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Enhancement of lamb meat quality: Effects of dietary supplementation of pistachio hulls

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Abstract

Pistachio hulls were evaluated as a nutrient source in the Sarda lamb finishing system in this study. Twenty male lambs (initial body weight $32.2 \pm \text{SD } 0.42$ kg) were fed for 40 days with either a concentrate diet (169 g/kg dry matter of crude protein, control group) or the same concentrate diet of the control group in which 20% (on a dry matter basis) of the cereals were replaced with pistachio hulls (PH20 group). Hay (*Sulla*, *Hedysarum coronarium*) was given ad libitum in a separate feeder. The results showed that no alterations in growth performance of lambs occurred following feeding with pistachio hulls; however, the feed conversion ratio (FCR) of lambs was slightly higher in the PH20 group than in the other dietary treatments ($P = 0.049$). Intramuscular fat content in lambs fed the PH20 diet was greater ($P = 0.032$) than in the control lambs. Lambs fed the PH20 diet had greater amounts of stearic acid ($P = 0.036$), rumenic acid ($P = 0.005$), and α -linolenic acid (C18:3 n-3; $P = 0.049$) in their *longissimus thoracis et lumborum* muscle (LTL). During 7 days of aerobic storage at 4 °C, thiobarbituric acid reactive substances (TBARS) increased in both groups ($P < 0.001$); however, concentrations of TBARS were significantly lower ($P < 0.001$) in PH20 meat compared to control meat. Lastly, the chroma (C^*) of lambs fed the PH20 diet were significantly greater than those fed the control diet ($P = 0.014$), indicating improved colour stability of PH20 meat compared to control meat.

59 Overall, replacing 20% of dietary dry matter with pistachio hulls preserved animal performance while
60 improving selected nutritional and oxidative stability traits of lamb meat.

61 **Keywords:** by-products, fatty acid, lipids, oxidation, sustainability.

62

63 **Introduction**

64 Interest in utilizing agro-industrial by-products as an alternative feed resource for livestock
65 production has grown due to these products' potential to enhance both economic sustainability of
66 animal production and to diminish the environmental negative consequences of products obtained
67 from animal sources. Ruminators are especially suited to utilize fibrous plant material and residues
68 produced from processing those plants inedible by humans and transform this residue into valuable
69 products for animals (Godfray et al., 2010; Simitzis and Deligeorgis, 2018). In addition, many by-
70 products have high concentrations of bioactive compounds, with polyphenols being the major class
71 of these compounds, that will potentially provide positive attributes to livestock health due to their
72 antioxidant, antimicrobial and anti-inflammatory properties; and can also increase the oxidative
73 stability of meat and milk products (López-Andrés et al., 2014; Routray and Orsat, 2017; Alasalvar
74 et al., 2020; Bešlo et al., 2022).

75 The processing of crops from the Mediterranean region such as fruits, olive, citrus and tree nuts
76 produces large quantities of lignocellulosic residues containing high levels of fibre and polyphenols.
77 Polyphenols included in ruminator diets at moderate levels can also influence rumen microbial
78 activity and improve nitrogen use, as several studies have reported (Min et al., 2003; Waghorn et al.,
79 1987; Makkar et al., 2007). Numerous studies have indicated that by-products with high
80 concentrations of polyphenols can enhance the oxidative stability of meat products and modulate the
81 biohydrogenation of polyunsaturated fatty acids in the rumen, without negatively affecting animal
82 performance (Scerra et al., 2018; Elhadeif et al., 2021; Scerra et al., 2021; Menci et al., 2023; Musati
83 et al., 2024b; Scerra et al., 2024; Zhao et al., 2023).

84

Pistachio hulls (which is the outer pericarp of the pistachio nut) are an abundant but underutilized by-product of the pistachio nut manufacturer; In Italy, pistachio cultivation is concentrated predominantly in Sicily, which accounts for more than 90% of the national production (FAOSTAT, 2022; Barone and Marra, 2004). Pistachio by-products are abundant in polyphenols, tannins and flavonoids, and have been reported to have positive health benefits to animals, improve product quality, and reduce the greenhouse gas methane (Gentile et al., 2007; Abid et al., 2019; Hassan et al., 2020; Noruzi et al., 2024). Research with lambs has demonstrated that feeding pistachio by-products (pistachio green hull aqueous extract or a mixture of soft hulls, leaves, and twigs) at levels of up to 30% in the diet does not negatively affect growth performance, and may even increase the antioxidant status of the meat and reduce the amount of lipid oxidation (Norouzian and Ghiasi, 2012; Kazemi et al., 2024; Noruzi et al., 2024). To date however, there is very limited information regarding the effect of pistachio hulls on growth performance and carcass characteristics, as well as the fatty acid profile of the meat and meat oxidative stability of growing lambs. Therefore, the objective of the current research was to evaluate the effects of adding 20% pistachio hulls to the diet of Sarda male lambs on growth performance, meat composition and oxidative stability of the meat.

