

PAPER

Lamb meat quality and intramuscular fatty acid composition as affected by concentrates including different legume seeds

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Abstract

The aim of this experiment was to study the effect of concentrates including legume seeds (*Vicia faba* var. *minor* or *Pisum sativum*) on lamb performances and meat quality, emphasizing the intramuscular fatty acid composition. Thirty lambs (14.5±3.45 kg live weight) were randomly assigned to three dietary treatments: i) group fed on concentrate including 400 g/kg of peas (PEA); ii) group fed on concentrate including 380 g/kg of faba bean (FB); iii) group fed on concentrate including 180 g/kg of soybean meal (SBM). Growth and slaughter performances were not affected by treatments as well as physical and proximate chemical meat characteristics. FB and SBM meat showed higher ($P<0.001$) vaccenic acid levels compared to PEA meat. Oleic acid was higher ($P<0.05$) in PEA meat compared to SBM meat while its level in FB meat was similar to counterparts. Linoleic acid levels tended to increase ($P<0.10$) in SBM lambs compared with PEA animals. PEA group showed higher ($P<0.001$) α -linolenic acid proportions compared with FB and SBM groups and a tendentially higher ($P<0.10$) eicosapentaenoic acid content compared with SBM meat. As a result, total $n-3$ fatty acids were higher ($P<0.05$) in PEA meat compared to SBM one while the proportions in FB meat were at intermediate level. These findings accounted for a lower and more favourable ($P<0.001$) $n-6/n-3$ ratio in PEA

group compared with counterparts. Peas based-concentrate seemed to be more effective than faba bean- or soybean meal-included concentrates to improve the acidic profile of meat leading to higher α -linolenic acid levels and a lower $n-6/n-3$ ratio.

Introduction

Recently, in Mediterranean areas there has been a growing interest in local alternative protein sources in animal nutrition for several reasons, as to reduce feeding costs arising from imports of expensive soybean, the most widely-used vegetable protein source; to improve organic agriculture systems, supporting the use of local feedstuffs according to a sustainability principle (European Commission, 1999); to substitute the animal protein sources banned in ruminant nutrition because of their role in bovine spongiform encephalopathy (BSE). Due to these constraints, great attention has been paid to vegetable protein sources in small ruminant diets (Vasta *et al.*, 2008).

In order to meet the demand for alternative protein sources, homegrown legume seeds were taken into account for their high protein [19-35% crude protein (CP) on as dry matter (DM) basis] and starch contents (over 40% DM), despite the presence of some anti-nutritional factors that could be deactivated by heat treatment (Yu *et al.*, 2002).

Moreover, the ecological role of legume crops to reduce nitrogen depletion in soil is consistent with organic rules (Caballero, 1999).

Literature on the use of alternative legume seeds in animal nutrition is copious, covers a long time period and has caused renewed interest in recent years (Purroy *et al.*, 1992; Hadjipanayiotou, 2002; Loe *et al.*, 2004; Christodoulou *et al.*, 2005, 2006; Lanza *et al.*, 2007; Liponi *et al.*, 2007; Stein *et al.*, 2006). Focusing the attention on the use of alternative legume seeds (*Faba bean* var. *minor*; *Pisum sativum*; *Cicer arietinum*) in lamb nutrition, several studies showed that their use did not negatively affect growth, slaughter performances or meat quality (Surra *et al.*, 1992; Purroy *et al.*, 1992; Hadjipanayiotou, 2002; Antongiovanni *et al.*, 2002; Lanza *et al.*, 1999, 2001, 2003a, 2003b).

Moreover, animal feeding strategies are oriented either in ruminants or in monogastrics towards decreasing saturated fatty acids and enhancing polyunsaturated fatty acids, especially of $n-3$ series, and conjugated linoleic acid

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in animal products (Wood *et al.*, 2008; Woods
and Fearon, 2009).

Despite the ability of the rumen microorganisms to partially hydrogenate dietary polyunsaturated fatty acids, increasing saturated fatty acids in animal tissue, diet could play an important role in changing intramuscular fatty acid composition (Wood and Enser, 1997; Wood *et al.*, 1999; Priolo *et al.*, 2001). An increased level of C12:0 lauric, C18:2 trans, C18:3 $n-6$, C18:2 CLA, C22:5 $n-3$ and total $n-3$ fatty acids was found in intramuscular fatty acid profile of meat from lambs fed chickpeas in total or partial substitution of soybean meal (Priolo *et al.*, 2003).

