1 **This is the peer reviewed version of the following article**

2 **Scerra M, Bognanno M, Foti F, Caparra P, Cilione C, Mangano F, Natalello A, Chies L, 2022.**

3 **Influence of almond hulls in lamb diets on animal performance and meat quality**

4 ***Meat Science, article 108903. ISSN: 0309-1740***

5 ***which has been published in final doi: 10.1016/j.meatsci.2022.108903***

6 ***The terms and conditions for the reuse of this version of the manuscript are specified in the***

7 ***publishing policy. For all terms of use and more information see the publisher's website***

8

9

10

11

12

13

14

15

16

17

18

19

20

21 **Influence of almond hulls in lamb diets on animal performance and meat quality**

22 Manuel Scerra*[a], Matteo Bognanno [a], Francesco Foti [a], Pasquale Caparra [a], Caterina Cilione [a],
23 Fabrizio Mangano [b], Antonio Natalello [b], Luigi Chies [a]

24

25 [a] *University of Reggio Calabria, Dipartimento di Agraria, Produzioni Animali, Via dell'Università,*
26 *25, 89124, Reggio Calabria, Italy*

27 [b] *University of Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Via*
28 *Valdisavoia 5, 95123, Catania, Italy*

29

30 *Corresponding author. E-mail address: manuel.scerra@unirc.it

31 **Abstract**

32 Almond hulls (AH) were fed to lambs to study effects on performance and meat quality. Thirty
33 Sarda lambs were allotted to three experimental groups and fed for 40 days either a cereal-based
34 concentrate diet (control) or diets in which cereals were replaced with 15% (AH15) or 30% (AH30)
35 almond hulls on a DM basis. Diets did not affect final body weight, dry matter intake, average daily
36 gain, feed conversion ratio or carcass weight. Replacing part of the cereal mix (i.e., barley and
37 maize) with AH did not affect meat fatty acid profiles. After 5 and 7 days of refrigerated storage
38 respectively for cooked and raw meat, AH15 and AH30 treatments reduced meat lipid oxidation (P
39 < 0.001). Our results suggest feeding almond hulls up to 30% in fattening lamb diets can improve
40 meat oxidative stability without compromising growth performance.

41 Keywords: by-product, lipid oxidation, fatty acids, phenolic compounds, antioxidants.

42

43

44 **Introduction**

45 Almond trees (*Prunus dulcis*) are deciduous, broad-leaved, slow growing, long-lived and can reach
46 5-7 m in height. The seeds of the almond tree, almonds, are oily, rich in vitamins and minerals and
47 the main nuts produced in subtropical countries (An et al., 2020). Almond production leads to
48 generation of by-products, such as almond hulls (AH), which is the outer covering that splits open
49 at maturity. As a byproduct, the almond industry produces 1.5 million tons of AH (Almond Board
50 of California, 2018), and in California (i.e. the leading producer of almonds), these are commonly
51 added to dairy cattle diets.

52 Almond hulls are low in protein but high in NDF, with a remarkable concentration of non-structural
53 carbohydrates. However, AH composition is influenced by factors such as the variety and the
54 fraction of debris (DePeters, Swanson, Bill, Asmus, & Heguy, 2020). In an early study, Takeoka
55 and Dao (2003) found extracts from AH had higher antioxidant activity than an equivalent
56 concentration of α-tocopherol. Almond hulls are particularly rich (up to 4.5%) in phenolic
57 compounds, including phenolic acids and flavonol glycosides (An et al., 2020). The biological
58 activities of AH phenolics are under current investigation (An et al., 2020; Najari, Khodaiyan,
59 Yarmand, & Hossein, 2022) as numerous studies have demonstrated phenolics can offer beneficial
60 effects, most importantly their antioxidant capacity (Hussain Tan, Yin, Blachier, Tossou, & Rahu,
61 2016; Oliveira et al., 2016; Li, Li, & Lin, 2018).

62 Effects of feeding AH to sheep on growth and carcass performance have been reported (Phillips,
63 Doyle, Harl, Carpenter, & Aschenbrener, 2015), but to the best our knowledge, no studies have
64 investigated any effects on meat quality. In addition, effects of AH on lamb fatty acid composition
65 have not be reported, although studies indicate phenolic compounds can impair ruminal
66 biohydrogenation of polyunsaturated fatty acids (PUFA) (Vasta & Luciano, 2011; Lanza et al.,
67 2015; Frutos et al., 2020).

68 The objectives of the present study were, therefore, to assess the effects of feeding AH on lamb
69 growth performance, meat quality traits, oxidative stability and fatty acid composition.

70

71 **2. Materials and methods**

72 The present trial was conducted from May to December 2021 and all procedures were approved
73 (prot. No. 8937) by the Animal Welfare Committee (O.P.B.A) of the University of Reggio Calabria.

