Food Bioscience 58 (2024) 103610

Contents lists available at ScienceDirect

Food Bioscience

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

Assessment of use of nutritional and organoleptic traits to differentiate the origin of Montesina lambs breed under three feeding regimes

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ARTICLE INFO

Keywords:
Authentication
Traceability
Grazing lamb
Indigenous breed
Sensorial traits
Biomarkers

ABSTRACT

In order to contribute to our knowledge of the meat quality of local lamb breeds in Spain, we carried out a characterization of the Montesina lamb breed, including nutritional (proximal composition, fatty acid profile, mineral content) and organoleptic (color, water holding capacity, shear force and volatile compounds) traits. The lambs were distributed in the following three production systems: spring grazing (SP; n = 10); winter grazing supplemented with concentrate (WP + c; n = 10) and concentrate supplemented with forage (C; n = 10). A combined statistical analysis using ANOVA, principal components and discriminant analysis by steps allowed us to select biomarkers in Montesina lambs' meat to identify the production system. The lambs' meat SP showed the highest moisture content (73.32%) and the lowest fat (1.05%). The lowest redness and yellowness color indices were observed in C lamb's meat (6.76 and 10.08, respectively). A higher content of aldehydes, C18:3n-3 and C22:5 n-3 DPA was associated to SP and WP + c, while alcohols were associated to C lamb's diet. Alcohol and aldehyde compounds, in combination with C18:3n-3 and C22:5 n-3 DPA fatty acids, could be proposed as potential biomarkers to differentiate the three production systems and as a tool to monitor the traceability of lambs' meat from the Montesina breed.

1. Introduction

According to a recent report issued by the United Nations Food and Agriculture Organization (FAO, 2020), there is an increasing interest worldwide in the genetic resources of indigenous animals found in particular regions around the globe because they bring benefits such as sustainable economic development and food security for local communities. Indigenous sheep breeds are characterized throughout the world by being adapted, by means of natural or artificial selection, to different environments and production systems. These local breeds deliver a wide range of products (meat, dairy products and wool, among others) and ecosystem services that support the local farmers' livelihoods. Generally, indigenous breeds offer lower production yields than genetically improved breeds. However, indigenous breeds present a higher ability to adapt to different feed systems (including grazing or concentrate), water availability, climate change and diseases (Hoffmann, 2011). In addition to the breed, in the sheep markets, we can find several commercial categories such as the feeding systems, origin of the breed, organic production or slaughter age of lambs (Campo et al., 2021). The results reported by Gracia and Maza (2015) indicated that consumers were willing to buy meat products from local breeds due to their social embeddedness with the local area of production. In addition to the quality features related to the nutritional and organoleptic (technological and sensory) characteristics of the meat that are valued by consumers at the time of deciding to purchase (Tomasevic et al., 2021), Gracia and Maza (2015), reported that 86% of consumer respondents said that they would probably/definitely buy lambs' meat from local sources if it was available at their usual butcher's shop.

In order to develop and support local breeds, a suitable food policy should include informing consumers about the relationship between meat quality and the origin of the lambs. In this context, as a first step to encourage autochthonous breed production, the Spanish government has announced several regulations, such as R.D. 45/2019 on the conservation, improvement and promotion of local animal breeds, followed by R.D. 505/2013 on the use of the trademark '100% autochthonous breed' for animal products. Besides, at the European level, meat from

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https://doi.org/10.1016/j.fbio.2024.103610
Received 27 August 2023; Received in revised form 12 December 2023; Accepted 10 January 2024
Available online 19 January 2024

local breeds is rigorously protected by Geographical Protected Indication (GPI) labels that guarantee the local breed origin and husbandry practices (Council Regulation (EC) No 510/2006).

The latest Official Catalog of Livestock Breeds of Spain (R.D. 45/2019) recognizes a total of 38 local autochthonous breeds of sheep used for their economic, zootechnical, productive, cultural, environmental or social interest. Among these local breeds can be found the 'Montesina' breed, which has its production area in the South of Spain (Andalusia). Due to the particular climatic conditions and the seasonality of available natural food resources, Montesina and other Spanish lambs are traditionally slaughtered at an early age. In fact, according to the traditional Montesina breed production system, the most common product in the market corresponds to the 'Ternasco lamb type', which are slaughtered at three months old, with a suckling diet during the first month of life and pasture and supplementing with grain plus forage for two more months before slaughter. The influence of the feeding system in ruminants on the characteristics of meat has been widely reported (Cabiddu et al., 2022). In this context, a novel feeding practice, based on grass feeding without concentrate during the spring season is gaining popularity in Montesina breeding locations, because it both reduces expenses on farms, and enhances the healthy qualities of the meat and the sustainability of rural areas.

To guarantee the origin of food, meat-omics is an emerging strategy to obtain comprehensive information about composition, nutritional value, safety and meat quality (Munekata et al., 2021). This discipline encompasses the use of biomarkers (e.g., lipidomes, proteomes, volatilomes, minerals, among other molecules) to identify the product origin, production system or genuine nutritive values of foodstuffs. Several biomarkers have been proposed to determine the animals' diet (Qie et al., 2021). Among these, nutritional compounds in meat, such as the fatty acid profile (for example C18:1cis9 or C18:2n-6; Cabiddu et al., 2022) or volatile compounds like hexanal or 1-octanol (Del Bianco et al., 2021) have been used as potential and practical biomarkers to provide information on animal feeding systems and to track the origin of food products from different production systems. However, to the best of our knowledge, other parameters such as organoleptic traits have never been explored as potential biomarkers, and these could be useful in order to determine the origin of the meat. In fact, the combined use of nutritional and organoleptic traits in meat could be an effective tool for the traceability of production systems of autochthonous breeds of lamb. Such knowledge will also expand our understanding of the meat quality from local lamb breeds under different production systems.

In the present work, we have included the nutritional and organoleptic traits of meat to identify the feeding system of the lambs. Volatile compounds may be classified as organoleptic traits due to their direct impact on key sensory aspects such as flavor and aroma. Thus, the aim of this study was characterize the nutritional and organoleptic traits in meat of the Montesina lamb breed and to identify the biomarkers in the meat, in order to classify the final products based on the three main production systems employed (spring pasture; winter pasture and grain or concentrate plus forage). This information could be used as a tool to enhance the traceability of system meat production in the Montesina breed and reinforce the control of the products and production systems protected by quality labels such as GPI or '100% autochthonous breed', thereby contributing to the development of local sheep breeds.

2. Materials and methods

The current research was conducted in the region of Andalusia (South of Spain), one of the areas with the greatest ecological biodiversity for autochthonous lamb breeds in Spain (R.D. 45/2019). All the procedures used in this experiment followed EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The ethical review and approval were waived for this study because the lambs were raised on local farms belonging to the "Asociación Nacional de Criadores de Oveja Montesina", according to the rules of Royal Decree 1312/2005, which recognizes sheep associations and the upkeep of the breed's Stud Book. The laboratory staff had B, C and D1 accreditation for animal experimentation and other scientific purposes, according to R.D. 1201/2005.

2.1. Selection of lambs

In the traditional area of production of the Montesina breed (Jaén, Spain), thirty single-birth male lambs of the Montesina breed were selected on two farms and fed with mother's milk until approximately one month of age. After that, the lambs were weaned and raised according to the traditional production systems, in the geographical region previously described, using pasture, grain and forage as follows: on Farm 1 (Otiñar, Latitude 37°46′43.2″N; Longitude 3°47′25.1″W), the first group of 10 lambs (SP) was raised on spring natural pasture grass from the Andalusia northeast area mountain, with *Quercus ilex* and *Q. faginea* canopy, including polyphytic meadows composed mainly of grasses (*Lolium multiforum*, *Lolium perenne* and *Dactylis glomerata*), leguminous (*Trifolium repens*, *Trifolium pratense* and *Medicago sativa*), and some minority species of asteraceae, cruciferae and umbelliferae families for two months in spring 2022 (March and April). The second group (n = 10) of lambs (WP + c) from Farm 1 was raised on winter pasture (grasses and leguminous), and green forage (oats, ryegrass and barley) for approximately 2 months (December 2021 and January 2022) and supplemented with concentrate (Table 1). Both of these groups were slaughtered when they had reached commercial weight (range 23–26 kg live weight). On Farm 2 (Valdepeñas de Jaén, Latitude 37°34′24.5″N; Longitude 3°45′23.0″W), a third group (n = 10) of stabled lambs (C) was raised on concentrate (Table 1) and barley straw for approximately 2 months until 25 kg live weight. The growth of the lambs was controlled by staff at the IFAPA center.

