-3	0.11	0.69	0.117	0.007
OBCFA[c]	3.57	4.13	0.250	0.275
Ratio				
C18:1 *t*10/*t*11 ratio	1.55	0.19	0.262	0.005

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; OBCFA, odd and branched chain fatty acids; PUFA, polyunsaturated fatty acids.

3.3. Intramuscular fatty acid composition

Intramuscular fat (IMF) content and muscle FA composition are presented in Table 7. The dietary inclusion of 35 % white lupin seeds affected the composition of most FA in lamb muscle tissue compared to the control diet, despite no difference in total IMF ($P = 0.241$). The LUP significantly increased C12:0 ($P = 0.026$), C15:0 *iso* ($P = 0.005$), C15:0 *anteiso* ($P = 0.004$), C15:0 ($P = 0.030$), and C17:0 *iso* ($P = 0.014$) compared to CON. However, no significant difference was observed for total SFA ($P = 0.230$). Among MUFA, LUP diet significantly decreased C16:1 *c*9 ($P = 0.009$), C17:1 *c*10 ($P < 0.001$), and C18:1 *t*10 ($P = 0.010$) and tended to decrease C18:1 *c*9 ($P = 0.054$), while increasing the VA proportion ($P = 0.031$) compared to the CON diet. Regarding FA groups, the total replacement of soybean meal and 30 % of maize with white lupin tended to increase the proportion of total PUFA ($P = 0.078$), while lowering the total MUFA proportion ($P < 0.029$) compared to CON. Lambs fed LUP displayed higher concentrations of α-LnA ($P = 0.008$), C20:5 n-3 ($P = 0.040$), C22:5 n-3 ($P = 0.022$), and C22:6 n-3 ($P = 0.036$) in meat than those on CON. These increases

Table 6
Effect of dietary treatment on the biohydrogenation (%) and completeness (%) indices in rumen and abomasal digesta of lambs.

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Biohydrogenation (%)[c]				
Rumen				
C18:1 *c*9 (OA)	57.0	62.5	3.34	0.262
C18:2 *c*9, *c*12 (LA)	69.9	87.9	2.38	< 0.001
C18:3 *c*9, *c*12, *c*15 (α-LnA)	98.7	88.0	1.80	< 0.001
Abomasum				
C18:1 *c*9 (OA)	55.5	72.6	3.42	0.003
C18:2 *c*9, *c*12 (LA)	68.9	87.1	2.07	< 0.001
C18:3 *c*9, *c*12, *c*15 (α-LnA)	97.5	90.5	2.01	0.028
Completeness (%)[d]				
Rumen	76.2	79.8	3.55	0.480
Abomasum	79.6	86.4	3.53	0.197

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c]Calculated as reported by Oliveira et al. (2016).
[d]Calculated as reported by Alves et al. (2017).

contributed to a higher total n-3 PUFA proportion (P = 0.014) and a lower n-6/n-3 PUFA ratio (P < 0.001) in LUP-fed lambs compared to CON. Additionally, RA proportion was greater in LUP than in CON muscle (P = 0.010). Feeding LUP increased the concentration of OBCFA acids in muscle compared to the CON treatment (P = 0.026). Finally, LUP decreased the desaturase index (SCDi17) in muscle (P < 0.001), while the atherogenic index was not affected by the dietary treatment (P = 0.441).

3.4. Fat-soluble vitamins, and antioxidant capacity of meat

As shown in Table 8, meat from LUP-fed lambs exhibited a higher cholesterol concentration (P = 0.020) and tended to have a higher pH (P = 0.081) compared to the CON group. Regarding fat-soluble vitamins, although the total tocopherol content did not differ significantly between groups (P = 0.561), LUP-fed animals had a lower α-tocopherol content (P < 0.001) and a concomitantly higher γ-tocopherol content (P < 0.001) in meat than the CON group. The dietary treatment did not influence the TEAC, Folin-Ciocalteu, and FICA antioxidant assays (P > 0.05). However, the FRAP value tended to be lower (P = 0.066) and the DPPH was greater (P = 0.05) in LUP compared to CON meat.

3.5. Meat oxidative stability

The effects of dietary treatment, time of storage, and their interaction on meat discolouration, and lipid oxidation over 7 days of refrigerated storage are reported in Table 9. The dietary inclusion of 35 % white lupin seeds did not affect (P > 0.050) the colour parameters and the lipid oxidation of meat, except for the h_{ab} value which was higher (P = 0.003) in LUP compared to CON treatment. All measured meat colour parameters were affected by time of storage (P ≤ 0.001). Particularly, *L**, *a**, *b**, *C**, and h_{ab}, reached maximum value on day 4 of storage and minimum on day 0 and 7 (P < 0.001). The percentages of metmyoglobin increased, indicating meat browning. The diet × time interaction was significant only for *L** (P < 0.01), h_{ab} (P = 0.002; Fig. 1), and metmyoglobin (P = 0.026; Fig. 2). Regarding lipid oxidation, both TBARS and Hydroperoxide values were not affected by the dietary treatment (P > 0.1), while the time of storage influenced only the TBARS values, increased over time of storage (P < 0.001). No significant diet × time interactions were found for TBARS and Hydroperoxide values.

4. Discussion

This study aimed at evaluating the effects of total replacement of soybean meal and partial of maize with white lupin seeds (*Lupinus albus* L.) in the diet of growing lambs, with a focus on performance, ruminal fermentation parameters, FA composition, and oxidative stability. We decided to replace these two ingredients to decrease reliance on these imported ingredients, and improve sustainability by supporting local crops production. Moreover, lupin cultivation is a valuable local protein resource in organic livestock systems, where soybean and its derivatives are excluded for the ban on foods derived from genetically modified organisms or produced with chemical solvents (Regulation EU, 2018/848). Taking into account the lupine chemical composition, especially the quinolizidine alkaloids content, a sweet (low-alkaloid) variety was chosen as feed in the lamb diet. In accordance with the literature, white lupin seeds have been effectively used in replacing dietary soybean meal at various doses: 1.9 % and 3.2 % for dairy cows (Joch and Kudrna, 2020), 20 % for young bulls (Vicenti et al., 2009), and 30 % for lactating sheep (Tefera et al., 2015).