2. Materials and Methods

2.1 Animal Management and Experimental Design

The experimental methods of this trial have been authorized by the Animal Welfare Committee of the University of Reggio Calabria (protocol No. 0000723).

The trial involved twenty Sarda male lambs. Lambs were weaned at 60 days of age and fed for 80 days with a commercial concentrate including, on a dry matter basis, 60% cereal mix, made up of equal quantities of maize and barley, 20% fava bean, 17% wheat bran, 3% vitamin mineral premix and with Sulla hay (*Hedysarum coronarium*) in a separate feeder (*ad libitum*). Subsequently, at the average initial body weight $32.2 \pm \text{SD } 0.42$ kg, the lambs were allocated in individual straw-bedded pens and randomly assigned to two dietary treatments (n=10), balanced for the initial body weight,

111 and adapted to the respective experimental diets for 6 days. At the end of the adaptation period, each
112 group was fed *ad libitum* with one of the following concentrate-based diet for 40 days: the same
113 commercial maize-barley-faba bean based concentrate used after weaning (control); control diet with
114 20 % of pistachio hulls inclusion (on a dry matter basis) as partial replacement of barley, maize and
115 faba bean (PH20). Diets were formulated to provide comparable levels of crude protein and
116 metabolizable energy. The pistachio by-product was supplied by the organic farm Di GaLa (San
117 Biagio Platani, AG). The by-product provided was free of twigs or leaves and therefore consisted
118 exclusively of pistachio hulls. Ingredients and the chemical composition of the experimental diets are
119 reported In Table 1. The ingredients of each concentrate mixture were ground to avoid the selection
120 of ingredients and pelleted. Sulla hay was given *ad libitum* in a separate feeder.

121 The experimental diets were provided twice daily, early morning and late afternoon; refused feeds
122 were collected from the individual pens and weighed to estimate the amount of feed intake. Daily
123 voluntary feed intake was measured by recording the amounts of feed offered and refused.

124 Once a week the animals were weighed to assess average daily gain. At the end of the trial, all the
125 lambs were slaughtered on the same day in a commercial abattoir according to the European Union
126 welfare guidelines (Directive 2010/63/EU; CR 1099/2009). The experimental diet and water were
127 available to lambs until approximately 4 h before slaughter. Lambs were firstly stunned by a captive
128 bolt and exsanguinated. Each carcass was immediately weighted and stored at 4 °C for 24 h.
129 Subsequently, the entire *longissimus thoracis et lumborum* muscle (LTL) was excised from both sides.
130 The right LTL was vacuum- sealed and stored at - 30 °C for later analysis of FA composition,
131 proximate composition and α -tocopherol. The left LTL was designated for shelf-life study and
132 subsequent evaluations of lipid oxidation.

133

134 **2.2 Analyses on feed and meat**

135 Throughout the feeding trial, feed samples were collected weekly from each experimental diet and
136 stored under vacuum at -30 °C. At the end of the trial, the samples were ground and pooled to obtain

137 representative composite samples of each dietary treatment. The proximate composition of the
138 representative samples of the experimental diets was evaluated through analysis of crude protein,
139 ether extract, and ash using the analysis methods developed by the Association of Official Analytical
140 Chemists (AOAC, 1995), which are identified by the references 984.13, 920.39, and 942.05
141 respectively. The fiber fraction was assessed for neutral detergent fiber (NDF) by using the analysis
142 method described by Van Soest et al. (1991). Metabolizable energy was estimated based on Cannas
143 et al. (2004). The total lipids used for fatty acid profiling were extracted and quantified using the
144 methods set forth by Gray et al. (1967).

145 Vitamin E was extracted from feedstuffs and quantified following the method of Rufino-Moya et al.
146 (2020). Briefly, 200 mg of diet or by-product were extracted with a 1:1:1 mixture of methanol,
147 acetone, and petroleum ether. After centrifugation ($1000 \times g$ for 5 min), the supernatant was collected
148 three times. The combined extracts were evaporated under a nitrogen stream at 40 °C, and the residue
149 was reconstituted in methanol and filtered through 0.22 μm PTFE filters prior to analysis. While, the
150 content of α -tocopherol in LTL was determined according to the method described by Bertolín et al.
151 (2018), with the modifications reported by Menci et al. (2022). Briefly, a 2.5 g sample was mixed
152 with 0.2 g of ascorbic acid and 7.5 mL of 10% KOH in ethanol:water and incubated overnight on an
153 orbital shaker. Lipids were then extracted twice with 5 mL of hexane:ethyl acetate (9:1, containing
154 25 mg/L BHT), followed by centrifugation. The combined supernatants were evaporated under a
155 nitrogen stream, and the residues were reconstituted in methanol and filtered prior to analysis. Vitamin
156 was separated and quantified by UHPLC (Nexera, Shimadzu, Japan) using a C18 column (Zorbax
157 ODS; 25 cm $\times$ 4.6 mm, 5 μm). Instrumental conditions followed Natalello et al. (2022). Quantification
158 was performed using external calibration curves prepared with pure standards (Merck Life Science
159 s.r.l., Milan, Italy).