2.2. Slaughter procedures and muscle sampling

The lambs were slaughtered, using standard commercial procedures, according to the guidelines of European Council Regulation (Regulation (EC) No.1099/2009) on the protection of animals at the time of killing. The carcasses were chilled for 24 h at 4 °C. After that, the ultimate pH (pHu) was measured in the caudal area of the *longissimus dorsi* (LD) muscle from the left half of the carcass, using a Crison pH meter (Crison Instruments, S.A., Barcelona, Spain). In the abattoir, the carcasses were weighed and taken to the IFAPA center (Granada, Spain). The LD muscle from the left half of the carcass was extracted in the IFAPA laboratory for analysis. From the LD muscle, several steaks of approximately 50 g were obtained, which were then vacuum-packed to take nutritional and instrumental measurements of the organoleptic attributes of the meat. The samples were frozen at −20 °C for approximatively two months until the analysis was carried out. The left half of the carcass leg was collected, vacuum-packed and frozen at −20 °C for approximatively five months for meat sensory evaluation.

Table 1
Chemical composition of concentrate fed to Montesina lambs in this trial.

	Chemical composition (%, Dry matter basis)				
	Dry matter	Crude protein	Crude fiber	Ether extract	Ash
Concentrate supplement[a]	87.78	15.19	5.22	5.66	4.73

[a] Ingredients (%): barley grain (30.4), oatmeal (16.0), peas in seed form (15.8), soybean cake (9.0), sunflower seeds (8.5), corn (17.0), vegetable oil (1.30), salt (0.6), urea (0.6), sodium bicarbonate (0.4) and mineral and vitamin correctors (0.4). Minerals: Ca (1.22%); P (0.41%); Na (0.52%); Fe (89 mg); I (0.05 mg); Cu (16.9 mg); Mn (40 mg); Zn (106 mg); Se (0,048 mg).

2.3. Nutritive traits

2.3.1. Chemical composition

Fresh samples of LD muscle were homogenized using a Veo Home moulin 200 W grinder (Veotech Inc., KWG-130 B, Vannes, France) for further analysis. The protein content was analyzed by the AOAC (1992) procedure, using a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden). A conversion factor of 6.25 (Nx6.25) was used for calculations. The fat content was determined in pre-dried samples using an MQC + benchtop nuclear magnetic resonance fat analyzer (BRUKER Corporation, Coventry, West Midlands CV4 9, UK), according to the Official AOAC Method 2008.06 (AOAC, 2008). The samples were weighed pre- and post-drying at 105 °C for 24 h in an Heraeus oven (Thermo electron Corp., Barcelona 08028, Spain) to determine the moisture content (AOAC, 1978). The ash content was determined according to AOAC 920.153 (AOAC, 1920), using an electric hot plate and muffle furnace (Carbolite ELF 11/14 B, Sheffield, England) at 550 °C for 12 h. The samples were analyzed in triplicate.

2.3.2. Fatty acid profile

The fatty acid (FA) profile of intramuscular fat from the LD muscle was analyzed according to Aldai et al. (2006) method using a chromatograph GC, Agilent 6890 N (Agilent technologies Inc., Santa Clara, California 95,051, USA) equipped with an FID and fitted with a BPX-70 capillary column (120 m, 0.25 mm i. d., 0.2 m film thickness, SGE, Postnova Analytics Inc., Salt Lake City, UT 84102, USA). Automatic injection of the samples was carried out using an HP 7683 injector. The analyses were performed in duplicate. Samples of approximately 1 g of LD were thawed at 4 °C for 12 h and saponified in 6 mL of 5 M KOH in methanol:water (50:50 v/v) with hydroxyquinone (1 g L-1) at 60 °C for 1 h, after flushing with nitrogen, after which the mixture was diluted with 12 mL of 0.5% NaCl and 5 mL of petroleum ether and the non-saponifiable fraction was removed. To neutralize the KOH, 3 mL of glacial acetic acid was added. Double petroleum ether clearance was used to extract the FAMEs, with nitrogen used to evaporate the solvent. The extracted FAMEs were then methylated using 200 μL of TMS-DM in methanol:toluene (2:1, v/v) at 40 °C for 10 min, dried under nitrogen and dissolved in 1 mL of n-hexane containing 50 ppm of butylated hydroxy toluene. The samples were centrifuged at 20,000 g force maximum under 4 °C for 5 min. The supernatant was transferred for analysis into 2 mL autosampler vials for chromatography. The chromatographic conditions were as follows: initial column temperature 100 °C, programmed to increase at a rate of 30 °C/min to 158 °C and then at 1.5 °C/min to 190 °C, maintaining this temperature for 15 min, then at 2 °C/min to 200 °C and then increasing again at 10 °C/min to a final temperature of 240 °C for 10 min. The injector and detector were kept at 300 and 320 °C, respectively. Hydrogen was used as the carrier gas at a flow rate of 2.7 mL/min. The split ratio was 17.7:1, and 1 μL of solution was injected. Nonadecanoic acid methyl ester (C19:0 ME) at 10 mg/mL was used as an internal standard. Individual FAMEs were identified by comparing their retention times with those of authenticated standards from Sigma (Sigma Chemical Co. Ltd., Poole, UK). CLA isomers (9c-11t CLA; 9c-11c CLA and 10t-12c CLA), which were purchased from Matreya (>98% purity; Matreya, LLC, Pleasant Gap 16,823, USA). Individual fatty acids from the intramuscular fat were expressed as a percentage of the total fatty acids detected. The contents of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFAs) and the n-6/n-3 and PUFA/SFA ratios were calculated from the individual FA concentrations.

2.3.3. Mineral profile

To determine the mineral content in the meat, samples of LD muscle were thawed at 15−17 °C for 1 h. Detection and quantification were performed by the ICP-OES method in a ICP Spectro blue (Spectro Analytical Instruments GmbH, Kleve 47,533, Germany) in the case of calcium, iron, potassium, magnesium, sodium, phosphorus, selenium and zinc (Türkmen & Ciminli, 2007). Previously, hydrolysis samples with nitric acid were digested as samples in a Milestone ETHOS ONE microwave (Milestone Systems, Sorisole 24,010, Italy). The mineral content of the meat was expressed as mg/100 g fresh meat.

2.4. Organoleptic traits

2.4.1. Color, WHC and WBSF

Muscle color was measured on fresh samples at 48 h after slaughter (1 h of blooming), using a Minolta CM-2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in the CIELAB space (CIE, 1986). The area diameter measure was 8 mm, including a standard illuminant D65, with an observer angle of 10° and zero and white calibration. Each sample was measured five times and then averaged. The lightness (L*), redness (a*) and yellowness (b*) were recorded. The hue angle (H*) and chroma (C*) indexes were calculated as follows: $H^* = \tan^{-1}(b^*/a^*) \times 57.29$ (expressed in degrees) and $C^* = (a^2 + b^2)^{1/2}$. To determine the water loss of the meat (WHC), the filter paper pressure method was used (Guzmán et al., 2019). To do this, 5.0 g of raw meat was manually minced (3 Claveles® mincer) and covered with two filter papers (Albet 238, 11.0 cm diameter) and two thin plates of glass material, and then pressed with a load of 2.25 kg for 5 min. WHC was expressed as a percentage of water released with respect to the weight of initial sample. To assess the toughness of the meat, Warner–Brätzler shear force (WBSF) was measured on the LD muscle using a Stevens QTS 25 texture analyzer equipped with a WB device, as described in Guzmán et al., 2019. To do this, a vacuum-packed LD sample was thawed at 4 °C for 12 h. After that, vacuum-packed including a portion of LD was heated in a 75 °C water bath to an internal temperature of 70 °C. The LD muscle was then cut into slices with a cross-section of 1 cm^2 parallel to the muscle fibers, and the maximum shear force (kg/cm^2) was assessed in at least three subsamples of LD.

