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Dietary olive cake reduces the oxidation of lipids, including cholesterol, in lamb meat enriched in polyunsaturated fatty acids

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ABSTRACT

Over 40 days, lambs were fed: concentrate (C), concentrate containing 20% linseed (L), concentrate containing 35% olive cake (OC), or concentrate containing 10% linseed and 17% olive cake (OCL). The polyunsaturated fatty acids (PUFA) and peroxidation index (PI) in phospholipids were increased by the L and OCL treatments ($P = 0.007$ and $P = 0.003$, respectively). The OC and OCL diets increased the concentration of tocopherol in muscle ($P < 0.001$). Compared to the OC and OCL diet, the L diet increased fatty acid oxidation, measured as conjugated dienes (CD; $P = 0.003$), peroxides (PV; $P < 0.001$) and TBARS ($P = 0.002$) in minced muscle over 11 days of storage in high-oxygen atmosphere. Also, the L diet increased ($P < 0.001$) the levels cholesterol oxidation products (COPs). In conclusion, feeding olive cake improved the oxidative stability of lamb meat and the combination of olive cake and linseed improved the fatty acid composition of meat without compromising its oxidative stability.

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1. Introduction

One of the main challenges in marketing meat is the retention of quality during storage and retail display by delaying the oxidative deterioration of muscle components, such as fatty acids and cholesterol, which compromises the sensory and nutritional quality of meat (Faustman, Sun, Mancini, & Suman, 2010; Hur, Park, & Joo, 2007). The oxidative stability of meat lipids is dependent on the balance between muscle antioxidant and pro-oxidant components. Muscle antioxidants comprise endogenous antioxidant systems, as well as molecules of dietary origin, such as tocopherols, carotenoids and others (Descalzo & Sancho, 2008). Conversely, polyunsaturated fatty acids (PUFA), especially those in the phospholipid fraction of cell membranes, are highly oxidizable substrates and may act as pro-oxidants (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). The diet of animals can significantly affect the inherent susceptibility of meat lipids to oxidative deterioration, by modifying both the antioxidant and the pro-oxidant components of muscle. Intensive concentrate-based feeding systems generally provide low amounts of dietary antioxidants, thus resulting in meat products characterized by a high susceptibility to oxidative deterioration compared to extensive pasture-based production systems (Luciano et al., 2012). The supplementation of concentrate-based diets with sources of PUFA, such as linseed, has been proposed as a strategy to increase the concentration of beneficial fatty acids, especially highly unsaturated *n-3* fatty acids, in muscle from different ruminant species. For example, Noci, French, Monahan, and Moloney (2007) observed a higher concentration of PUFA in intramuscular lipids from grazing cattle supplemented with 15% linseed oil. Similar results were obtained in lambs by supplementing concentrate-based diets with 6% linseed oil or 17.9% linseed (Jerónimo, Alves, Prates, Santos-Silva, & Bessa, 2009; Moloney, Kennedy, Noci, Monahan, & Kerry, 2012). However, feeding sources of PUFA without implementing adequate antioxidant interventions in parallel may be deleterious in terms of the oxidative stability of meat (Jacobsen, Let, Nielsen, & Meyer, 2008).

Meat production in Europe faces several challenges linked to increased production costs, competition from cheaper imports and adhering to strict regulations applying to the entire production chain. In this context, the promotion of sustainable and safe production systems that deliver high-quality products is encouraged. Low-input strategies in small ruminants farming can be implemented by using alternative feeding resources, such as agri-industrial by-products, which often contain remarkable amounts of bioactive molecules able to positively affect meat quality (Vasta & Luciano, 2011). In Mediterranean areas, the olive oil industry produces substantial amounts of by-products, with one of the most important being the

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olive cake (OC). The potential use of OC as a replacer of part of the cereal concentrate in diets for small ruminants has been explored, as this might partially contribute both to solving the problem of the disposal of OC and to reducing production costs for livestock feeding. Although the study of the effect of OC in small ruminants diets on feed digestibility and animal performances has been relatively commonplace (Molina-Alcaide & Yáñez Ruiz, 2008), there is a little information on the impact of dietary OC on lamb meat quality (Foti, Caparra, Giuffrida, Scerra, & Chies, 2003). In relation to the oil extraction procedure, OC may present an interesting chemical composition. Particularly, the removal of stones can improve the nutritional properties of OC, while the use of mild drying temperatures avoids severe losses of lipophilic antioxidants and other bioactive compounds such as phenolic compounds (Amro, Aburjai, & Al-Khalil, 2002; Martín-García, Moumen, Yáñez-Ruiz, & Molina-Alcaide, 2003). These compounds may influence the oxidative stability of meat. For instance, the dietary administration of OC was shown to affect the oxidative status in rabbits (Dal Bosco et al., 2007); however, the results were less definitive when meat oxidative stability was investigated (Dal Bosco et al., 2012).

The aim of the present study was to assess whether the inclusion of destoned OC in concentrate-based diets of lambs can improve the oxidative stability of lipids in the resultant meat. Specifically, the use of OC in combination with linseed in the diet was tested in order to enhance the oxidative stability of meat enriched in PUFA. Furthermore, the contribution of tocopherols and of the PUFA in both total intramuscular lipids and polar lipids to meat lipid oxidation was studied, including also the oxidation of cholesterol which has been seldom reported in lamb meat.