160 To determine the total amount of total polyphenols present in a feed sample, an aqueous/acetic acid
161 (acetone) solvent system was employed. Quantification of total polyphenols was carried out using the
162 Folin-Ciocalteu colorimetric method (Makkar et al., 1993).

163 Meat sample analyses were performed on *LTL* muscle. Moisture, crude fat, ash and crude protein
164 levels were evaluated according to standard AOAC analyses (AOAC, 1995; methods 950.46, 991.36,
165 920.153, and 984.13). The meat colour properties measured included L* (lightness), a* (red), b*
166 (yellow), chroma (C*), and hue angle(H*) based on the CIELAB system and were determined by
167 conducting three separate measurements with a Minolta CR-300 colorimeter (CR-300 Model,
168 Minolta Co., Ltd., Osaka, Japan) and by using the D65 light source for the measurement after
169 calibration with a standard white tile.

170

171 **2.3 Oxidative stability of lipids and fatty acid composition in meat samples**

172 The *LTL* sections were kept in a refrigerator at 4 °C under an environment that is uninterrupted by
173 oxygen through cover sheets and underwent analysis on the fresh item after different storage times;
174 T0 (0 days), T4 (4 days) and T7 (7 days).

175 The thiobarbituric acid reactive substances (TBARS) assay is an indicator of lipid oxidation and was
176 measured using the Siu and Draper procedure (978). For this analysis, 2.5 g of meat was homogenized
177 (with Ultraturrax, model T25, IKA-Werke GmbH & Co.KG, Staufen, Germany) in 12.5 mL of
178 distilled water. The resulting homogenate was then treated with 12.5 mL of 5% (w/v) trichloroacetic
179 acid, thoroughly vortexed, and subsequently filtered through Whatman No. 1 filter paper. Four mL of
180 a tested homogenate was aliquotted and mixed with 1 mL thiobarbituric Acid (0.02 M) and incubated
181 at 80 °C for 90 minutes. The absorbance was read at 532 nm using a double beam UV
182 Spectrophotometer (Shimadzu Corp., Milan, Italy, UV-1800). The data was expressed in mg of
183 malondialdehyde (MDA)/kg of meat.

184 Using the method proposed by Folch, Lees and Sloane Stanley (1957), intramuscular fat was obtained
185 from 5 g of fresh meat by using chloroform/methanol (2:1, v/v). Then, the lipid extract was
186 methylated with 1 ml of hexane and 0.05 ml of 2 N methanolic KOH (IUPAC, 1987), and C19:0 was
187 used as the internal standard.

188 Fatty acid methyl esters were analyzed with the use of a Varian CP-3900 gas chromatogram (Mitchell
189 Drive Walnut Creek CA USA). This chromatogram has a CP-Sil 88 column (100M × 0.25 mm i.d.,
190 film thickness 0.25µm; Agilent J&W) and one microliter of sample was injected using helium as the
191 carrier gas at a flow rate of 0.7 mL/min. The chromatograph conditions are as follows: the flame
192 ionization detector (FID) temperature is set at 260 °C; the injection port (split/splitless mode) is at
193 220 °C with an injection flow of 120 mL/min; column oven temperature begins with 4 minutes at 140
194 °C before increasing to a final temperature of 220 °C at 4 °C/min. The identification of individual
195 fatty acids is done by comparing the retention time of the fatty acid methyl esters (FAME) with that
196 of the standard FAME mixture (Supelco Inc., Bellefonte PA USA) and are quantified in terms of
197 mg/100 g of total fatty acids.

198

199 **2.4 Statistical analysis**

200 A one-way ANOVA was used to statistically analyze the effects of dietary treatments on animal
201 performance, feed intake, chemical composition of muscle, and intramuscular fatty acid composition,
202 considering the individual lambs as the experimental units. The assumption of normality of the data
203 distribution was assessed using the Shapiro–Wilk test. All statistical analyses were performed using
204 Minitab software (version 14, Minitab Inc., State College, PA). For testing the effect of the dietary
205 treatment, time of storage, and their interaction as fixed factors on colour and lipid oxidation
206 parameters was applied a mixed model for repeated measures, with individual lambs as random factor.
207 The Tukey test for multiple comparisons was performed when $P \leq 0.05$, whereas tendencies toward
208 significance were considered when $0.05 < P \leq 0.10$.