2.4.2. Volatile compounds

The profile of volatile compounds in the meat was obtained from the LD muscle (20 g), using the solid phase microextraction analysis technique. The samples were thawed at 4 °C overnight before analysis and were then cooked at 200 °C on a griddle (Jatta electro, GR266 1000 W, Abadiano, Vizcaya, Spain). The griddle was switched on for 15 min before the sample was grilled. Each sample was placed in the middle of the griddle to be grilled uniformly, and was cooked for approximately 3 min to a core temperature of 70 °C. Directly after cooking, the meat and all the fat released from the steak during cooking was chopped finely in an electric laboratory grinder (Janke and Kunkel A-10, IKA Labortechnik, Germany). Straight after chopping, 10 g of the cooked sample was placed in a headspace vial (Tekmar, 100 mL) and equilibrated for 40 min at 40 °C, prior to exposing the SPME fiber (Fiber Assembly 50/30 μm DVB/CAR/PDMS, StableFlex, 2 cm, 23 Ga, Gray-Notched; Bellefonte, Pennsylvania, U.S.A.) and then placed over the sample for a further 20 min. The analysis of volatile compounds was performed using a Thermo Scientific TRACE 1300 series gas chromatograph (Milan, Italy) equipped with a Thermo Scientific TRIPLUS RSH autosampler (Milan, Italy) for injection and coupled to an ion trap mass spectrometer (Thermo Scientific ISQ QD Single Quadrupole Mass Spectrometer; Milan, Italy). The desorption process, purges, injection, temperature ranges in column and other analytical conditions are reported in Gutiérrez et al. (2022). The volatile compounds were separated using a VF-WAXms fused silica capillary column (30 m length x 0.25 mm id x 0.50 μm film thickness, Agilent Technologies, Inc. 2012, USA). The mass spectra of the volatile compounds were generated by a MS equipped with an Ion trap. The data acquisition was performed by scanning the mass range 29–400 amu. in EI mode (70 eV with an emission current of 50 mA) at 1.9 microscans. The LRI (Stashenko & Martínez, 2010) were calculated by previous injection of standards of saturated n-alkanes (C6-C22) under the same GC–MS conditions. The volatile compounds were expressed as a percentage of the total volatile compounds

identified.

2.5. Sensorial traits

For the sensorial analysis, the left legs of the lambs were used after being thawed under chilled conditions (4 °C for 24 h). The samples were evaluated by 30 untrained panelists consisting of members over the age of 18 from 10 families (three panelists from each family). Each family randomly received one leg from each diet treatment (SP; WP + c and C). According to the recommendations for cooking the samples, the legs were roasted with olive oil, water and salt in a standard oven at 220 °C for approximately 50 min. Next, a sensorial analysis was carried out to rate the attributes of tenderness, juiciness, flavor quality, lamb smell and overall appraisal on a 10-point scale according the scale prescribed by Martínez-Cerezo et al. (2005) with a trained sensory panel. The tenderness was rated from 1 = very tough to 10 = very tender; juiciness from 1 = very dry to 10 = very juicy; flavor quality from 1 = no flavor to 10 = very intense flavor, and lamb smell from 1 = low characteristic lamb smell to 10 = high characteristic lamb smell. Finally, the overall appraisal was rated from 1 = unpleasant to 10 = very pleasant.

2.6. Statistical analysis

The effect of the production system (SP, WP + c and C) on the nutritive, organoleptic and sensory traits in meat from the Montesina breed was analyzed using an ANOVA procedure. The models included the feeding system as a fixed effect among the subjects, according to the following statistical model:

$$Yijk = \mu + Wi + e\ ijk$$

where: Yijk = nutritional, organoleptic and sensorial traits; μ = least squares mean value, and Wi = fixed effect of feeding system (i = 1: SP; i = 2: WP + c; i = 3: C; eijk = random residual). The carcass weight was used as a linear covariate. A post hoc Duncan Test was employed with a confidence level of 95% to compare the means. In all cases, differences with $p < 0.05$ were considered significant. Principal component analysis was performed using significant variables to determine the number of independent traits that account for most of the variation in the carcass traits. Next, a stepwise forward discriminant analysis was carried out in order to classify the feeding systems according to the study variables. The discriminant analysis utilized a stepwise selection algorithm to discriminate between the three group levels (SP, WP + c and C). Independent variables that were significant in the ANOVA analysis were employed. For the stepwise regression, a value of 4.2 was used in the F statistic for inclusion, while a value of 3 was used for elimination. Wilks' lambda method shows which variables contributed significantly to the discriminant function. All the data were analyzed using STATISTICA software (data analysis software system), version 12 (www.statsoft.com, accessed on September 15, 2022, Tulsa, OK, USA)

3. Results and discussion

While consumers of North Europe prefer meat from lambs slaughtered at a greater weight, the market in Mediterranean countries prefers lean lamb carcasses, which correspond to light lambs (Gracia & De-Magistris, 2013). The Montesina lambs used in our study were therefore reared using spring grass (SP), winter grass plus concentrate (WP + c) and concentrate plus forage (C) and then slaughtered at 81 d ± 5.15 (mean ± s. e.), ranging from 23 to 26 kg of live weight, as corresponds to light lamb ('Ternasco lambs') in the Spanish market. No significant differences in carcass weight were observed (Table 2), with a range of 12.60–13.94 kg, corresponding to light carcasses in Spanish market (Campo et al., 2021).

At a commercial and scientific level, the pHu is an important indicator of meat quality. Here, the pHu values measured in meat from the

Table 2
Descriptive statistics (mean ± standard error) for nutritive traits in Montesino lambs grazing in spring pasture (SP), winter pasture supplemented with grain (WP + c), and concentrate and forage (C).

