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21 **Effect of high levels of almond hulls supplementation on performance and meat oxidative**  
22 **stability in lambs**

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32 **Abstract**

33 The main objective of this study was to evaluate if high dietary levels of almond hulls could  
34 influence performance and meat oxidative stability in lambs. Twenty lambs, at an average body  
35 weight of  $28.8 \pm 0.30$  (SD) kg, were divided into two experimental groups and fed *ad libitum* for 40  
36 days either with a control diet (cereal-based concentrate, control group) or with a similar diet in  
37 which part of the cereals was replaced with 40% of almond hulls on a dry matter basis (AH40  
38 group). Dietary AH did not affect dry matter intake but reduced final body weight, average daily  
39 gain, feed conversion ratio or carcass weight. In meat, TBARS values were lower ( $P < 0.001$ ) in the  
40 AH40 group than in control group. The partial replacement of cereals with 40% almond hulls in the  
41 diet negatively affecting the growth parameters of the animals but can improve meat shelf-life by  
42 reducing lipid oxidation.

43 **Keywords:** by-product, lipid oxidation, phenolic compounds, antioxidants, shelf-life.

## 44 **Introduction**

45 To date, the economic emergency and the increase of the cost of raw materials is putting many  
46 livestock farms in difficulty. In Italy, for the livestock sector, in the first quarter of 2022, farmer  
47 disbursements increased by 16.6% on an annual basis, registering a further boost after + 6.4% in  
48 2021, reflecting the price increases of farm animals (+ 9.8%) and feed (+ 21%) as well as energy  
49 products (+ 61.5%) (Ismea, 2022). Therefore, the use of alternative feeds available at lower costs  
50 than conventional feeds has become essential for the sustenance of the sector (Ponnampalam &  
51 Holman, 2022).

52 Almond hulls (AH) are by-products from almond fruit available in large quantities in the market. In  
53 2019, the almond industry produced 2 million tons of AH, and this amount is expected to increase  
54 in the coming years (Almond Board of California, 2019). Recent study (Scerra et al., 2022) has  
55 shown that the replacement of cereals with AH up to 30% in lamb diets improved meat oxidative  
56 stability without compromising the animals' growth performance, proving to be an interesting  
57 strategy to reduce use of cereals. AH are cheap by-products, low in protein but with a fair amount of  
58 nonfiber carbohydrates (De Peters, Swanson, Bill, Asmus & Heguy, 2020). Furthermore, in almond  
59 hulls a high level of phenols such as phenolic acids and tannins has been observed (An et al., 2020),  
60 compounds that have shown health-related properties, especially to their antioxidant activity (Li, Li  
61 & Lin, 2018). Considering the interesting results obtained by Scerra et al. (2022), the main  
62 objective of the present trial was to investigate the effect of AH inclusion at 40% in lamb diets on  
63 growth performance and meat oxidative stability.

64

## 65 **2. Materials and methods**

### 66 *2.1. Animals and Diets*

67 The experimental trial was carried out from February to November 2022 and the Animal Welfare  
68 Committee (O.P.B.A) of the University of Reggio Calabria approved all procedures (prot. No.  
69 8937).