209

210 **Results**

211 Table 2 summarizes how dietary treatments impacted *in vivo* performance, and chemical composition
212 of the *LTL* muscle of Sarda lambs. The inclusion of 20% pistachio hulls had little effect on the final
213 body weight, carcass weight, dry matter intake and average daily gain of lambs ($P > 0.05$). The final

body weight of the control and PH20 lambs was not significantly different, and no differences in carcass yields or total feed intake were observed between lambs fed the two diets. The average daily gain of lambs on the PH20 group was numerically lower than that of the control group, but this difference was not statistically significant ($P > 0.05$). Conversely, the feed conversion ratio (FCR) was significantly affected by dietary treatment ($P < 0.05$), with lambs fed the PH20 diet showing higher FCR values, indicating lower feed efficiency compared with control lambs.

Regarding the chemical composition, the levels of alpha-tocopherol and ether extract were significantly higher ($P < 0.001$) in the PH20 group compared with the control group (Table 2).

The inclusion of pistachio hulls in the diet markedly influenced the fatty acid profile of muscle tissue (Table 3). In lambs, the experimental diet (20% pistachio hulls) resulted in a significant accumulation of several fatty acids in muscle compared with the control group. Furthermore, the intramuscular fat (IMF) level was also higher ($P < 0.05$) in meat from PH20 group than in control group. Fatty acids that increased significantly included stearic acid (C18:0, $P < 0.05$), *trans*-9-octadecenoic acid (C18:1 *trans*-9, $P < 0.01$), rumenic acid (C18:2 *cis*-9, *trans*-11, $P < 0.01$) and α -linolenic acid (C18:3 n-3, $P < 0.05$); furthermore vaccenic acid (C18:1 *trans*-11) was tendentially higher ($P = 0.057$; trend toward significance) in meat from PH20 group compared with the control group. However, no significant differences were observed in oleic acid (C18:1 *cis*-9), total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), or total polyunsaturated fatty acids (PUFA) levels ($P > 0.05$). Total n-6 and n-3 fatty acid composition, as well as the n-6/n-3 ratio, were not affected by dietary treatment.

The pattern of lipid oxidation over time and diet was evaluated in Figure 1, through changes in TBARS value during refrigerated storage. The dietary treatment affected secondary products of lipid oxidation, with an average higher ($P < 0.05$) TBARS value in control meat than in PH20 meat. A similar trend was shown by interaction diet $\times$ time in TBARS, with notable differences only starting from day 4 of storage ($P < 0.01$). In particular, at day 0 the mean MDA values did not differ between the control and PH20 groups ($P > 0.05$). However, after four days of storage, MDA levels increased

240 in both groups. By day 7, the control group exhibited significantly higher MDA values than the PH20
241 group ($P < 0.001$), indicating greater lipid oxidation.

242 Dietary treatment and storage time influenced meat colour parameters. Muscles from lambs fed the
243 pistachio hulls diet (PH20) showed tendentially higher a^* (redness) values ($P = 0.052$) than those
244 from the control group. However, redness (a^*) values decreased over storage time ($P < 0.001$) in both
245 groups, with a less pronounced decline in the PH20 group compared with control group (diet $\times$ time
246 interaction, $P < 0.025$). b^* (yellowness) values were not affected by dietary treatment ($P > 0.05$), but
247 decreased during storage ($P < 0.001$). Lightness (L^*) was also unaffected by diet but decreased
248 progressively during refrigeration ($P < 0.001$). Chroma (C^*) values were higher in the PH20 group
249 at all time points ($P < 0.05$), although no diet $\times$ time interaction was observed ($P > 0.05$). No
250 significant effect of diet was found on *hue* angle (H^*) values; however, H^* decreased over the storage
251 period ($P < 0.001$).

252

253 Discussion

254 The present study evaluated the effects of partially replacing conventional cereals with pistachio hulls
255 at a 20% inclusion level in Sarda lamb diets. The current trend towards sustainable production and
256 circular economy models supports the utilization of agro-industrial waste products instead of
257 traditional feedstuffs in order to improve resource use efficiency, with no reduction in the quality of
258 the final animal products (Vastolo et al., 2022; Musati et al., 2023; Shah et al., 2025). The high
259 availability of pistachio processing residues, together with its high content of fermentable
260 carbohydrates, fibre, and polyphenols (including flavonoids, anthocyanins and their related
261 compounds), and their antioxidant properties, supports their potential use as feed ingredients capable
262 of reducing feed costs while improving meat oxidative stability (Tomaino et al., 2010; Shakeri et al.,
263 2017; Arjeh et al., 2020; Koyuncu et al., 2025).