To the best of our knowledge, few data are available on the effects of using faba bean (*Vicia faba* var. *minor*) or peas (*Pisum sativum*) on lamb intramuscular fatty acid composition.

Recently, Scerra *et al.* (2011) found an increase in polyunsaturated $n-3$ fatty acids content in intramuscular fat of lambs fed a diet with peas and faba bean (24% on as feed basis for both legume seeds) in total and partial replacement of soybean meal and wheat, respectively.

To give a further contribution to confirm the results of Scerra *et al.* (2011), we planned an experiment aiming at studying the effect of total replacement of soybean meal and partial replacement of cereal grains with faba bean (*Vicia faba* var. *minor*) or peas (*Pisum*

sativum) on growth and slaughter performances and on meat quality, with an emphasis on intramuscular fatty acid composition.

Materials and methods

Experimental design, animals and diets

The trial was carried out on a farm located in the Catania province (Sicily, Italy) at a latitude of 37° 23' N and 15° 02' E and at the sea level. Thirty Comisana x Valle del Belice lambs, born on the same farm and weaned at 60 days of age, were stratified according to weight (kg 14.5±3.45, average live weight), housed in collective pens and randomly assigned to one of three dietary treatments [peas (PEA), 10 animals; faba bean (FB), 10 animals; soybean meal (SBM), 10 animals]. All the diets included maize, barley, dehydrated lucerne, carob pulp, brewer's yeast, limestone, dicalcium phosphate and mineral-vitamin premix. PEA diet included 400 g/kg (on as fed basis) of peas, in total replacement of soybean meal and in partial replacement of maize and barley. FB diet included 380 g/kg (on as fed basis) of faba bean in replacement of soybean meal and maize and barley as above. SBM diet was considered as the control diet and included soybean meal as the main protein source (180 g/kg, on as fed basis).

All the ingredients were ground and compounded into pellets (6 mm diameter) by a feed mill. The ingredients and the chemical composition of the diets are reported in Tables 1 and 2. Lambs had *ad libitum* access to the complete pelleted diets and water allowed. Fresh feed was offered once daily every morning in order to improve palatability and feed refusals were removed and recorded. Lambs spent a 7 days-pre experimental period. Initial weight was recorded and then once a week lambs were weighed in the morning before fresh feed supply. Before inclusion into concentrate mixtures, five subsamples of peas, faba bean and soybean meal were collected and vacuum-packed and stored at -30 °C until analyses. Once a week throughout the trial, subsamples of concentrate mixtures were collected and vacuum-packed and stored at -30 °C until analyses. Before chemical analyses all subsamples of each protein source and of each concentrate mixture were freeze-dried and finely ground (1-mm screen) and thoroughly mixed into a final sample representative for each protein source and for each concentrate mixture.

Neutral (Van Soest *et al.*, 1991) and acid (AOAC, 1995, method no. 973.18) detergent fibre (NDF, ADF) were determined using an Raw Fiber Extractor (Velp Scientifica srl, Usmate, MB, Italy). Feed samples were also analyzed for lignin (AOAC, 1995, method no. 973.18), crude protein (method no. 984.13), ash (method no. 942.04) and ether extract (EE, method no. 920.39) according to AOAC (1995).

Fatty acid composition of peas, faba bean, soybean meal and of each experimental diet were determined according to Sukhija and Palmquist (1988) procedure modified by Palmquist and Jenkins (2003). Total lipids were extracted from 0.5 g of each concentrate

samples into 20×150 mm test tubes with Teflonlined screw caps. Nonadecanoic acid (C19:0, NuChek Prep, Elysian, MN; 2.0 mg/mL in heptane) was used as internal standard. Fatty acids were methylated using HCL in methanol prepared by adding 20 mL of acetyl chloride to 100 mL of methanol. Fatty acid methyl esters were analysed by a gas-chromatography apparatus (Thermo Finnigan, Rodano, Mi, Italy, TRACE with the software Chrom-Card) equipped with a flame ionization detector, a capillary column SP-2340 in fused silica (60 m × 0.25 mm i.d.; 0.20 µm film thickness). The gas-chromatography GC conditions were as following: the oven temperature was

Table 1. Dietary ingredients and chemical composition.