4.1. Animal performance and rumen fermentation

Regarding growth performance, in the present trial the white lupin-fed lambs ingested about a third of the feed compared to the

Table 7
Effect of dietary treatment on intramuscular fat (g/100 g of meat) and muscle fatty acid composition (g/100 g of total fatty acids).

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
Intramuscular fat (IMF)	1.59	1.34	0.104	0.241
C10:0	0.15	0.15	0.011	0.859
C12:0	0.13	0.26	0.031	0.026
C14:0	2.12	2.96	0.265	0.115
C14:1 *t*9	0.04	0.03	0.008	0.684
C15:0 *iso*	0.06	0.13	0.014	0.005
C15:0 *anteiso*	0.09	0.17	0.014	0.004
C14:1 *c*9	0.10	0.10	0.013	0.885
C15:0	0.31	0.39	0.019	0.030
C15:1 *t*10	0.08	0.15	0.015	0.017
C16:0	23.21	21.08	1.451	0.484
C16:1 *t*9	0.26	0.31	0.037	0.553
C17:0 *iso*	0.32	0.47	0.031	0.014
C16:1 *c*9	1.60	1.06	0.110	0.009
C17:0 *anteiso*	0.38	0.43	0.023	0.299
C17:0	1.17	1.07	0.043	0.298
C17:1 *t*10	0.10	0.11	0.013	0.848
C17:1 *c*10	0.78	0.54	0.040	< 0.001
C18:0	14.36	20.48	1.120	0.002
C18:1 *t*6, *t*7, *t*8	0.08	0.14	0.020	0.165
C18:1 *t*9	0.25	0.37	0.037	0.090
C18:1 *t*10	0.64	0.28	0.073	0.010
C18:1 *t*11 (VA)	0.54	1.09	0.130	0.031
C18:1 *c*6	0.32	0.43	0.032	0.112
C18:1 *c*9 (OA)	38.60	29.37	2.433	0.054
C18:1 *c*11	1.89	1.46	0.100	0.027
C19:1 *t*7	0.13	0.14	0.022	0.800
C18:2 *c*9, *c*12 (LA)	8.38	9.05	0.602	0.592
C20:0	0.10	0.49	0.088	0.021
C18:3 *c*6, *c*9, *c*12	0.10	0.10	0.022	0.932
C20:1 *c*11	0.65	0.60	0.035	0.430
C18:3 *c*9, *c*12, *c*15 (α-LnA)	0.13	0.30	0.035	0.008
C18:2 *c*9, *t*11 (RA)	0.30	0.50	0.043	0.010
C20:2 *c*11, *c*14	0.09	0.12	0.014	0.280
C22:0	0.01	0.09	0.015	0.004
C20:3 n-6	0.33	0.73	0.087	0.014
C23:0	0.20	0.19	0.024	0.795
C20:5 n-3	0.48	1.17	0.172	0.040
C22:4 n-6	0.29	0.49	0.059	0.086
C22:5 n-6	0.12	0.13	0.019	0.816
C22:5 n-3	0.82	2.04	0.280	0.022
C22:6 n-3	0.27	0.52	0.062	0.036
Fatty acid groups (%)				
SFA[c]	42.64	48.58	2.414	0.230
MUFA[c]	46.07	36.18	2.339	0.029
PUFA[c]	11.29	15.24	1.124	0.078
OBCFA[c]	2.56	3.05	0.114	0.026
n-3 PUFA	1.21	2.86	0.356	0.014
n-6 PUFA	9.30	10.69	0.725	0.356
Ratio				
PUFA n-6/n-3 ratio	7.86	4.21	0.577	< 0.001
PUFA/SFA	0.27	0.34	0.030	0.247
C18:1 *t*10/*t*11 ratio	1.33	0.31	0.165	< 0.001
Indexes				
AI[d]	0.54	0.74	0.128	0.441
Desaturase index (SCDi17)[e]	0.40	0.33	0.011	< 0.001

[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; OBCFA, odd and branched chain fatty acids; PUFA, polyunsaturated fatty acids.
[d]AI and TI calculated as reported by Ulbricht and Southgate (1991).
[e]SCDi17 calculated as C17:1 c9/(C17:0 + C17:1 c9).

control. This substantial reduction of over 65 % in DMI suggests lower palatability or voluntary feed intake associated with the inclusion of 350 g/kg white lupin in the diet. Consequently, the final BW also differed significantly between treatments with animals fed lupin being 12.6 kg lighter compared to control. Despite the lupine cultivar used being sweet, the antinutritional compounds, such as alkaloids, inherent in lupin seeds may have negatively impacted palatability, accentuating a strong bitter and astringent taste that

Table 8
Effect of dietary treatment on pH, vitamins, cholesterol, and antioxidant capacity of fresh lamb.

	Dietary treatment[a]		SEM[b]	P-value
	CON	LUP		
pH	5.89	6.31	0.157	0.081
Cholesterol (g/kg)	0.73	0.91	0.048	0.020
Fat-soluble vitamins (mg/kg)				
Total tocopherols	1.18	1.37	0.228	0.561
α-tocopherol (%)	74.2	34.4	3.06	< 0.001
γ-tocopherol (%)	25.8	65.6	3.06	< 0.001
Retinol	0.16	0.15	0.012	0.548
Antioxidant capacity, mg/g				
TEAC (Trolox eq)	46.3	49.7	5.02	0.644
FRAP (Fe^{2+} eq)	26.9	23.8	1.09	0.066
Folin-Ciocalteau (TA eq)	0.62	0.60	0.039	0.687
FICA (EDTA eq)	0.77	0.78	0.053	0.876
DPPH (Trolox eq)	0.69	0.92	0.046	0.005

Abbreviations: DPPH: 1,1-diphenyl-2-picrylhydrazyl; EDTA: ethylenediaminetetraacetic acid; FICA: ferrous ion chelating activity; FRAP: ferric reducing antioxidant power; TA eq: tannic acid equivalents; TEAC: Trolox equivalent antioxidant capacity.
[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.

Table 9
Effect of diet on color stability in lamb over 7 days of refrigerated storage.