74 The trial included twenty-seven Sarda male lambs born in May. Lambs were weaned at 60 days of
75 age and then fed a commercial concentrate (30% maize, 30% barley, 20% fava bean, 17% wheat
76 bran, 3% vitamin mineral premix on a DM basis) for 80 days. When lambs reached an average
77 initial body weight 27.79 ± 0.40 (SD) kg, they were allocated to individual straw-bedded pens and
78 randomly assigned to one of three dietary treatments (n = 9) and adapted to diets for 10 days. At the
79 end of the adaptation period, lambs were fed diets *ad libitum* for 40 days. The control group
80 received the same commercial concentrate given after weaning whereas the two experimental
81 groups received the concentrate diet with 15 % (AH15 group) and 30 % (AH30 group) AH
82 substituted for barley and maize. To maintain a similar crude protein concentration between
83 treatments, the AH15 and AH30 also had a higher fava bean content (table 1). The ingredients of
84 each concentrate mixture were ground (5-mm screen) to avoid the selection of ingredients. Hay was
85 provided *ad libitum* in a separate feeder.
86 The experimental diets were provided twice daily, early morning and late afternoon at a rate of
87 110% of ad libitum intake calculated by weighing-back refusals daily.
88 Once a week the animals were weighed to assess average daily gain. At the end of the trial, all the
89 lambs were slaughtered on the same day at a commercial abattoir according to the European Union
90 welfare guidelines. Experimental diets and water were available to lambs until approximately 4 h

91 before slaughter. Lambs were firstly stunned by a captive bolt and exsanguinated. Each carcass was
92 immediately weighted and stored at 4° C for 24 h.
93

94 *2.1. Muscle sampling procedures*

95 Samples of *longissimus thoracis et lumborum* muscle (*LTL*) were collected from the left side of
96 each carcass, vacuum-packed and stored at -30°C in darkness. One sample of *LTL* from each animal
97 was vacuum-packed and stored at 4 °C in darkness for 24 hours to evaluate lipid oxidation and
98 colour stability in raw muscle slices.
99

100 *2.2. Analyses of Feedstuffs and meat proximate analysis*

101 Samples of the feeds offered were collected over the duration of the trial and sub-samples of each
102 feed were pooled, freeze-dried, ground (1 mm screen) and stored at −30 °C pending analyses.
103 Feed samples were analysed for neutral detergent fibre (NDF) (Van Soest, Robertson, & Lewis,
104 1991), crude protein (AOAC, 1995a; method 984.13; Kjeldahl method), crude fat (AOAC, 1995b;
105 method 920.39) and ash (AOAC, 1995c; method 942.05). Following the procedure described by
106 Makkar, Blümmel, Borowy, and Becker (1993) total phenolic compounds and total tannins were
107 analysed.
108 Fatty acid composition of AH and diets were extracted using chloroform and converted to fatty acid
109 methyl esters (FAME) with 2 % (v/v) sulfuric acid in methanol (Priolo, Valenti, Natalello, Bella,
110 Luciano, & Pauselli, 2021), using tridecanoic acid as an internal standard. Gas chromatographic
111 (GC) analysis was performed as described later for the analysis of intramuscular fatty acid.
112 Tocopherols were extracted from 200 mg of almond hulls and concentrates, as reported by Rufino-
113 Moya, Joy, Lobón, Bertolín, & Blanco (2020).
114 After thawing *LTL* samples for 24 h at 4 °C, moisture, crude fat, ash and protein (method no.
115 950.46, 991.36, 920.153 and 984.13 respectively) were assessed according to AOAC procedures
116 (AOAC, 1995).

117

118 *2.3. Analysis of fatty acids, vitamin E and cholesterol in muscle*

119 Muscle lipids were extracted from 10 g of tissue with chloroform/methanol (2:1, v/v) and 100 mg of
120 lipids were methylated (15 min at 50 °C) using 1 ml of 0.5 M sodium methoxide in methanol and 2
121 ml of hexane, which included nonadecanoic acid as internal standard. GC analysis was performed
122 on a ThermoQuest gas chromatograph (ThermoQuest, Milan, Italy) with a high-polar fused silica
123 column (100 m, i.d. 0.25 mm, film thickness 0.25 μm; SP 24056; Supelco Inc., Bellefonte, PA) and
124 flame ionization detector (FID). Gas-chromatography conditions and identification of FAME was
125 performed as reported by Natalello et al. (2019). Atherogenic and thrombogenic indexes were
126 evaluated using formulas reported by Ulbricht and Southgate (1991).
127 Tocopherols, retinol, and cholesterol from muscle were extracted and quantified as reported by
128 Natalello et al. (2022), using an UHPLC system.

129

130 *2.4. Meat oxidative stability measurements*

131 Lipid oxidation was monitored in raw and cooked meat by measuring thiobarbituric acid reactive
132 substances (TBARS) at each day of storage as reported by Luciano et al. (2019). In brief, three
133 slices from *LTL* (2 cm thick) were overwrapped with a commercial PVC film, placed in a tray and
134 kept at 4°C. Using one slice for each day of storage, colour and lipid oxidation measurements were
135 performed at day 0 (after 2 h of blooming) and after 3, 7 days of storage. Three more slices were
136 cooked at 75 °C for 30 min in a water bath and used to assess the extent of lipid oxidation at 0, 2
137 and 5 days in cooked meat. The two slices used to assess lipid oxidation after 2 and 5 days were
138 stored at +4°C.
139 From each slice, 2.5 g of LTL were homogenized with 12.5 ml of distilled water. Then, 12.5 ml of
140 10% (w/v) trichloroacetic acid were added to precipitate proteins, after which samples were filtered.
141 In a screw-cap glass tubes, 4 ml of clear filtrate was mixed with 1 ml of 0.06 1M aqueous
142 thiobarbituric acid and samples were incubated in a water bath at 80 °C for 90 min. The absorbance

of the samples at 532 nm was measured using a Shimadzu double beam spectrophotometer (model UV-1800; Shimadzu Corporation, Milan, Italy). The assay was calibrated using solutions of known concentrations of TEP (1,1,3,3, -tetra-ethoxypropane) in 5% (w/v) trichloroacetic acid ranging from 5 to 65 nmoles/4 ml. Results were expressed as TBARS values (mg of malonaldehyde (MDA)/kg of meat).