Ingredients	Treatment		
	PEA	FB	SBM
Lucerne dehydrated, g/kg	160	170	170
Maize, g/kg	140	170	260
Barley, g/kg	200	180	290
Carob pulp, g/kg	40	40	40
Brewer's yeast, g/kg	30	30	30
Peas, g/kg	400	-	-
Faba bean, g/kg	-	380	-
Soybean meal, g/kg	-	-	180
Limestone, g/kg	14	14	14
Dicalcium phosphate, g/kg	12	12	12
Vitamin-mineral premix, g/kg	4	4	4
Chemical composition			
Dry matter, g/kg	892.2	896	882.1
Crude protein, g/kg DM	158.2	174.7	181.1
NDF, g/kg DM	198.2	223.3	253.2
ADF, g/kg DM	110.4	127.6	124.8
Lignin (sa) ³ , g/kg DM	45.4	10.1	32.3
Ether extract, g/kg DM	21.8	19.3	22.8
Ash, g/kg DM	67.1	64	67.1
Net energy for gain, MJ/kg DM	7.68	8.53	8.75

PEA, peas diet; FB, faba bean diet; SBM, soybean meal diet; DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ³lignin determined by solubilization of cellulose with sulphuric acid.

Table 2. Fatty acid composition of protein sources and of the complete diets. Data are expressed as mg fatty acid /g DM of feed sample.

Fatty acids	Protein source			Dietary treatment		
	Peas	Faba	Soybean meal	PEA	FB	SBM
C12:0	0.085	0.085	nd	0.073	0.084	0.106
C14:0	0.085	0.065	0.021	0.074	0.074	0.150
C15:0	0.064	0.043	0.021	0.032	0.042	0.043
C16:0	3.750	3.955	2.886	3.493	3.671	4.636
C16:1	0.075	0.076	0.042	0.075	0.053	0.075
C18:0	0.702	0.654	0.900	0.664	0.565	0.832
C18:1 <i>n</i> -9	4.566	4.889	2.318	4.313	4.434	5.133
C18:2 <i>n</i> -6	13.597	14.499	12.296	13.148	13.533	17.372
C18:3 <i>n</i> -6	0.108	0.120	0.116	0.107	0.096	0.108
C18:3 <i>n</i> -3	2.783	1.665	2.137	2.823	1.566	1.998
Total fatty acids	25.582	25.858	20.695	24.802	24.117	30.453

PEA, peas diet; FB, faba bean diet; SBM, soybean meal diet; nd, not detected.

programmed at 160°C and held for 1 min; then increased up to 230 °C at a rate of 1 °C/min and held for 3 min. The injector and detector temperatures were set up at 220°C and 250°C, respectively. Helium was used as carrier gas with a flow of 1.5 mL/min.

Fatty acids were identified using a mixture of standard fatty acids (Larodan Fine Chemicals AB, Malmö, Sweden) and quantified using the peak areas, and were expressed as mg fatty acid/g dry matter of feed sample.

Slaughter procedures, meat physical and proximate chemical analyses

The animals were slaughtered after stunning by captive bolt, at 139 d of age. At the abattoir, slaughter weight, hot carcass weight and dressing percentage were recorded. Carcass fatness and muscular conformation were estimated using a 1-15 points scale according to Dransfield *et al.* (1990) on the basis of EU grid (European Commission, 1992; European Commission, 1993). Carcass fat colour was measured by a MINOLTA CM 2022 colour meter (Illuminant D65; standard observer 10°) according to CIEL*a*b* system (CIE, 1986). Three measurements were taken at caudal level and the average value was reported. After 6 h at room temperature, the carcasses were then stored in a refrigerated room set to 4°C.

Twenty-four h after slaughter carcasses were halved and from the right side samples of *longissimus dorsi* muscle were taken between the 6th and 13th thoracic rib to measure some meat characteristics.

Ultimate pH was assessed using a pH meter equipped with a penetrating glass electrode on samples of *longissimus dorsi* taken as described above. 2.5 cm-thick steaks of *longissimus dorsi* muscle (11th-13th thoracic vertebra) were placed in polystyrene trays and wrapped with PVC oxygen-permeable film and kept in the dark at 3°C. After 2-hour blooming period in order to improve oxymyoglobin on meat surface, CIEL*a*b* colour was measured by a Minolta CM 2022 (Illuminant D65; 10° standard observer). Triplicate readings were made for each sample and average values were recorded.