	Dietary Treatments[a] (D)		Time (T), d			SEM[b]	P-value		
	CON	LUP	0	4	7		D	T	D x T
Meat									
*L** (lightness)	45.31	45.26	44.14[b]	46.45[a]	44.38[b]	0.379	0.153	< 0.01	< 0.01
*a** (redness)	14.26	14.33	14.38[a]	14.94[a]	13.32[b]	0.305	0.133	< 0.01	0.543
*b** (yellowness)	11.94	12.51	9.88[c]	13.72[a]	12.19[b]	0.314	0.969	< 0.001	0.208
*C** (saturation)	18.67	19.07	17.44[b]	20.31[a]	18.08[b]	0.395	0.353	< 0.001	0.409
h_{ab} (Hue angle; deg)	39.64	41.05	34.38[b]	42.58[a]	42.45[a]	0.594	0.003	< 0.001	0.002
Metmyoglobin, %	50.03	52.07	44.63[c]	51.79[b]	55.27[a]	0.711	0.142	< 0.001	0.026
Lipid oxidation, mg/kg meat									
Hydroperoxide	7.29	10.98	10.06	9.57	7.78	2.121	0.261	0.573	0.111
TBARS[c]	0.72	1.20	0.60[b]	0.90[b]	1.39[a]	0.184	0.122	< 0.001	0.554

a, b, c For the Time factor, means within a row that do not share a superscript letter are statistically different.
[a]CON: control maize-soybean based concentrate diet. LUP: diet including 350 g/kg of lupin seeds.
[b]SEM, standard error of the mean.
[c]TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).
a,b,c Within a row, different superscripts indicate significant differences within days of storage ($P < 0.05$).

made the diet unpleasant for animals (Khan et al., 2015; Hill and Roberts, 2020), particularly due to the high level of white lupin inclusion in the experimental diet. This was in accordance with Lestingi et al. (2016), who highlighted a lower intake in lambs fed sweet lupin seeds (150 g/kg) compared with other various protein sources, probably due to the high alkaloid content of lupin seeds. However, the lower performance might also be due to the higher degradability of lupin proteins by rumen microflora compared to those of soybean, leading to lower bioavailability of proteins in the intestine (Masoero et al., 2005). This interpretation is supported by the protein fraction profile reported in Table 1, where the white lupin diet contained more soluble protein (A1) and less of the slowly degradable B2 fraction, together with higher B3 and C fractions, indicating a greater ruminal degradability and a reduced intestinal availability of dietary protein (Masoero et al., 2005). Additionally, the low ruminal degradability of maize starch (Offner et al., 2003) could have further impaired ruminal microbial protein synthesis. Similar reductions in growth performance with lupin inclusion have been reported in previous studies when the substitution level was high or not adequate, reducing the palatability (Kung et al., 1991; Facciolongo et al., 2014). The inclusion level of 35 % white lupin seeds adopted in the present study was selected to evaluate the biological feasibility of a complete replacement of soybean meal rather than to identify the optimal level of inclusion. Although this high proportion negatively affected feed intake and growth performance, these outcomes are still informative, as they define the upper biological and practical limits of lupin utilization in lamb diets. It should also be noted that the inclusion of white lupin seeds in this study influenced both the protein and energy supply of the diet. Although the primary objective was to achieve a full replacement of soybean meal, the partial reduction in maize was a formulation adjustment required to maintain the overall protein-to-energy balance.

The rumen fermentation patterns were significantly modulated with white lupin inclusion, notably by increasing the acetate: propionate ratio and butyrate concentrations, while decreasing propionate and valerate levels. This pattern may reflect a shift in microbial populations favoring butyrogenic over valerate-producing species, potentially influenced by the composition of fermentable protein fractions or microbial competition dynamics (Guduk et al., 2023). Despite the evidenced lower intake in lambs fed white lupin

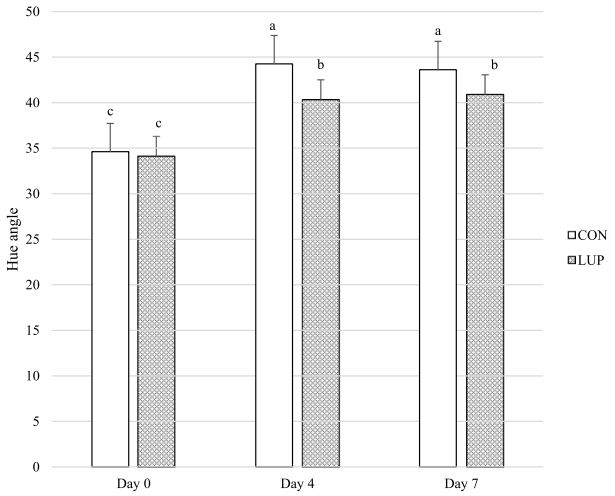

Fig. 1. Interaction between diet and storage time on Hue angle in lamb. CON, control diet; LUP, diet containing 350 g/kg of lupine seeds; Error bars represent the standard error of the mean. a, b, c, Bars that do not share a letter are statistically different at $P < 0.01$.

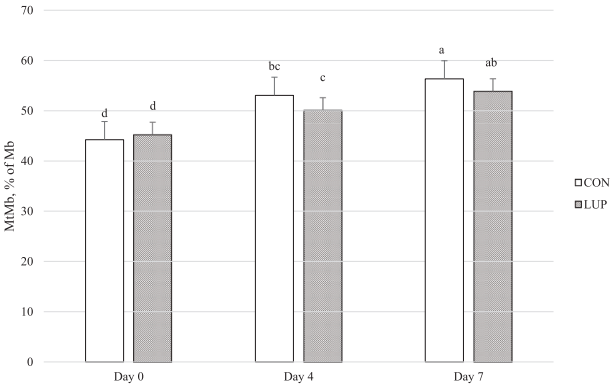

Fig. 2. Interaction between diet and storage time on Metmyoglobin (MtMb, %) in lamb. CON, control diet; LUP, diet containing 350 g/kg of lupine seeds; Error bars represent the standard error of the mean. a, b, c, d, Bars that do not share a letter are statistically different at $P < 0.01$.

diet, these shifts could be consistent with the high content of dietary fiber in lupin, particularly cellulose and pectins, and its low lignin content (Keller et al., 2022), which may promote cellulolytic over amylolytic fermentation pathways (Um and Park, 2022; Morsy et al., 2024). Moreover, it cannot be excluded that variations in protein fractions between the diets may have affected the production of butyrate and valerate in the rumen (Putri et al., 2021).