264 In the present trial, no effect on growth performance and carcass yield, in agreement with previous
265 findings on nut-derived by-products in small ruminants, where moderate inclusion level did not affect

266 growth or feed intake (Ghasemi et al., 2012; SoltaniNezhad et al., 2016). Similarly, pistachio by-
267 product inclusion (up to 30%) has been reported not to impair growth performance while promoting
268 improved antioxidant status and reduced lipid oxidation in lamb meat (Norouzzian and Ghiasi, 2012;
269 Noruzi et al., 2024; Kazemi et al., 2024). Comparable results have also been reported for almond
270 hulls, which can be safely included in balanced diets despite their high lignin and fibre content (Scerra
271 et al., 2022; Scerra et al., 2023). A slight deterioration of feed conversion ratio was observed in lambs
272 fed the PH20 diet, likely related to lower digestibility and energy density of fibrous residues, has been
273 previously reported and is consistent with the present findings, underscoring the importance of
274 appropriate diet formulation when high-fibre by-products are used (Recalde et al., 2024; Cachucho
275 et al., 2025; Sirakaya, 2023; Zoabi et al., 2025). Similar benefits on intestinal morphology, antioxidant
276 capacity, rumen fermentation, and methane mitigation have been described following dietary
277 supplementation of pistachio hulls, generally without compromising digestibility (Abid et al., 2019;
278 Hassan et al., 2020). Comparable results with olive cake and citrus pulp further support their role as
279 sustainable feed components in small ruminants (Gravador et al., 2014; Caparra et al., 2023; De Caria
280 et al., 2025).

281 The increase in muscle ether extract observed in PH20 lambs suggests a modest enhancement of lipid
282 deposition, consistent with responses to almond hulls (Scerra et al., 2022; Scerra et al., 2023),
283 plausibly mediated by plant secondary metabolites that influence rumen fermentation and lipid
284 metabolism (Musati et al., 2023; Shah et al., 2025). In this context, polyphenols-rich agro-industrial
285 by-products have been increasingly recognized as functional feed ingredients capable of modulating
286 lipid metabolism and improving animal performance (Elhadeif et al., 2021; Zhao et al., 2023), and
287 pistachio hulls appear to fit within this broader category.

288 Regarding the fatty acid composition, the higher α -linolenic acid content in PH20 meat likely reflects
289 partial protection of dietary PUFA from ruminal hydrogenation and modulation of microbial lipid
290 metabolism driven by tannins and other polyphenolic compounds in pistachio hulls. These
291 mechanisms have been demonstrated in studies employing tannin-rich feeds or plant extracts that

292 either inhibit specific steps of the biohydrogenation pathway or shift microbial populations toward
293 species associated with incomplete hydrogenation (Vasta and Luciano, 2011; Buccioni et al., 2012;
294 Kahraman et al., 2023; Musati et al., 2025).

295 The increase in rumenic acid is of nutritional relevance, given its reported beneficial effects on lipid
296 metabolism, inflammation, and oxidative status (Givens, 2010). Its higher concentration in meat from
297 the PH20 group may result from increased formation from vaccenic acid and reduced further
298 hydrogenation to stearic acid. Comprehensive reviews on pistachio by-products confirm that their
299 polyphenolic profile, rich in flavan-3-ols, phenolic acids and condensed tannins, can interact with
300 lipid metabolism and microbial consortia in the rumen, ultimately influencing the pattern of
301 biohydrogenation intermediates (Kahraman et al., 2023). The higher C18:0 levels in PH20 meat are
302 likely attributable to its higher levels in the PH20 diet.

303 The rise in α -linolenic acid further suggests that pistachio hulls contribute not only as fibre source but
304 also as a carrier of bioactive compounds capable that enhance meat nutritional quality. Similar effects
305 have been observed in lambs fed hazelnut skin or linseed-based diets, where increased α -linolenic
306 acid levels were attributed to reduced ruminal degradation rather than *de-novo* synthesis (Ciliberti et
307 al., 2024; Musati et al., 2024a; Cattivelli et al., 2025).

308 From a comparative standpoint, almond, hazelnut and pistachio by-products share compositional
309 traits such as residual unsaturated lipid and polyphenols. However, differences in tannin structure,
310 fibre composition, and flavonoid profile likely explain variability in animal responses (Monagas et
311 al., 2009; Arjeh et al., 2020; Ivanović et al., 2020; Koç and Atar Kayabaşı, 2023; Silva et al., 2025).

312 In particular, pistachio by-products used in previous studies often contained mixed fractions (hulls,
313 leaves, and shells) with higher tannin content and lower fermentable carbohydrate availability, which
314 may negatively affect intake and performance at high inclusion levels (Ghaffari et al., 2014; Soltani
315 Nezhad et al., 2016; Cachucho et al., 2024; Recalde et al., 2024). By contrast, the pistachio hulls used
316 in the present study exhibited a more balanced composition, which likely contributed to the absence
317 of negative effects on intake (Ghaffari et al., 2014; Soltani Nezhad et al., 2016).