Colour stability of raw meat was measured with a Minolta CR300 colour-meter (Minolta Co. Ltd. Osaka, Japan), set with illuminant A and 10° standard observer. The colour descriptors L* (lightness), a* (redness), b* (yellowness), C (saturation), and H (hue angle) were measured in the CIE L* a* b* colour space. To determine changes in muscle colour during storage, the coefficient ΔE was calculated using the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$, where ΔL*, Δa* and Δb* denote the difference in the values of lightness, redness and yellowness, respectively, in individual muscles between day 0 and day 3 or 7.

2.5. Statistical analysis

The effect of the dietary treatment (Control, AH15 and AH30) on animal performance, feed intake, tocopherols and intramuscular FA was analysed using a one-way ANOVA. A GLM procedure for repeated measures was used to analyse oxidative stability parameters to assess the effect of dietary treatment (Diet), the time of storage (Time) and their interaction (Diet x Time) as fixed factors, while individual animal was included as a random factor. Differences between means were assessed using Tukey's multiple-comparison test. Significance was declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$. Data were analysed using the statistical software Minitab, version 14 (Minitab Inc, State College, PA).

3. Results

169 *3.1. Animals Performance and intakes*

170 As shown in Table 2, the replacement of 15 % and 30 % of cereal mix with AH did not affect
171 animal voluntary dry matter intake (DMI), feed conversion ratio (FCR), final body weight, average
172 daily gain (ADG) or hot carcass weight. Animals from the three groups ingested the same amount
173 of total fatty acids ($P > 0.05$). Among the individual fatty acid intakes, no statistical differences
174 were observed between the experimental groups. However, the lambs from the AH30 group tended
175 to ingeste a higher quantity of steric acid and oleic acid than lambs from the control group.

176

177

178 *3.2. Fatty Acid Composition of Intramuscular fat and antioxidant vitamins*

179 The intramuscular fatty acid composition and the concentration of tocopherols and retinol in meat
180 are reported in Table 3. Vitamin E (VE) was mainly represented by α-tocopherol and its
181 concentration was not affected by supplementing almond hulls in the diet of lambs. Also, the
182 concentration of retinol (vitamin A) was not affected by the dietary treatment.

183 Feeding with AH did not affect the content of total saturated fatty acids ($P > 0.05$), MUFA ($P >$
184 0.05) and PUFA ($P > 0.05$) in the meat. The contents of *n-3* PUFA and *n-6* PUFA were also
185 comparable between groups. As a consequence, the *n-6/n-3* ratio was also comparable between
186 groups. No statistical differences were observed for each fatty acid identified. The atherogenic (AI)
187 and thrombogenic index (AI) were not affected by the dietary treatment, as well as the
188 concentrations in meat of the highly peroxidizable (HP) PUFA, with unsaturation degree ≥ 3, and of
189 the HP-PUFA ÷ VE ratio.

190

191 *3.3. Meat colour and oxidative stability*

192 Figure 1 reports effects of dietary treatment and time of storage on lipid oxidation measured in raw
193 and cooked meat. In both raw and cooked meat during the storage days the TBARS values
194 increased ($P < 0.001$). Feeding almond hulls positively influenced this parameter. In fact, both AH

195 treatments reduced the extent of the overall lipid oxidation measured over time ($P < 0.001$). A
196 significant diet × time interaction was found in raw and cooked meat ($P < 0.001$).
197 Specifically, compared to day 0, while the TBARS values already increased after 3 days in raw
198 meat (Fig. 1a) of the control-fed lambs ($P < 0.01$), lipid oxidation increased after 7 days in raw meat
199 from animals fed both AH diets ($P < 0.01$). Raw meat from AH15 and AH30 treatments had lower
200 TBARS values compared to raw meat from control treatment after 7 days of storage ($P < 0.001$;
201 1.14, 0.59 and 0.55 respectively for control, AH15 and AH30 groups). In cooked meat (Fig. 1 b),
202 the TBARS values were lower ($P < 0.001$) for each observation day (Day 0, 3 and 5) in meat from
203 both AH treatments compared to control.
204 The time of storage influenced some of the colour parameters, with L*, b* and H* values increasing
205 ($P < 0.01$) from 0 to 3 days and stabilizing thereafter (table 4). No significant difference was
206 observed for ΔE index ($P > 0.05$).
207
208
209 ***4. Discussion***
210 The inclusion of locally available industrial by-products in animal diets to replace conventional
211 ingredients may contribute to improve the sustainability of livestock industry. This practice could
212 help farmers to reduce the costs of animal feeding and lead to the reduction of costs related to the
213 disposal of waste biomass by agro-industries, as well as reducing feed-to-food competition in
214 livestock production. Moreover, the dietary inclusion of by-products naturally rich in bioactive
215 compounds could lead to the transfer of these compounds to the animal products, improving their
216 nutritional and functional properties.
217 Almond hulls have been successfully included in dairy cow (Aguilar, Smith, & Baldwin, 1983;
218 Swanson, Bill, Asmus, Heguy, & DePeters, 2021) and broilers diets (Wang, Singh, Kong, & Kim,
219 2021) but data on effects of AH in lamb finishing diets on lamb performance and carcass
220 characteristics have been limited. In this study, AH diets had no effect relative to control on the

