Chestnut shells in the diet of lamb: Effects on growth performance, fatty acid metabolism, and meat quality

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Chestnut shells in the diet of lamb: Effects on growth performance, fatty acid metabolism, and meat quality

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ABSTRACT

This study aimed to formulate a diet for finishing lambs that included chestnut shells, an underexploited by-product of chestnut industry, and evaluate its effects on in vivo performance and meat quality. Twenty-eight male lambs (race Romane; 27.9 ± 2.7 kg bodyweight) were divided into 4 groups and fed 4 different pelleted diets: one control, one containing chestnut shells (CNS), one containing sainfoin, and one containing both. After 21 days of feeding trial, at slaughter, rumen and abomasum digesta were sampled for the analysis of fatty acid (FA) profile, and meat was analysed for FA profile, vitamins content, and oxidative stability. All lambs showed similar growth performance and carcass characteristics. The CNS diet limited ($P = 0.001$) ruminal biohydrogenation, increasing ($P < 0.050$) the C18:1 *trans*11 proportion in both rumen and abomasum. Consequently, the C18:1 *trans*11 content of CNS meat was more than 50% higher ($P = 0.006$) than in the other groups. No differences in the discolouration and lipid oxidation of raw meat were observed over 9 days of refrigerated storage. The phenolic compounds of chestnut shells may have preserved the low native α-tocopherol level of the CNS diet. Chestnut shells can be fed to lambs without detrimental effect on performance, potentially improving meat FA profile.

1. Introduction

Chestnut is the fruit of deciduous trees in the genus *Castanea*. The world production of chestnuts is about 2.3 millions of tonnes (in 2020, FAOSTAT), with China accounting for 75% of global production, followed by the EU (14%), Bolivia (3.5%), and Turkey (3.2%). Chestnut fruit is composed of an edible kernel protected by a thin inner shell (integument) and a hard outer shell (pericarp). Chestnut shells (meant as integument and pericarp) are discarded as a by-product of the peeling process. In sweet chestnut (*Castanea sativa* Miller), integument and pericarp represent respectively 6.3–10.1% and 8.9–13.5% of the whole fruit, depending on the chestnut cultivar (de Vasconcelos et al., 2010). Thus, the annual world production of chestnut shells likely averages 500 thousand tonnes. Chestnut shells are a fibrous material with a remarkable content of phenols (up to 52 mg gallic acid equivalents per kg), especially phenolic acids, flavonoids, and tannins (Pinto et al., 2021a). In particular, chestnut pericarp is rich in procyanidins and hydrolysable tannins, such as castalagin, whereas chestnut integument is rich in prodelphinidins (de Vasconcelos et al., 2010, Pinto et al., 2021b). However, the phenolic composition of chestnut shells changes depending on cultivar and environmental conditions, as well as the technique used to obtain the phenolic extract (Pinto et al., 2021a). Phenolic compounds are acknowledged for a wide range of positive effects on animal health, such as antimicrobial, anti-inflammatory, and anti-thrombotic effects, thanks to their bioactive properties, first and foremost the antioxidant activity (Hajam et al., 2020).

Research has so far focused on industrial applications for chestnut shells (Vázquez et al., 2009, Zhao et al., 2014, Morana et al., 2017, Morales et al., 2018), however, to the best of our knowledge, no direct application in livestock diet has ever been studied. Livestock farming is one of the human activities with the highest environmental impact, and feed production, including cultivation, processing, and transport, accounts for about half of the greenhouse gas emission of livestock industry (Gerber et al., 2013). In this scenario, feeding livestock with

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alternative agro-industrial by-products has the potential to reduce the environmental impact of feed production and up-cycle industry waste, while limiting feed-to-food competition and promoting circular economy (Salami et al., 2019). Indeed, resorting to locally available by-products for the formulation of animal diets can reduce the need for cultivated feed, which, in some cases, could be destined to human consumption.

Chestnut shells could be included in the diet of ruminants as a fibrous feed, while also representing an interesting source of phenols and tannins. Despite being known for their anti-nutritional effects, dietary tannins have been attracting increasing interest in research for the past 20 years thanks to their positive effects on animal health (Patra and Saxena, 2011), animal pollutant emissions (Herremans et al., 2020, Cardoso-Gutierrez et al., 2021), and the quality of animal products. Indeed, dietary tannins can increase the oxidative stability of meat and milk (Soldado et al., 2021), as well as improve their fatty acid (FA) profile by modulating ruminal biohydrogenation (BH) (Biondi et al., 2019; Frutos et al., 2020). For example, feeding lambs with quebracho (*Schinopsis lorentzii* Engl.) tannins (40 g of tannic acid equivalents per kg of diet DM) limited colour oxidation and metmyoglobin development in raw meat (Luciano et al., 2009). Moreover, dairy goats fed 700 g/d of sainfoin (*Onobrychis viciifolia* Scop.), a well-known tanniferous forage, produced cheese richer in C18:3 *n*-3 and with a lower *n*-6 to *n*-3 polyunsaturated fatty acids (PUFA) ratio compared to goats fed alfalfa (Menci et al., 2022a). In fact, changes in ruminal BH often lead to changes in the FA profile of products, which may have particular implications in terms of healthiness and oxidative stability. However, the complexity of biochemical reactions combined with the diversity of tannin structures makes any prediction difficult.

On the basis of the above, chestnut shells were integrated into a commercial prototype of pelleted feed for lambs, as part of a balanced diet. Our hypothesis was that chestnut shells could be included in the diet of lamb without detrimental effects on growth performance and meat quality. Furthermore, we hypothesized that the phenolic compounds contained in chestnut shells may modulate ruminal BH and therefore affect meat FA profile and oxidative stability. For the purpose, we assessed the FA profile of rumen content, abomasum content, and meat, the antioxidant fat-soluble vitamins of meat, and colour and lipid stability in meat under a simulated retail condition. Considering that different tannins from different plant sources may interact in biological systems leading to unforeseeable synergistic effects (Luciano et al., 2019; Menci et al., 2021), we included sainfoin in the experimental design, in order to compare different tannin sources and highlight potential synergistic effects.

2. Materials and methods

2.1. Animals, diets, and experimental design

The experiment took place in the facilities of INRAE Clermont-Auvergne-Rhône-Alpes, in central France. The experimental procedures were conducted in accordance with the European Union Directive 2010/63/EU, reviewed by the local ethics committee (C2E2A, "Comité d'Ethique pour l'Expérimentation Animale en Auvergne") and authorised by the French Ministry for Research under agreement number 22514–2019101821388910.

Twenty-eight pure-bred Romane male lambs were sourced from the same farm, and used in a randomized block experimental design. The lambs were individually penned indoors; building had no temperature control, and the animals received no artificial light. Before the beginning of the trial, the lambs were fed with the same basal diet consisting of hay and a conventional pellet. At 90 d of age (27.9 ± 2.7 kg bodyweight), the lambs were divided into 4 experimental groups (n = 7), each fed with hay and a different pelleted feed: the same conventional pellet as before the beginning of the trial (CON), a pellet including chestnut (*C. sativa*) shells (CNS), a pellet including sainfoin (SFN), and a pellet including both chestnut shells and sainfoin (C+S). The ingredients of the experimental pellets are reported in Table 1. The experimental feeds were formulated to maximise the inclusion of CNS while ensuring their "pelletability". Maximum temperature reached during pelleting was 64 °C, and pellets diameter was 4.5 mm. Basing on individual bodyweight, the lambs were further assigned to 4 blocks (n = 4, 8, 8, 8 for block 1, 2, 3, 4, respectively), with equal representation of the 4 experimental groups. The 4 blocks started the feeding trial progressively, at intervals of 1 week. This was done to spread slaughtering over a 4-week period, in order to not overload the experimental abattoir.

To ensure the complete ingestion of the pellets, diets (pellet and hay) were restricted to 95% of protein and energy (as feed units for maintenance and meat production; UFV; INRA, 1988) requirements, according to the feed intake recorded before the beginning of the trial. Reference requirement was based on an average daily gain (ADG) of 150 g/d. The offer of pellets and hay was periodically adjusted for the individual body weight. As a consequence of the different energy and protein levels of the experimental pellets (Table 1), the CNS and C+S groups received a higher amount of pellet compared to the CON and SFN groups for all diets to be isonitrogenous and isoenergetic. In practice, the CON and SFN lambs received 640 g DM/d of pellet while the CNS and C+S groups received 900 g DM/d of pellet, on average. All the lambs received 300 g DM/d of hay, on average. All the lambs were fed individually twice a day (0830 and 1530), and any residues of pellet and hay were weighted daily. The feeding trial lasted 21 d, at the end of which the animals were sacrificed at the experimental slaughterhouse.