4.2. Fatty acid metabolism

Concerning the rumen FA profiles, dietary inclusion of white lupin resulted in a substantial reduction in the concentrations of SFA, particularly C12:0, C14:0, and C16:0. Concurrently, an increase in MUFA beneficial to human health, especially VA and OA, was observed, whereas ruminal C18:1 *t*10 concentration was reduced by lupin inclusion. These findings suggest a shift in the BH pathways, favoring the C18:1 *t*11 pathway over the C18:1 *t*10 one, as confirmed by the reduction in the C18:1 *t*10/*t*11 ratio (from 1.56 to 0.21). Both ruminal and abomasal digesta from LUP lambs contained a greater concentration of α-LnA, consistent with the slightly lower extent of BH observed compared to the CON group, suggesting a partial protection α-LnA from extensive microbial hydrogenation. These changes may be due to the lower intake in lambs fed white lupin diet, which in turn might alter the ruminal flora, influencing the BH process. Moreover, lupin could modulate ruminal microbiota or alter microbial access to dietary lipids, likely due to its fermentable fibre or secondary plant metabolites, such as monoterpenes including camphor and eucalyptol (Glasser et al., 2008; Morsy et al., 2024). Similar modulatory effects of plant-derived secondary compounds on ruminal fermentation and BH have been reported in lambs fed polyphenol-rich diets (Aderao et al., 2025).

In the current experiment, the elevated levels of VA in the rumen and abomasal digesta resulted in more than a 50 % increase of this fatty acid in LUP meat. This transfer of VA from the rumen to muscle tissue aligns with previous findings (Priolo et al., 2021; Menci et al., 2023). Consequently, dietary white lupin led a higher proportion of RA in meat compared to the control, further enhancing the

health-promoting profile of the meat. Indeed, higher levels of RA have been associated with anticarcinogenic, anti-diabetic, and immune-modulating effects in animal models and human studies (Benjamin and Spener, 2009). This was expected, as the majority of RA in meat is synthesized from VA via the action of stearoyl-CoA 9-desaturase (SCD) in muscle (Bessa et al., 2015). However, the observed decrease in the desaturase index (SCDi17) suggests altered lipid metabolism at the tissue level, possibly due to down-regulation of desaturase gene expression in response to metabolic stress in lambs (Ntambi and Miyazaki, 2004).

The absence of differences in IMF between CON and LUP lambs, despite the higher final body weight of the CON group, is physiologically plausible. In young lambs, IMF deposition does not increase proportionally with body weight or overall carcass fat, as subcutaneous and visceral depots respond more readily to changes in energy intake (Pethick et al., 2005). By contrast, IMF accrual is slower, more genetically regulated, and less sensitive to short-term growth variation (Hocquette et al., 2010; Wood et al., 2008). Consequently, the greater body weight of CON lambs did not translate into higher IMF levels, consistent with previous evidence showing that IMF deposition in growing ruminants is relatively independent of rapid changes in adiposity (Hocquette et al., 2010).

Dietary inclusion of white lupin increased the intramuscular proportion of n-3 PUFA, including C20:5 n-3, C22:5 n-3 and C22:6 n-3, and significantly lowed the n-6/n-3 ratio. This could be the result of the activity of the elongase enzyme, which produced significantly higher amounts of long-chain n-3 (C20:5 n-3, C22:5 n-3 and C22:6 n-3) due to higher amounts of ALA substrate (Scollan et al., 2001). Furthermore, the results of the present study are in line with those of previous works showing that replacing soybean meal with lupin or other alternative feeds enhances the PUFA profile of meat (Vicenti et al., 2009; Vasta et al., 2008; Scerra et al., 2011). Future studies should explore gene expression and microbial ecology to further elucidate the mechanisms underpinning these compositional changes.

4.3. Oxidative stability of meat

The oxidative stability of meat is influenced by a complex interplay between pro-oxidant factors, such as PUFA, and antioxidant components, e.g. tocopherols (Bekhit et al., 2013). In this study, dietary treatment did not significantly alter the lipids oxidation in terms of hydroperoxides and TBARS.

The α-tocopherol, the most bioavailable isoform of vitamin E with powerful antioxidant properties (Bellés et al., 2019), although had similar concentration in both experimental diets, was significantly lower in the meat from lambs fed the LUP diet compared to CON, likely due to the lower intake by lambs.

The γ-tocopherol was surprisingly higher in the LUP meat sample than in CON, with a higher concentration than that of α-tocopherol. The underlying reasons for these variations, however, remain unclear. To the best of our knowledge, α-tocopherol is the most abundant form of vitamin E found in various tissues of both animals and humans. Indeed, the peripheral system of the body preferentially absorbs α-tocopherol over γ-tocopherol with consequent higher levels in the muscle (Ponnampalam et al., 2012). Therefore, due to the lack of studies on this topic, our results cannot be compared with the scientific literature. Nevertheless, considering the 5-fold higher γ-tocopherol of the LUP than the CON diet and the higher intake by the animals, it is reasonable to assume that the adsorption in the muscle tissue could inevitably be affected as well.

Concerning hydrophilic antioxidants, the DPPH assay showed a higher activity in the LUP group, suggesting a possible antioxidant effect exerted by the experimental diet. The DPPH assay, which reflects hydrophilic antioxidant activity, may capture subtle dietary effects not detected by assays more specific to hydrophilic antioxidants. Indeed, the antioxidant assays provided mixed results, with no consistent differences in FRAP, TEAC, or Folin-Ciocalteu values, indicating that the overall hydrophilic antioxidant capacity was only marginally influenced by the dietary treatment.

