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

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Abstract

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

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

Introduction

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

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

2. Materials and methods

2.1. Animals and Diets

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

Twenty entire male lambs (*Sarda* breed) were weaned at 60 days of age and then received a conventional concentrate-based diet composed of maize and barley (both at 30% on a DM basis), fava bean (20%), wheat bran (17%) and vitamin mineral premix (3%) for about three months. At an average body weight of 28.8 ± 0.30 (SD) kg, lambs were penned individually and randomly divided into two groups (n=10, control and AH40 groups) and, after an adaptation period (7 days) to the treatments, fed with their respective diet *ad libitum* for 40 days. Control group were fed with the same conventional concentrate-based given after weaning; whereas the AH40 group received the concentrate diet in which most of the barley and maize was replaced with 40% AH (AH40 group; 9% maize, 9% barley, 32% fava bean, 7% wheat bran, 40% AH, 3% vitamin mineral premix on a dry matter basis). Hay was provided *ad libitum* in a separate feeder. The diet of the AH40 group had a higher fava bean content to maintain a similar crude protein concentration between treatments (considering the low protein content of the AH). The chemical composition of the two experimental diets was (g/kg DM): DM 896 (g/Kg wet weight), crude protein 146, ether extract 28.2, ash 32.2, NDF 221, total phenolic, in g of tannic acid equivalent (TAe)/Kg DM, 4.63, total tannins (g TAe/Kg DM) 1.54, α-Tocopherol (μg/g DM) 54.6, γ-Tocopherol (μg/g DM) 60.5, δ-Tocopherol (μg/g DM) 13.1, metabolizable energy (Mcal/kg DM) 2.37 for control diet; DM 891 (g/Kg wet weight), crude protein 139, ether extract 13.9, ash 59.4, NDF 309, total phenolic (g TAe/Kg DM) 28.1, total tannins (g TAe/Kg DM) 16.9, α-Tocopherol (μg/g DM) 34.4, γ-Tocopherol (μg/g DM) 32.2, δ-Tocopherol (μg/g DM) 12.1, metabolizable energy (Mcal/kg DM) 2.07 for AH40 diet.
The experimental diets were provided twice daily (0730 and 1630 h) *ad libitum*, and refusals were weighted daily to measure voluntary intake. The lambs were weighed fasting every 10 days.

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

2.2. Analyses of Feedstuffs

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

2.3. Meat quality analysis

Each carcass was immediately weighed and stored at 4°C for 24h. From the left side of each carcass, a portion of *Longissimus thoracis et lumborum* (LTL) muscle were collected from the 13th rib, vacuum-packed and stored at -30°C, pending fatty acid and vitamins analyses. Another portion of LTL muscle from each carcass was stored at 4°C and used for the analyses of lipid oxidation.

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

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

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

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

2.3. Statistical analysis

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

3. Results and Discussion

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

inclusion rates the starch reduction due to lower dietary inclusion of maize and barley was probably not compensated. In fact, the partial replacement of corn and barley with 40% almond hulls in the diet led to a 13 % reduction in the metabolizable energy in the AH40 diet (2.37 vs 2.07, for control and AH40 groups respectively), negatively affecting the growth performance of the animals. Furthermore, phenols intake, of which more than 60% are represented by tannins, from the lambs of AH40 group probably negatively influenced these parameters, considering that a high level of these compounds in the diets could lead to a slowdown in the animals' growth rates (Priolo, Waghorn, Lanza, Biondi & Pennisi, 2000). In a study of Vasta et al. (2019), it is pointed out that tannins show antimicrobial activity and protein-binding ability, make them able to affect rumen digestion and reducing biohydrogenation activity. In an *in vitro* study, Durmic et al. (2014) highlighted a reduction in methane and NH_3 production when AH were incubated with sheep rumen fluid microbes. In this trial, lambs from AH40 group daily ingested an amount of phenols and tannins respectively 6 and 11 times higher (P<0.001) than that from control lambs. However, considering the lower cost of the diet of the AH40 group (data not shown, *198 vs 320 €/t* for AH40 and control groups, respectively), the cost per 100 g of body weight gain was equivalent.

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

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

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

Conclusions

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

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

[1]BW=Body weight ; [2]ADG=average daily gain; [3]FCR=feed conversion ratio, DMI/ADG; [4]DMI=dry matter intake; [5]Tannic acid equivalent; [6]SFA=saturated fatty acids; [7]MUFA: monounsaturated fatty acids; [8]PUFA: polyunsaturated fatty acids; [9]OBCFA: odd and branched chain fatty acids; [10]SEM= standard error of means.

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