Table 1
Ingredients and chemical composition of experimental feedstuffs.

Item[a]	Pellet[b]				Hay
	CON	CNS	SFN	C+S	
Ingredients of pellet, g/kg					
Alfalfa	640	450	-	-	
Barley	127	60	65	105	
Soybean 46.6 CP	100	45	95	33	
Flaxseed	133	100	135	100	
Chestnut shells	-	321	-	228	
Sainfoin, dehydrated	-	-	705	509	
Hydrogenated vegetable oil	-	24	-	-	
$CaCO_3$	-	-	-	25	
Chemical composition, g/kg DM					
DM, g/kg FM	894	902	899	903	930
CP	209	155	209	156	132
EE	67	86	61	54	10
NDF	376	415	367	386	518
ADF	270	185	251	182	275
Lignin	59	43	63	47	27
Starch	88	174	71	177	na[c]
Phenols (TA eq)	14.7	16.9	21.0	21.3	16.6
Tannins (TA eq)	1.4	4.6	8.0	7.8	4.5
Ash	84	80	92	110	89
UFV, g/kg DM	857	646	914	685	na[c]
Fatty acids, g/kg DM					
C16:0	3.6	3.0	2.6	2.4	1.3
C18:0	1.1	1.0	0.8	0.7	0.2
C18:1 *cis*9	4.0	6.0	3.8	3.4	0.2
C18:2 *n*-6	5.1	8.3	4.1	1.8	0.6
C18:3 *n*-3	10.0	7.4	8.0	5.8	1.8
Vitamins, mg/kg DM					
α-tocopherol	37.9	14.8	66.3	24.7	35.4
γ-tocopherol	11.1	11.0	21.5	24.0	5.5
δ-tocopherol	16.1	6.2	14.1	12.4	4.5

[a] DM, dry matter; FM, fresh matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; TA eq, tannic acid equivalents; UFV, feed unit for maintenance and meat production, as g of standard air-dried barley equivalent (INRA, 1988).

[b] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

[c] Not assessed.

2.2. Weighing and carcass evaluation

Final body weight was recorded right before slaughtering. Hot carcass weight was recorded immediately after slaughtering, and cold carcass weight was recorded after 24 h of refrigerated storage. Carcasses were graded for conformation (SEUROP classification, from S "superior" to P "poor") and fatness (from 1 "low" to 5 "very high") by a trained assessor, according to Commission Delegated Regulation (EU) 2017/1182. The firmness of subcutaneous dorsal fat was measured by a trained assessor on a 7 points scale (from 1 "very soft" to 7 "very hard") using a finger test (Prache et al., 2011).

2.3. Analyses on feedstuffs

Samples of pellets and hay were collected weekly during the trial and then pooled to get a representative sample of each feedstuff. The samples were oven-dried at 60 °C for 72 h and then stored refrigerated.

The DM content was determined by oven-drying at 103 °C for 48 h and ash content at 550 °C for 6 h in a muffle furnace (European Union Commission Regulation EC n. 152/2009). The NDF, ADF and lignin contents were determined according to the method described by Van Soest et al. (1991), using a Fibre Analyser (Ankom Technology Corporation, Fairport, NY, USA). The N content was determined by the Dumas combustion method (AOAC International, 2005; method 968.06) using a rapid N-cube protein/N apparatus (Elementar Americas Inc., Mt Laurel, NJ, USA), and CP content was calculated as N content × 6.25. Starch was analysed using an enzymatic method (Faisant et al., 1995). The fat content was determined after acid hydrolysis (AOAC International, 2005; method 954.02). Total phenolic compounds and total tannins were analysed according to the procedure of Makkar et al. (1993), as modified by Luciano et al. (2019).

Fatty acid profile was assessed through a one-step extraction-transesterification (Valenti et al., 2018). In brief, 100 mg of ground sample was mixed with 1.5 mL of chloroform and 2.5 mL of 2% methanolic sulfuric acid. After incubation in a water bath at 70 °C for 2 h, 1.5 mL of chloroform and 2.5 mL of 6% K_2CO_3 were added, and the sample was centrifuged at 2500 ×g for 10 min at 4 °C. The underlying organic phase (1 mL) was collected, evaporated under N flow, and dissolved in 1 mL of hexane. Gas-chromatographic analysis was performed as later described for rumen and abomasum contents, using C13:0 as internal standard.

Fat-soluble vitamins were extracted according to Rufino-Moya et al. (2020). Briefly, 200 mg of ground sample was mixed with 3 mL of methanol:acetone:petroleum ether (1:1:1, v-v:v) with BHT (0.1 g/L), and vortexed 1 min. Supernatant was collected after centrifugation at 1000g for 5 min: this operation was repeated twice. After evaporation under N flow, the residue of the supernatant was dissolved in 1 mL of methanol. The sample was filtered with a 0.22 μm PTFE filter and placed into a 2 mL vial. Analytes were quantified using a Nexera UHPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a C18 phase column (Zorbax ODS, Supelco, Bellefonte, PA; length: 25 cm; i.d.: 4.6 mm; particle size: 5 μm). Settings and temperatures were the same described by Natalello et al. (2022). Tocopherols were detected by fluorescence (RF-20AXS, Shimadzu; excitation wavelength: 295 nm; emission wavelength: 330 nm), whereas β-carotene was detected using a photodiode array detector (SPD-M40, Shimadzu; absorbance wavelength: 450 nm). The comparison with the retention time of pure standards (Merck Life Science s.r.l., Milano, Italy) was used for analytes identification. External calibration curves with pure standards were created for each analyte.

2.4. Fatty acid profile of rumen and abomasum contents

At slaughtering, the rumen and abomasum contents of each lamb were sampled and immediately frozen in liquid nitrogen. The samples were then freeze-dried and stored at − 20 °C.

The FA profile of rumen and abomasum contents was analysed after basic-acid transesterification (Alves et al., 2013), following the method described by Menci et al. (2021). Briefly, 250 mg of freeze-dried sample was mixed with 2 mL of 0.5 M methanolic CH_3ONa in a glass tube and incubated at 50 °C for 10 min. Then, 3 mL of 10% methanolic HCl was added and the tube was incubated at 50 °C for 15 min. The solution was then vortexed with 4 mL of 6% aqueous K_2CO_3. After adding 2 mL of hexane, the tube was centrifuged (1500 ×*g*, 10 min, 4 °C) and the supernatant extract was collected; this phase was repeated once. The extract was evaporated under N_2 and the residue was dissolved in 1 mL of hexane (GC grade). A Thermo Finnigan Trace gas chromatograph featuring a flame ionization detector (FID; ThermoQuest, Milan, Italy) and a high-polar fused silica capillary column (SP-2560 fused silica, Supelco, Bellafonte, PA; length: 100 m; i.d.: 0.25 mm; film thickness: 0.25 μm) was used for the separation of FA methyl esters. Oven, injector, and detector were set as described by Natalello et al. (2019), and helium was used as carrier gas at constant flow (1 mL/min). C18:1 isomers were separated through isothermal analysis at 165 °C. Methyl nonadecanoate (C19:0) was used as internal standard. The comparison with the retention time of standard FA methyl esters mixtures (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmo, Sweden) and with published chromatograms (Alves and Bessa, 2007, Kramer et al., 2008) was used to identify individual FA.

2.5. Analyses on meat

After storing the carcasses for 24 h at 0–4 °C, the *longissimus thoracis et lumborum* muscle was excised from the right side of each carcass, and pH was measured with a pH-meter equipped with probe (average of 3 measurements). Meat samples were then cut in different aliquots: the aliquots for the analyses of fatty acid profile, fat-soluble vitamins, and cholesterol were vacuum-packaged and stored at − 80 °C, whereas the aliquot for oxidative stability analysis was processed as described below (Section 2.5.2).

2.5.1. Fatty acid profile, fat-soluble vitamins, and cholesterol

The intramuscular fat was extracted from 10 g meat samples using 2:1 (v:v) chloroform:methanol (Folch method). The FA contained in the fat extract were converted to FA methyl esters through basic transesterification with methanolic CH_3ONa (Christie, 1982). The FA profile of meat was determined by gas chromatography as described above for rumen and abomasum contents.