318 In ruminants, change in tissue fatty acids composition are inherently limited by rumen
319 biohydrogenation and by the physicochemical properties of dietary lipid, which restrict PUFA transfer
320 to tissues (Chilliard et al., 2007; Sinclair et al., 2005; Chikunya et al., 2004). Within these constraints,
321 the moderate increases in α -linolenic and rumenic acids observed in the present study can nevertheless
322 be considered nutritionally meaningful improvements in meat lipid quality (Cal-Pereyra et al., 2023).
323 The antioxidant responses observed at muscle level further support the potential of pistachio hulls as
324 a functional feed ingredient. Among the secondary compounds generated during lipid peroxidation,
325 malondialdehyde is one of the most abundant and widely used indicators of oxidative deterioration,
326 being measured, along with other reactive aldehydes, by the TBARS assay. Fresh meat from lambs
327 fed the PH20 diet exhibited lower levels of secondary lipid oxidation products than meat from control
328 lambs. Although TBARS values increased during storage in both groups (diet $\times$ time interaction),
329 PH20 meat exhibited lower TBARS values than control meat at the final storage time point. On day
330 7 of refrigerated storage, the control treatment showed 1.99 mg MDA/kg meat, whereas PH20
331 samples remained below 1.5 mg MDA/kg. Campo et al. (2006) reported 2 mg MDA/kg meat as the
332 sensory threshold for rancid off-flavour perception in fresh meat. Accordingly, TBARS values in
333 control samples approached this threshold, suggesting a potential negative impact on flavour and
334 aroma perception by consumers. The lower MDA formation in meat from the PH20 group, along with
335 stabilized redness and chroma values during storage, are consistent with the antioxidant capacity of
336 pistachio green hull extracts in meat systems, where lipid and pigment oxidation are delayed
337 (Noorolahi et al., 2020). This result was likely due to the increased α -tocopherol content in PH20
338 meat, which contributed to its superior antioxidant capacity. The α -tocopherol has been extensively
339 studied because it protects membrane fatty acids from lipid peroxidation (Ponnampalam et al., 2022).
340 However, α -tocopherol levels in PH20 meat compared to control meat were unexpectedly higher,
341 considering the α -tocopherol content of the experimental diets. Drawing on data from previous studies
342 regarding the potential influence of polyphenol-rich by-products on nutrient absorption (Viveros et
343 al., 2011; Iglesias et al., 2012; Abu Hafsa and Ibrahim, 2018), we hypothesized that the

344 supplementation of pistachio hulls may have positively influenced vitamin E absorption. Evidence
345 on the effects of dietary polyphenols on meat antioxidant status in ruminants remains inconsistent
346 (Luciano et al., 2011a, b), likely reflecting their structural diversity and limited bioavailability, which
347 constrains intestinal uptake and tissue deposition, as reported for condensed tannins (Soldado et al.,
348 2021). Nonetheless, indirect antioxidant effects have been described. Lobón et al. (2017), for
349 example, found that quebracho tannins increased muscle α -tocopherol and reduced lipid oxidation in
350 lambs, despite unchanged vitamin E intake. In this context, pistachio hulls supplementation may have
351 improved antioxidant status by enhancing tocopherol intake and by limiting their oxidation through
352 polyphenol-mediated protection. The elevated content of polyphenols in pistachio hulls increased the
353 levels of these compounds in the PH20 treatment, resulting in a concentration 3–4 folds higher than
354 in the control treatment. Recent studies conducted by Elakremi et al (2020; 2022) have shown that
355 processing wastes from pistachios exhibit a very high concentration of polyphenolic compounds, and
356 a larger portion of those compounds have significant antioxidant ability. Phenolic acids, anthocyanins
357 and flavonoids have the ability to stabilize membranes and scavenge reactive oxygen species
358 (Bellomo and Fallico, 2007; Hayes et al., 2010). In addition, several studies have emphasized the
359 capacity of polyphenol-rich agro-industrial by-products to enhance the oxidative stability of meat and
360 animal products, thereby improving shelf life and product quality (Scerra et al., 2023; Cifuni et al.,
361 2023; Duncan et al., 2024; Bouzaïene et al., 2024). Likewise, hazelnut skins and almond hulls
362 decreased TBARS and improved oxidative stability in lambs, reflecting their richness in polyphenols
363 and tocopherols (Della Malva et al., 2023; Ciliberti et al., 2024; Musati et al., 2024a; Cachucho et al.,
364 2024; Recalde et al., 2024; Cattivelli et al., 2025).