After 3 d ageing period at 3°C, another portion of *longissimus thoracis* (6th-10th thoracic vertebra) was used to evaluate water-holding capacity measured as cooking losses. About 100 g of muscle sample were held in plastic bags and immersed in a water-bath set to 80°C until the internal temperature reached 75°C. The bags were then cooled under running water for 30 min and blotted dry with paper

towels and re-weighed. Cooking losses were then measured by difference between weights of uncooked and cooked samples and expressed as a percentage (Honikel, 1998).

Warner-Bratzler shear force (WBS) was measured on *longissimus thoracis* samples cooked as above and chilled overnight at 3°C. Six cores (10×10 mm) were removed from each sample parallel to longitudinal orientation of muscle fibres and sheared perpendicularly to the fibre direction by INSTRON 4411 equipped with Warner-Bratzler shearing device. The crosshead speed was 200 mm/min. Maximum peak force values were expressed in Newtons.

On *longissimus lumborum* samples, vacuum-packed and frozen at -24°C until analyses, moisture, crude fat, ash and protein were assessed according to AOAC procedures (AOAC, 1995), after 24 h thawing at 4°C.

Intramuscular fatty acid composition

The intramuscular fatty acid analysis was performed on a slice of the *longissimus dorsi* muscle obtained from each right half-carcass between the 1st and 3rd lumbar vertebra. Total lipids were extracted in duplicate, from a 5 g ground meat sample, according to Folch *et al.* (1957). Fatty acids were methylated as described by Raes *et al.* (2001) with NaOH/MeOH followed by HCl/MeOH and analysed by gas-chromatography apparatus (Thermo Finnigan) equipped with a flame ionization detector and a capillary column SP-2560 in fused silica (100 m × 0.25 mm i.d.; 0.20 µm film thickness). The gas-chromatograph operated under the following conditions: initial temperature, 150°C; first ramp, 25 min;

temperature increasing, 2°C/min up to 170°C; second ramp, 25 min, then temperature increasing 3°C/min to 230°C. The injector temperature was set up at 230°C and the detector temperature at 250°C. Helium was used as carrier gas with a flow of 1.5 mL/min. Peaks were identified by comparing retention times with those of a mixture of corresponding fatty acid standards (Larodan Fine Chemicals AB) and fatty acids were quantified using nonadecanoic acid (C19:0) as internal standard. Individual fatty acids were expressed as g /100 g fatty acid methyl esters (FAMES) while total FAMES were expressed as mg/g fresh tissue.

Statistical analysis

All data were analysed according to a completely randomized design using GLM procedure of Minitab statistical software (Minitab, 2003). The statistical model included diet treatment effect and experimental error. When the diet effect was significant (P<0.05) means were compared by pairwise comparison using the Tukey test (Minitab, 2003).

Results

Growth and slaughter performances

Table 3 reports growth and slaughter performances. No significant differences were found among treatments. Average daily gain was over 250 g/day in all groups thus resulting in final weights of around 35-37 kg with small differences among groups. Carcass weights were over 13 kg, which is the borderline to

Table 3. Lamb performances *in vivo* and at slaughter.

	PEA	Treatment FB	SBM	SEM	Significance
Initial weight, kg	17.5	17.28	16.69	0.672	ns
Final weight, kg	35.58	37.12	36.73	1.070	ns
Average daily gain 67-139 d, g/d	251	275.60	278.40	8.420	ns
Voluntary feed intake, g DM/d	1370	1500	1470		
Feed conversion ratio, g DM/g gain	5.45	5.44	5.28		
Hot carcass weight, kg	17.45	18.5	18.11	0.548	ns
Dressing, %	48.99	49.93	49.26	0.377	ns
Carcass conformation, score 1-15	9.2 (R+)	9.8 (U-)	9.56 (U-)	0.251	ns
Carcass fatness, score 1-15	8.3 (3)	7.7 (3-)	8.33 (3)	0.139	ns
Caudal fat lightness, L*	73.19	69.15	69.36	1.06	ns
Caudal fat redness, a*	3.78	3.86	4.56	0.311	ns
Caudal fat yellowness, b*	3.77	4.54	4.32	0.435	ns
Caudal fat chroma, C*	5.39	6.29	6.36	0.507	ns
Caudal fat hue, H*	41.56	44.22	39.6	2.22	ns