221 final body weight, voluntary DMI, ADG or feed conversion rate. The composition of the three diets
222 probably influenced these results. The higher NDF/non-structural carbohydrate of AH (Swanson et
223 al., 2021) compensate for reduced starch from maize and barley, and lower protein of AH was
224 compensated from by additions of fava beans. Similar results were observed in an experimental trial
225 with lambs carried out by Phillips et al. (2015), where almond hulls were integrated into the diet at
226 5% and 10%. Rad, Rouzbehan, and Rezaei (2016) also observed no statistical differences on DMI
227 and ADG when alfalfa was replaced with urea-almond hulls in a diet for fattening lambs.
228 To the best of our knowledge, this is the first experiment in which the effects of feeding AH on
229 meat quality in growing lambs has been evaluated. The present trial was planned to replace part of
230 the cereals, which are commonly used as dietary ingredients for growing ruminants with AH.
231 Replacing part of the cereal mix (i.e., barley and maize) with AH did not affect meat fatty acid
232 profiles. These results were unexpected, because AH are rich in bioactive compounds that are
233 believed to be able to modulate the rumen biohydrogenation process. In our experiment, animals
234 from the three experimental groups had a similar intakes of C18:2 *n-6* and C18:3 *n-3*, as well as
235 other fatty acids identified. Nonetheless, we expected some changes in the concentration of several
236 intramuscular fatty acids such as C18:3 *n-3*, C18:1 *trans-11* and C18:2 *cis-9 trans-11* as a result of
237 supplementing AH. This expectation was linked to the higher amount of phenolic compounds in the
238 AH compared to concentrate (Frutos et al., 2020; Valenti et al., 2021). In the rumen, rumenic acid is
239 mainly produced by the isomerization of dietary linoleic acid, while vaccenic acid derives mainly
240 from the biohydrogenation of both C18:2 *n-6* and C18:3 *n-3* (Shingfield, Bernard, Leroux, &
241 Chilliard, 2010). Dietary polyphenols may alter the rumen microbial population and metabolism
242 (Scerra et al., 2018; Salami et al., 2019; Vasta et al., 2019; Scerra et al., 2021). In fact, different
243 studies reported that some secondary metabolites of plants such as tannins, polyphenolic
244 compounds, could impair ruminal biohydrogenation of PUFA via modification of the bacterial
245 population in the rumen and the inhibition of biohydrogenation steps, such as the conversion of
246 vaccenic acid to stearic acid. In our experiment, the results observed did not suggest an inhibition of

247 the biohydrogenation process due to AH phenols, although the lambs of both AH groups received
248 diets with 4-5 times more total phenols than the control group lambs.
249 However, although the effect of dietary phenolic compounds on ruminal biohydrogenation has been
250 long studied (Vasta et al., 2009; Vasta & Luciano, 2011; Vasta et al., 2019; Frutos et al., 2020),
251 their ability to modulate biohydrogenation is still a controversial issue.
252 The effects of feeding almond hulls on oxidative stability of raw and cooked meat are reported in
253 fig. 1. The time of storage and dietary treatment affected TBARS values measured in raw *LTL* slices
254 during 7 days of refrigerated storage (Fig. 1a). In raw meat, diet effects were most strongly noted
255 after 7 days of refrigerated storage, with a TBARS of control *LTL* being double that of AH.
256 However, TBARS values in raw meat remained below the value of 2 mg MDA/Kg, which is
257 considered a threshold for the sensory perception of rancidity (Campo, Nute, Hughes, Enser, Wood
258 & Richardson, 2006), for the entire monitoring period for all experimental groups.
259 In the present study, to further highlight diet-related differences of meat oxidative stability under
260 more pro-oxidant conditions, TBARS values in cooked *LTL* during 5 days of storage were
261 evaluated. The effects of diet on oxidative stability were more evident when the meat was subjected
262 to more stressful conditions, and already on the first day of studying the oxidative stability in
263 cooked meat, the TBARS values were significantly lower in both AH treatments compared to the
264 control treatment.
265 Different authors reported that the increase of antioxidant compounds in the diet is commonly
266 associated with the improvement of the antioxidant capacity of meat (Descalzo & Sancho, 2008;
267 Luciano et al., 2017). In this study, the level of vitamin E (α-Tocopherol and γ-Tocopherol) was
268 higher in the control diet than in both AH diets. However, differences between treatments in
269 vitamin E intake probably were not enough to result in differences in meat oxidative stability.
270 In fact, despite the data reported on vitamin E and considering that vitamin E has been extensively
271 proven to play a major role in protecting animal tissues against lipid peroxidation (Burton & Traber,
272 1990; Luciano et al., 2017), after 7 and 5 days of storage, respectively for raw and cooked meat,