365 Another important determinant of consumer acceptance is meat colour. Regarding storage time,
366 almost all investigated parameters were affected over the 7-day period. The colour descriptors
367 exhibited a discoloration pattern consistent with meat browning over time (Faustman et al., 2010),
368 characterized by a decrease in redness (a^*) and concomitant increases in yellowness (b^*) and hue
369 angle, in agreement with previous studies conducted under similar experimental conditions (Luciano

et al., 2019; Scerra et al., 2022; Menci et al., 2023). However, redness (a^*) and chroma values in the PH20 group indicate better colour stability and consumer appeal, likely due to the preservation of oxymyoglobin and protection of membrane lipids from peroxidation. In fact, fresh meat from lambs fed the PH20 diet exhibited a tendentially higher redness (a^*) value than meat from control lambs. Although redness (a^*) values decreased linearly during storage in both groups, PH20 meat exhibited higher values than control meat at the final storage time point (13.6 vs 11.8 in PH20 and control groups, respectively). This is in line with evidence for natural carotenoids and polyphenols improving colour stability in beef and poultry products (Bellomo and Fallico, 2007; Hayes et al., 2010; Rajput et al., 2014; Natalello et al., 2020; Terevinto et al., 2023). Similar improvements have been described with almond hulls (Cachucho et al., 2024; Recalde et al., 2024), hazelnut skins (Della Malva et al., 2023; Ciliberti et al., 2024; Musati et al., 2024a; Cattivelli et al., 2025), which enhances redness and mitigates discoloration during storage.

382

383 **Conclusions**

384 The present study demonstrates that partial replacement of conventional cereals with pistachio hulls
385 at a 20% inclusion level in Sarda lamb diets can be implemented without compromising growth
386 performance or carcass yield. A slight deterioration of feed conversion ratio was observed in lambs
387 fed the PH20 diet, likely related to lower digestibility and energy density of fibrous residues,
388 underscoring the importance of appropriate diet formulation when high-fibre by-products are used.
389 The dietary use of this polyphenol-rich agro-industrial residue selectively modulated meat lipid
390 composition, enhancing rumenic and α -linolenic acids, while maintaining overall fatty acid balance
391 and quality indices. These modifications are consistent with the recognized ability of nut-derived by-
392 products to influence rumen biohydrogenation dynamics and promote the accumulation of health
393 promoting intermediates such as CLA and n-3 fatty acids. In parallel, the observed reduction in lipid

394 peroxidation and the improvement in color vividness confirm the antioxidant efficacy of pistachio
395 hulls.

396 Taken together, these findings support the feasibility of incorporating pistachio hulls as a functional
397 feed ingredient for small ruminants, offering dual benefits in terms of oxidative stability and
398 nutritional enhancement of meat. The cross-species consistency of antioxidant and lipid responses
399 observed with other nut and fruit residues, such as almond hulls, hazelnut skins, and olive cake,
400 further highlights the robustness of this strategy within circular livestock systems. The valorization
401 of pistachio processing by-products therefore represents a practical route to reduce feed costs, lower
402 environmental impact, and replace synthetic antioxidants with natural alternatives of regional origin.

403

404

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Table 1. Ingredients (% on DM basis) and chemical composition of the experimental diets.

	Pistachio hulls	Dietary treatment ¹	
		Control	PH20
<i>Ingredients, % as fed basis</i>			
Maize		30	18
Barley		30	18
Fava bean		20	24
Bran		17	17
Pistachio hulls		-	20
Vitamin mineral premix ²		3	3
<i>Chemical composition</i>			
Dry matter (DM; g/kg wet weight)	923	913	912
Crude protein (g/kg DM)	97.0	169	168
Ether extract (g/kg DM)	56.7	23.3	26.1
Ash (g/kg DM)	149	53.6	58.4
Neutral detergent fiber (NDF; g/kg DM)	316	269	306
Metabolizable energy (MJ/kg DM)	-	11.3	11.0
Total extractable polyphenols (mg Tae ³ /g DM)	37.6	1.48	4.01
α-Tocopherol (μg/g DM)	10.90	13.5	13.3
<i>Fatty acids (g/100g of total fatty acid)</i>			
C12:0	0.17	0.16	0.16
C14:0	0.43	0.20	0.24
C16:0	18.9	19.2	18.8
C18:0	7.25	2.60	3.00
C18:1 n-9	35.0	24.6	25.8
C18:2 n-6	17.2	45.1	37.2
C18:3 n-3	3.00	1.84	2.48

¹Control: maize-barley-fava bean based concentrate diet. PH20: diet including 20 % of pistachio hulls.

² The mineral vitamin premix consisted of vitamin A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg.

³ Tannic acid equivalents.

Table 2

Animal performance and chemical composition of muscle (g/100g wet weight).