The tocopherols, retinol, and cholesterol contents in meat were assessed following the method of Bertolín et al. (2018), with some modifications as described by Menci et al. (2022b). Briefly, 2.5 g of meat sample was mixed with 200 mg of L-ascorbic acid and 7.5 mL of KOH (10% in 1:1 ethanol:water), and let saponify overnight in an incubator shaker. Then, the extraction was carried out adding 5 mL of 9:1 hexane: ethyl acetate (with 25 mg/L of BHT), and the supernatant extract was collected after centrifugation (2000 ×*g*, 5 min, 10 °C); this phase was repeated once. The extract was evaporated under N_2 and the residue was dissolved in 1 mL of methanol (HPLC grade) and then filtered through PTFE syringe filters (0.2 μm/13 mm). The analytes were quantified by UHPLC as described above for feeds. Cholesterol and retinol were detected using a photodiode array detector (SPD-M40, Shimadzu) at the absorbance wavelength of 220 nm and 325 nm, respectively.

2.5.2. Oxidative stability

Each aliquot of meat for oxidative stability analysis was cut into four 1.5 cm thick slices. The 4 slices were placed on polystyrene trays overwrapped with cling film and stored at 0–4 °C for 0 d (2 h), 3 d, 6 d, or 9 d, respectively. At the end of each storage time, the colour parameters were assessed through reading with a portable spectrophotometer (CM-2022, Minolta Co. Ltd. Osaka, Japan; SCE mode; illuminant: A; 10° standard observer) on the slice surface (average of 3 readings on non-overlapping areas). The colour descriptors L* (lightness), a* (redness), b* (yellowness), C* (saturation), and h_{ab} (hue angle), as well as the reflectance

spectrum between 400 nm and 700 nm were measured in the CIE L*a*b* colour space. The meat slices were then vacuum-packaged and stored at − 80 °C before lipid oxidation analysis.

The thiobarbituric acid reactive substances (TBARS) were measured to assess the extent of lipid oxidation in meat, following the procedure described by Natalello et al. (2020), with some modification. Briefly, 2.5 g of meat was placed in a tube with 12.5 mL of water and homogenized (9500 rpm, 1 min) keeping the tube in a water-ice bath. Then, 12.5 mL of 10% trichloroacetic acid was mixed with the homogenate, and the sample was filtered (Whatman 541 paper). Four mL of the filtrate was reacted with 1 mL of 0.06 M aqueous thiobarbituric acid in a water bath at 80 °C for 90 min. A blank was prepared with 5% trichloroacetic acid in place of the filtrate. The absorbance at 532 nm was read and the result was expressed as mg of malondialdehyde per kg of meat, through comparison with a TEP (1,1,3,3-tetraethoxypropane) calibration curve (points ranging from 1.25 to 16.25 mmol/L).

2.6. Calculations and statistics

The biohydrogenation of C18:1 cis9, C18:2 *n*-6, and C18:3 *n*-3 was estimated according to Oliveira et al. (2016). Biohydrogenation completeness (BHC) was calculated according to Alves et al. (2017). Atherogenicity index (AI) and thrombogenicity index (TI) of meat were calculated according to Ulbricht and Southgate (1991). The hypocholesterolemic to hypercholesterolemic FA ratio (h:H) of meat was calculated according to the formula of Santos-Silva et al. (2002), modified as follows: h:H= (C18:1 cis9 +PUFA)/(C12:0 +C14:0 +C16:0). The activity of stearoyl-CoA 9-desaturase in muscle was calculated basing on the desaturation of C14:0. Concerning colour parameters, total colour change (ΔE) of meat after 3, 6, and 9 days of storage was calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL* , Δa* , and Δb* are the differences in L* , a* , and b* , respectively, between day 0 and day 3, day 6, or day 9. The ratio between the reflectance of meat at 630 nm and 580 nm (630/580) was calculated as indicator of myoglobin oxidation (Khliji et al., 2010). The integral value of the reflectance spectrum of meat at wavelengths between 450 nm and 530 nm ($I_{450–530}$) was calculated as indicator of carotenoids presence (Priolo et al., 2002).

Statistical analysis was performed with the software Minitab 19 (Minitab, LLC) using the single animal as statistical unit. Mixed ANOVA was applied to the data of feed intake, growth performance, carcass weights, intramuscular fat, FA profile, fat-soluble vitamins, and cholesterol, with the dietary treatment as fixed factor and the block as random factor. Kruskal-Wallis test was applied to the scores of carcass conformation, carcass fatness, and subcutaneous fat firmness, to highlight differences among diets. After verifying that the block did not have a significant effect, a mixed ANOVA for repeated measures was applied to the data of oxidative stability, with the single animal as random factor and the dietary treatment, the storage time, and their interaction as fixed factors. Differences were considered significant when $P \leq 0.050$ and the Tukey post hoc test was performed for multiple comparisons.

3. Results

3.1. In vivo performance and carcass traits

The daily intake of feedstuffs and nutrients by the experimental lambs is reported in Table 2. As CNS and C+S pellets were poorer in protein and energy and considering that diet was restrained to 95% of nutritional requirements, the CNS and C+S lambs had higher ($P < 0.001$) pellet DMI compared to the CON and SFN lambs. The CNS and C+S groups had the highest ($P < 0.001$) NDF and starch daily intake. Concerning lipids, the CNS group showed the highest intake of all groups, while the C+S group had a higher value than the SFN ($P < 0.001$). This, combined with the different FA content of pellets, led to differences ($P < 0.001$) in all individual FA intake. For example, the CNS group had the highest daily intake of C18:1 *cis*9 and C18:2 *n*-6, whereas the C+S group had the lowest intake of C18:2 *n*-6. Furthermore, the CON and CNS groups showed a similar C18:3 *n*-3 intake, which was higher than the SFN and C+S groups ($P < 0.001$). The phenols and tannins intakes were different ($P < 0.001$) among feeding groups, according to the order C+S>CNS>SFN>CON and C+S>SFN>CNS>CON, respectively. Finally, the daily intake of tocopherols was different ($P < 0.001$) among feeding groups. Notably, the SFN group had the highest α-tocopherol intake while the CNS group had the lowest.

Table 2
Daily intake of experimental feedstuffs and nutrients (g/d).

Item[1]	Diet[2]				SEM	P-value
	CON	CNS	SFN	C+S		
DMI, pellet	656[b]	926[a]	634[b]	886[a]	27.4	< 0.001
DMI, hay	289.5	309.2	298.0	300.2	4.26	0.462
UFV[3]	629.2	662.5	644.4	671.7	9.68	0.377
CP	175.8	185.0	172.0	178.5	2.64	0.347
EE	46.7[bc]	82.7[a]	41.5[c]	50.7[b]	3.19	< 0.001
NDF	397[b]	544[a]	387[b]	497[a]	14.3	< 0.001
ADF	256.8	257.1	241.5	244.5	3.76	0.302
Lignin	46.35	48.31	47.89	49.96	0.714	0.339
Starch	58[b]	161[a]	45[c]	157[a]	10.5	< 0.001
Phenols (TA eq)	14.40[d]	20.72[b]	18.18[c]	23.80[a]	0.711	< 0.001
Tannins (TA eq)	2.23[d]	5.65[c]	6.38[b]	8.22[a]	0.424	< 0.001
Ash	80.7[c]	101.9[b]	84.9[c]	124.1[a]	3.55	< 0.001
Fatty acids						
C16:0	2.761[b]	3.211[a]	2.052[c]	2.499[b]	0.0890	< 0.001
C18:0	0.776[b]	0.969[a]	0.564[d]	0.690[c]	0.0303	< 0.001
C18:1 *cis*9	2.69[bc]	5.60[a]	2.45[c]	3.04[b]	0.248	< 0.001
C18:2 *n*-6	3.54[b]	7.85[a]	2.80[c]	1.82[d]	0.448	< 0.001
C18:3 *n*-3	7.07[a]	7.46[a]	5.66[b]	5.73[b]	0.180	< 0.001
Vitamins (mg/d)						
α-tocopherol	31.7[b]	22.6[c]	47.5[a]	29.7[b]	1.82	< 0.001
γ-tocopherol	7.60[d]	10.70[c]	13.70[b]	20.69[a]	0.931	< 0.001
δ-tocopherol	10.65[a]	6.48[c]	9.30[b]	11.21[a]	0.379	< 0.001

[a, b, c, d] Means within a row that do not share a superscript letter are statistically different.

[1] DMI, dry matter intake; UFV, feed unit for maintenance and meat production, as g of standard air-dried barley equivalent (INRA, 1988); CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; TA eq, tannic acid equivalents.

[2] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

[3] Hay excluded.

Table 3
Growth performance and carcass measurements.