273 TBARS values of meat from control group were higher than the TBARS values observed in meat
274 from both almond hull groups.
275 Some authors (Takeoka & Dao, 2003) reported that the extracts from almond hulls presented higher
276 antioxidant capacity than the equivalent concentration of α-tocopherol. Safarian et al. (2016)
277 observed a high content of antioxidants, including flavonoids, terpenoids, and other phenolic
278 compounds in a powdered extract of almond hulls, indicating that this by-product is a potential
279 source of dietary antioxidants.
280 Therefore, the protective effects of the diets containing 15 and 30 % of almond hulls against lipid
281 oxidation observed in our experiment could be attributed to the presence of high levels of
282 antioxidant compounds (e.g., flavonoids), which were about 5 times higher in the AH30 diet than in
283 the control diet. Other authors (Luciano et al., 2012; Gravador et al., 2015) have also attributed the
284 improvement in the oxidative stability of lamb meat to the antioxidant action of the phenolic
285 compounds present in the studied feed.
286 Colour stability is another important aspect to consider when meat shelf life is studied. Some
287 authors report as fatty acid oxidation could promote oxidation of myoglobin (Alderton, Faustman,
288 Liebler, & Hill, 2003; Faustman, Sun, Mancini, & Suman, 2010) and consequently, strategies to
289 reduce lipid oxidation should bring positive effects in terms of colour stability. In this experimental
290 trial, while the dietary treatment affected the lipid oxidation in raw and cooked meat, no effect was
291 observed on colour stability. Moreover, Diet x Time interactions were not significant for any of the
292 colour stability parameters, indicating that dietary treatment did not affect the trend of variation of
293 the colour descriptors.
294
295
296 **Conclusions**

297 Partial replacement of cereals in concentrate with almond hulls had no effect on lamb meat fatty
298 acid profiles. Higher oxidative stability was, however, observed in meat from animals fed AH.

299 The inclusion of almond hulls at up to 30% in diets for fattening lamb, therefore, might be an
300 excellent strategy to reduce cereals feeding without compromising animal growth performances,
301 while improving meat oxidative stability.
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319

320 **References**

321 Aguilar, A. A., Smith, N. E., & Baldwin, R.L. (1983). Nutritional Value of Almond Hulls for Dairy
322 Cows. *Journal of Dairy Science*, 67, 97-103.

323 Almond Board of California (2018). Almond almanac 2018 (annual report).

324 An, J., Liu, J., Liang, Y., Ma, Y., Chen, C., Cheng, Y., Peng, P., Zhou, N., Zhang, R., Addy, M.,
325 Chen, P., Liu, Y., Huang, G., Rena, D., & Ruan, R. (2020). Characterization, bioavailability
326 and protective effects of phenolic-rich extracts from almond hulls against pro-oxidant induced
327 toxicity in Caco-2 cells. *Food Chemistry,* 322. Article 126742.

328 Alderton, A.L., Faustman, C., Liebler, D.C., & Hill, D.W. (2003). Induction of redox instability of
329 bovine myoglobin by adduction with 4-Hydroxy-2-Nonenal. *Biochemistry*, 42 (15), 4398–
330 4405.

331 AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis (16th
332 ed.). Washington, DC:AOAC.

333 Burton G.W., & Traber, M.G. (1990). Vitamin E: antioxidant activity, biokinetics and
334 bioavailability. *Annual Review of Nutrition,* 10, 357–382.

335 Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006).
336 Flavour perception of oxidation in beef. *Meat Science, 72*, 303–311.

337 DePeters, E. J., Swanson, K. L., Bill, H. M., Asmus, J., & Heguy, J. M. (2020). Nutritional
338 composition of almond hulls. *Applied Animal Science*, 36, 761–770.

339 Descalzo, A.M., & Sancho, A.M. (2008). A review of natural antioxidants and their effects on
340 oxidative status, odour and quality of fresh beef produced in Argentina. *Meat Science*, 79, 423–
341 436.

342 Faustman, C., Sun, Q., Mancini, R., & Suman, S.P. (2010). Myoglobin and lipid oxidation
343 interactions: mechanistic bases and control. *Meat Science*, 86, 86–94.

344 Frutos, P., Hervas, G., Natalello, A., Luciano, G., Fondevila, M., Priolo, A., & Toral, P. (2020).
345 Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles.
346 *Animal Feed Science and Technology*, 269, 114623.
347 Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M.C., & Rahu, N. (2016). Oxidative stress and
348 inflammation: What polyphenols can do for us?. *Oxidative Medicine and Cellular Longevity*,
349 7432797.
350 Gravador, R.S., Luciano, G., Jongberg, S., Bognanno, M., Scerra, M., Andersen, M.G., Lund, M.N.,
351 & Priolo, A. (2015). Fatty acids and oxidative stability of meat from lambs fed carob-
352 containing diets. *Food Chemistry*, 182, 27–34.
353 Lanza, M., Scerra, M., Bognanno, M., Buccioni, A., Cilione, C., Biondi, L., Priolo, A., & Luciano,
354 G. (2015). Fatty acid metabolism in lambs fed citrus pulp. *Journal of Animal Science*, 93, 3179-
355 3188.
356 Li, Y.R., Li, S., & Lin, C.C. (2018). Effect of resveratrol and pterostilbene on aging and longevity.
357 *BioFactors*, 44 (1), 69-82.
358 Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., & Priolo, A.
359 (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of
360 longissimus dorsi muscle from lambs fed a tannin-containing diet. *Food Chemistry*, 124, 1036-
361 1042.
362 Luciano, G., Roscini, V., Mattioli, S., Ruggeri, S., Gravador, R. S., Natalello, A., …Priolo, A.
363 (2017). Vitamin E is the major contributor to the antioxidant capacity in lambs fed whole dried
364 citrus pulp. *Animal*, 11(3), 411–417.
365 Luciano, G., Natalello, A., Mattioli, S., Pauselli, M., Sebastiani, B., Niderkorn, V., …Valenti., B.
366 (2019). Feeding lambs with silage mixtures of grass, sainfoin and red clover improves meat
367 oxidative stability under high oxidative challenge. *Meat Science*, 156, 59-67.