PEA, diet including peas (400 g/kg on as fed basis); FB, diet including faba bean (380 g/kg on as fed basis); SBM, diet including soy-bean meal (180 g/kg on as fed basis); (R+), more than good muscularity; (U-), less than optimal muscularity; (3), medium fatness; (3-), less than medium fatness; ns, not significant.

classify carcasses according to EUROP grid (EEC 1992, 1993). All carcasses showed similar muscular conformation (R+ good or U- optimal expressed as 9.2-9.8 scores, respectively) and fatness (3- and 3 medium, expressed as 7.7-8.3 scores). Also, caudal fat colour was comparable among treatments.

Meat physical and proximate analyses

Meat physical and chemical parameters are shown in Table 4. Dietary treatments did not affect any parameters. Ultimate pH was comparable among groups as well colour, cooking losses and Warner-Bratzler shear force. Also, proximate analyses did not discriminate dietary treatments, showing very low levels (<2%) of intramuscular fat in all groups.

Intramuscular fatty acid composition

Intramuscular fatty acid composition is reported in Table 5. PEA and FB lambs showed higher ($P<0.05$) amounts of C15:0 compared to SBM group. Meat of PEA group showed higher ($P<0.01$) proportion of C17:1 *cis*-9 compared to SBM one while the content in FB meat was at an intermediate level. Dietary treatment significantly ($P<0.001$) affected *trans*-vaccenic acid (C18:1 *trans*-11) lower in meat from PEA group compared to the other groups.

The levels of oleic acid (C18:1 *cis*-9), the major fatty acid found in meat of all treatments, resulted significantly ($P<0.05$) different between PEA and SBM groups with a higher concentration in PEA meat. The level of the same fatty acid in FB meat was comparable with those found in meat of the other groups. Meat from SBM group showed a significantly ($P<0.001$) higher proportion of C20:1 compared to the levels found in the other two groups. With regard to polyunsaturated fatty acids, PEA diet tended ($P<0.10$) to decrease linoleic acid content compared to SBM diet while determined comparable proportions to those found in FB group. No differences between FB and SBM were found concerning the latter fatty acid.

The levels of α -linolenic acid (C18:3 *n*-3) were higher ($P<0.001$) in meat from animals receiving PEA diet than in meat from lambs receiving FB and SBM diets. The level of C18:3 *n*-3 in FB meat resulted higher in tendency ($P<0.10$) than levels found in SBM meat.

Among *n*-3 derivatives PEA meat showed higher ($P<0.001$) C20:3 *n*-3 content compared to the counterparts. Moreover C20:5 eicosapentaenoic acid (EPA) levels were slightly higher ($P<0.10$) in PEA meat than in SBM meat while comparable to the proportions found in FB meat.

Overall, the sum of *n*-3 fatty acids resulted

Table 4. Physical and chemical characteristics of meat.

	PEA	Treatment FB	SBM	SEM	Significance
pH	5.78	5.72	5.86	0.029	ns
Lightness, L*	41.70	41.12	41.47	0.409	ns
Redness, a*	10.56	11.39	10.46	0.322	ns
Yellowness, b*	4.13	4.39	3.77	0.201	ns
Chroma, C*	11.35	12.22	11.14	0.364	ns
Hue angle, H*	21.29	20.77	19.33	0.561	ns
Cooking losses, %	18.49	17.68	16.76	0.587	ns
WBS, N°	88.70	75.52	76.00	0.470	ns
Moisture, %	74.05	75.04	74.05	0.336	ns
Fat, %	1.26	1.33	1.2	0.066	ns
Protein, %	23.34	22.27	23.34	0.317	ns
Ash, %	1.35	1.37	1.41	0.048	ns

PEA, peas diet; FB, faba bean diet; SBM, soybean meal diet; WBS, Warner-Bratzler shear force; N°, Newton (equal to 0.10197 kgF); ns, not significant.

Table 5. Fatty acid composition of longissimus dorsi muscle (g /100 g fatty acid methyl esters).