Item	Diet[a]				SEM	P-value
	CON	CNS	SFN	C+S		
Initial bodyweight, kg	26.57	28.86	27.39	27.95	0.529	0.391
Average daily gain, g/d	172	163	140	156	12.4	0.304
Final bodyweight, kg	30.14	32.28	30.29	31.18	0.520	0.451
Hot carcass weight, kg	16.52	16.89	15.98	16.07	0.712	0.632
Cold carcass weight, kg	16.04	16.38	15.51	15.58	0.694	0.643
Chilling loss, %	2.92	2.96	2.92	3.06	0.224	0.707
Meat pH (24 h)	5.635	5.681	5.669	5.666	0.0230	0.364
Conformation[b] (SEUROP)	R- (17.1)	R- (17.4)	O+ (9.5)	O+ (13.9)	-	0.238
Fatness[b] (1–5 scale)	3 (16.0)	3 (18.4)	2 (13.2)	2 (10.4)	-	0.301
Fat firmness[b] (1–7 scale)	5 (16.1)	5 (17.1)	4 (10.9)	5 (14.0)	-	0.505

[a] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

[b] Values are medians with mean ranks in brackets. The overall mean rank is 14.5.

No difference on growth performance and carcass traits were observed among experimental groups (Table 3).

3.2. Fatty acid profile of rumen and abomasum contents and biohydrogenation

The diet affected the FA profile of rumen content, as shown in Table 4. Concerning branched-chain FA (BCFA), the rumen content of CNS lambs showed a lower ($P \leq 0.050$) proportion than the CON group, especially regarding *anteiso* FA. The SFN group had a higher ($P < 0.050$) proportion of *anteiso* C15:0 and *iso* C15:0 than the CNS group, and the lowest ($P < 0.001$) proportion of *anteiso* C17:0. Also, the proportion of odd-chain FA (OCFA), particularly C11:0, C13:0, and C17:0, was higher ($P < 0.050$) in the rumen content of SFN lambs compared with CNS lambs. Concerning BH intermediates, the CNS rumen had a higher ($P = 0.009$) proportion of C18:1 *trans*11 than the CON and SFN groups, with an intermediate level for the C+S group. In addition, the C18:1 *trans*10 proportion was higher ($P = 0.031$) in the CNS group than in the SFN group. Finally, the C+S group showed a higher ($P = 0.015$) proportion of PUFA than the CNS and SFN groups.

The FA profile of abomasum content is showed in Table 5. The CON group had the highest ($P \leq 0.001$) proportion of BCFA and *anteiso* FA. In particular, the CNS group had the lowest ($P = 0.001$) proportion of *anteiso* C15:0, whereas the SFN group had the lowest ($P < 0.001$) proportion of *anteiso* C17:0. Moreover, the CNS group showed the lowest ($P = 0.006$) value of *iso* C15:0 proportion. Concerning OCFA, the CNS group had the lowest ($P = 0.001$) proportion of C17:0, and the SFN group had the highest ($P < 0.001$) proportion of C15:0. As a result, OCFA proportion was lower ($P = 0.001$) in the CNS abomasum than in the SFN. The CNS group showed the lowest C16:0 and C16:1 *cis*9 values ($P < 0.001$ and $P = 0.003$, respectively). Similar to rumen content, the CNS abomasum content had a higher ($P = 0.017$) proportion of C18:1 *trans*11 than the CON and SFN groups, with an intermediate level for the C+S group. Furthermore, the CNS group showed higher C18:1 *cis*13 ($P = 0.016$) and C18:2 *n*-6 ($P = 0.034$) proportions compared with the SFN group.

The analysis of BH indices (Table 6) highlighted a higher C18:1 *cis*9 BH rate in the CNS and C+S groups than in the CON group, with intermediate levels in the SFN group, in both rumen ($P = 0.003$) and abomasum ($P = 0.001$) contents. The C+S group stood out for the lowest ($P < 0.001$) C18:2 *n*-6 BH rate, in both rumen and abomasum contents. According to the FA profile of abomasum content, the least complete BH ($P = 0.001$) occurred with the CNS diet.

3.3. Meat quality

Concerning meat FA profile (Table 7), the CNS diet increased ($P = 0.006$) the C18:1 *trans*11 concentration in lamb of 70% (on average) compared to the other diets. The concentration of C18:2 *cis*9*trans*11 was higher ($P = 0.008$) in CNS meat than in SFN and C+S meats, but no difference with the CON group was observed. Moreover, the CNS group showed a higher ($P < 0.001$) concentration of C18:1 *cis*12 in meat than the CON group. The sum of *trans* FA in CNS meat was higher ($P = 0.001$) than in other meats; the difference disappeared ($P > 0.050$) when C18:1 *trans*11 was excluded from the count. The diet had no effect ($P > 0.050$) on the activity of muscle stearoyl-CoA 9-desaturase.

The contents in α-tocopherol, γ-tocopherol, and retinol of lamb are showed in Table 8. The SFN and C+S groups had higher ($P < 0.001$) α-tocopherol content than the CON and CNS groups. The lambs fed with conventional pellet had the lowest ($P < 0.001$) γ-tocopherol content in meat. Finally, the content of retinol was lower ($P = 0.009$) in SFN and C+S meat than in CON meat.

The diet did not affect ($P > 0.050$) the colour parameters and the lipid oxidation of meat (Table 9). All measured oxidative stability parameters changed during the storage time, without significant interaction with the diet. In particular, L^*, b^*, and h_{ab} increased, whereas a^*, 630/580, and $I_{450–530}$ decreased from 0 to 9 days of storage ($P < 0.001$). The value of ΔE was lower ($P < 0.001$) at day 3 compared with day 6 and 9. The content of malondialdehyde in meat was similar between day 0 and day 3, and then progressively increased after 6 days and 9 days of storage ($P < 0.001$).

4. Discussion

4.1. In vivo performance

According to scientific literature, this is the first time that chestnut shells, a by-product of chestnut industry, have been tested in livestock feeding (Musati et al., 2023). In the present experiment, chestnut shells were included in a pelleted feed as part of a balanced diet for finishing lambs. The different chemical composition of the experimental pellets was balanced by the feeding restriction, as confirmed by the similar UFV and CP intakes, growth performance, and carcass traits among groups. However, it cannot be ignored that achieving the same growth performance with a larger quantity of feed would necessarily lower feed efficiency and increase excretions.

Despite the relatively higher starch intake of the lambs fed pellets containing chestnut shells, all the diets used in the present experiment had no risk of developing rumen acidosis. Indeed, the starch content was lower than 200 g/kg DM and the NDF content was higher than 300 g/kg DM, both parameters that prevent the risk of acidosis (INRA, 2018). This was proven by the ruminal pH values of experimental lambs, which were in the range of 6.6–7 (data not shown).

The high fat intake observed in the CNS group was due to the inclusion of hydrogenated vegetable oil in the formulation of the CNS pellet. This was necessary for two reasons: to overcome the poor "pelletability" of chestnut shells and to balance the energy value of the pellet. Anyway, the presence of hydrogenated vegetable oil did not affect the in vivo performance of the CNS lambs, likely because all the diets consumed were isoenergetic. Similar results were obtained by Castro et al. (2005) when supplementing the diet of lambs with different vegetable oil supplements. Nonetheless, we cannot exclude that a feeding period longer than 21 d may result in different carcass characteristics, such as the fatness score.

The tannin content of agro-industrial by-products may set a limit to their use in animal diet, as these phenolic compounds may have antinutritional properties if a certain dose is exceeded. For example, Shakeri (2016) tested the inclusion of the by-product of pistachio hulling in the diet of lambs and observed that a dose of 300 g/kg (corresponding to a level of tannins of 2.3% in the diet) had a negative effect on growth performance because of dietary tannins. Despite dietary levels of about 1–2% are generally considered not detrimental for ruminant performance (Vasta et al., 2019), the great variability of tannin structures of different plants often leads to conflicting results. For instance, Valenti et al. (2021) observed that supplementing the diet of lambs with 2.3% of chestnut bark tannins resulted in lower feed intake and/or bodyweight gain, whereas mimosa, gambier, and tara tannins at the same dose did not exert any detrimental effects. However, in the present experiment, the composition of pelleted feeds resulted in a "harmless" dietary tannins level of less than 1%, even when chestnut shells and sainfoin were fed together.

Consistent with our results, Copani et al. (2016) found no difference in ADG and carcass weight of lambs when replacing timothy (*Phleum pratense*) with sainfoin (about 460 g/d) in a diet of red clover (*Trifolium pratense*) silage. Indeed, the feeding value of sainfoin can be considered similar to common forages such as alfalfa, provided that its tannin content remains below an indicative threshold of 50 g/kg DM (Wang et al., 2015).

4.2. Fatty acid metabolism

As expected, the diets including chestnut shells and/or sainfoin

Table 4
Fatty acid profile of rumen content (g/100 g of fatty acids).