70 Twenty entire male lambs (*Sarda* breed) were weaned at 60 days of age and then received a  
71 conventional concentrate-based diet composed of maize and barley (both at 30% on a DM basis),  
72 fava bean (20%), wheat bran (17%) and vitamin mineral premix (3%) for about three months. At an  
73 average body weight of  $28.8 \pm 0.30$  (SD) kg, lambs were penned individually and randomly divided  
74 into two groups (n=10, control and AH40 groups) and, after an adaptation period (7 days) to the  
75 treatments, fed with their respective diet *ad libitum* for 40 days. Control group were fed with the  
76 same conventional concentrate-based given after weaning; whereas the AH40 group received the  
77 concentrate diet in which most of the barley and maize was replaced with 40% AH (AH40 group;  
78 9% maize, 9% barley, 32% fava bean, 7% wheat bran, 40% AH, 3% vitamin mineral premix on a  
79 dry matter basis). Hay was provided *ad libitum* in a separate feeder. The diet of the AH40 group had  
80 a higher fava bean content to maintain a similar crude protein concentration between treatments  
81 (considering the low protein content of the AH). The chemical composition of the two experimental  
82 diets was (g/kg DM): DM 896 (g/Kg wet weight), crude protein 146, ether extract 28.2, ash 32.2,  
83 NDF 221, total phenolic, in g of tannic acid equivalent (TAe)/Kg DM, 4.63, total tannins (g  
84 TAe/Kg DM) 1.54,  $\alpha$ -Tocopherol ( $\mu\text{g/g DM}$ ) 54.6,  $\gamma$ -Tocopherol ( $\mu\text{g/g DM}$ ) 60.5,  $\delta$ -Tocopherol  
85 ( $\mu\text{g/g DM}$ ) 13.1, metabolizable energy (Mcal/kg DM) 2.37 for control diet; DM 891 (g/Kg wet  
86 weight), crude protein 139, ether extract 13.9, ash 59.4, NDF 309, total phenolic (g TAe/Kg DM)  
87 28.1, total tannins (g TAe/Kg DM) 16.9,  $\alpha$ -Tocopherol ( $\mu\text{g/g DM}$ ) 34.4,  $\gamma$ -Tocopherol ( $\mu\text{g/g DM}$ )  
88 32.2,  $\delta$ -Tocopherol ( $\mu\text{g/g DM}$ ) 12.1, metabolizable energy (Mcal/kg DM) 2.07 for AH40 diet.

89 The experimental diets were provided twice daily (0730 and 1630 h) *ad libitum*, and refusals were  
90 weighted daily to measure voluntary intake. The lambs were weighed fasting every 10 days.

91 After 40 days of experimental trial, after fasting for 8 h, all animals were weighted, transported to a  
92 commercial abattoir (25 min from experimental farm) and immediately slaughtered (stunned by a  
93 captive bolt) according to EU welfare guidelines.

94

## 95 2.2. Analyses of Feedstuffs

96 Subsamples of the feedstuffs were collected 4 times during the trial (at the beginning, after 10 and  
97 20 days and at the end of experimental trial), and vacuum-stored at  $-30^{\circ}\text{C}$ . After grounding (1  
98 mm), the subsamples were pooled to obtain one representative samples of each feedstuff. The  
99 representative samples of each feedstuff were analyzed for neutral detergent fibre (NDF) (Van  
100 Soest, Robertson & Lewis, 1991), ether extract, crude protein and ash (AOAC, 1995; methods  
101 920.39, 984.13 and 942.05, respectively). Total phenolic compounds and total tannins were  
102 analyzed as reported by Makkar, Blümmel, Borowy and Becker (1993) while tocopherols were  
103 determined following the method reported by Rufino-Moya, Joy, Lobón, Bertolín and Blanco  
104 (2020).

105

## 106 2.3. Meat quality analysis

107 Each carcass was immediately weighed and stored at  $4^{\circ}\text{C}$  for 24h. From the left side of each  
108 carcass, a portion of *Longissimus thoracis et lumborum* (LTL) muscle were collected from the 13<sup>th</sup>  
109 rib, vacuum-packed and stored at  $-30^{\circ}\text{C}$ , pending fatty acid and vitamins analyses. Another portion  
110 of LTL muscle from each carcass was stored at  $4^{\circ}\text{C}$  and used for the analyses of lipid oxidation.

111 Intramuscular fat where lipids was extracted from 5 g of wet meat samples with  
112 chloroform/methanol 2:1 v:v (Folch, Lees & Stanley, 1957), methylated adding 1 mL of hexane and  
113 0.05 mL of 2 N methanolic KOH (I.U.P.A.C., 1987), containing C19:0 as an internal standard, and  
114 analyzed using a gas chromatograph ThermoQuest (Milan, Italy) equipped with a 100 m high-polar  
115 fused silica capillary column (5 mm i.d., 0.25  $\mu\text{m}$  film thickness; Supelco Inc., Bellefonte, PA).

116 Gas-chromatography conditions and identification of FAME was performed as described by  
117 Natalello et al. (2019).