2. Materials and methods

2.1. Animals and dietary treatments

The study was conducted at the experimental station of the Department of Applied Biology of the University of Perugia (Italy). Thirty-two Appenninica male lambs were weaned at 40 ± 5 days (body weight 17.8 ± 1.6 kg) and were randomly distributed into 4 groups of 8 lambs each. After 20 days of adaptation to the experimental diets, each group was fed one of the following concentrate-based diets for 40 days: a barley- and oat-based concentrate with a low level of lipids and a high content of non structural carbohydrate (diet C); a concentrate containing 20% on a dry matter basis (DM) of rolled linseed (diet L); a concentrate containing 35% DM of stoned olive cake (diet OC); a diet containing a mixture of rolled linseed (10% DM) and stoned olive cake (17% on DM; diet OCL). All ingredients were incorporated into pellets using a pelleting machine (CMS IEM—Colognola ai Colli Verona Italy): pellet diameter was 3 mm and pelleting temperature ranged between 35 and 40 °C. Fresh olive cake was obtained after mechanical extraction of virgin olive oil using the following operative conditions: the olives were stoned and malaxed for 40 min at 25 °C and the oil extraction was performed using an RCM Rapanelli three phases decanter mod. 400 eco. After storage at room temperature for 36 hours, stoned olive cake was dried using a fluid bed dryer; the initial temperature of the drying air flow was 120 °C and the maximum temperature of olive cake during the drying process was 45 °C. The dried stoned olive cake was stored at room temperature.

The composition of the experimental concentrates is given in Table 1. All concentrates were administered together with grass hay (forage) at a 30:70 forage:concentrate ratio, on the basis of the expected DM intake. Lambs were weighed at the beginning of the experiment and every 10 days until the day before slaughter. For each group, feed given and refused were recorded daily, while samples of the feeds offered and of the refusals were collected weekly and stored at −30 °C for analyses. Crude protein (CP) and ether extract (EE) of the experimental feeds were determined according to AOAC methods (AOAC, 1995). Fiber fractions (NDF, ADF and ADL) were analyzed according to the method described by Van Soest, Robertson, and Lewis (1991). The net energy of the concentrates was estimated according to INRA (1989).

2.2. Slaughter procedures and muscle sampling

At the end of the experiment lambs were slaughtered. Carcasses were immediately weighed to obtain hot carcass weight and, then, kept at 4 °C for 24 hours. The *longissimus dorsi* muscle (LM) was removed between the 2th and the 13th rib, vacuum packed and stored at −80 °C until analysis.

2.3. Fatty acid composition of feeds and of total intramuscular lipids and phospholipid fraction

Total intramuscular lipids (TL) were extracted according to Rodriguez-Estrada, Penazzi, Caboni, Bertacco, and Lercker (1997). Triacylglycerols (TG) and phospholipids (PL) were separated according to Juaneda and Roquelin (1985) using Sep-Pak® silica cartridges (Warters, Milford-Massachusetts, USA). For each lipid fraction (i.e. TL, TG and PL), fatty acid methyl esters (FAME) were prepared according to Christie (1982) and, before methylation, C9:0 and C23:0 FAME were added together as internal standards. The FAMEs were analyzed by a gas-chromatograph (GC) equipped with a flame ionization detector (FID; ThermoQuest, Milan, Italy) and a high polar fused silica capillary column was used (WCOT fused silica CP-Select CB for FAME Varian, Middelburg, Netherland; 100 m × 0.25 mm i.d.; film thickness 0.25 μm). Helium was used as the carrier gas at a flow of 1 mL/min. The split ratio was 1:80. The GC conditions were as follows: the oven temperature was programmed at 150 °C and held for 1 min, then increased up to 175 °C at a rate of 0.8 °C/min, held for 14 min, then increased up to 188 °C at 2 °C/min, held for 18 min, and then increased up to 230 °C at a rate of 2 °C/min, held for 13 min. The injector and detector temperatures were set at 270 °C and 300 °C, respectively. A mixture of 52 component FAME Mix (Nu-Chek Prep. Inc., Elysian, MN, USA) and 77 individual FAME standards (Larodan Fine Chemicals, Malmo, Sweden) were used for the identification of individual FAME. For each fatty acid, the response factors to flame ionization detector and inter- and intra-assay coefficients of variation were calculated by using a reference standard butter (CRM 164, Community Bureau of Reference, Brussels, Belgium).

The peroxidation index (PI) of polyunsaturated fatty acids (PUFA) in both the total lipids (PI-TL) and the phospholipid (PI-PL) fractions was calculated in order to account for the increasing susceptibility of PUFA to peroxidation with increasing unsaturation degree of their molecules. The PI was calculated according to Scislowski, Bauchart, Gruffat, Laplaud, and Durand (2005) using the following equation:

$$\mathrm{PI} = (\%\ \text{dienoic} \times 1) + (\%\ \text{trienoic} \times 2) + (\%\ \text{tetraenoic} \times 3) + (\%\ \text{pentaenoic} \times 4) + (\%\ \text{hexaenoic} \times 5)$$

With regard to feed samples, fat was extracted according to the method described by Folch, Lees, and Stanley (1957). Fatty acids were esterified according to Christie (1982) with C19:0 as the internal standard, and were identified using the same procedure described above for meat samples. The fatty acid composition of the four experimental diets is presented in Table 1.

2.4. Preparation and storage of muscle samples for oxidative stability measurements

For each animal, a 150 g portion of LM was removed from the freezer and, while still partially frozen, was trimmed of the outer fat and chopped into small cubes using a knife. The muscle was finely blended using a homogenizer. The minced muscle was divided into

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