Item[1]	Diet[2]				SEM	P-value
	CON	CNS	SFN	C+S		
C10:0	0.028	0.021	0.026	0.033	0.0023	0.278
C11:0	0.024^{a}	0.012^{b}	0.024^{a}	0.019^{ab}	0.0016	0.006
C12:0	0.163	0.111	0.142	0.116	0.0158	0.654
C13:0	0.26^{ab}	0.22^{b}	0.32^{a}	0.34^{a}	0.127	0.009
C14:0	0.356^{ab}	0.258^{b}	0.385^{a}	0.345^{ab}	0.0173	0.046
iso C14:0	0.184	0.159	0.241	0.216	0.0190	0.064
C14:1 *trans*9	0.114	0.071	0.123	0.133	0.0117	0.252
C14:1 *cis*9	0.248	0.202	0.266	0.224	0.0197	0.710
C15:0	1.030	0.893	1.228	1.043	0.0451	0.060
anteiso C15:0	1.184^{ab}	0.974^{b}	1.317^{a}	1.041^{ab}	0.0434	0.014
iso C15:0	0.436^{ab}	0.328^{b}	0.528^{a}	0.507^{a}	0.0256	0.010
C15:1 *trans*10	0.848^{b}	0.715^{b}	1.052^{a}	0.869^{b}	0.0322	< 0.001
C15:1 *cis*10	0.038	0.043	0.073	0.047	0.0072	0.309
C16:0	16.07	15.39	16.25	17.25	0.364	0.360
iso C16:0	0.614	0.505	0.618	0.602	0.0301	0.527
C16:1 *trans*9	0.367	0.233	0.334	0.361	0.0243	0.174
C16:1 *cis*9	0.650	0.527	0.700	0.669	0.0357	0.355
C17:0	0.514^{ab}	0.435^{b}	0.542^{a}	0.514^{ab}	0.0144	0.030
anteiso C17:0	0.979^{a}	0.868^{ab}	0.570^{c}	0.637^{bc}	0.0455	< 0.001
iso C17:0	0.190	0.202	0.281	0.228	0.0301	0.739
C17:1 *trans*10	0.080	0.072	0.103	0.076	0.0056	0.176
C17:1 *cis*10	0.135	0.130	0.165	0.119	0.0097	0.284
C18:0	40.07	39.67	41.76	36.37	0.920	0.126
C18:1 *trans*6 + 7 + 8	0.719	0.825	0.715	0.815	0.0425	0.715
C18:1 *trans*9	0.444	0.489	0.450	0.491	0.0251	0.859
C18:1 *trans*10	0.704^{ab}	1.103^{a}	0.630^{b}	0.674^{ab}	0.0775	0.031
C18:1 *trans*11	8.57^{b}	14.36^{a}	9.46^{b}	10.43^{ab}	0.681	0.009
C18:1 *cis*9	4.17	4.06	3.67	3.54	0.176	0.544
C18:1 *cis*11	5.33	5.45	5.00	5.77	0.172	0.487
C18:1 *cis*12	0.601	0.553	0.840	0.891	0.0570	0.160
C18:1 *cis*13	0.781^{a}	0.777^{ab}	0.581^{b}	0.623^{ab}	0.0729	0.019
C18:1 *cis*14	1.307	1.220	1.226	1.148	0.0439	0.701
C18:2 *cis*9*trans*11	0.184	0.151	0.121	0.174	0.0142	0.425
C18:2 *n*-6	2.222	2.400	2.191	2.456	0.0524	0.212
C18:3 *n*-6	0.030	0.022	0.017	0.031	0.0039	0.461
C18:3 *n*-3	2.069	1.939	2.208	2.418	0.0745	0.099
C19:1 *trans*7	0.099	0.089	0.110	0.101	0.0087	0.539
C19:1 *trans*10	0.302	0.296	0.258	0.244	0.0130	0.285
C20:0	0.512^{ab}	0.459^{b}	0.561^{a}	0.551^{ab}	0.0141	0.033
C20:1 *trans*11	0.034	0.024	0.011	0.097	0.0147	0.136
C20:1 *cis*11	0.039	0.037	0.034	0.039	0.0044	0.979
C20:2 *n*-6	0.199	0.206	0.243	0.184	0.0142	0.491
C20:3 *n*-6	0.059	0.061	0.056	0.062	0.0067	0.992
C20:3 *n*-3	0.089	0.030	0.054	0.029	0.0752	0.770
C20:5 *n*-3	0.490	0.487	0.498	0.552	0.0126	0.206
C21:0	0.309	0.296	0.345	0.306	0.0192	0.742
C22:0	0.457	0.534	0.518	0.564	0.0179	0.192
C22:1 *trans*13	0.017	0.036	0.014	0.036	0.0059	0.393
C22:1 *cis*13	0.077	0.063	0.039	0.025	0.0160	0.686
C22:2 *n*-6	0.180	0.119	0.128	0.100	0.0609	0.865
C22:4 *n*-6	0.085	0.064	0.086	0.085	0.0051	0.396
C22:5 *n*-6	0.110^{ab}	0.097^{b}	0.161^{a}	0.146^{ab}	0.0112	0.047
C22:5 *n*-3	0.032	0.020	0.026	0.038	0.0113	0.742
C22:6 *n*-3	0.037	0.018	0.027	0.022	0.0028	0.083
C23:0	0.242	0.212	0.291	0.301	0.0350	0.807
C24:0	0.012^{b}	0.021^{ab}	0.031^{a}	0.026^{ab}	0.0023	0.024
C24:1 *cis*9	0.053	0.031	0.027	0.046	0.0095	0.761
Sums and calculations						
SFA	57.85	56.46	59.67	55.25	0.776	0.104
OCFA	2.38^{ab}	2.07^{b}	2.75^{a}	3.12^{a}	0.149	0.005
BCFA	3.586^{a}	3.036^{b}	3.505^{ab}	3.071^{ab}	0.0922	0.050
anteiso FA	2.162^{a}	1.724^{b}	1.887^{ab}	1.678^{b}	0.0582	0.002
iso FA	1.424	1.194	1.491	1.447	0.0710	0.125
MUFA	27.69	30.43	25.92	27.70	0.689	0.055
PUFA	8.50^{ab}	8.01^{b}	8.15^{b}	9.58^{a}	0.210	0.015
PUFA *n*-6/*n*-3	1.759	1.866	1.702	1.739	0.0484	0.660
C18:1 *trans*10/11	0.075	0.084	0.067	0.082	0.0047	0.601

$^{a, b, c}$ Means within a row that do not share a superscript letter are statistically different.

[1] SFA, saturated fatty acids; OCFA, odd-chain fatty acids; BCFA, branched-chain fatty acids; FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[2] CON, control; CNS, chestnut peels; SFN, sainfoin; C+S, chestnut peels and sainfoin.

Table 5
Fatty acid profile of abomasum content (g/100 g of fatty acids).

