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Influence of stall finishing duration of Italian Merino lambs raised on pasture on intramuscular fatty acid composition

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ABSTRACT

Forty male Italian Merino lambs were used to study the effects of four feeding systems on muscle fatty acids composition: S group—ten lambs were kept indoors, and fed with concentrate for all experimental period (89 days); P group—ten lambs were allowed to graze a pasture for all experimental period; PS37 group—ten lambs were allowed to graze a pasture for 52 days and shifted indoor, fed with concentrate, 37 days before slaughtered; PS14 group, where 10 lambs were fed on pasture for 75 days and shifted indoor, fed with concentrate, 14 days before slaughtered. Grazing lowered the levels of C12:0, C14:0, C16:0 and n-6 PUFA and increased n-3 PUFA and CLA isomer compared to concentrate feeding. After a short period of indoor finishing with concentrate, the fatty acid characteristics of the meat retain a part of the benefits occurring from grazing, while a longer period seems to erase almost all the benefits from grazing.

1. Introduction

Never as now consumers require meat which is safe, of consistent eating quality, healthy and convenient. Numerous studies (Gidding et al., 2005; Keys, 1970; Mensink et al., 2003) have confirmed that there is a strong relationship between the lipids consumed in the human diet and serum total cholesterol. Constituent of red meat that have been proposed to be responsible for this association include the fat content and fatty acid composition.

Dietary recommendations for humans promoting the consumption of less saturated fat (World Health, 2003) have led to an increased interest in meats containing more unsaturated fatty acids.

Ruminant fat has a higher SFA and a lower polyunsaturated fatty acids than non-ruminant fat, due to hydrogenation of unsaturated fatty acids in the rumen (French et al., 2000). However, manipulation of animal feed has been used as an alternative method to improve meat quality.

New feeding strategies in animal nutrition in both ruminants and monogastrics have as principal objective to increase polyunsaturated fatty acids, especially of n-3 series, increase conjugated linoleic acid level and reduce saturated fatty acids in zootechnical products (Scollan et al., 2006; Wood et al., 2008).

Recent studies have demonstrated that ruminants fed to the pasture show a higher level of n-3 polyunsaturated fatty acids in meat than animals fed with grain based diets (Enser, 2000; Realini et al., 2004; Vasta et al., 2009; Yang et al., 2002).

Ruminant animals grazing pasture may have the ability through enzymatic activity to synthesize the long-chain n-3 FA (EPA and DHA) from their precursor α-linolenic acid, although the conversion efficiency is relatively low (Enser et al., 1998; French et al., 2000). Moreover, diets rich in forage favor the growth of fibrolytic micro-organisms that are principally responsible of the high hydrogenation activity in the rumen and consequently of CLA and vaccenic acid (C18:1 trans 11, precursor of CLA in tissue) production (Baumann et al., 1999).

Unfortunately environmental conditions do not allow often the continuous use of fresh grass, so that it becomes necessary to finish some lambs on concentrate. Concentrate show a higher percentage of C18:2 n-6 and a lower C18:3 n-3 than fresh grass and consequently can alter meat fatty acid composition. In this way the effect of use of the pasture in animal diets on fatty acid composition could be removed.

The objective of the present trial is to evaluate the effect of the use of concentrate before slaughter in lambs raised to the pasture on meat fatty acid composition.

The effect of the use of concentrate before slaughter, considering two different periods of stall-finishing indoor with concentrate, on fatty acid composition of lamb meat has been considered (Aurousseau et al., 2007). However, there are some differences.

In the trial of Aurousseau et al. the lambs were slaughtered when they reached the specified body live weight of 34.5 kg and therefore at different age, whereas in our experiment all the lambs were slaughtered to the same age, at about 170 days. Furthermore in our study, the experiment started (first separation in groups) after that

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the lambs have been weaned, at 60 days of age, while in the trial of Aurousseau et al. the experiment started (first separation in groups) when the animals had around 7–15 days of age and therefore were separated with the corresponding dams.