	PEA	Treatment FB	SBM	SEM	Significance
Fatty acids					
C10:0	0.178	0.176	0.145	0.009	ns
C12:0	0.172	0.170	0.141	0.011	ns
C14:0	2.352	2.447	2.195	0.081	ns
C14:1 <i>cis</i> -9	0.081	0.092	0.064	0.005	0.1
C15:0	0.393 ^a	0.392 ^a	0.234 ^b	0.026	0.012
C15:1	0.101	0.102	0.153	0.018	ns
C16:0	22.701	22.532	22.122	0.361	ns
C16:1 <i>cis</i> -9	1.856	1.519	1.746	0.098	ns
C17:0	1.436	1.250	1.241	0.060	ns
C17:1 <i>cis</i> -9	1.036 ^A	0.787 ^{AB}	0.531 ^B	0.067	0.005
C18:0	12.972	13.426	12.984	0.462	ns
C18:1 <i>trans</i> -9	0.309	0.118	0.253	0.070	ns
C18:1 <i>trans</i> -11	1.924 ^A	3.568 ^B	4.033 ^B	0.265	0.001
C18:1 <i>cis</i> -9	37.559 ^a	34.612 ^{ab}	33.633 ^b	0.674	0.040
C18:1 <i>cis</i> -11	1.670	1.584	1.638	0.102	ns
C18:2 <i>trans</i> 9- <i>trans</i> -12	0.045	0.044	0.046	0.002	ns
C18:2 <i>cis</i> 9- <i>cis</i> 12 <i>n</i> -6	8.325 ^x	10.081 ^{xy}	10.961 ^y	0.487	0.076
C18:2 <i>cis</i> 9- <i>trans</i> 11 CLA	0.939	0.933	0.779	0.065	ns
C18:2 <i>trans</i> 10- <i>cis</i> 12 CLA	0.084	0.111	0.154	0.019	ns
C18:3 <i>n</i> -6	0.134	0.159	0.145	0.009	ns
C18:3 <i>n</i> -3	0.364 ^A	0.164 ^{Bx}	0.114 ^{By}	0.022	0.000
C20:1	0.539 ^a	0.532 ^a	0.96b	0.049	0.006
C20:2 <i>n</i> -6	0.103	0.110	0.127	0.007	ns
C20:3 <i>n</i> -3	0.136 ^A	0.042 ^B	0.043 ^B	0.009	0.000
C20:4 <i>n</i> -6	2.974	3.574	3.618	0.198	ns
C20:5 <i>n</i> -3	0.421 ^x	0.348 ^{xy}	0.232 ^y	0.035	0.083
C22:1	0.037	0.034	0.023	0.003	ns
C22:5 <i>n</i> -3	0.825	0.806	0.791	0.044	ns
C22:6 <i>n</i> -3	0.253	0.283	0.283	0.019	ns
Total FAMES, mg/g fresh tissue	12.84	13.90	10.53	0.975	ns
Saturated	40.205	40.394	39.061	0.440	ns
Monounsaturated	48.050	46.489	46.628	0.586	ns
Polyunsaturated	11.665	13.113	13.698	0.725	ns
P/S	0.293	0.328	0.355	0.021	ns
S <i>n</i> -6	11.540	13.920	14.850	0.677	ns
S <i>n</i> -3	1.998 ^a	1.642 ^{ab}	1.463 ^b	0.092	0.047
<i>n</i> -6/ <i>n</i> -3	5.814 ^C	8.557 ^B	10.293 ^A	0.414	0.000

PEA, peas diet; FB, faba bean diet; SBM, soybean meal diet; FAMES, fatty acid methyl esters; ^{A,B,C}within the same row differs ($P<0.01$); ^{a,b}within the same row differs ($P<0.05$); ^{x,y}within the same row differs ($P<0.10$); ns, not significant.

higher ($P < 0.05$) in PEA meat than in SBM one. Otherwise, meat from lambs fed FB diet showed intermediate levels although statistically comparable with the counterparts.

As a result of previous findings, $n-6/n-3$ ratio was significantly ($P < 0.001$) lower in meat of lambs receiving PEA diet compared to lambs given FB- and SBM-based diets. In addition, FB meat showed lower ($P < 0.001$) levels of the $n-6/n-3$ ratio compared to SBM one.