Item[1]	Diet[2]				SEM	P-value
	CON	CNS	SFN	C+S		
C10:0	0.026	0.018	0.025	0.022	0.0036	0.904
C11:0	0.006	0.003	0.010	0.015	0.0030	0.559
C12:0	0.140^{ab}	0.065^{b}	0.168^{a}	0.091^{b}	0.0120	0.004
C13:0	0.039	0.025	0.048	0.044	0.0051	0.425
C14:0	0.486^{ab}	0.348^{b}	0.575^{a}	0.513^{a}	0.0252	0.006
iso C14:0	0.278	0.277	0.419	0.371	0.0233	0.065
C14:1 *trans*9	0.043	0.021	0.056	0.031	0.0063	0.229
C14:1 *cis*9	0.083	0.049	0.084	0.058	0.0075	0.247
C15:0	0.731^{b}	0.616^{b}	0.893^{a}	0.727^{b}	0.0254	< 0.001
anteiso C15:0	0.773^{a}	0.570^{b}	0.833^{a}	0.711^{ab}	0.0269	0.001
iso C15:0	0.415^{a}	0.229^{b}	0.369^{a}	0.367^{a}	0.0220	0.006
C15:1 *trans*10	0.745^{a}	0.506^{b}	0.774^{a}	0.671^{ab}	0.0342	0.016
C15:1 *cis*10	0.015	0.008	0.024	0.021	0.0040	0.518
C16:0	11.84^{a}	10.91^{b}	11.79^{a}	12.27^{a}	0.124	< 0.001
iso C16:0	0.190	0.225	0.190	0.176	0.0207	0.869
C16:1 *trans*9	0.274	0.163	0.238	0.260	0.0161	0.053
C16:1 *cis*9	0.263^{a}	0.181^{b}	0.290^{a}	0.260^{a}	0.0119	0.003
C17:0	0.514^{a}	0.422^{b}	0.547^{a}	0.515^{a}	0.0128	0.001
anteiso C17:0	1.108^{a}	0.952^{ab}	0.606^{c}	0.691^{bc}	0.0535	< 0.001
iso C17:0	0.126	0.124	0.153	0.131	0.0107	0.782
C17:1 *trans*10	0.087	0.050	0.097	0.070	0.0069	0.064
C17:1 *cis*10	0.007	0.004	0.014	0.017	0.0038	0.602
C18:0	47.00	44.43	48.00	45.45	0.766	0.315
C18:1 *trans*6 + 7 + 8	0.852	1.055	0.905	0.997	0.0437	0.308
C18:1 *trans*9	0.519	0.573	0.535	0.592	0.0216	0.610
C18:1 *trans*10	0.759	1.049	0.643	0.889	0.0571	0.061
C18:1 *trans*11	11.31^{b}	16.83^{a}	10.49^{b}	11.45^{ab}	0.797	0.017
C18:1 *cis*9	5.53	5.38	5.18	5.43	0.169	0.915
C18:1 *cis*11	3.48	4.08	3.64	4.12	0.140	0.274
C18:1 *cis*12	0.655	0.474	0.581	0.550	0.0395	0.455
C18:1 *cis*13	0.721^{ab}	1.249^{a}	0.621^{b}	0.700^{b}	0.0816	0.016
C18:1 *cis*14	1.691	1.395	1.506	1.549	0.0429	0.099
C18:2 *cis*9*trans*11	0.125	0.107	0.168	0.109	0.0185	0.647
C18:2 *n*-6	1.734^{ab}	1.921^{a}	1.621^{b}	1.952^{a}	0.0480	0.034
C18:3 *n*-6	0.010	0.059	0.014	0.010	0.0135	0.530
C18:3 *n*-3	1.908	1.580	1.765	1.883	0.0714	0.365
C19:1 *trans*7	0.019	0.025	0.038	0.044	0.0087	0.745
C19:1 *trans*10	0.418	0.351	0.375	0.432	0.0189	0.416
C20:0	0.572^{ab}	0.548^{b}	0.603^{ab}	0.661^{a}	0.0154	0.040
C20:1 *trans*11	0.017	0.011	0.011	0.025	0.0038	0.520
C20:1 *cis*11	0.070	0.066	0.065	0.060	0.0038	0.842
C20:2 *n*-6	0.129	0.132	0.088	0.131	0.0130	0.589
C20:3 *n*-6	0.027	0.075	0.117	0.068	0.0172	0.338
C20:3 *n*-3	0.013	0.008	0.014	0.007	0.0014	0.232
C20:5 *n*-3	0.532^{ab}	0.523^{b}	0.648^{ab}	0.666^{a}	0.0231	0.036
C21:0	0.235	0.236	0.328	0.271	0.0222	0.430
C22:0	0.587	0.605	0.610	0.649	0.0153	0.539
C22:1 *trans*13	0.045	0.100	0.197	0.040	0.0236	0.054
C22:1 *cis*13	0.019	0.032	0.042	0.017	0.0051	0.275
C22:2 *n*-6	0.011	0.016	0.022	0.017	0.0030	0.649
C22:4 *n*-6	0.035	0.020	0.025	0.028	0.0029	0.363
C22:5 *n*-6	0.015	0.012	0.037	0.054	0.0087	0.278
C22:5 *n*-3	0.000	0.005	0.014	0.012	0.0032	0.437
C22:6 *n*-3	0.024	0.010	0.030	0.022	0.0048	0.403
C23:0	0.138	0.124	0.143	0.143	0.0031	0.099
C24:0	0.045	0.061	0.040	0.219	0.0355	0.228
C24:1 *cis*9	0.057	0.056	0.080	0.065	0.0048	0.220
Sums and calculations						
SFA	60.69	56.98	61.82	59.88	0.800	0.127
OCFA	1.664^{ab}	1.427^{b}	1.970^{a}	1.715^{ab}	0.0537	0.001
BCFA	3.029^{a}	2.249^{c}	2.569^{b}	2.447^{bc}	0.0623	< 0.001
anteiso FA	1.881^{a}	1.395^{b}	1.438^{b}	1.402^{b}	0.0553	0.001
iso FA	1.010	0.855	1.131	1.045	0.0427	0.135
MUFA	27.68^{ab}	32.13^{a}	26.51^{b}	28.38^{ab}	0.761	0.029
PUFA	7.08	7.08	7.13	7.58	0.161	0.655
PUFA *n*-6/*n*-3	1.486	1.811	1.459	1.500	0.0566	0.079
C18:1 *trans*10/11	0.073	0.075	0.063	0.081	0.0045	0.571

[a, b, c] Means within a row that do not share a superscript letter are statistically different.

[1] SFA, saturated fatty acids; OCFA, odd-chain fatty acids; BCFA, branched-chain fatty acids; FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[2] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

Table 6
Biohydrogenation indices in rumen and abomasum contents (%).

Item	Diet[1]				SEM	P-value
	CON	CNS	SFN	C+S		
Rumen						
C18:1 *cis*9[2]	69.0[b]	78.5[a]	75.6[ab]	80.6[a]	1.30	0.003
C18:2 *n*-6[2]	87.4[a]	90.9[a]	87.1[a]	74.9[b]	1.43	< 0.001
C18:3 *n*-3[2]	94.13	92.23	93.58	92.87	0.282	0.061
Completeness[3]	64.7	60.9	67.4	61.6	1.28	0.287
Abomasum						
C18:1 *cis*9[2]	62.7[b]	73.8[a]	68.5[ab]	73.7[a]	1.28	0.001
C18:2 *n*-6[2]	91.11[a]	93.32[a]	91.34[a]	84.10[b]	0.730	< 0.001
C18:3 *n*-3[2]	95.10	94.20	95.33	95.16	0.225	0.293
Completeness[3]	71.4[a]	60.5[b]	70.4[a]	67.6[a]	1.18	0.001

[a, b, c] Means within a row that do not share a superscript letter are statistically different.
[1] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.
[2] Calculated according to Oliveira et al. (2016).
[3] Calculated according to Alves et al. (2017).

affected the FA profiles of digesta and meat in the present experiment. This was probably due to the increased dietary intake of tannins, which are known to affect microbial activity and change the proportion of FA in the rumen (Frutos et al., 2020). In particular, tannins affect the presence of two main classes of FA of microbial origin: the odd- and branched-chain FA (OBCFA), which constitute microbial cells, and the FA involved in ruminal BH. Moreover, the different chemical composition of the experimental pellets, such as the high starch and fat content of CNS pellet, may have further affected the composition of rumen microbiota (Mizrahi et al., 2021; Vargas-Bello-Pérez et al., 2016).

Concerning OBCFA, these are particularly related to microbial species, which in turn depend on the composition of the diet. Indeed, cellulolytic bacteria are rich in *iso* FA, whereas amylolytic bacteria contain high amount of *anteiso* FA and OCFA (Vlaeminck et al., 2006). In the present experiment, the tannin-containing diets (i.e., CNS, SFN, C+S) particularly reduced the proportion of *anteiso* FA in rumen and abomasum, suggesting a probable targeted action of tannins against amylolytic bacteria. On the contrary, tannins are generally found to inhibit cellulolytic bacteria and reduce the presence of *iso* FA in the rumen (Alves et al., 2017). However, the great variability of rumen microbiota makes it difficult to draw univocal conclusion, also considering that some important cellulolytic bacteria such as those of *Prevotella* strains are particularly rich in *anteiso* FA (Vlaeminck et al., 2006). Interestingly, feeding the CNS diet had a different effect against ruminal OBCFA compared to the SFN diet, suggesting once again the variability of the action mechanisms of different tannins (Costa et al., 2018, Menci et al., 2021). However, it cannot be ignored that the different ingredients used in the formulation of pellets may have further affected the FA profile of rumen and abomasum contents. For instance, the presence of hydrogenated vegetable oil in the CNS diet may have modified rumen microbiota composition and, thus, rumen FA profile, as already observed by Vargas-Bello-Pérez et al. (2016) in dairy cows fed 27 g DM/kg of hydrogenated palm oil. Consistently with Alves et al. (2017), the different OBCFA content in the abomasum did not lead to a different deposition in meat among treatments. This could have health implications considering that dietary BCFA have shown anti-cancer and anti-inflammatory properties (Vahmani et al., 2020).

Ruminal BH is the process in which rumen microbiota converts dietary unsaturated FA (UFA) such as C18:2 *n*-6, C18:3 *n*-3, and C18:1 *cis*9 to C18:0, producing a number of intermediate FA, including *trans* FA and conjugated FA (Chilliard et al., 2007). In the present experiment, the BH rate of the main UFA reflected the differences in the dietary intake among treatments: the CNS and C+S groups had the highest intake and BH rate of C18:1 *cis*9, and the C+S group had the lowest intake and BH rate of C18:2 *n*-6. Indeed, the greater the quantity of an UFA, the greater its potential toxicity to rumen microorganisms, which leads to a higher BH rate (Maia et al., 2007). The different dietary intake of C18:3 *n*-3 among treatments did not result in a different BH rate probably because even the lowest intake exceeded the toxicity threshold for rumen microorganisms.