The colour of meat is a significant factor influencing consumer acceptance, and it is commonly evaluated through reflectance-based colour parameters (Faustman et al., 2010). In this study, the experimental diets did not affect the initial colour characteristics. However, storage time significantly influenced all measured parameters over the 7-day shelf-life trial. As expected, colour descriptors followed the typical meat discoloration pattern (Faustman et al., 2010; Chikwanha et al., 2019). Indeed, as reported in similar studies (Luciano et al., 2019; Menci et al., 2023), redness (a^*) and saturation (C^*) declined, while hue angle (hab) increased. Yellowness (b^*) also rose with extended storage time due to the meat browning (Menci et al., 2023; Natalello et al., 2020). Meat from white lupine-fed lambs exhibited a lower increase in hue angle over the investigated storage time compared to the control group, suggesting a potential delayed discoloration process. Hue angle serves as an effective indicator of meat browning and has been shown to strongly correlate with sensory panel evaluations of meat discoloration (Lee et al., 2005). This parameter could affect the meat purchasing decisions since consumers use discoloration as an indicator of freshness (Mancini and Hunt, 2005). A significant interaction between diet and storage time was observed also for lightness (L*), although this parameter is not strictly linked to lipid oxidation but rather to the oxidation of oxymyoglobin into metmyoglobin (Faustman et al., 2010). Slaughter body weight is also known to influence meat colour, with heavier lambs tending to have darker meat (Santos-Silva et al., 2002). In the present study, lambs in the LUP group had a significantly lower slaughter weight than those in the CON group. However, this difference did not negatively affect the colour parameters, which remained within the range of light pink meat (Sañudo et al., 1992).

Accordingly, metmyoglobin, a key marker of meat discoloration, peaked at day 7, confirming the progression of pigment oxidation and meat discoloration throughout storage. However, metmyoglobin accumulation over storage was significantly moderated in lambs fed white lupin seeds. This could be attributed to the lupine-derived secondary metabolites with antioxidant properties, such as eucalyptol (Ranucci et al., 2019; Morsy et al., 2024), that may contribute to greater oxidative stability. Oxidation of myoglobin to metmyoglobin imparts a brownish color, which reduces the visual appeal of meat and negatively impacts consumer acceptance (Faustman et al., 2010). The observed reduction in metmyoglobin formation aligns with findings from other studies where polyphenol-rich feeds reduced discoloration in meat improving color stability (Luciano et al., 2009; Serra et al., 2021).

5. Conclusion

This study demonstrates that the complete replacement of soybean meal and 30 % of maize of the total diet with 350 g/kg DM of white lupin seeds (*Lupinus albus* L.) in the diet of growing lambs significantly impairs feed intake and growth performance, likely due to reduced palatability and the nutritional value of lupin-based diet. Despite these limitations, the lambs fed white lupin-based diet elicited modifications in ruminal fermentation patterns and fatty acid profiles, both in the digestive tract and in meat, including increased levels of health-promoting MUFA and n-3 PUFA, and a substantial improvement in the n-6/n-3 ratio. Furthermore, dietary lupin increased the meat concentration of RA and VA, suggesting potential benefits for meat quality and human health. The oxidative and color stability were not negatively affected by the inclusion of lupin seeds in lamb diet.

Limitations and future prospects

The decline in performance associated to the white lupin diet underscores the need for further formulation adjustments. Future studies should prioritize strategies to improve palatability, as reduced feed intake appears to be the major contributor to the lower growth performance of lambs, defining the optimal inclusion levels and assessing the economic feasibility of partial substitution strategies under practical production conditions. Moreover, determining the concentration of antinutritional factors such as alkaloids in white lupin seeds and identifying effective treatments to reduce their impact will be essential for enhancing the nutritive value of white lupin-based diets. Additionally, investigating microbial and gene expression responses may provide deeper insights into the mechanisms underlying the observed changes in fermentation and lipid metabolism.

CRediT authorship contribution statement

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

Ethics approval

Experimental procedures with animals were conducted in accordance with European Union (Council Directive 2010/63/EU) legislation for the protection of animals used for experimental and other scientific purposes and reviewed by the Research Ethics Committees of the University of Catania (protocol number: 169878).

Consent for publication

The authors declare to consent for publications.

Funding

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D. D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. Part of this research was funded by the projects "Gen.Al.Tec." (Linea 1—PIAno di inCEntivi per la RIcerca di Ateneo 2024/2026; P.I. Andrea Criscione) and "GreenFeed" (Linea 3 Starting Grant - PIAno di inCEntivi per la RIcerca di Ateneo 2020–2022; P.I. Antonio Natalello).

Declaration of Competing Interest

The authors declare no competing interests.

References

Aderao, G.N., Sahoo, A., Bhatt, R.S., Soni, L.K., Gadekar, Y.P., Kumawat, P.K., 2025. Ziziphus nummularia and acacia nilotica leaves based polyphenols rich diet modulates fatty acid profile and keeping quality of lamb meat. Small Rumin. Res. https://doi.org/10.1016/j.smallrumres.2024.107406.

Alves, S.P., Francisco, A., Costa, M., Santos-Silva, J., Bessa, R.J., 2017. Biohydrogenation patterns in digestive contents and plasma of lambs fed increasing levels of a tanniferous bush (Cistus ladanifer L.) and vegetable oils. Anim. Feed Sci. Technol. 225, 157–172. https://doi.org/10.1016/j.anifeedsci.2017.01.018.

AOAC (Association of Official Analytical Chemists), 1995. Official Methods of Analysis, 16th edition. AOAC, Washington, DC, USA.

Aouadi, D., Luciano, G., Vasta, V., Nasri, S., Brogna, D.M.R., Abidi, S., Salem, H.B., 2014. The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of Artemisia herba alba and Rosmarinus officinalis essential oils. Meat Sci. 97 (2), 237–243. https://doi.org/10.1016/j.meatsci.2014.02.005.

Bekhit, A.E.-D.A., Hopkins, D.L., Fahri, F.T., Ponnampalam, E.N., 2013. Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. Compr. Rev. Food Sci. Food Saf. 12 (5), 565–597. https://doi.org/10.1111/1541-4337.12027.