368 Makkar, H.P.S., Blümmel, M., Borowy, N.K., & Becker, K. (1993). Gravimetric determination of
369 tannins and their correlations with chemical and protein precipitation methods. *Journal of*
370 *Environmental Science and Health B*, 61, 161–165.
371 Oliveira, B. D., Rodrigues, A. C., Cardoso, B. M. I., Ramos, A. L. C. C., Bertoldi, M. C., Taylor, J.
372 G., ... Pinto, U. M. (2016). Antioxidant, antimicrobial and anti-quorum sensing activities of
373 Rubus rosaefolius phenolic extract. *Industrial Crops and Products*, 84, 59–66.
374 Natalello, A., Luciano, G., Morbidini, L., Valenti, B., Pauselli, M., Frutos, P., … Priolo, A. (2019).
375 Effect of feeding pomegranate by-product on fatty acid composition of ruminal digesta, liver
376 and muscle in lambs. *Journal of Agricultural and Food Chemistry, 67*, 4472–4482.
377 Zahra Najari, Z., Khodaiyan, F., Yarmand, M. S., & Hossein, S. S. (2022). Almond hulls waste
378 valorization towards sustainable agricultural development: Production of pectin, phenolics,
379 pullulan, and single cell protein. *Waste Management*, 141, 208-219.
380 Natalello, A., Khelil-Arfa, H., Luciano, G., Zoon, M., Menci, R., Scerra, M., Blanchard, A.,
381 Mangano, F., Biondi, L., & Priolo, A. (2022). Effect of different levels of organic zinc
382 supplementation on pork quality. *Meat Science*, 186, 108731.
383 Phillips, C.R., Doyle, S.P., Harl, A., Carpenter, C.B., & Aschenbrener, M. (2015). Effect of
384 Almond Hull Level in a Finishing Diet on Lamb Growth and Carcass Performance. *Journal of*
385 *Agriculture and Life Science*, 2 (2), 11-15.
386 Priolo, A., Valenti, B., Natalello, A., Bella, M., Luciano, G., & Pauselli, M. (2021). Fatty acid
387 metabolism in lambs fed hazelnut skin as a partial replacer of maize. *Animal Feed Science and*
388 *Technology,* 272, 114794.
389 Rad, M.I., Rouzbehan, Y., & Rezaei, J. (2016). Effect of dietary replacement of alfalfa with urea-
390 treated almond hulls on intake, growth, digestibility, microbial nitrogen, nitrogen retention,
391 ruminal fermentation, and blood parameters in fattening lambs. *Journal of Animal Science*, 94,
392 349–358.

393 Rufino-Moya, P.J., Joy, M., Lobón, S., Bertolín, J.R., & Blanco, M. (2020). Carotenoids and
394 liposoluble vitamins in the plasma and tissues of light lambs given different maternal feedings
395 and fattening concentrates. *Animals*, 10, 1813.
396 Safarian, S., Azarmi, Y., Jahanban-Esfahlan, A., & Jahanban-Esfahlan, H. (2016). The beneficial
397 effects of almond (Prunus amygdalus Batsch) hull on serum lipid profile and antioxidant
398 capacity in male rats. *Turkish Journal of Medical Sciences*, 46(4), 1223–1232.
399 Salami, S.A., Luciano, G., O'Grady, M.N., Biondi, L., Newbold, C.J., Kerry, J.P., & Priolo, A.
400 (2019). Sustainability of feeding plant by-products: a review of the implications for ruminant
401 meat production. *Animal Feed Science and Technology*, 251, 37–55.
402 Scerra, M., Foti, F., Caparra, P., Cilione, C., Violi, L., Fiammingo, G., D'Agui', G., & Chies, L.
403 (2018). Effects of feeding fresh bergamot (Citrus bergamia Risso) pulp at up to 35% of dietary
404 dry matter on growth performance and meat quality from lambs. *Small Ruminant Research*,
405 169, 160–166.
406 Scerra, M., Foti, F., Caparra, P., Lanza, M., Natalello, A., Cilione, C., Rosa, R., D'Aguì, G., &
407 Chies, L. (2021). The effect of fresh bergamot pulp on fatty acid composition of suckling kids.
408 *Small Ruminant Research*, 203, Article 106483.
409 Shingfield, K.J., Bernard, L., Leroux, C., & Chilliard, Y. (2010). Role of trans fatty acids in the
410 nutritional regulation of mammary lipogenesis in ruminants. *Animal,* 4, 1140–1166.
411 Swanson, K. L., Bill, H. M., Asmus, J., Heguy, J. M., & DePeters, E. J. (2021). Feeding high
412 amounts of almond hulls to lactating cows. *Journal of Dairy Science*, 104, 8846-8856.
413 Takeoka, G. R., & Dao, L. T. (2003). Antioxidant constituents of almond [Prunus dulcis (Mill.) DA
414 Webb] hulls. *Journal of Agricultural and Food Chemistry*, 51(2), 496–501.
415 Ulbricht, T.L.V., & Southgate, D.A.T. (1991). Coronary heart disease: Seven dietary factors.
416 *Lancet,* 338, 985–992.