2. Materials and methods

2.1. Experimental design, animals and diets

The study was carried out on 40 male Italian Merino lambs in a farm located in Calabria (Italy, 38°38′ N, 16°04′ E). Single male lambs born on the 16th (±10 days) of October were considered. On December 15th, at about 60 days of age, all the lambs have been separate from their mothers and gradually adapted to the experimental diets during an 18-day transition period: all the animals received a decreasing amount of the weaning concentrate, 100 g/d of oats (*Avena sativa*) hay and the experimental diet. Starting from the 2nd of January (day 1 of the experimental feeding), for the whole period of 89 days of experimental trial, 10 lambs were kept indoors and fed with the stall diet (stall group, S) and 10 lambs were allowed to graze (pasture group, P). The other 20 lambs were divided into two groups and allowed to graze the same pasture of the P group before their abrupt switching to the stall fed period: pasture-stall 37 days lambs (PS37 group) were fed on pasture for 52 days and in barn for 37 days; pasture-stall 14 days lambs (PS14 group) were fed on pasture for 75 days and switched to stall diet for 14 days. All the animals were weekly weighed. Stall animals, PS37 and PS14 lambs during the stall-fed phase, were individually penned and received concentrate (64% barley, 34% chickpea and 2% mineral and vitamin mix) and oats hay in a ratio of 80/20 of the diet on an as fed basis. Feed quantities were weekly adjusted to obtain similar daily gain compared to pasture fed lambs. Water was always freely available in the stall. Pasture group, and PS37 and PS14 groups during the pasture feeding period, grazed for 6–7 h/d on a natural pasture composed by 80% of Graminaceous (*Avena barbata, Bromus ramosus, Cynodon dactylon, Dactylis hispanica, Lolium perenne*) and 20% of Leguminous (*Biturnimaria bituminosa, Melilotus sulcata, Trifolium nigrescens, Trifolium phleoides, Trifolium repens, Vicia sativa L., Vicia villosa*), approximately. The grazing area for the experimental lambs was fenced, in order to prevent from entering the other animals; continuous grazing system has been applied, according to the traditional use in southern Italy. In the remaining hours of the day, lambs were kept in multiple boxes within the stall without receiving any feed supplementation.

Animals were slaughtered in a commercial abattoir at 167 days of age. Carcasses were stored at 4 °C for 24 h after which were halved and the *longissimus dorsi* muscle was excised from the right side between 6th thoracic rib and 4th lumbar rib, vacuum packaged and stored at −30 °C for subsequent analyses.

2.2. Feed analysis

Feed samples were analyzed for neutral detergent fiber (NDF) (Van Soest et al., 1991), crude protein ((AOAC (Association of Official Analytical Chemists), 1995); method 984.13) and fatty acid composition (Gray et al., 1967; fatty acids were expressed as percent of total methylated fatty acids).

2.3. Proximate analysis on longissimus dorsi samples

A 50 g portion of the of the *longissimus dorsi* between the 1st and 3rd was allowed to thaw for 24 h at 4 °C and was used to measure moisture (method no. 950.46), crude protein (method no. 984.13) and ash (method no. 920.153) according to AOAC procedures (AOAC (Association of Official Analytical Chemists), 1995).

2.4. Intramuscular fatty acid determination

Samples of *longissimus thoracis* muscle were taken at the level of the 13th thoracic rib, minced and vacuum-packed (50 g for each animal) and stored at −25 °C until needed. Lipids were extracted according to the method used by Folch, Lees and Stanley (Folch et al., 1957). Briefly, a 5 g homogenized meat sample was blended with extraction solvent chloroform/methanol (2:1, v/v) twice, filtered, placed in separator funnels and mixed with saline solution (0.88% KCl). After separation in two phases, the aqueous methanol fraction was discarded, whereas the chloroform lipid fraction was washed with distilled water/methanol (1:1, v/v). After a further filtration and evaporation by means of a rotary evaporator, lipid extracts were transferred to test tubes for subsequent gas chromatographic analysis. Duplicates of 100 mg of lipid were methylated adding 1 ml of hexane and 0.05 ml of 2 N methanolic KOH (I.U.P.A.C., 1987), and nonanoic acid (C9:0) was used as an internal standard. Gas chromatograph analysis was performed on a Varian model Star 3400 CX instrument equipped with a CP 88 capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25 lm). Operating conditions were a helium flow rate of 0.7 ml/min, an FID detector at 260 °C, a split–splitless injector at 220 °C with an injection rate of 120 ml/min, an injection volume of 1 μl. The temperature program of the column was 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (37 component FAME mix, 18919-1 AMP, Supelco, Bellefonte, PA) run under the same operating conditions. Fatty acids were expressed as percent of total methylated fatty acids.

2.5. Statistical analysis

All data were analyzed according to a completely randomized design using GLM procedure of Minitab statistical software. The statistical model included diet treatment effect and experimental error. Differences between treatments were determined by using the Tukey's test.

3. Results

3.1. Chemical composition of diets and feed fatty acids

Chemical composition and fatty acid composition of the feeds is presented in Table 1.

Table 1
Chemical composition (g/kg dry matter) and fatty acid profile (weight % of total fatty acid methyl esters) of feed offered to lambs.

	Food		
	Pasture	Hay	Barley/chickpea (64/34)
Chemical composition			
Dry matter (g/kg wet weight)	210.9	917.0	878.3
Crude protein	109.3	63.0	148.6
Ether extract	7.5	17.9	25.3
Ash	105.8	88.4	23.4
NDF	477.0	641.6	147.0
Main fatty acids			
C16:0	18.99	24.46	20.89
C18:0	1.65	4.31	1.72
C18:1 *n*-9	4.80	15.36	21.37
C18:2 *n*-6	16.55	34.14	51.01
C18:3 *n*-3	58.01	21.73	5.01

All the analyses were performed in triplicates.
NDF, neutral detergent fiber.