Feeding lambs the CNS diet reduced the completeness of BH according to the FA profile of abomasum content, which can be considered the end point of BH (Alves et al., 2017). Indeed, the higher UFA intake of CNS lambs did not result in a higher proportion of C18:0 in digesta, and we observed an accumulation of C18:1 *trans*11 in rumen and abomasum digesta. This confirms the hypothesis of an inhibitory effect of dietary tannins on the last step of ruminal BH, as already reported in vitro (Khiaosa-Ard et al., 2009) and in vivo (Vasta et al., 2009). Instead, we observed no difference in BH completeness when feeding the SFN and C+S diets, suggesting a lack of effect of sainfoin tannins on ruminal BH. However, dietary sainfoin is reported to affect ruminal BH in both lambs (Campidonico et al., 2016) and cows (Huyen et al., 2020), by reducing the BH of C18:3 *n*-3 and promoting the accumulation of conjugated FA. Interestingly, Jerónimo et al. (2010) and Alves et al. (2017) observed that tannins of *Cistus ladanifer* L. slowed down ruminal BH in lambs only when the diet was supplemented with vegetable oils. We cannot therefore rule out that an effect on rumen BH could also have been observed in the SFN and C+S groups if the diets had been supplemented with vegetable oil as was the CNS diet.

The modification of rumen FA profile as a consequence of the slowdown of rumen BH by dietary tannins is not steadily reflected in meat FA profile (Frutos et al., 2020). However, in the present experiment, the higher proportion of C18:1 *trans*11 in digesta led to an increase in this FA in CNS meat of more than 50%. The transfer of C18:1 *trans*11 from rumen to meat is consistent with the findings of Priolo et al. (2021). In the present study, this resulted in a higher proportion of C18:2 *cis*9*trans*11 in CNS meat, even if only compared to the SFN and C+S groups. Indeed, most of the C18:2 *cis*9*trans*11 in meat originates from C18:1 *trans*11 through the action of stearoyl-CoA 9-desaturase in muscle (Bessa et al., 2015). The CNS diet also led to the accumulation of *trans* FA in meat, the consumption of which is associated with an increased risk of coronary heart disease (Mozaffarian et al., 2009). However, the difference in the *trans* FA content of meat among treatments was only due to C18:1 *trans*11, which in turn is reported to have beneficial effects on human health (Vahmani et al., 2020). In particular, C18:1 *trans*11 showed anti-inflammatory, anti-carcinogenic, anti-atherosclerotic, and anti-diabetic effects, both directly and indirectly, after desaturation to C18:2 *cis*9*trans*11 (Vahmani et al., 2020).

4.3. Oxidative stability of meat

The oxidative stability of meat depends on the complex balance between pro-oxidant factors, such as PUFA, and antioxidant factors, such as α-tocopherol. In the present experiment, the diet did not affect the peroxidability of lipids, according to the FA profile of meat, whereas the α-tocopherol content was higher in the meat from lambs fed sainfoin. However, all α-tocopherol values were well below the 3 μg/g indicated by Ponnampalam et al. (2014) as a threshold to ensure good control over lipid oxidation. This probably made the differences in α-tocopherol content irrelevant in slowing down the oxidation of meat.

The inclusion of chestnut shells in the diet (i.e., CNS and C+S) of lambs reduced the daily intake of α-tocopherol, as a consequence of the lack of vitamin E in this by-product (de Vasconcelos et al., 2010).

Table 7
Intramuscular fat content, cholesterol content, and fatty acid profile of lamb.

Item[1]	Diet[2]				SEM	P-value
	CON	CNS	SFN	C+S		
Intramuscular fat, g/kg	15.81	16.78	16.64	15.62	0.746	0.937
Cholesterol, g/kg	0.639	0.654	0.640	0.651	0.0100	0.944
FA profile, g/100 g FA						
C10:0	0.135	0.163	0.191	0.164	0.0113	0.392
C12:0	0.196	0.239	0.342	0.316	0.0232	0.087
C13:0	0.021	0.025	0.031	0.028	0.0020	0.343
C14:0	2.29	2.50	3.09	2.99	0.172	0.251
iso C14:0	0.041	0.038	0.050	0.044	0.0033	0.597
C14:1 *cis*9	0.083	0.089	0.129	0.104	0.0079	0.167
C15:0	0.402	0.394	0.488	0.448	0.0200	0.319
anteiso C15:0	0.149	0.143	0.184	0.163	0.0085	0.318
iso C15:0	0.094	0.089	0.126	0.106	0.0061	0.117
C15:1 *trans*10	0.029	0.021	0.021	0.020	0.0025	0.507
C16:0	20.31	20.15	21.03	21.42	0.238	0.176
iso C16:0	0.181	0.174	0.207	0.186	0.0079	0.455
C16:1 *trans*9	0.187	0.236	0.176	0.165	0.0126	0.185
C16:1 *cis*7	0.265	0.254	0.307	0.287	0.0076	0.058
C16:1 *cis*9	1.235	1.246	1.379	1.288	0.0299	0.321
C17:0	1.289	1.120	1.257	1.174	0.0286	0.088
anteiso C17:0	0.531	0.466	0.531	0.501	0.0138	0.297
iso C17:0	0.411	0.357	0.425	0.413	0.0139	0.312
C17:1 *trans*10	0.021	0.021	0.018	0.012	0.0015	0.074
C18:0	16.29	15.39	15.88	15.32	0.178	0.171
C18:1 *trans*5	0.030	0.036	0.028	0.028	0.0023	0.539
C18:1 *trans*6 + 7 + 8	0.223	0.259	0.210	0.212	0.0117	0.253
C18:1 *trans*9	0.317	0.366	0.400	0.304	0.0227	0.399
C18:1 *trans*10	1.03	1.05	0.82	0.85	0.129	0.885
C18:1 *trans*11	2.28[b]	3.59[a]	2.13[b]	1.87[b]	0.172	0.006
C18:1 *cis*6	0.784	0.736	0.753	0.855	0.0259	0.284
C18:1 *cis*9	31.03	30.53	30.94	30.45	0.364	0.930
C18:1 *cis*11	1.447	1.382	1.391	1.414	0.0395	0.920
C18:1 *cis*12	0.554[b]	0.798[a]	0.475[ab]	0.576[ab]	0.0299	< 0.001
C18:1 *cis*13	0.113	0.111	0.105	0.108	0.0027	0.746
C18:1 *cis*14	0.290	0.258	0.272	0.278	0.0070	0.463
C18:2 *cis*9*trans*11	0.603[ab]	0.784[a]	0.563[b]	0.508[b]	0.0330	0.008
C18:2 *n*-6	6.72	7.27	6.76	7.06	0.233	0.833
C18:3 *n*-6	0.052	0.047	0.045	0.051	0.0020	0.653
C18:3 *n*-3	1.950	1.699	1.980	2.043	0.0733	0.381
C19:1 *trans*7	0.127	0.110	0.101	0.120	0.0050	0.221
C19:1 *trans*10	0.103	0.098	0.071	0.098	0.0061	0.133
C20:0	0.113	0.117	0.138	0.118	0.0037	0.075
C20:1 *cis*11	0.141	0.132	0.154	0.138	0.0053	0.535
C20:2 *n*-6	0.058	0.068	0.054	0.057	0.0030	0.380
C20:3 *n*-6	0.195	0.193	0.155	0.185	0.0101	0.499
C20:3 *n*-3	0.039	0.035	0.032	0.033	0.0025	0.822
C21:0	0.062	0.058	0.067	0.057	0.0033	0.741
C22:0	0.023[b]	0.027[ab]	0.064[a]	0.030[b]	0.0059	0.042
C22:4 *n*-6	0.058	0.042	0.035	0.042	0.0045	0.295
C22:5 *n*-6	0.599	0.573	0.483	0.557	0.0359	0.718
C22:5 *n*-3	0.128	0.137	0.111	0.134	0.0133	0.901
C24:0	0.383	0.351	0.328	0.374	0.0250	0.878
C24:1 *cis*9	0.144	0.135	0.127	0.130	0.0089	0.931
Sums and calculations						
SFA	42.92	41.80	44.42	43.85	0.451	0.153
OCFA	1.775	1.597	1.843	1.707	0.0429	0.207
BCFA	1.406	1.266	1.524	1.413	0.0488	0.334
anteiso FA	0.680	0.609	0.715	0.664	0.0210	0.346
iso FA	0.726	0.657	0.809	0.749	0.0288	0.326
MUFA	40.45	41.21	40.00	39.30	0.442	0.506
PUFA	12.25	12.70	11.81	12.49	0.392	0.886
PUFA *n*-6/*n*-3	4.80	5.85	4.63	4.84	0.176	0.051
PUFA/SFA	0.288	0.305	0.267	0.287	0.0108	0.692
TFA	4.39[b]	5.59[a]	4.00[b]	3.72[b]	0.219	0.001
TFA - C18:1 *trans*11	2.11	2.24	1.86	1.85	0.155	0.732
AI	0.564	0.566	0.655	0.658	0.0223	0.200
TI	1.227	1.198	1.277	1.264	0.0213	0.552
h:H	1.905	1.903	1.758	1.767	0.0411	0.356
DSI_{C14}	0.035	0.034	0.035	0.034	0.0010	0.978
PI	20.40	20.49	19.00	20.51	0.730	0.876

[a, b, c] Means within a row that do not share a superscript letter are statistically different.