	SP (n = 10)	WP + c (n = 10)	C (n = 10)	*p*-values
Carcass weight (kg)	12.60 ± 1.22	13.22 ± 2.11	13.96 ± 2.24	ns
$pH_{24\ h}$	5.62 ± 0.119	5.69 ± 0.138	5.62 ± 0.133	ns
Chemical composition (% fresh meat)				
Moisture	73.32^{a}±0.74	72.05^{b} ± 0.70	72.37^{b} ± 1.00	0.012
Protein	24.55 ± 0.89	25.06 ± 0.31	24.94 ± 0.91	ns
Fat	1.05^{b} ± 0.33	1.93^{a}±0.59	1.64^{a}±0.68	0.010
Ash	1.09 ± 0.33	0.96 ± 0.18	1.05 ± 0.16	ns
Fatty acid profile of intramuscular fat (% of total fatty acids detected)				
TOTAL SFA	44.89^{b} ± 5.34	55.45^{a}±5.06	52.68^{a}±6.33	<0.001
Caprylic acid (C8:0)	0.57^{a}±0.04	0.11^{b} ± 0.02	0.14^{b} ± 0.02	ns
Caproic acid (C10:0)	0.28^{b} ± 0.03	0.38^{a}±0.03	0.35^{a}±0.05	0.007
Undecanoic acid (C11:0)	0.13 ± 0.03	0.05 ± 0.01	0.07 ± 0.01	ns
Lauric acid (C12:0)	0.86^{b} ± 0.06	0.93$^{a.b}$±0.06	0.99^{a}±0.04	0.002
Tridecanoic acid (C13:0)	0.28 ± 0.02	0.04 ± 0.01	0.07 ± 0.02	ns
Myristic acid (C14:0)	3.84^{b} ± 0.97	6.18^{a}±2.33	5.79^{a}±2.01	<0.001
Pentadecanoic acid (C15:0)	0.57^{b} ± 0.04	0.81^{a}±0.07	0.78^{a}±0.07	<0.001
Palmitic acid (C16:0)	20.17^{b} ± 4.77	26.88^{a}±5.98	25.28^{a}±5.11	<0.001
Margaric acid (C17:0)	0.71^{b} ± 0.02	1.08^{a}±0.08	0.99^{a}±0.07	<0.001
Stearic acid (C18:0)	16.34^{b} ± 3.24	18.05^{a}±3.33	17.30^{a}±4.12	0.011
Arachidic acid (C20:0)	0.43 ± 0.04	0.33 ± 0.06	0.34 ± 0.08	ns
Henicosanoic acid (C21:0)	0.15 ± 0.01	0.22 ± 0.03	0.14 ± 0.02	ns
Behenic acid (C22:0)	0.13 ± 0.02	0.10 ± 0.01	0.07 ± 0.03	ns
Tricosanoic acid (C23:0)	0.12 ± 0.04	0.12 ± 0.04	0.08 ± 0.01	ns
Lignoceric acid (C24:0)	0.30 ± 0.05	0.16 ± 0.05	0.28 ± 0.05	ns
TOTAL MUFA	33.81 ± 6.66	32.85 ± 7.31	34.96 ± 7.11	ns
Myristoleic (C14:1)	0.18^{b} ± 0.02	0.22^{a}±0.05	0.21^{a}±0.06	0.001
Pentadecenoic acid (C15:1)	0.10 ± 0.01	0.11 ± 0.03	0.07 ± 0.01	ns
Palmitoleic acid (C16:1)	1.11^{b} ± 0.07	1.57^{a}±0.06	1.55^{a}±0.07	0.006
Heptadecenoic acid (C17:1)	0.43^{b} ± 0.01	0.65^{a}±0.01	0.64^{a}±0.02	<0.001
Elaidic acid (C18:1n-9t)	0.71 ± 0.03	0.49 ± 0.02	0.71 ± 0.05	ns
Vaccenic acid (C18:1n-11t)	2.98^{a}±0.09	1.95^{b} ± 0.09	2.82^{a}±1.01	0.024
Oleic acid (C18:1n-9c)	27.62^{b} ± 5.75	27.32^{b} ± 6.02	28.39^{a}±6.33	0.039
Gadoleic acid (C20:1n-9)	0.28^{a}±0.01	0.22^{b} ± 0.02	0.21^{b} ± 0.02	0.006
Erucic acid (C22:1n-9	0.14 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	ns
Nervonic acid (C24:1)	0.28 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	ns
TOTAL PUFA	21.31^{a}±2.89	11.71^{b} ± 2.44	12.36^{b} ± 3.11	0.005
Linoleic acid *trans* (C18:2 n-6t)	0.71^{a}±0.07	0.54^{b} ± 0.05	0.42^{b} ± 0.07	0.038
Linoleic acid *cis* (C18:2 n-6c)	10.37^{a}±2.07	5.05^{b} ± 1.11	6.21^{b} ± 2.04	<0.001
γ Linolenic acid (C18:3 n-6)	0.13 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	ns
α Linolenic acid (C18:3 n-3)	2.42^{a}±1.03	1.41^{b} ± 0.55	0.99^{c}±0.36	<0.001
Rumenic acid (9c-11t CLA)	0.85 ± 0.37	0.49 ± 0.03	0.56 ± 0.05	ns
9c-11c Conjugate linoleic acid (9c-11c CLA)	0.16 ± 0.04	0.06 ± 0.01	0.06 ± 0.01	ns

(*continued on next page*)

Table 2 (*continued*)

	SP (n = 10)	WP + c (n = 10)	C (n = 10)	*p*-values
10t-12c Conjugate linoleic acid (10t-12c CLA)	0.12 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	ns
Eicosadienoic acid (C20:2)	0.13 ± 0.06	0.10 ± 0.2	0.07 ± 0.01	ns
γ-dihomolinolenic acid (C20:3 n-6)	0.29[a]±0.04	0.23[b] ± 0.01	0.28[a]±0.01	0.022
Arachidonic acid (C20:4 n-6)	2.56[a]±1.09	1.63[b] ± 1.11	1.69[b] ± 1.05	0.029
Eicosapentaenoic acid (C20:5 n-3 EPA)	0.85[a]±0.04	0.54[b] ± 0.01	0.56[b] ± 0.02	0.027
Docosadienoic acid (C22:2)	0.14 ± 0.09	0.05 ± 0.01	0.07 ± 0.01	ns
Docosapentaenoic acid (C22:5 n-3 DPA)	1.85[a]±0.55	1.08[b] ± 0.87	0.92[b] ± 0.79	0.019
Docosahexaenoic acid (C22:6 n-3 DHA)	0.71[a]±0.09	0.38[b] ± 0.01	0.35[b] ± 0.02	0.049
n-6/n-3	2.37[b] ± 0.950	2.25[b] ± 1.069	3.18[a]±0.765	0.004
PUFA/SFA	0.49[a]±0.175	0.23[b] ± 0.054	0.25[b] ± 0.103	<0.001
Mineral composition (mg/100g fresh meat)				
Calcium	5.28 ± 1.75	6.05 ± 1.89	6.16 ± 1.66	ns
Iron	1.44 ± 0.13	1.72 ± 0.56	2.16 ± 1.04	ns
Potassium	230.27 ± 41.01	251.09 ± 105.72	295.66 ± 116.96	ns
Magnesium	23.55[a]±0.65	19.30[b] ± 5.89	18.05[b] ± 7.62	0.003
Sodium	60.23 ± 11.77	52.38 ± 18.68	60.24 ± 17.59	ns
Phosphorus	256.32 ± 8.63	290.33 ± 105.58	325.24 ± 122.30	ns
Selenium	traces			
Zinc	1.87 ± 0.17	2.25 ± 0.69	2.15 ± 0.70	ns

Different superscripts ([a, b, c]) indicate significant differences ($p < 0.05$) among treatments; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ns: not significant.

Montesina lambs were in the range of 5.62–5.69 (Table 2). With reference to the extensive literature on the pH value of meat (Holman, Kerr, et al., 2021), technological and organoleptic problems were not expected in the sample used in this trial.

In this study, we used the usual nutritive, organoleptic and sensorial traits described in the literature to characterize lamb meat quality, and the results are given below.

3.1. Chemical composition, fatty acid profile and mineral composition

The nutritional traits of Montesina lambs' meat (chemical composition, fatty acid profile and mineral composition) for the three feeding models proposed in this study are provided in Table 2. The proximal and chemical composition of Montesina lambs' meat was compared with other local European lamb breeds. In this regard, moisture for Montesina meat (ranging from 72.05% to 73.32%) was lower than that observed by Echegaray et al. (2021) in the Portuguese Bordaleira breed (range 75.91%–78.00%); and by Santos et al. (2018) in the Spanish Merino breed (average 76.45%). The average values of intramuscular fat content in the Montesina breed (range from 1.05% to 1.93%) were lower than Spanish Merino (2.18%) and higher than the Portuguese breed (1.00%; Echegaray et al., 2018). On the other hand, protein content in meat from Montesina breed was found to be higher compared with other European breeds. For instance, the range of protein content in Montesina lambs was of 24.55%–25.06%, while local, Portuguese (range 19.32%–20.92%; Echegaray et al., 2021) and Merino Spanish breeds (range 19.3%–19.5%) were significantly lower. Regarding the total mineral content in meat, the values for the Montesina breed were lower (average 1.02%) than those reported in Portuguese (1.28%; Echegaray et al., 2021) and Spanish Merino breeds (1.53%; Santos et al., 2018). Our results agree with those reported by other authors who observed that lambs' meat with the highest fat content also showed the highest moisture content (Polidori et al., 2017). The only effect of the production system observed in Montesina lambs was for moisture ($p = 0.012$) and fat percentage in meat ($p = 0.010$) (Table 2). Lamb meat produced under the SP system had a significantly higher moisture percentage (73.32%) than meat from WP + c and C production systems (72.05% and 72.37%, respectively; $p < 0.05$). Observing values found for the moisture of meat in Montesina breed, it could be verified that the diet with the higher fibre content provided the highest averages for meat's moisture as also was reported by Germano et al. (2009) in Morada Nova and Santa Inês native lamb breeds or Karaca et al. (2016) in male lambs from Norduz breed. In contrast, meat from SP showed significantly lower amounts of intramuscular fat (1.05%) than meat from WP + c and C lambs (1.93% and 1.64%, respectively; $p < 0.05$). These results agree with other studies reporting that concentrate feeding increased intramuscular fat in meat (Cadavez et al., 2020; Cividini et al., 2014), who reported that the lambs reared in intensive production systems, using grain, showed higher intramuscular fat content than those reared in semi-extensive or extensive systems using mainly fresh pasture.