Discussion

Diets, animal performances

Diets with alternative legume seeds, such as peas and faba bean, did not adversely affect growth and slaughter performances compared to soybean meal diet.

The different fatty acid profile of legume seeds affected to some extent the fatty acid composition of experimental diets. The higher C18:3 linolenic acid content in PEA diet compared to FB and SBM diets reflected the higher C18:3 levels found in peas compared to faba bean and soybean meal, as main protein sources (Table 2).

Although soybean meal revealed lower proportions of C18:2 linoleic acid compared to the alternative legume seeds, SBM diet showed a higher C18:2 concentration than PEA and FB diets. The higher proportions of maize and barley, rich in linoleic acid, in SBM diet could probably justify this finding.

The levels of C18:3 linolenic acid found in peas were twice higher than the levels reported by Grela and Günther (1995) in a specific pea cultivar. The same authors reported lower C18:2 linoleic acid contents in peas as well as in faba bean compared with values found in our experiment in both alternative legume seeds. Although peas played an important role to increase C18:3 in PEA diet compared to FB and SBM diets, the overall levels (< 3 mg FA/g DM) of this favourable fatty acid were clearly lower in comparison with concentrations reported in lamb diets including linseed oil, protected soybean and linseed or algae (Cooper *et al.*, 2004). Priolo *et al.* (2003) reported very low levels (3.22 g/100 g methyl esters) of linolenic acid in chickpeas compared to values found in peas and faba bean in our trial.

Overall average daily gain (around 250-270 g/d) was substantially comparable with the one found in similar previous experiments (Lanza *et al.* 2003b; Loe *et al.* 2004; Caballero *et al.* 1992). Also carcass weights were not affected by dietary treatments. The values (17-18 kg)

were higher compared to those (< 17 kg) reported in previous similar experiments but these differences can be probably attributed to different slaughter ages (Lanza *et al.*, 2007). Also Loe *et al.* (2004) did not observe significant differences in carcass weights from lambs fed diets with different peas proportions as well as Surra *et al.* (1992) and Purroy *et al.* (1992) between lambs fed diets including different proportions of faba bean and those fed soybean meal-based diets.

Carcass classification according to European regulations showed favorable acceptability by local markets with medium fat coverage and good or optimal muscular conformation.

Meat physical and chemical characteristics

No evidences of dietary effects on meat physical and chemical characteristics were found. Ultimate pH values were higher than those reported by Lanza *et al.* (2003a, 2003b) in lambs fed peas based- or chickpeas based-diets (5.78 *vs* 5.5-5.6). The final pH values reflected that animals were not exposed to severe stress during pre-slaughter handling which is of major concern (Geesink *et al.*, 2001). Nevertheless SBM lambs in comparison with PEA and FB showed average pH-value > 5.8 which is considered undesirable (Devine *et al.*, 1993). The lack of significant differences in ultimate pH probably explained the absence of differences among groups in meat colour. Lightness values were lower than those found in similar trials in meat from lambs fed different proportions either of peas or of faba beans (Lanza *et al.*, 1999, 2003b). Probably these differences could be attributed to the lower slaughter ages (around 100 days) compared to the age in the present trial (139 days). Increasing the slaughter age is a well-recognized cause of lowering meat lightness (Santos-Silva *et al.*, 2002).

Cooking loss values were lower than those reported by Lanza *et al.* (2003b) in meat from lambs fed different percentages of peas (19 and 39% on as fed basis) probably due to higher pH values found in this trial compared to the latter. WBS did not show significant differences among groups. Absolute values referred to tough meat according to De Stefanis *et al.* (2008) who divided into five categories WBS values from beef cooked by roasting in electric oven. The latter authors attributed tough judgement to meat that showed shear force values included into categories 1 and 2 (WBS > 52.78 N). Certainly the different cooking method (waterbath) could have negatively influenced WBS values obtained in our experi-

ment (from 75.5 to 88.7 N).

The fatty acid composition of *longissimus lumborum* muscle partially reflected the dietary fatty acid composition. In ruminants, the biohydrogenation occurring in the rumen (i.e. saturation of the dietary unsaturated fatty acids) is responsible for the variations in intramuscular fatty acid composition (Harfoot and Hazlewood, 1988).