417 Valenti, B., Campidonico, L., Natalello, A., Lanza, M., Salami, S. A., Priolo, A., ... & Luciano, G.
418 (2021). Fatty acid metabolism in lambs supplemented with different condensed and
419 hydrolysable tannin extracts. *Plos one,* 16(10), e0258265.
420 Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent
421 fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*,
422 74, 3583-3597.
423 Vasta, V., Makkar, H.P.S., Mele, M., & Priolo, A. (2009). Ruminal biohydrogenation as affected by
424 tannins in vitro. *British Journal of Nutrition*, 102, 82–92.
425 Vasta, V., & Luciano, G. (2011). The effects of dietary consumption of plants secondary
426 compounds on small ruminants' products quality. *Small Ruminant Research,* 101, 150-159.
427 Vasta, V., Daghio, M., Cappucci, A., Buccioni, A., Serra, A., Viti, C., & Mele, M. (2019). Invited
428 review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation,
429 fiber digestion, and methane emission: Experimental evidence and methodological approaches.
430 *Journal of Dairy Science,* 102, 3781–3804.
431 Wang, J., Singh, A. K., Kong, F., & Kim, W. K. (2021). Effect of almond hulls as an alternative
432 ingredient on broiler performance, nutrient digestibility, and cecal microbiota diversity. *Poultry*
433 *Science,* 100, 100853.
434
435
436
437
438
439
440
441
442

443

444

445

Table 1

Ingredients (% on DM basis) and chemical composition of diets offered

	Control diet	AH15 diet	AH30 diet	Almond hulls	Hay
Barley	30	21	12		
Maize	30	21	12		
Bran	17	17	15		
Fava bean	20	23	28		
Almond hulls	-	15	30		
Vitamin mineral premix[1]	3	3	3		
Chemical composition					
Dry matter (DM) g/kg wet weight	873	863	855	784	902
Crude protein g/kg DM	140	138	136	54.4	53.6
Ether extract g/kg DM	19.3	19.4	20.3	8.70	4.23
Ash g/kg DM	22.1	26.1	32.5	107	87.8
NDF g/kg DM	228	244	294	337	740
Total phenolic (g TAe[2]/kg DM)	3.34	13.5	15.6	64.7	5.81
Total tannins (g TAe[2]/kg DM)	1.16	7.22	7.50	38.1	2.20
Tocopherols, µg/g dry matter					
α-Tocopherol	61.1	35.1	35.6	33.5	5.25
γ-Tocopherol	70.8	58.5	59.3	11.7	12.5
δ-Tocopherol	14.4	8.08	8.03	12.1	9.58
fatty acids (g/100g of total fatty acid)					
C14:0	0.20	0.18	0.30	0.54	2.20
C16:0	16.9	17.0	17.4	18.7	53.6
C18:0	2.10	2.60	3.01	6.30	8.30
C18:1 *n*-9	21.1	24.7	26.8	45.3	9.70
C18:2 *n*-6	51.8	49.2	47.5	18.8	12.5
C18:3 *n*-3	3.50	3.61	4.20	5.80	5.50

[1]Supplied per kilogram: 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.

[2]tannic acid equivalent.

446

447

448

449

450

451

452

Table 2

Lamb performances and chemical composition of *LTL* muscle (g/100g wet weight).

	Dietary treatments[1]			SEM[6]	*P* values
	Control	AH15	AH30		
Final BW[2], kg	36.6	36.1	36.2	0.683	0.445
Hot carcass weight, kg	17.8	17.5	17.4	0.454	0.865
Total DMI[3], kg/d	1.65	1.57	1.63	34.07	0.465
Concentrate intake, g/d	956	931	963	28.07	0.398
Hay intake, g/d	694	631	667	20.31	0.432
ADG[4], g/d	220	207	210	4.432	0.612
FCR[5], g DMI[3]/g ADG[5]	7.50	7.58	7.73	0.362	0.234
Total FA[7] intake, g/d	19.3	18.4	19.2	1.645	0.679
SA[8] intake, g/d	0.41	0.48	0.58	0.087	0.087
OA[9] intake, g/d	4.07	4.54	5.17	0.121	0.071
LA[10] intake, g/d	10.0	9.04	9.14	0.263	0.139
ALA[11] intake, g/d	0.68	0.66	0.81	0.121	0.109
Chemical composition					
Moisture	73.6	73.7	74.0	0.168	0.676
Crude protein	22.1	21.7	21.6	0.220	0.269
Ether extract	3.56	3.65	3.89	0.227	0.579
Ash	1.11	1.06	1.11	0.012	0.133

[1]Treatments were: only concentrate (control), concentrate integrated with almond hulls at the level of 15% (AH15) or at the level of 30% (AH30) of dry matter on the diet fed.

[2]BW=Body weight; [3]DMI=dry matter intake; [4]ADG=average daily gain;[5]FCR=feed conversion ratio; [6]SEM= standard error of means; [7]FA=Fatty acid; [8]SA=Stearic acid; [9]OA=Oleic acid; [10]LA=Linoleic acid; [11]ALA=α-linolenic acid.