The descriptive statistics of the main FAs of intramuscular fat in the Montesina breed are presented in Table 2. A total of 39 FAs were identified and grouped into saturated FAs (SFA; n = 15), monounsaturated FAs (MUFA; n = 10) and polyunsaturated FAs (PUFA; n = 14). The FA profile obtained was similar to those reported by Bravo-Lamas et al. (2016) in meat from local Mediterranean lamb breeds, such as Rasa Aragonesa, sheep breed raised under Spanish traditional production systems. The most abundant FA in meat was oleic acid (C18:1n-9c), ranging from 27.32% to 28.39 % of the total FA detected, followed by palmitic acid (C16:0) and stearic acid (C18:0). These results are consistent with those reported in other breeds of Mediterranean lambs raised in similar production systems (Cividini et al., 2014). As expected, due to the ruminal process in the digestive tract, meat from ruminants is characterized by high SFA, which was observed here in Montesina lambs' meat, regardless of the production system (Wood et al., 2008).

The nutritional value of meat is closely related to the FA profile, which is mostly affected by the feeding strategies applied to the animals (Cadavez et al., 2020). In the present study, it is particularly noticeable that the abundance of SFAs ($p < 0.001$) and PUFAs ($p = 0.005$) in Montesina lambs' meat was significantly affected by the production system (Table 2); however, no impact was observed for the abundance of MUFAs. In fact, a significantly higher SFA percentage was detected in meat from lambs with concentrate in their diet ($p < 0.05$) (WP + c and C) than SP lambs. In contrast, SP lambs had significantly higher content of PUFAs, especially the n-3 fatty acids α linolenic acid (C18:3 n-3), eicosapentaenoic acid (C20:5 n-3 EPA), docosapentaenoic acid (C22:5 n-3 DPA), docosahexaenoic acid (C22:6 n-3 DHA).

The relevance of FA ratios for human health has been well documented (Warren et al., 2008). For example, low n-6/n-3 (lower than 4) or high PUFA/SFA ratios (higher than 0.4) in human diet are considered to prevent coronary disease, diabetes and some types of cancer (Wood et al., 2004). In our study, more desirable lower n-6/n-3 ($p = 0.004$) and higher PUFA/SFA ($p < 0.001$) ratios were found in lambs raised using grass in the diet compared with the concentrate-fed lambs WP + c and C). These results are in agreement with those reported by Boughalmi and Araba (2016) in the Tiamhdite lambs breed, raised on a grazing system. From a nutritional point of view, one positive feature of the grass feeding of lambs is that levels of the long chain fatty acids n-3 PUFA, which are desirable for human health, are increased: C20:5 n-3 (EPA) and C22:6 n-3 (DHA) (Wood et al., 2008).

The mineral content in meat from Montesina lambs showed (Table 2) ranges of calcium, potassium, sodium and zinc lower than those

previously described by Campo et al. (2021) in meat from several local Spanish breeds (Churra, Castellana, Manchega, Merina, Rasa Aragonesa and Segurena breeds), while levels of iron, magnesium and, especially, phosphorus in meat from Montesina lambs were superior to the above-mentioned breeds. Furthermore, while Campo et al. (2021) were able to detect reasonable selenium levels in meat from local Spanish breeds (with a range of 6.54–12.5 ng/100 g fresh meat), in our study, Montesina meat showed very low levels of this mineral. When compared with other non-Spanish autochthonous breeds, our results are in line with iron, zinc and magnesium content in meat from Australian lambs (Holman, Hayes, et al., 2021). In general, no effect of the production system on mineral content in meat from Montesina lambs was observed, except for magnesium content ($p = 0.003$), whose levels were significantly higher in SP-raised lambs compared to WP + c and C lambs ($p < 0.05$). As reported by Loudon et al. (2021), magnesium imbalances in pasture tend to be widespread during winter, thus putting grazing stock at risk of deficiency, while the spring grass provides magnesium, helping to improve muscle glycogen concentration and contributing to a reduced incidence of dark cutting in meat.

3.2. Color, WHC, WBSF and volatile compounds

After showing the nutritional traits of Montesina lambs' meat, we performed an instrumental analysis for the following organoleptic characteristics of the meat: color (CieLab), WHC, WBSF and volatile compounds grouped in families (Table 3). A more detailed description of the individual volatile compounds is provided in Supplementary Table S1.

Table 3
Descriptive statistics (mean ± standard error) for organoleptic traits in Montesino lambs grazing in spring pasture (SP), winter pasture supplemented with grain (WP + c), and concentrate and forage (C).

	SP (n = 10)	WP + c (n = 10)	C (n = 10)	*p*-values
Color				
Lightness (L*)	40.16 ± 1.72	38.61 ± 1.46	40.91 ± 2.56	nd
Redness (a*)	9.53^{a}±2.0	9.47^{a}±0.56	6.76^{b} ± 0.98	0.002
Yellowness (b*)	11.96^{a}±0.61	12.22^{a}±0.48	10.08^{b} ± 1.01	<0.001
Chroma (C*)	15.34^{a}±1.61	15.46^{a}±0.64	12.16^{b} ± 1.26	<0.001
Hue angle (H*)	51.98^{b} ± 5.17	52.36^{b} ± 1.35	56.33^{a}±2.99	0.015
Water-holding capacity (WHC)	19.76 ± 1.76	20.65 ± 1.20	16.94 ± 5.55	ns
Shear force (kg/cm^2)	6.71 ± 0.56	6.68 ± 0.32	6.31 ± 0.21	ns
Volatile compound species identified (% of the total volatile compounds detected)				
Aldehydes	32.41^{a}±17.32	8.75^{b} ± 2.45	15.11^{b} ± 8.85	<0.001
Ketones	5.75 ^{b} ± 2.02	12.32^{a}±11.57	11.24^{a}±8.89	0.039
Aliphatic hydrocarbons	1.54 ± 1.30	1.21 ± 0.24	2.03 ± 0.37	ns
Alcohols	11.23^{b} ± 3.77	40.42^{a}±9.11	42.39^{a}±5.60	<0.001
Furans	1.57 ± 0.81	2.26 ± 1.36	3.05 ± 1.74	ns
Sulfur compounds	0.19 ± 0.80	0.73 ± 0.27	0.76 ± 1.50	ns
Lactones	4.42^{a}±1.53	1.03^{b} ± 0.25	1.26^{b} ± 0.39	<0.001
Acid compounds	28.57^{a}±0.63	26.00^{a}±0.87	18.30^{b} ± 2.63	0.002
Nitrogen compounds	13.16^{a}±12.16	4.46^{b} ± 1.95	4.29^{b} ± 1.68	<0.001
Aromatic hydrocarbons	1.15^{b} ± 0.40	2.83^{a}±0.48	1.57^{a,b}±0.202	0.033

Different superscripts (^{a,b}) indicate significant differences ($p < 0.05$) among treatments. WHC: Water-holding capacity, expressed in percentage of liquid expelled; ns: not significant.

