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The effect of dietary alfalfa and flax sprouts on rabbit meat antioxidant content, lipid oxidation and fatty acid composition

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ABSTRACT

The aim of this study was to determine the effect of dietary supplementation with flax and alfalfa sprouts on fatty acid, tocopherol and phytochemical contents of rabbit meat. Ninety weaned New Zealand White rabbits were assigned to three dietary groups: standard diet (S); standard diet + 20 g/d of alfalfa sprouts (A); and standard diet + 20 g/d of flax sprouts (F). In the F rabbits the *Longissimus dorsi* muscle showed a higher thio-barbituric acid-reactive value and at the same time significantly higher values of alpha-linolenic acid, total polyunsaturated and n-3 fatty acids. Additionally n-3/n-6 ratio and thrombogenic indices were improved. The meat of A rabbits showed intermediate values of the previously reported examined parameters. Dietary supplementation with sprouts produced meat with a higher total phytoestrogen content.
The addition of fresh alfalfa and flax sprouts to commercial feed modified the fat content, fatty acid and phytochemical profile of the meat, but the flax ones worsened the oxidative status of meat.

1. Introduction

Sprouts, i.e., the young seedlings obtained from seed germination, are deemed to be healthy foods because they contain high levels of nutrients that may have positive effects on human health such as preventing cardiovascular diseases and cancer (Finley, 2005; Marton, Mandoki, Csapò-Kiss & Csapç, 2010; Schenker, 2002; Webb, 2006). Compared to sound seeds, sprouts are low in antinutritive compounds (e.g., trypsin inhibitor, phytic acid, tannins; Marton et al., 2010) and high in oligo- and monosaccharides (Koehler, Hartmann, Wieser & Rychlik, 2007), free fatty acids (Kim, Kim & Park, 2004), oligopeptides, amino acids (Urbano et al. 2005), vitamins and phytochemicals such as polyphenols (flavonoids, phenolic acids, lignans, phytoestrogens), glucosinolates and carotenoids (Amici, Bonli, Spina, Cecarini, Calzuola & Marsili, 2008; Fernandez-Orozco et al., 2006 and Krishna, Paridhavi & Patel, 2008).

Flax sprouts have been found to be a better source of protein, crude fiber, simple sugars and essential micronutrients than sound seeds (Narina, Hamama & Bhardwaj, 2012). Flax sprouts are characterized by higher levels of water soluble proteins and free amino acids (Wanasundara, Shahidi & Brosnan, 1999a) higher levels of free fatty acids, glycolipid fractions, lysophosphatidylcholine, phosphatidic acid (from negligible amounts to 46% of the total) and similar phospholipid levels (Wanasundara, Wanasundara & Shahidi, 1999b).

Alfalfa sprouts contain high amounts of vitamins, phytoestrogens and saponins. Sprouts have higher levels of vitamin A and C (1250- and 10-fold increase with respect to the sound seed, respectively; Plaza, De Ancos & Cano, 2003), coumestrol, liquiritigenin, isoliquiritigenin, loliolide (Hong et al. 2011 and Horn-Ross et al. 2000) and saponins than raw seeds (Oleszek, 1998).

Despite the increasing popularity of sprouts as a '*healthy food*' in western countries, the risk of bacterial contamination (e.g., *Escherichia coli*, *Salmonella enteritidis*, *Vibrio cholerae*) represents a public health concern, as sprouts are normally home grown and used as components of salads and therefore undergo no thermal or other sanitation treatment (Taormina, Beuchat & Slutsker, 1999). Dried sprout powder has been proposed as dietary supplement that could be blended at proportions similar to those used in other conventional foods (e.g., wheat sprout powder in wheat bakery flours) to increase the nutritional value of foods without changing dietary behavior (Koehler et al., 2007); however, certain phytochemicals may be lost during processing.

An alternative could be the use of sprouts in animal feeding: bioactive compounds from sprouts could be transferred to livestock products, which, in turn, would be transferred to humans. This scheme represents an attractive way of improving the quality and safety of food destined for human consumption and animal health.

Some studies examined the effect of sprouts on the performance and health of animals (Jegede et al. 2008; Peer & Leeson, 1985) with controversial result; however, to the best of our knowledge, no specific data on the possibility of transferring bioactive compounds from sprouts to animal products are available. Evidence of bioactive transfer from sprouts to animal tissues could be presumed by some papers. Winarso,

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Purwo & Kusuma (2011) assessed that the improvement in spermatozoa quality of goat was due to the transfer of high vitamin E amount from legumes sprouts.

Thus, given the lack of scientific data, the aim of the present study was to evaluate the effect of dietary alfalfa and flax sprouts on rabbit meat antioxidant content, lipid oxidation and fatty acid composition.

2. Materials and methods

2.1. Animals and diets

Ninety New Zealand White mixed-sex rabbits were weaned at 30 days of age, allocated into three homogeneous groups (weight, sex) and subjected to the following dietary treatments until they were 80 days old:

- Standard (S) diet;
- Standard diet + 20 g/d of alfalfa sprouts (A);
- Standard diet + 20 g/d of flax sprouts (F).

Fresh sprouts were placed daily near the feeders.