Bellés, M., Del Mar Campo, M., Roncalés, P., Beltrán, J.A., 2019. Supranutritional doses of vitamin E to improve lamb meat quality. Meat Sci. 149, 14–23. https://doi.org/10.1016/j.meatsci.2018.11.002.
Benjamin, S., Spener, F., 2009. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr. Metab. 6, 36. https://doi.org/10.1186/1743-7075-6-36.
Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as ameasure of "antioxidant power": The FRAP assay. Anal. Biochem 239 (1), 70–76. https://doi.org/10.1006/abio.1996.0292.
Bernhoft, A., Siem, H., Bjertness, E., Meltzer, M., Flaten, T., Holmsen, E., 2010. Bioactive compounds in plants–benefits and risks for man and animals. Nor. Acad. Sci. Lett. Oslo 13–14.
Bertolín, J.R., Joy, M., Rufino-Moya, P.J., Lobón, S., Blanco, M., 2018. Simultaneous determination of carotenoids, tocopherols, retinol and cholesterol in ovine lyophilised samples of milk, meat, and liver and in unprocessed/raw samples of fat. Food Chem. 257, 182–188. https://doi.org/10.1016/j.foodchem.2018.02.139.
Bessa, R.J.B., Alves, S.P., Santos-Silva, J., 2015. Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. Eur. J. Lipid Sci. Technol. 117, 1325–1344. https://doi.org/10.1002/ejlt.201400468.
Boudalia, S., Smeti, S., Dawit, M., Senbeta, E.K., Gueroui, Y., Dotas, V., Bousbia, A., Symeon, G.K., 2024. Alternative Approaches to Feeding Small Ruminants and Their Potential Benefits. Animals 14 (6), 904. https://doi.org/10.3390/ani14060904.
Cannas, A., Tedeschi, L., Fox, D., Pell, A.N., Van Soest, P., 2004. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. J. Anim. Sci. 82, 149–169. https://doi.org/10.2527/2004.821149x.
Caramona, A., Martins, A.M., Seixas, J., Marto, J., 2024. The use, reuse and valorization of lupin and its industry by-products for dermocosmetics applications. Sustain. Chem. Pharm. 38, 101477. https://doi.org/10.1016/j.scp.2024.101477.
Chikwanha, O.C., Moelich, E., Gouws, P., Muchenje, V., Nolte, J.V.E., Dugan, M.E.R., Mapiye, C., 2019. Effects of feeding increasing levels of grape (Vitis vinifera cv. Pinotage) pomace on lamb shelf-life and eating quality (Article). Meat Sci. 157, 107887. https://doi.org/10.1016/j.meatsci.2019.107887.
Chiofalo, B., Presti, V.L., Chiofalo, V., Gresta, F., 2012. The productive traits, fatty acid profile and nutritional indices of three lupin (Lupinus spp.) species cultivated in a Mediterranean environment for the livestock. Anim. Feed Sci. Technol. 171 (2-4), 230–239. https://doi.org/10.1016/j.anifeedsci.2011.11.005.
da Silva, R.F.B., Moran, E.F., Millington, J.D.A., Viña, A., Liu, J., 2023. Complex relationships between soybean trade destination and tropical deforestation. Sci. Rep. 13. https://doi.org/10.1038/s41598-023-38405-1.
Dronne, Y., 2003. L'approvisionnement en protéines de la France dans son contexte européen et mondial. Fourrages 174, 107–128.
Eurostat, European Union. 2024. Eu Agricultural Outlook ⟨https://agriculture.ec.europa.eu/data-and-analysis/markets/outlook/medium-term_en⟩ (accessed April 26, 2025).
Facciolongo, A.M., Rubino, G., Zarrilli, A., Vicenti, A., Ragni, M., Toteda, F., 2014. Alternative protein sources in lamb feeding 1. Effects on productive performances, carcass characteristics and energy and protein metabolism. Prog. Nutr. 16 (2), 105–115.
Fadel, A.H., Kamarudin, M.S., Romano, N., Ebrahimi, M., Saad, C.R., Samsudin, A.A., 2017. Carob seed germ meal as a partial soybean meal replacement in the diets of red hybrid tilapia. Egypt. J. Aquat. Res. 43 (4), 337–343. https://doi.org/10.1016/j.ejar.2017.09.007.
Faustman, C., Sun, Q., Mancini, R., Suman, S.P., 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86 (1), 86–94. https://doi.org/10.1016/j.meatsci.2010.04.025.
Food and Agriculture Organization of the United Nations. 2024. "Yearly per capita supply of all meat" [dataset] – processed by Our World in Data. ⟨https://ourworldindata.org/grapher/meat-supply-per-person⟩ (Retrieved April 18, 2025).
Froidmont, E., Bartiaux-Thill, N., 2003. Utilisation du lupin et du pois dans l'alimentation des vaches laitières hautes productrices. Fourrag. (Fr.) (174).
Froidmont, E., Bartiaux-Thill, N., 2004. Suitability of lupin and pea seeds as a substitute for soybean meal in high-producing dairy cow feed. Anim. Res. 53, 475–487.
Glasser, F., Ferlay, A., Chilliard, Y., 2008. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J. Dairy Sci. 91 (12), 4687–4703. https://doi.org/10.3168/jds.2008-0987.
Gresta, F., Oteri, M., Scordia, D., Costale, A., Armone, R., Meineri, G., Chiofalo, B., 2023. White lupin (Lupinus albus L.), an alternative legume for animal feeding in the Mediterranean area. Agriculture 13 (2), 434. https://doi.org/10.3390/agriculture13020434.
Guduk, E., Hall, M.B., Zanton, G.I., Steinberger, A.J., Weimer, P.J., Suen, G., Weigel, K.A., 2023. Characterization of rumen microbiota in lactating Holstein cows fed molasses versus corn grain at two levels of rumen-degradable protein (Article). Front. Micro 2, 1204988. https://doi.org/10.3389/frmbi.2023.1204988.
Hill, N.S., Roberts, C.A., 2020. Plant Chemistry and Antiquality Components in Forage. In: Moore, K.J., Collins, M., Nelson, C.J., Redfearn, D.D. (Eds.), In Forages. https://doi.org/10.1002/9781119436669.ch35.
Hocquette, J.F., Gondret, F., Baéza, E., Médale, F., Jurie, C., Pethick, D.W., 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4 (2), 303–319. https://doi.org/10.1017/S1751731109991091.
Joch, M., Kudrna, V., 2020. Partial replacement of soybean meal by white lupine seeds in the diet of dairy cows. AsianAustralas. J. Anim. Sci. 33 (6), 957–964. https://doi.org/10.5713/ajas.19.0457.
Keller, J., Marmit, S.P., Bunzel, M., 2022. Structural Characterization of Dietary Fiber from Different Lupin Species (*Lupinus* sp. J. Agric. Food Chem. 70 (27), 8430–8440. https://doi.org/10.1021/acs.jafc.2c02028.
Khan, M.K., Karnpanit, W., Nasar-Abbas, S.M., Huma, Z.E., Jayasena, V., 2015. Phytochemical composition and bioactivities of lupin: a review. Int. J. Food Sci. Tech. 50 (9), 2004–2012. https://doi.org/10.1111/ijfs.12796.
Krzywicki, K., 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci. 3 (1), 1–10. https://doi.org/10.1016/0309-1740(79)90019-6.
Kung Jr, L., Maciorowski, K., Powell, K.M., Weidner, S., Eley, C.L., 1991. Lupin as a protein supplement for growing lambs. J. Anim. Sci. 69 (8), 3398–3405. https://doi.org/10.2527/1991.6983398x.
Lee, S., Decker, E.A., Faustman, C., Mancini, R.A., 2005. The effects of antioxidant combinations on color and lipid oxidation in n-3 oil fortified ground beef patties. Meat Sci. 70 (4), 683–689. https://doi.org/10.1016/j.meatsci.2005.02.017.
Lestingi, A., Facciolongo, A.M., Jambrenghi, A.C., Ragni, M., Toteda, F., 2016. The use of peas and sweet lupin seeds alone or in association for fattening lambs: Effects on performance, blood parameters and meat quality. Small Rumin. Res. 143, 15–23. https://doi.org/10.1016/j.smallrumres.2016.08.006.
Licitra, G., Hernandez, T., Van Soest, P., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3.
Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A., 2009. Dietary tannins improve lamb meat colour stability. Meat Sci. 81 (1), 120–125. https://doi.org/10.1016/j.meatsci.2008.07.006.
Luciano, G., Natalello, A., Mattioli, S., Pauselli, M., Sebastiani, B., Niderkorn, V., Valenti, B., 2019. Feeding lambs with silage mixtures of grass, sainfoin and red clover improves meat oxidative stability under high oxidative challenge. Meat Sci. 156, 59–67.
Makkar, H.P.S., Blümmel, M., Borowy, N.K., Becker, K., 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61 (2), 161–165. https://doi.org/10.1002/jsfa.2740610205.
Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Sci. 71 (1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003.
Maqsood, S., Benjakul, S., Balange, A.K., 2012. Effect of tannic acid and kiam wood extract on lipid oxidation and textural properties of fish emulsion sausages during refrigerated storage. Food Chem. 130 (2), 408–416. https://doi.org/10.1016/j.foodchem.2011.07.065.
Masoero, F., Pulimeno, A.M., Rossi, F., 2005. Effect of extrusion, espansion and toasting on the nutritional value of peas, faba beans and lupins. Ital. J. Anim. Sci. 4 (2), 177–189. https://doi.org/10.4081/ijas.2005.177.
Menci, R., Biondi, L., Natalello, A., Lanza, M., Priolo, A., Valenti, B., Bertino, A., Scerra, M., Luciano, G., 2023. Feeding hazelnut skin to lambs delays lipid oxidation in meat (Article). Meat Sci. 202, 109218. https://doi.org/10.1016/j.meatsci.2023.109218.