At the moment of purchase, the main trait preference among consumers is meat color. According to Ripoll et al. (2012), the CieLab results for the Montesina breed in this trial (Table 3) reveal a meat ranging from pale pink to pink rose, similar to the preferences of Spanish consumers. As can be seen, the production system had a significant effect on redness ($p = 0.002$), yellowness ($p < 0.001$), Chroma ($p < 0.001$) and Hue angle ($p = 0.015$). In fact, an increase in redness, yellowness and Chroma (SP and WP + c) were observed in meat where grass was included in the diet, while the highest Hue angle value was observed in meat from lambs fed mainly with concentrate (C), providing strong evidence that including concentrate in the diet of the animals impacts the color parameters.

The water holding capacity in meat is an important organoleptic attribute related with mouthfeel. In fact, higher WHC values could be related with a decrease in the initial meat juiciness. WHC values (Table 3) in the present work were not affected by the lamb production system ($p > 0.05$), with values in the range of those reported by Aguayo-Ulloa et al. (2013) (average 17.90% expelled water) in lambs' meat from other popular local Spanish breeds, such as Rasa Aragonesa and Spanish Merino slaughtered at three months old.

Texture can probably be considered as the most important sensorial trait that consumers value when chewing the cooked meat. The WBSF values observed in meat from the Montesina lambs averaged 6.57 kg/cm^2, which was a higher range than that described by Carrasco et al. (2009) for Churra Tensina lambs (Spain) slaughtered at 22–24 kg (range 3.06–4.07 kg/cm^2). There were no significant differences ($p > 0.05$) (Table 3) in WBSF values among the production systems of Montesina lambs, demonstrating that in these young lambs with a low body weight, the feeding system does not affect the hardness of the meat.

The odor when swallowing the meat is related to the volatile compounds generated after cooking, especially with lamb (Mottram, 1998). A large number of volatile compounds have been described in meat in order to define the characteristic odor of different species of animals intended for human consumption. In fact, a complex combination of volatile compounds determines the specific aroma of each animal species (Bassam et al., 2022). As reported by Bleicher et al. (2022) several factors (e.g., sex, age at slaughter, breed, fat content in muscle, or way of cooking the meat) can affect the composition of the volatile compounds in meat. Specifically, research has shown that the animal's diet can influence the flavor of ovine meat (Borton et al., 2005).

In total, 109 volatile compounds were identified in the meat from Montesina lambs, which were grouped into ten chemical families as follows (Table S1): aldehydes (22); ketones (12); aliphatic hydrocarbons (5); alcohols (26); furans (2); sulfur compounds (3); lactones (5); acids compounds (13), nitrogen compounds (15) and aromatic hydrocarbons (6). The aldehydes, ketones, alcohols and nitrogen family compounds were the most abundant ones identified in the meat of Montesina lambs. The impact of the production system on the ten different families of volatile compounds was statistically analyzed, and the results are shown in Table 3. Significant differences were observed for the proportion of aldehydes ($p < 0.001$), ketones ($p = 0.039$), alcohol ($p < 0.001$), lactones ($p < 0.001$), acid compounds ($p = 0.002$), nitrogen compounds ($p < 0.001$) and aromatic hydrocarbons ($p = 0.033$) among the different feeding systems.

The percentage of aldehydes, lactones, and nitrogen compounds were notably higher ($p < 0.05$) in meat from SP than WP + c and C, while higher percentages ($p < 0.05$) of ketones and alcohols were observed in meat from WP + c and C lambs compared to SP lambs (Table 3). The highest percentage of volatile compounds belonging to acid molecule families was observed in the meat from lambs raised using grass in the diet (SP and WP + C) ($p < 0.05$), while the highest percentage of aromatic hydrocarbon molecules was observed in meat from lambs where concentrate was included in the diet (WP + c and C) (Table 3; $p < 0.05$). Several volatile compounds derived from lipid degradation have been reported, including aldehydes, ketones, alcohols, carboxylic acids, and lactones (Sohail et al., 2022). The higher content in

ketones and alcohols in meat from WP + c and C lambs, in contrast to SP lambs, can be related to the higher fat content observed in lambs raised using concentrate in the diet (WP + c and C), because aromatic compounds such as ketones and alcohols result from oxidation of the fatty acid components of intramuscular fat. Since unsaturated fatty acids undergo autoxidation much more readily than SFAs (Bleicher et al., 2022), higher percentages of aldehydes and lactones were observed in meat from grass-fed lambs (SP and WP + c) than C lambs, due to the fact that meat from lambs raised on grass showed a significantly higher PUFA content than meat from lambs fed with concentrate (i.e., C; see Table 2). The higher content of acid compounds observed in meat from lambs raised using grass (SP and WP + C) compared to C lambs could be explained by a higher degradation of carbohydrates from grass through the Maillard reaction (Bleicher et al., 2022). Lambs' meat from spring grass (SP) showed a higher content in nitrogen containing heterocyclic compounds than meat from lambs fed with concentrate, probably due to the fact that meat from C lambs included a higher content of precursors which contribute to Maillard reactions (Sohail et al., 2022).

The contribution of the individual volatile compounds to differentiation among the three production systems studied is reported in Supplementary Table S1. These compounds have been ranked according to the level of significance obtained from the analysis of variance. In the aldehydes family, the effect of the production system was significant in twelve volatile compounds. Among these, hexanal, heptanal, benzaldehyde and 17-octadecenal were the most abundant and more frequent in SP lambs than in WP + c and C lambs. 3-methylbutanal, furfural and 2,4-heptanedienal were detected only in meat from grass-fed lambs. In the ketones family, the effect of the production system was significant in three of twelve of ketones detected. The acetoin compound percentage in meat was higher in meat from lambs raised using concentrate in the diet than grass-fed lambs ($p < 0.05$). However, 2-tridecanone and 2,5-octanedione content were higher in meat from grass-fed lambs than meat from lambs raised including concentrate in the diet. 2-butanone, 2,3-pentanedione and 2-undecanone content were only observed in meat from SP lambs. In the aliphatic hydrocarbons family, two volatile compounds (namely, hexadecane and nonadecane) showed significant differences among treatments. In fact, a higher hexadecane content was observed in meat from lambs raised using concentrate in the diet ($p < 0.05$), while a higher nonadecane content was detected in meat from SP lambs than in meat from WP + c and C lambs ($p < 0.05$). Importantly, in the aliphatic hydrocarbons family, tetradecane was only observed when concentrate was included in the lambs' diet. In the alcohols family, nine of twenty-six compounds detected showed a significant effect on the production system. However, in this list, the most abundant alcohol (1-octen-3-ol) was not included, because no significant differences were observed among the treatments ($p > 0.05$). 1-butanol, 1,3-propanediol contents were higher in meat from grass-fed animals than meat from lambs raised with concentrate included in the diet, while 1-nonanol, 1-hexanol, heptanol and 4-decen-1-ol contents were higher in meat from lambs raised using concentrate than in meat from lambs fed only on grass. As shown in Table S1, 1-penten-3-ol, benzenemethanol, benzeneethanol, tetradecanol, 2-hexanol, 2,4-decadienol and 1,2-propanediol compounds were only detected in meat from lambs raised using concentrate.