	Dietary treatment ¹		SEM ⁶	<i>P</i> value
	Control	PH20		
Final BW ² , kg	40.2	39.0	1.370	0.127
Carcass weight, g	17.7	16.9	0.695	0.441
DMI ³ , g/d	909	898	8.000	0.499
ADG ⁴ , g/d	223	198	6.760	0.069
FCR ⁵ , g DMI ³ /g ADG ⁴	4.10	4.50	0.161	0.049
<i>Chemical composition</i>				
α-Tocopherol, µg/g muscle	0.535	0.659	0.019	0.001
Moisture	74.3	74.2	0.400	0.951
Crude protein	20.8	20.9	0.129	0.943
Ether extract	2.17	2.40	0.039	0.001
Ash	1.07	1.08	0.013	0.636

¹ Control: maize-barley-fava bean based concentrate diet. PH20: diet including 20 % of pistachio hulls.²BW=Body weight; ³DMI=dry matter intake; ⁴ADG=average daily gain; ⁵FCR=feed conversion ratio; ⁶SEM=standard error of means.

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Table 3

Effect of the dietary treatments on fatty acid composition of muscle
(mg/100 g of meat)

Item	Dietary Treatment ¹		SEM ³	P values
	Control	PH20		
intramuscular fat, mg/100 g of muscle	1997	2198	142	0.032
C12:0	0.07	0.08	0.009	0.802
C14:0	1.30	1.60	0.119	0.338
C15:0 <i>iso</i>	0.10	0.06	0.004	0.323
C15:0 <i>anteiso</i>	0.10	0.07	0.005	0.485
C14:1 <i>cis-9</i>	0.05	0.05	0.005	0.821
C16:0	19.0	21.0	0.719	0.116
C17:0 <i>iso</i>	0.38	0.37	0.021	0.960
C16:1 <i>cis-9</i>	1.38	1.36	0.047	0.823
C17:0	0.87	0.98	0.029	0.056
C18:0	14.1	15.8	0.416	0.036
C18:1 <i>cis-9</i>	32.0	35.1	1160	0.196
C18:1 <i>trans-9</i>	0.16	0.24	0.016	0.009
C18:1 <i>trans-11</i>	0.65	0.77	0.039	0.057
C18:2 <i>cis-9, cis-12</i> LA ²	10.7	8.06	0.847	0.127
C18:3 n-3 ALA ²	0.11	0.25	0.008	0.049
C18:2 <i>cis-9 trans-11</i>	0.32	0.44	0.022	0.005
C 20:1 <i>cis-11</i>	0.50	0.41	0.039	0.264
C20:2 <i>cis 11,14</i>	0.29	0.08	0.114	0.387
C20:3 n-6	0.34	0.25	0.026	0.112
C20:4 n-6	4.82	3.71	0.454	0.235
C20:5 n-3 EPA ²	0.28	0.22	0.045	0.518
C22:4 n-6	0.39	0.35	0.029	0.550
C22:5 n-6	0.15	0.13	0.014	0.337
C22:5 n-3 DPA ²	0.60	0.56	0.061	0.734
C22:6 n-3 DHA ²	0.21	0.15	0.040	0.465
Σ n-6	16.7	12.6	1.410	0.156
Σ n-3	1.21	1.17	0.127	0.620
n-6/n-3	14.8	10.7	0.875	0.109
Σ SFA ²	35.5	39.8	1.160	0.058
Σ MUFA ²	34.8	37.9	1.160	0.182
Σ PUFA ²	18.2	14.2	1.500	0.101

¹ Control: maize-barley-fava bean based concentrate diet. PH20: diet including 20 % of pistachio hulls.

²LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

³SEM= Standard error of the mean.

Table 4

Effect of the dietary treatments and time of refrigerated storage on meat colour stability

	Dietary treatment ¹		Time (T) ³			SEM ⁴	<i>P</i> values		
	Control	PH20	0	1	2		Diet	Time	Diet x Time
L* values ²	41.9	42.5	40.4 ^c	42.51 ^b	43.73 ^a	0.276	0.206	< 0.001	0.135
a* values ²	14.3	14.9	16.0 ^a	15.1 ^a	12.7 ^b	0.242	0.052	< 0.001	0.025
b* values ²	12.1	12.8	10.6 ^c	12.6 ^b	14.1 ^a	0.271	0.067	< 0.001	0.561
C* values ²	18.9	19.7	19.2	19.6	19.1	0.178	0.014	0.382	0.119
H* values ²	40.4	40.4	33.5 ^c	39.8 ^b	47.91 ^a	0.947	0.985	< 0.001	0.075
TBARS ⁵	1.77	1.26	0.27 ^c	1.69 ^b	2.59 ^a	0.148	0.023	< 0.001	0.004

^{a,b,c} Within row, different superscripts indicate differences between days of storage ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.

¹Control: maize-barley-fava bean based concentrate diet. PH20: diet including 20 % of pistachio hulls

²L*=lightness; a*=redness; b*=yellowness; C*=Chrome; h*=hue angle, measured in degrees.

³Times 0, 1, 2 = days 0, 4, 7 for raw meat at 4 °C under aerobic conditions (meat slices).

⁴SEM= Standard error of the mean.

⁵TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).