Morsy, T.A., Kholif, A.E., Adegbeye, M.J., Olafadehan, O.A., Gouda, G.A., Fahmy, M., Chahine, M., 2024. Lupin seed supplementation as a functional feed additive: in vitro ruminal gas, methane and carbon dioxide production, fermentation kinetics, and nutrient degradability. Animals 14 (14), 2119. https://doi.org/10.3390/ani14142119.

Musati, M., Bertino, A., Cannone, M.S., Mangano, F., Luciano, G., Priolo, A., Bella, M.S., Biondi, L., Scerra, M., Mangione, G., Natalello, A., 2025. Dietary hazelnut skin prevents lipid oxidation in lamb enriched in omega-3 polyunsaturated fatty acids (Article). Meat Sci. 225, 109811. https://doi.org/10.1016/j.meatsci.2025.109811.

Natalello, A., Khelil-Arfa, H., Luciano, G., Zoon, M., Menci, R., Scerra, M., Blanchard, A., Mangano, F., Biondi, L., Priolo, A., 2022. Effect of different levels of organic zinc supplementation on pork quality. Meat sci. 186, 108731. https://doi.org/10.1016/j.meatsci.2021.108731.

Natalello, A., Luciano, G., Morbidini, L., Valenti, B., Pauselli, M., Frutos, P., Biondi, L., Rufino-Moya, P.J., Lanza, M., Priolo, A., 2019. Effect of Feeding Pomegranate Byproduct on Fatty Acid Composition of Ruminal Digesta, Liver, and Muscle in Lambs. J. Agric. Food Chem. 67 (16), 4472–4482. https://doi.org/10.1021/acs.jafc.9b00307.

Natalello, A., Priolo, A., Valenti, B., Codini, M., Mattioli, S., Pauselli, M., Puccio, M., Lanza, M., Stergiadis, S., Luciano, G., 2020. Dietary pomegranate by-product improves oxidative stability of lamb meat (Article). Meat Sci. 162, 108037. https://doi.org/10.1016/j.meatsci.2019.108037.

Ntambi, J.M., Miyazaki, M., 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 43 (2), 91–104. https://doi.org/10.1016/s0163-7827(03)00039-0.

Offner, A., Bach, A., Sauvant, D., 2003. Quantitative review of in situ starch degradation in the rumen. Anim. Feed Sci. Technol. 106 (1-4), 81–93. https://doi.org/10.1016/S0377-8401(03)00038-5.

Oliveira, M.A., Alves, S.P., Santos-Silva, J., Bessa, R.J.B., 2016. Effects of clays used as oil adsorbents in lamb diets on fatty acid composition of abomasal digesta and meat. Anim. Feed Sci. Technol. 213, 64–73. https://doi.org/10.1016/j.anifeedsci.2016.01.006.

Parrini, S., Aquilani, C., Pugliese, C., Bozzi, R., Sirtori, F., 2023. Soybean replacement by alternative protein sources in pig nutrition and its effect on meat quality. Animals 13 (3), 494. https://doi.org/10.3390/ani13030494.

Pethick, D.W., Davidson, R., Hopkins, D.L., Jacob, R.H., D'Souza, D.N., Thompson, J.M., Walker, P.J., 2005. The effect of dietary treatment on meat quality and on consumer perception of sheep meat eating quality. Aust. J. Exp. Agric. 45, 517–524. https://doi.org/10.1071/EA03255.