[1] FA, fatty acid; SFA, saturated fatty acids; OCFA, odd-chain fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids; AI, atherogenic index (Ulbricht & Southgate, 1991); TI, thrombogenic index (Ulbricht & Southgate, 1991); h:H,

hypocholesterolemic index, calculated as h:H= (C18:1 *cis*9 +PUFA)/(C12:0 +C14:0 +C16:0); DSI_{C14}, desaturation index, calculated as DSI_{C14}=C14:1 *cis*9/(C14:0 +C14:1 *cis*9); PI, peroxidability index, calculated as PI= Σdienoic+ Σtrienoic× 2 + Σtetraenoic× 3 + Σpentaenoic× 4 + Σhexaenoic× 5.

[2] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

Table 8
Fat-soluble vitamins contents of lamb (μg/kg fresh matter).

Item	Diet[1]				SEM	P-value
	CON	CNS	SFN	C+S		
α-tocopherol	798[b]	866[b]	1350[a]	1158[a]	51.7	< 0.001
γ-tocopherol	86.7[b]	133.5[a]	124.1[a]	152.3[a]	5.94	< 0.001
Retinol	21.58[a]	18.57[ab]	17.18[b]	18.06[b]	0.559	0.009

[a, b, c] Means within a row that do not share a superscript letter are statistically different.

[1] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

However, this did not limit the deposition of α-tocopherol in meat, which is crucial to protect lipids from oxidation. Indeed, the α-tocopherol content of CNS and C+S meats was similar to their respective counterparts from lambs that were not fed chestnut shells, namely CON and SFN meats. Probably, the phenolic compounds of chestnut shells exerted their antioxidant effect in the gastrointestinal tract, protecting tocopherols from the oxidation that naturally occurs during digestion (Soldado et al., 2021). Thus, a higher quota of tocopherols was available to build up in tissues and muscles, balancing the lower dietary intake. Concerning the lambs fed sainfoin (i.e., SFN and C+S groups), the high α-tocopherol content in meat was expected considering the higher intake of this vitamin. This could be due to either a higher content of α-tocopherol in sainfoin than in alfalfa or a higher preservation of the vitamin by the phenolic compounds of sainfoin, or even to the combination of these two factors.

Retinol is a vitamin of secondary importance in terms of antioxidant power that originates from the bioconversion of dietary carotenoids (Nozière et al., 2006). Probably, in the present experiment the native β-carotene content of feeds was drastically reduced by the pelletizing process (Nozière et al., 2006) and this, combined with the absence of vitamin supplements in the diet, led to the accumulation of a low amount of retinol in the muscle, compared to previous studies (Valenti et al., 2018; Luciano et al., 2019). Our results seem to suggest that dietary sainfoin might limit the accumulation of retinol in the muscle. Dietary phenolic compounds, such as those contained in sainfoin, have already been hypothesized to have a negative effect on vitamin accumulation in lamb (Luciano et al., 2019) and pork (Menci et al., 2022b), but targeted research is still needed to confirm this effect.

In the present experiment, the colour parameters changed during storage following the typical meat discoloration pattern (Luciano et al., 2009), and the malondialdehyde content of meat increased over time. Although the lambs of CNS, SFN, and C+S groups had a higher intake of phenolic compounds, the colour oxidation and TBARS development in raw meat were similar among treatments. This seems in contrast to the fact that dietary tannins would improve the oxidative stability of animal products (Soldado et al., 2021). However, the complexity of the biological mechanisms underlying oxidative stability often leads to contradictory results. For example, Luciano et al. (2019) observed no differences in TBARS development in raw meat over 7 days of refrigerated storage when feeding lambs with different tanniferous silages, including sainfoin. Chikwanha et al. (2019) studied dietary supplementation of up to 200 g/kg of grape pomace (containing 14 g/kg of total tannins) in lamb and reported no effect on raw meat colour over 9 days of retail display. Furthermore, Valenti et al. (2019) demonstrated that feeding either hydrolysable or condensed tannins at the dose of 2.3% had no effect on the oxidative stability of meat.

5. Conclusion

In the present experiment, chestnut shells, by-product of chestnut industry, were included in a pelleted feed as part of a balanced diet for finishing lambs. Chestnut shells can be fed to lambs with no adverse effects on growth performance and carcass characteristics. However, lower inclusion levels than those used in this study are recommended to avoid a decrease in feed efficiency. The inclusion of chestnut shells in the diet of finishing lambs improved the fatty acid profile of meat by increasing the content of C18:1 *trans*11, a fatty acid recognised for its beneficial effect on human health. This is likely the result of the modulation of ruminal biohydrogenation by the tannins contained in chestnut shells, although a vegetable oil supplement might be needed to highlight this effect. Furthermore, feeding lambs with chestnut shells did not affect the shelf-life of meat, considering discolouration and lipid oxidation. Basing on our results, no synergistic effect between the bioactive compounds of chestnut shells and sainfoin occurred. Farmers should be aware of the low content of crude protein and α-tocopherol in this by-product.

Declaration of Competing Interest

The authors declare that they have no known competing financial

Table 9
Evolution of colour and lipid oxidation in lamb over 9 days of refrigerated storage.

Item[1]	Diet[2] (D)				Storage time, d (T)				SEM	P-value		
	CON	CNS	SFN	C+S	0 (2 h)	3	6	9		D	T	D×T
Colour												
L*	38.36	39.10	37.76	39.37	37.35[c]	38.57[b]	39.56[a]	39.10[ab]	0.245	0.155	< 0.001	0.283
a*	15.00	15.22	15.29	15.20	17.27[a]	15.60[b]	13.89[c]	13.96[c]	0.285	0.907	< 0.001	0.498
b*	16.57	16.42	16.20	16.65	13.59[c]	17.15[b]	16.89[b]	18.22[a]	0.206	0.389	< 0.001	0.970
C*	22.55	22.59	22.47	22.74	22.15[bc]	23.25[a]	21.92[c]	23.02[ab]	0.231	0.910	0.034	0.838
h_{ab}, rad	0.842	0.829	0.822	0.835	0.684[d]	0.837[c]	0.884[b]	0.922[a]	0.0117	0.494	< 0.001	0.297
$I_{450–530}$	95.1	111.7	105.2	103.3	216.1[a]	97.4[b]	62.2[c]	39.6[d]	6.85	0.409	< 0.001	0.093
630/580	2.557	2.641	2.725	2.552	3.888[a]	2.563[b]	2.035[c]	1.989[c]	0.0847	0.392	< 0.001	0.423
ΔE	6.14	6.22	6.61	6.16	-	5.25[b]	6.48[a]	7.11[a]	0.201	0.911	< 0.001	0.669
Lipid oxidation												
MDA, mg/kg	1.278	1.505	1.571	1.440	0.642[c]	0.985[c]	1.843[b]	2.480[a]	0.0861	0.455	< 0.001	0.964

[a, b, c, d] Means within a row that do not share a superscript letter are statistically different for the storage time.

[1] L* , lightness; a* , redness; b* , yellowness; C* , chroma; h_{ab}, hue angle; $I_{450–530}$, integral value of the reflectance spectrum between 450 nm and 530 nm wavelengths; 630/580, ratio between the reflectance at 630 nm and 580 nm; ΔE, total colour change between each day of storage and the day 0, calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL* , Δa* and Δb* are the differences in L* , a* , and b* , respectively, between day 0 and day 3, 6, or 9; MDA, malondialdehyde.

[2] CON, control; CNS, chestnut shells; SFN, sainfoin; C+S, chestnut shells and sainfoin.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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