118 Muscle tocopherols were extracted and quantified using an UHPLC system as reported by Natalello  
119 et al. (2022).

120 Thiobarbituric acid reactive substances (TBARS) were determined to evaluate lipid oxidation in  
121 meat as reported by Luciano et al. (2017) on three slices of LTL (2 cm thick), one slice for each day  
122 of storage (0, 4 and 7 days), where the absorbance (532 nm) was measured using a double beam  
123 spectrophotometer (Shimadzu Corporation, Milan, Italy; model UV-1800).

124

### 125 *2.3. Statistical analysis*

126 A one-way ANOVA was applied to analyzed the effect of the dietary treatments on animal  
127 performance, feed intake, tocopherols and intramuscular fatty acids, with the diet as fixed factor  
128 (the Minitab 14 software was used for all statistical analyses). A GLM procedure for repeated  
129 measures was applied to analyzed the data of oxidative stability, with diet, time of storage and their  
130 interaction as fixed factors, while individual animal was included as a random factor. Differences  
131 between means were assessed using Tukey's multiple-comparison test. Differences were considered  
132 significant at  $P \leq 0.05$ , whereas trends toward significance were considered when  $0.05 < P \leq 0.10$ .

## 133 **3. Results and Discussion**

134 As shown in Table 1, the replacement of part of the maize and barley with AH did not affect dry  
135 matter intake (DMI) of the lambs, but negatively influenced feed conversion ratio (FCR,  $P < 0.05$ ),  
136 average daily gain (ADG,  $P < 0.05$ ), carcass weight ( $P < 0.05$ ) and consequently tendentially  
137 negatively influenced final body weight ( $P = 0.069$ ). In several previous studies (Scerra et al., 2022;  
138 Phillips et al. 2015; Rad et al., 2016), the inclusion of AH in lamb diets did not affect these  
139 parameters. In the present experimental trial, the inclusion of the AH was brought at 40%, the  
140 highest inclusion than those tested in previous experimental trials. Although AH is an excellent  
141 source of highly fermentable carbohydrates (Offeman, Holtman, Covello & Orts, 2014), at high

142 inclusion rates the starch reduction due to lower dietary inclusion of maize and barley was probably  
143 not compensated. In fact, the partial replacement of corn and barley with 40% almond hulls in the  
144 diet led to a 13 % reduction in the metabolizable energy in the AH40 diet (2.37 vs 2.07, for control  
145 and AH40 groups respectively), negatively affecting the growth performance of the animals.  
146 Furthermore, phenols intake, of which more than 60% are represented by tannins, from the lambs of  
147 AH40 group probably negatively influenced these parameters, considering that a high level of these  
148 compounds in the diets could lead to a slowdown in the animals' growth rates (Priolo, Waghorn,  
149 Lanza, Biondi & Pennisi, 2000). In a study of Vasta et al. (2019), it is pointed out that tannins show  
150 antimicrobial activity and protein-binding ability, make them able to affect rumen digestion and  
151 reducing biohydrogenation activity. In an *in vitro* study, Durmic et al. (2014) highlighted a  
152 reduction in methane and NH<sub>3</sub> production when AH were incubated with sheep rumen fluid  
153 microbes. In this trial, lambs from AH40 group daily ingested an amount of phenols and tannins  
154 respectively 6 and 11 times higher ( $P<0.001$ ) than that from control lambs. However, considering  
155 the lower cost of the diet of the AH40 group (data not shown, 198 vs 320 €/t for AH40 and control  
156 groups, respectively), the cost per 100 g of body weight gain was equivalent.

157 The inclusion of AH in the diet had significant effects on lipids oxidation measured in raw meat  
158 during refrigerated storage (Fig.1). In fact, the meat from animals fed with the AH40 diet showed  
159 clearly lower ( $P<0.001$ ) TBARS values than the meat from lambs of the control group. During the  
160 monitoring period, TBARS values increased ( $P<0.001$ ) and a significant diet × time interaction was  
161 observed ( $P<0.05$ ). In particular, compared to day 0, while the TBARS values increased already  
162 after 4 days in the raw meat of the lambs from control group ( $P<0.01$ ), the lipid oxidation was  
163 evident only after 7 days of storage in the meat of lambs whose diet was supplemented with 40%  
164 AH ( $P<0.01$ ). Moreover, the TBARS values in raw meat after 7 days of storage were markedly  
165 lower in the AH40 group than in the control one ( $P<0.001$ ).