The experimental protocol was devised according to the Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes, and the research was carried out at the experimental farm of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Italy). All of the rabbits were housed individually in flat-deck cages (600 × 250 × 330 mm). The feeding program was adjusted according to previous studies (Martens & Villamide, 1998). Water was supplied ad libitum. The applied temperature and lighting schedules in the rabbit house were 15–18 °C and 16 L:8 D, respectively.

2.2. Production of alfalfa and flax sprouts

Alfalfa (*Medicago sativa L.*) and flax (*Linum usitatissimum L.*) seeds were germinated on a substrate consisting of moistened tissue paper lying on a layer of silica sand sterilized at 105 °C in aluminum trays (22 cm × 30 cm for alfalfa and 30 cm × 36 cm for flax). In each tray, the sand (600 g for the alfalfa and 1 kg for the flax) was distributed to create a uniform layer on the bottom of the tray and moistened with demineralized water. The trays were placed in a temperature-controlled room at 20 °C in the dark and kept in these conditions for three days. Water was added periodically to compensate for sand water loss due to evaporation. In contrast to the usual sprouting procedure used for alfalfa in which sand water content is restored once a day, flaxseed requires several separate additions of water, as the seeds tend to produce a glue-like mucilaginous exudate in the presence of high water content that would hamper seedling development. For each species, the sprouts obtained on the third day from different trays were combined to prevent a possible tray effect and stored at 4 °C in plastic bags until use (i.e., within three days).

2.3. Sampling and analytical determination

At 80 days of age, 20 rabbits per group with weights close to the average of the group (±10%) were selected and slaughtered in the departmental processing plant 12 h after feed withdrawal under the supervision of veterinarians from the University of Perugia; none of the animals underwent any form of transportation. The rabbits were sacrificed by severing the carotid arteries and jugular veins following electro-stunning and the carcasses were prepared according to the methods described by Blasco and Ouhayoun (1993). Following carcass chilling (24 h at + 4 °C), the two *Longissimus lumborum* muscles were removed and carefully freed from connective and adipose tissues.

2.3.1. Chemical composition of the feed and sprouts

The chemical composition of the feed and sprouts was determined according to the method of the AOAC (1995).

2.3.2. Fatty acid profile of the feed, sprouts and meat

The fatty acid profile of the feed, sprouts and meat was determined by gas chromatography following lipid extraction according to the method described by Folch, Lees & Sloane-Stanley (1957). In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen and the residue was derived by adding 3 mL of sulfuric acid (3% in methanol). Following incubation at 80 °C for 1 h, the methyl esters were extracted with petroleum ether, and 1 μL was injected into a gas chromatograph (Mega 2 — model HRGC; Carlo Erba, Milan, Italy) equipped with a flame ionization detector. The fatty acid methyl esters (FAMEs) were separated with an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions used during the column injection of the 1 mL sample volume were as follows: the temperatures of the injector and detector were set at 270 °C and 280 °C, respectively, and the detector gas flows were H_2 at 50 mL min^{-1} and air at 100 mL min^{-1}. The oven temperature was programmed to provide a good peak separation as follows: the initial oven temperature was set at 130 °C; this temperature increased at a rate of 4.0 °C min^{-1} to 180 °C and was held for 5 min; the temperature was then increased at a rate of 5.0 °C min^{-1} to 230 °C; the final temperature was held for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL min^{-1}. Individual fatty acid methyl esters were identified by referring to the retention time of FAME authentic standards.

The average amount of each fatty acid was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated (PUFA) fatty acids.

2.3.3. Nutritional indexes

The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986): PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8).

The amount of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI) as proposed by Ulbricht and Southgate (1991) and the hypocholesterolemic/hypercholesterolemic ratio (HH) as suggested by Santos-Silva, Bessa & Santos-Silva (2002).

2.3.4. Oxidative status of meat

The extent of muscle lipid oxidation was evaluated by a spectrophotometer set at 532 nm (Shimadzu Corporation UV-2550, Kyoto, Japan), according to the modified method of Ke, Ackman, Linke, and Nash (1977), which measured the absorbance of thio-barbituric acid-reactive substances (TBARS). Briefly 5 g of meat, was homogenized in a 75 g/L trichloroacetic acid solution. After centrifugation 5 mL of extract was reacted with 2.88 g/L of fresh thio-barbituric acid (TBA), with a ratio of 1:2 (v/v). Oxidation products were quantified as malondialdehyde equivalents (mg MDA/kg muscle) through a 1,1,3,3-tetraethoxypropane calibration curve. The tocopherol (α-tocopherol and its isomers β + γ and δ) and retinol contents of the feed, sprouts and meat were quantified by HPLC according to the method described by Hewavitharana, Lanari & Becu (2004). In particular, 5 ml of distilled water and 4 ml of ethanol were added to 2 g of sample and vortexed for 10 s. After mixing, 4 ml of hexane containing BHT (200 mg/l) was added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (3 mL) was dried under a stream of nitrogen and dissolved in 300 μL of acetonitrile; 50 μL were injected into the HPLC (pump model Perkin Elmer series 200, equipped with an autosampler system, model AS 950-10, Tokyo, Japan) on an Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 μm particle size; CPS analitica, Milan, Italy). Tocopherols were identified using a FD detector (model Jasco, FP-1525 — excitation and emission