Ponnampalam, E.N., Burnett, V.F., Norng, S., Warner, R.D., Jacobs, J.L., 2012. Vitamin E and fatty acid content of lamb meat from perennial pasture or annual pasture systems with supplements. Anim. Prod. Sci. 52, 255–262. https://doi.org/10.1071/AN11054.

Priolo, A., Valenti, B., Natalello, A., Bella, M., Luciano, G., Pauselli, M., 2021. Fatty acid metabolism in lambs fed hazelnut skin as a partial replacer of maize (Article). Anim. Feed Sci. Technol. 272, 114794. https://doi.org/10.1016/j.anifeedsci.2020.114794.

Putri, E.M., Zain, M., Warly, L., Hermon, H., 2021. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on in vitro digestibility, rumen fermentation, and microbial protein synthesis. Vet. World 14 (3), 640–648. https://doi.org/10.14202/vetworld.2021.640-648.

Ranucci, D., Branciari, R., Cobellis, G., Acuti, G., Miraglia, D., Olivieri, O., Roila, R., Trabalza-Marinucci, M., 2019. Dietary essential oil mix improves oxidative stability and hygienic characteristic of lamb meat. Small Rumin. Res. 175, 104–109. https://doi.org/10.1016/j.smallrumres.2019.04.012.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Reardon, J., Foreman, J.A., Searcy, R.L., 1966. New reactants for the colorimetric determination of ammonia. Clin. Chim. Acta 14 (3), 203–205. https://doi.org/10.1016/0009-8981(66)90120-3.

Regulation E.U. 2018/848. ⟨https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0848⟩.

Rufino-Moya, P.J., Joy, M., Lobón, S., Bertolín, J.R., Blanco, M., 2020. Carotenoids and Liposoluble vitamins in the plasma and tissues of light lambs given different maternal feedings and fattening concentrates. Animals 10 (10), 1813. https://doi.org/10.3390/ani10101813.

Santos-Silva, J., Bessa, R.J.B., Santos-Silva, F.J.L.P.S., 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. Livest. Prod. Sci. 77 (2-3), 187–194. https://doi.org/10.1016/S0301-6226(02)00059-3.

Sañudo, C., Delfa, R., Gonzalez, C., Alcalde, M.J., Casas, M., Santolaria, P., Vigil, E., 1992. Calidad de la carne del ternasco. ITEA 88 (3), 221–227.

Scerra, M., Caparra, P., Foti, F., Cilione, C., Zappia, G., Motta, C., Scerra, V., 2011. Intramuscular fatty acid composition of lambs fed diets containing alternative protein sources. Meat Sci. 87 (3), 229–233. https://doi.org/10.1016/j.meatsci.2010.10.015.

Scollan, N.D., Choi, N.-J., Kurt, E., Fisher, A.V., Enser, M., Wood, J.D., 2001. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. Br. J. Nutr. 85, 115–124. https://doi.org/10.1079/BJN2000223.

Serra, V., Salvatori, G., Pastorelli, G., 2021. Dietary polyphenol supplementation in food producing animals: effects on the quality of derived products. Animals 11 (2), 401. https://doi.org/10.3390/ani11020401.

Shingfield, K.J., Ahvenjarvi, S., Toivonen, V., Arola, A., Nurmela, K.V.V., Huhtanen, P., Griinari, J.M., 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim. Sci. 77, 165–179. https://doi.org/10.1017/S1357729800053765.

Siu, G.M., Draper, H.H., 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43, 1147–1149. https://doi.org/10.1111/j.1365-2621.1978.tb15256.x.

Šufliarský, P., Volek, Z., Ebeid, T.A., Zita, L., 2024. Dietary inclusion of white lupine seeds and their byproducts in rabbits can contribute to EU agricultural sustainability: a review. World Rabbit Sci. 32 (3), 145–160.

Tefera, G., Tegegne, F., Mekuriaw, Y., Melaku, S., Tsunekawa, A., 2015. Effects of different forms of white lupin (*Lupinus albus*) grain supplementation on feed intake, digestibility, growth performance and carcass characteristics of Washera sheep fed Rhodes grass (Chloris gayana) hay-based diets. Trop. Anim. Health Prod. 47 (8), 1581–1590. https://doi.org/10.1007/s11250-015-0901-9.

Ulbricht, T.L., Southgate, D.A., 1991. Coronary heart disease: seven dietary factors. Lancet 338 (8773), 985–992. https://doi.org/10.1016/0140-6736(91)91846-m.

Um, K.H., Park, B.K., 2022. Study on the rumen fermentation, growth performance and carcass characteristics according to the supplementation of lupin flake in Hanwoo steers. J. Anim. Sci. Technol. 64 (6), 1077–1091. https://doi.org/10.5187/jast.2022.e79.

Valenti, B., Luciano, G., Pauselli, M., Mattioli, S., Biondi, L., Priolo, A., Natalello, A., Morbidini, L., Lanza, M., 2018. Dried tomato pomace supplementation to reduce lamb concentrate intake: effects on growth performance and meat quality. Meat Sci. 145, 63–70. https://doi.org/10.1016/j.meatsci.2018.06.009.

Van Soest, Pv, Robertson, J., Lewis, B., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vasta, V., Nudda, A., Cannas, A., Lanza, M., Priolo, A., 2008. Alternative feed resources and their effects on the quality of meat and milk from small ruminants. Anim. Feed Sci. Technol. 147 (1-3), 223–246. https://doi.org/10.1016/j.anifeedsci.2007.09.020.

Vicenti, A., Toteda, F., Di Turi, L., Cocca, C., Perrucci, M., Melodia, L., Ragni, M., 2009. Use of sweet lupin (Lupinus albus L. var. Multitalia) in feeding for Podolian young bulls and influence on productive performances and meat quality traits. Meat Sci. 82 (2), 247–251. https://doi.org/10.1016/j.meatsci.2009.01.018.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78 (4), 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019.

Yen, G.C., Wu, J.Y., 1999. Antioxidant and radical scavenging properties of extracts from Ganoderma tsugae. Food Chem. 65 (3), 375–379. https://doi.org/10.1016/S0308-8146(98)00239-8.