166

167 Different authors reported that the increase of antioxidants compounds, such as vitamin E, is  
168 associated with improved antioxidant stability (Luciano et al., 2017), while a higher PUFA  
169 deposition makes meat more susceptible to lipid oxidation (Moloney, Kennedy, Noci, Monahan, &  
170 Kerry, 2012).

171 In lamb meat, vitamin E is considered one of the compounds with the greatest antioxidant capacity  
172 (Luciano et al., 2017). In the present study, although the vitamin E level was lower in the diet  
173 received from lambs of AH40 group and although the level of PUFA in meat from AH40 group  
174 tended to be higher ( $P = 0.083$ ) than in meat from the control group, we observed a greater  
175 oxidative stability in meat from this group. These results are in agreement with those observed in  
176 our previous study (Scerra et al., 2022), where the lambs were fed a diet containing 30% AH.  
177 However, in that experimental trial the differences in TBARS values between the groups were  
178 observed only after 7 days of storage. Despite the higher vitamin E level in the control diet, no  
179 differences were observed in meat for the vitamin E levels between treatments. The latter data could  
180 be influenced by phenolic compounds present in the AH, which, due to their antioxidant activity,  
181 could have indirectly preserved vitamin E during digestion, allowing the animal to absorb a greater  
182 amount of it (López-Andrés et al., 2013). Furthermore, some authors suggest that dietary phenolic  
183 compounds could directly contribute to the antioxidant capacity of meat through their metabolites  
184 absorbed in the intestine (Soldado, Bessa & Jerónimo, 2021).

185

## 186 **Conclusions**

187 The results of the present study show that the inclusion of 40% almond hulls in the diet negatively  
188 affects feed conversion ratio, average daily gain, carcass weight and final body weight in lambs.  
189 However, the inclusion of high percentage of this by-product can improve meat shelf-life by  
190 reducing lipid oxidation. This is probably influenced by antioxidant molecules contained in AH,  
191 primarily phenolic compounds.

192

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196

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**Table 1**

Growth performance and intakes of experimental lambs, vitamins and fatty acids of the meat

	Control diet	AH40 diet	SEM <sup>10</sup>	P value
<i>Growth performance</i>				
Final BW <sup>1</sup> , kg	37.3	33.7	0.882	0.069
Carcass weight, kg	18	16	0.472	0.025
ADG <sup>2</sup> , g/d	207	127	16.70	0.050
FCR <sup>3</sup> , g	8.17	13.1	0.937	0.015
<i>Intake</i>				
total DMI <sup>4</sup> , g/d	1691	1657	35.16	0.391
Total phenols, g TAe <sup>5</sup> /d	7.83	46.5	3.63	<0.001
Total tannins, g TAe <sup>5</sup> /d	2.60	28.1	1.92	<0.001
α-Tocopherol, mg/kg DM	92.3	52.0	5.54	<0.01
<i>Tocopherols, µg/g muscle</i>				
α-Tocopherol	3.37	3.80	0.070	0.763
γ-Tocopherol	0.81	0.70	0.011	0.210
<i>Fatty acids, mg/100g muscle</i>				
SFA <sup>6</sup>	1177	1293	36.50	0.193
MUFA <sup>7</sup>	1064	1123	40.61	0.276
PUFA <sup>8</sup>	122	149	5.901	0.083
OBCFA <sup>9</sup>	31.1	41.7	1.192	0.091

<sup>1</sup>BW=Body weight ; <sup>2</sup>ADG=average daily gain; <sup>3</sup>FCR=feed conversion ratio, DMI/ADG; <sup>4</sup>DMI=dry matter intake; <sup>5</sup>Tannic acid equivalent;

<sup>6</sup>SFA=saturated fatty acids; <sup>7</sup>MUFA: monounsaturated fatty acids; <sup>8</sup>PUFA: polyunsaturated fatty acids; <sup>9</sup>OBCFA: odd and branched chain fatty acids;

<sup>10</sup>SEM= standard error of means.