In reference to sulfur compounds detected in meat from Montesina lambs, the benzothiazole content was the highest in meat from lambs including grass in their diet, with a significant decrease as grass is replaced by concentrate, while 2-acetyl-thiazoline and dimethyl sulphone content was detected in meat from lambs raised using concentrate. In the lactones family, pentalactone compound was not observed in meat when concentrate was included in the lambs' diet. Only four of the total acid compounds identified in Montesino lambs showed significant differences (namely, 2,4-hexadienoic acid; hexadecanoic acid; acetic acid and butanoic acid). Higher 2,4-hexadienoic acid and hexadecanoic acid concentrations were detected in meat when grass was included in the lambs' diet, while acetic acid content in the grilled meat was higher when concentrate was included in the diet. An effect on the butanoic acid content in the meat was observed when grass was included in the lambs' diet ($p = 0.05$). In fact, a tendency was observed to an increase of butanoic acid in diets including grass (Table S1). Among the fifteen nitrogenous compounds detected in the lambs' meat, seven showed significant differences among the three treatments. The content of all of them (i.e., 2,5-dimethylpirazine; trimethylpyrazine; 2-acethyl pyrrole; amide; undecylamide; benzenamine and 2-ethyl-6methylpyrazine) were higher in meat from lambs raised using grass than in meat from lambs raised using concentrate in the diet. Pyrazine, ethylpyridine and 2,3-dimethylpyrazine were only detected in meat from grass-fed (SP) lambs, while nonadecanamide was detected only in meat from lambs where concentrate was included in the diet. Finally, a higher toluene and p-Xylene (aromatic hydrocarbons family) content in meat from grass-fed lambs was observed when compared to meat from lambs including concentrate in the diet, while a higher styrene content in grilled lambs' meat was detected in concentrate-fed lambs. The aromatic compound p-Cymene was observed in meat from lambs including concentrate in the diet, while aromatic limonene was detected in meat from lambs raised using mainly grass (SP).

3.3. Sensorial traits

Table 4 shows the means and standard errors for palatability traits of lambs' meat from the Montesina breed. The sensorial consumers test showed no significant differences in tenderness, juiciness and flavor quality of grilled meat among all three treatments. Nevertheless, there were significant differences among treatments for lamb smell ($p = 0.003$) and overall appraisal ($p = 0.005$). In fact, consumers detected a more intense lamb smell in meat from SP and C than in meat from animals raised using a blend of grass and concentrate diet (WP + c), probably due in SP lambs to higher concentrations of aldehydes, lactones and nitrogen volatile compounds (Table 3), and in C lambs to the higher fat content (Table 2) and alcohol volatile compounds (Table 3) observed in the meat.

According to Mottram (1998), flavor is an important aspect of meat quality criteria to determine the acceptance or rejection of the product during swallowing, especially in lamb. In our study, consumers reported higher sensorial scores for overall appraisal in meat from C and SP lambs than WP + c. This observation could be correlated with the higher lamb smell score observed previously in C and SP. Bueno et al. (2013), emphasized the link between cultural background and meat acceptability in several countries, and reported that the main acceptability of lambs' meat is contingent to an appraisal of lamb flavor related to fat content.

3.4. Discriminant analysis

In order to identify potential biomarkers to discriminate between the three production systems (SP, WP + c and C) in Montesina lambs, a principal component analysis (PCA) was first proposed. Because of the

Table 4
Evaluations of sensorial analysis (mean ± standard error) by untrained panelists for meat quality characteristics in Montesino lambs grazing in spring pasture (SP), winter pasture supplemented with grain (WP + c), and concentrate and forage (C).

	SP (n = 10)	WP + c (n = 10)	C (n = 10)	*p*-values
Tenderness	7.67 ± 0.79	7.08 ± 1.47	8.43 ± 1.08	ns
Juiciness	7.59 ± 0.94	7.49 ± 1.46	8.61 ± 1.06	ns
Flavor quality	7.60 ± 0.80	7.34 ± 0.92	8.32 ± 0.91	ns
Lamb smell	7.68^{a}±0.97	6.33^{b} ± 1.11	8.19^{a}±0.98	0.003
Overall appraisal	$6.71^{a,b}$±0.47	6.30^{b}b ± 0.45	7.14^{a}±0.49	0.005

Different superscripts ($^{a,\ b}$) indicate significant differences ($p < 0.05$) among treatments; Scale values (1–10): 1 = low sensory score to 10 = high sensory score; ns: not significant.

high number of nutritional, organoleptic and sensorial variables traits available in this study, only significant variables from the ANOVA analysis (shown in Tables 2–4 for nutritive, organoleptic and sensorial traits of meat, respectively) were included. A graphical representation of the principal component analysis results is shown in Fig. 1. Two principal components obtained from three production systems for the Montesina breed accounted for 70.71% of data variability. The first principal component explained 57.99% of the variability, including mainly color variables (a*, b* and C) and fat traits, as % fat content in meat and fatty acid profile variables on the right-hand side. Next to the fat variable, the alcohols family was located on the right-hand side of the plot. According to Bleicher et al. (2022), compounds derived from lipid degradation have been found in cooked meat, including the alcohols family. On the left-hand side of the plot, organoleptic traits (aldehydes, lactones, nitrogen compounds, aliphatic hydrocarbons and aromatic hydrocarbons) and sensorial traits (lamb smell and overall appraisal) in relationship to meat flavor were located. The fat percentage in meat found on the right-hand side of the plot was opposite to the moisture percentage on the left (Fig. 1). The second principal component accounted for 12.72% of the variability of lambs' meat from Montesina lambs and discriminated PUFAs located at the top from the other SFAs and MUFAs located at the bottom of the plot. This difference was mainly noticeable in long-chain PUFA (i.e., C22:5 n-3 DPA and C20:3 n-6), compared with short-chain FAs on the bottom right-hand side. The effect of the feeding system of lambs on the lipid profile of meat has been widely reported (Cividini et al., 2014). In fact, the research has frequently reported increased PUFAs in meat from grass-fed animals as opposed to higher unsaturated and monounsaturated fatty acid content in meat from lambs raised on concentrate (Cabiddu et al., 2022). Sensory variables such as lamb smell and overall appraisal were located in isolation in the top left-hand side, away from the rest of the variables. For this reason, of all the sensory attributes perceived by consumers, it can be considered that the variables related to meat aroma could be of most help in differentiating the three production systems proposed in this work. The PCA result in our study points to the idea that nutritional variables such as PUFAs and organoleptic variables related to meat odor could be proposed as potential biomarkers to differentiate Montesina lamb production systems according to different feeding systems.

Despite the existence of a long list of nutritional, organoleptic, and sensory traits that could potentially serve as key parameters to identify the meat production system of Montesina lambs (Tables 2–4, and S1), only the traits showing significant differences in Tables 2–4 were included in the discriminant analysis. This approach was taken because a high number of variables included in the statistical model could have lowered the confidence level of the results. The results of the discriminant analysis, including significant data variables, are shown in Fig. 2. The plot shows how individual lambs are grouped according to their system production. Root 1 clearly separates Montesina lambs raised on grass (SP) from lambs whose diet included concentrate (WP + c and C). In fact, WP + c and C lambs are situated at the opposite end of the plot to grass-fed lambs (SP). The effect of including concentrate in the diet of the animals is clearly evidenced by the sample group distribution, as was also reported by Cabiddu et al., 2022. In addition, root 2 separates WP + c from group C, while SP lambs were also clearly grouped by root 2. Root 2 shows evidence that grass combined with concentrate in the diet has an effect on the location of the lambs' group, despite WP + c and C lambs being close. Our results are in line with those reported by Ripoll et al. (2008) in light lambs from the Rasa Aragonesa breed. In fact, using variables associated with the fat color of lambs, Ripoll et al. (2008) showed clear evidence that discriminant analysis could discriminate between grass-fed lambs and concentrate-fed lambs, while the classification of lambs was not clear-cut when grass-fed lambs were compared with lambs raised using grass combined with concentrate.

Finally, Table 5 shows the best selection of variables chosen to discriminate meat from the Montesina breed raised on grass in spring (SP), grass in winter combined with concentrate (WP + c), and concentrate with forage (C). Four variables (alcohols, C18:3n-3, C22:5 n-3 DPA and aldehydes) were identified as potential predictors of the origin of the SP, WP + c and C groups.