453

454

455

456

457

458

Table 3

Effect of the dietary treatments on the antioxidant vitamins (µg/g meat) and fatty acid composition of *LTL* (mg/100 g of meat)

	Dietary Treatment				
Item	Control	AH15	AH30	SEM	*P* values
Tocopherols and retinol, µg/g muscle					
α-Tocopherol	1.75	1.70	2.02	0.153	0.690
γ-Tocopherol	0.48	0.42	0.52	0.034	0.491
Retinol	0.64	0.36	0.56	0.080	0.369
Cholesterol (mg/g)	1.17	1.13	1.23	0.057	0.836
C10:0	4.08	3.78	4.05	0.205	0.826
C12:0	1.88	1.74	2.51	0.233	0.369
C14:0	48.2	49.21	55.7	2.890	0.535
C14:1 *cis-9*	2.03	1.63	1.90	0.153	0.585
C15:0 *iso*	2.09	1.89	2.02	0.156	0.887
C15:0 *anteiso*	1.81	1.65	2.03	0.144	0.583
C15:0	6.98	5.16	6.06	0.399	0.393
C16:0 *iso*	3.34	2.97	3.99	0.219	0.157
C16:0	566	606	624	24.10	0.623
C16:1 *cis-9*	40.6	40.7	39.3	1.700	0.932
C16:1 *cis-7*	4.83	5.06	5.77	0.303	0.433
C17:0 *anteiso*	10.9	9.01	10.8	0.586	0.350
C17:0 *iso*	9.57	7.82	10.5	0.647	0.226
C17:0	26.2	21.4	24.9	1.07	0.169
C17:1 *trans-10*	4.47	3.82	5.28	0.328	0.197
C17:1 *cis-9*	17.3	13.6	13.5	0.775	0.097
C18:0	364	394	439	16.90	0.111
C18:1 *trans-9*	8.05	8.51	9.23	0.465	0.603
C18:1 *trans-10*	14.1	11.4	17.7	1.910	0.426
C18:1 *trans-11* VA[1]	16.0	16.6	19.6	1.880	0.591
C18:1 *cis-9*	1011	1049	1117	42.90	0.619
C18:1 *cis-11*	33.5	30.2	30.9	1.320	0.586
C18:1 *cis-12*	5.49	5.35	5.92	0.262	0.668
C18:1 *cis-13*	3.03	2.66	3.01	0.120	0.380
C18:1 *cis-14*	4.37	4.40	5.76	0.311	0.109
C18:2 *trans-9 trans*-12	0.13	0.17	0.43	0.078	0.252
C18:2 *cis-9 cis-12* LA[1]	133	105	116	7.800	0.369
C18:3 *n-3* ALA[1]	6.24	4.69	7.92	0.743	0.212
C18:2 *cis-9 trans-11*	8.76	8.11	10.6	0.749	0.375
C 20:0	3.20	2.82	3.43	0.173	0.369
C 20:1 *cis-11*	3.00	3.26	3.55	0.159	0.387

C20:2 *cis-11 cis-14*	1.50	0.99	1.40	0.160	0.416
C20:3 *n-6*	4.51	3.65	3.47	0.390	0.532
C20:3 *n-3*	0.03	0.04	0.07	0.022	0.768
C20:4 *n-6*	35.8	29.7	25.2	3.630	0.459
C20:5 *n-3* EPA[1]	3.67	2.02	2.27	0.372	0.151
C22:5 *n-6*	5.99	4.94	4.11	0.585	0.443
C22:5 *n-3* DPA[1]	1.52	1.05	1.02	0.223	0.610
C22:6 *n-3* DHA[1]	0.19	0.15	0.21	0.043	0.888
∑ SFA[1]	987	1058	1129	39.70	0.361
∑ MUFA[1]	1186	1218	1303	47.70	0.605
∑ PUFA[1]	203	162	175	12.90	0.437
OBCFA[1]	61.7	50.4	61.0	2.910	0.214
∑ *n-3*	11.6	7.95	11.5	1.020	0.261
∑ *n-6*	189	152	159	12.00	0.438
n-6/n-3	17.8	19.3	14.8	1.020	0.208
Thrombogenic index[2]	1.07	1.18	1.24	0.041	0.238
Atherogenic index[3]	0.55	0.59	0.57	0.014	0.426
HP-PUFA[4](mg/g muscle)	0.60	0.49	0.46	0.050	0.504
HP-PUFA ÷ VE [5]	2.39	2.35	2.26	0.047	0.510

[1]VA: vaaccenic acid; LA: 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; OBCFA: odd and branched chain fatty acids.

[2]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6).

[3]Atherogenix index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3).

[4]Highly peroxidizable (HP) PUFA: calculated as the sum of PUFA with ≥ 3.

[5]Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10

459

460

461

462

463

464

465

466

467

468

469

Table 4

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

	Dietary treatment[1]			Time (T)[3]			SEM	*P* values		
	Control	AH15	AH30	Day 0	Day 3	Day 7		Diet	Time	Diet x Time
L* values[2]	42.0	43.2	42.8	40.7[b]	43.5[a]	43.8[a]	0.399	0.458	0.002	0.846
a* values[2]	16.6	14.6	14.3	16.0	15.3	14.2	0.505	0.126	0.319	0.193
b* values[2]	12.0	12.2	11.4	10.7[b]	12.2[a]	12.6[a]	0.245	0.399	0.004	0.930
C* values[2]	20.7	19.0	18.4	19.3	19.9	19.0	0.480	0.116	0.751	0.226
H* values[2]	37.2	39.8	38.7	33.8[b]	40.2[a]	41.6[a]	0.714	0.160	0.001	0.199
ΔE	4.69	3.99	5.85	-	4.68	5.01	0.306	0.171	0.347	0.674

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

[1]Treatments were: only concentrate (Control); concentrate and almond hulls at the level of 15% (AH15) or at the level of 30% dry matter on the diet fed (AH30).

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

[3]Time days of storage at 4 °C under aerobic conditions (meat slices).

470

471

472

473

474

475