Discriminant functions were statistically significant, with p-values less than 0.05 and a confidence level of 99.0%. Using the four predictor variables, a set of functions was developed to classify observations. A separate function was determined for each of the three group levels. These four variables were included in a multinomial logistic regression to calculate the probability of a lamb belonging to each production system. According to the matrix classification proposed including these four combined variables, 100.0%, 97.83% and 99.00% of the lambs were correctly classified into SP, WP + c and C lambs, respectively. Clearly, the variable that contributes the most (higher F value) to discriminate and to predict the Montesina lamb production system was the alcohol compounds family (Table 5), probably including nine significant compounds (in the following order: 1-butanol; 2,3-butanediol; 1-nonanol; 1,3-propanediol; 1-hexanol; 2-decenol; heptanol; 4-decen-1-ol; heptadecanol; see Table S1) ($p < 0.001$). In second place comes the PUFAs C18:3n-3 ($p < 0.001$) and C22:5 n-3 DPA ($p < 0.001$), and finally

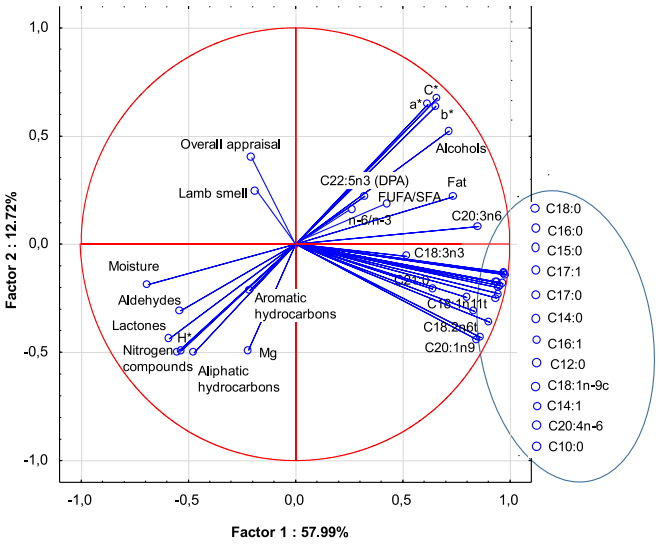

Fig. 1. Principal Component Analysis of production systems of Montesina lambs according to nutritive traits (proximal composition, fatty acid profile and mineral composition), organoleptic traits (color and volatile compound profile) and sensorial traits (lamb smell and overall appraisal) of the meat.

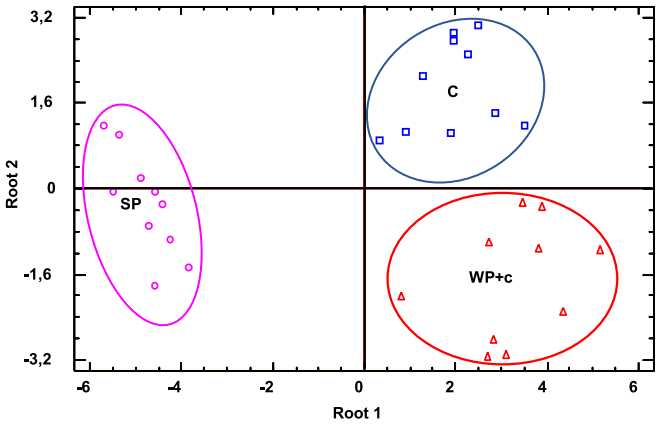

Fig. 2. Plot of canonical discriminant analysis among lambs of Montesina breed raised using SP (spring pasture), WP + c (winter pasture supplemented with grain), and C (concentrate and forage) in their diet.

Table 5
Summary of stepwise regression for nutritional, organoleptic and sensorial variables to discriminate the production system in Montesino lambs.

	F-values	Lambda Wilk	p-values
Alcohols	105.985	0.0978852	<0.001
C18:3n-3	13.4409	0.0440547	<0.001
C22:5 n-3 (DPA)	5.64975	0.0286428	<0.001
Aldehydes	4.76649	0.0193972	<0.001

the aldehydes family compounds, including probably twelve compounds (in the following order: hexanal; heptanal; benzaldehyde; 4 -ethylbenzaldehyde; 2-undecenal; (E,E)-2,4-octadienal; hexadecanal; 17-octadecenal; dodecanal; 2-nonenal; 2, 4-decadienal; (Z,Z)-3,6-nonadienal; see Table S1). These results are in agreement with Yang et al. (2022), who reported higher concentration of alcohol compounds in meat from concentrate-lamb than meat from pasture-lambs in Sunit sheep. Moreover, according to Wu et al. (2021), a high content of PUFAs (including C18:3n-3 and C22:5 n-3 DPA) has been described in meat from animals raised on pasture. Among all the variables analyzed in this work, the organoleptic parameters related to meat flavor (alcohols and aldehydes), along with nutritive traits related to its lipid profile (C18:3n-3 and C22:5 n-3 DPA) could be the best variables for discriminating the production system of meat from lambs of the Montesina breed and for using as biomarkers to identify the origin of lamb's meat. As was reported by Fisher et al. (2000) in British lambs' meat, our results in the Montesina breed provide further evidence of the importance of the production system addressing consumer preferences when it comes to lamb flavor and PUFA profiles, which can influence the consumer's purchasing decisions. Important efforts to obtain novel analytical technologies are being made by the industry and governments to prevent fraud and achieve a more transparent market in essential foods such as meat products (Gagaoua et al., 2017). To achieve this, the biomarkers proposed in meat lamb (alcohols, aldehydes, C18:3n-3 and C22:5 n-3 DPA) could be used as a tool to identify the product's origin and to improve traceability systems in the industry to guarantee the food safety demanded by consumers.

4. Conclusion

From a nutritional and organoleptic approach, we have shown how the main keys to differentiate lambs' meat production systems based on the use of grass and concentrate feed are located in several traits - nutritional (i.e., fatty acids) and organoleptic (i.e., volatile compounds and odor) - in the relationship with the fat contained in the meat. A combination of organoleptic flavor traits such as alcohols (mainly 1-butanol; 2,3-butanediol; 1-nonanol; 1,3-propanediol; 1-hexanol; 2-decenol; heptanol; 4-decen-1-ol; heptadecanol) and aldehydes (mainly hexanal; heptanal; benzaldehyde; 4 -ethylbenzaldehyde; 2-undecenal; (E,E)-2,4-octadienal; hexadecanal; 17-octadecenal; dodecanal; 2-nonenal; 2, 4-decadienal; (Z,Z)-3,6-nonadienal), in combination with nutritive biomarkers (C18:3n-3 and C22:5 n-3, DPA) in the meat could be proposed to differentiate the production system of the Montesina breed of lambs, whether based on fresh grass, concentrate feed or a mixture of both. A diet including concentrate contains a higher content in compounds from the alcohol family, while a higher level of the aldehyde family could be associated with grass-fed lambs. Moreover, a higher percentage of long-chain fatty acids (C18:3n-3 and C22:5 n-3 DPA) could help to identify the origin of grass-fed lambs in the Montesina breed. The combined use of two fatty acid and volatile compounds could be used as a tool by private companies and official control institutions to monitor the traceability of the lambs' meat and to disseminate information about the quality of the meat of Montesina lambs. Future studies to identify the volatile compounds from the alcohol and aldehyde families look promising, and are required to the identify specific biomarkers in lambs' meat in order to differentiate the production systems used in the Montesina breed.

Funding sources

The present research was funded by the Government of Andalusia-Spain (FEDER, PR. TRA.TRA2019.008. RESGAP) and Research Foundation of the University of Seville (PRJ202304693).

CRediT authorship contribution statement

Alberto Horcada: Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Luis Pablo Ureña:** Resources, Methodology. **Carlos Álvarez:** Writing – review & editing, Supervision. **Manuel García-Infante:** Software, Methodology. **Francisco de Asís Ruiz:** Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the Montensina Sheep Breeders' Association (Spain), and the staff of SGIA of University of Seville (Spain).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.103610.

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