The major saturated fatty acid in meat (myristic acid C14:0, palmitic acid C16:0 and stearic acid C18:0) have each been found to be significantly associated with CHD risk (Valsta *et al.*, 2005), although a distinction should be made for stearic acid (C18:0), which has been found to have little cholesterol-raising effects in humans (Mensink *et al.*, 2003). The levels of these fatty acids were similar in the meat of lambs of all experimental groups. Different trend was observed by Scerra *et al.* (2011), where the levels of saturated fatty acids myristic and palmitic were lower ($P < 0.001$) in meat from lambs fed with concentrate where peas was the main protein source; moreover in that trial palmitic acid was higher in meat from lambs fed with concentrate where soybean meal was the main protein source than in meat from lambs fed a concentrate including faba bean as the main protein source. The latter authors attributed these differences to the different dietary levels of these fatty acids. In our trial the higher C14:0 myristic, C16:0 palmitic and C18:0 stearic in SBM diet compared to PEA and FB diets did not lead to different levels in meat of all the groups.

The higher *trans*-vaccenic acid content (C18:1 *trans*-11) in meat from SBM lambs compared to meat from PEA lambs was probably linked to the higher level of linoleic acid in SBM diets. Increasing the concentration of C18:2 *n*-6 in the diet has been shown to increase C18:1 *trans*-11 proportion in the rumen (Lock and Garnsworthy, 2002) where, through the microbial hydrogenation of linoleic acid to stearic acid, there is a formation of *trans*-vaccenic acid as an intermediate (Harfoot and Hazlewood, 1988).

Although the similar proportions in linoleic acid, the higher *trans*-vaccenic acid content in FB meat compared to PEA meat could be attributed to a potential higher tannin content in FB diet compared to PEA diet (Hickling, 2003). Tannins are found as means to accumulate *trans*-vaccenic acid in the rumen both *in vitro* and *in vivo* because of their capability to inhibit bacteria from vaccenic acid to stearic acid conversion (Vasta *et al.*, 2009a; 2009b).

As expected, oleic acid was the most represented fatty acid in all the groups with higher levels in PEA meat than in SBM meat (37.56 *vs*

33.63 g/100 g FAMES; $P < 0.05$) whereas it was found at an intermediate level in FB meat. It is well known that this fatty acid originates from the desaturation, through $\Delta 9$ desaturase activity in muscle tissue, of stearic acid originating from the biohydrogenation of linoleic and linolenic acids in the rumen (Woods and Fearon, 2009). The efficiency of $\Delta 9$ desaturase activity was probably more effective in meat from PEA group compared to SBM one. Overall, literature showed a strong effect of ruminant dietary regimen on oleic acid levels in meat with concentrate-based diet increasing oleic acid levels either in beef (Hidiroglu *et al.*, 1987; Mitchell *et al.*, 1991) or in sheep tissues (Rowe *et al.*, 1999) partially dependent on an increased activity of $\Delta 9$ desaturase.

The differences in dietary linoleic acid contents probably accounted for a slightly higher level in meat from lambs fed SBM diet compared to both legume seed-diets.

Linolenic acid (C18:3 *n*-3) as the precursor of long chain *n*-3 fatty acids that have a wide range of biological effects as anti-inflammatory and cardioprotective effects (Kromhout, 1989; Barlow *et al.*, 1990; Howe *et al.*, 2006), was higher in intramuscular fat from PEA lambs than in their counterparts. Also this result was probably linear with the highest level of this fatty acid in PEA diet (2.823 vs 1.566 and 1.998 mg/g DM, respectively) (Table 2). On absolute value, C18:3 *n*-3 content in meat from all the groups was lower (< 0.500 g/100 g FAME) compared with values (1.16-1.25 g/100 g FAME) found in a similar trial where lambs fed a chickpeas included-concentrate (Priolo *et al.*, 2003) and with values (0.73-1.39 g/100 g FAME) reported by Scerra *et al.* (2011) in meat from lambs fed on faba bean- and on peas-included diets. Legume seeds show a fatty acid composition mainly characterized by high level of C18:2 linoleic among PUFA and C16:0 palmitic among SFA while the C18:3 linolenic content is low although quite variable according to varieties (Grela and Günther, 1995). Recent literature reported low and quite comparable C18:3 linolenic content in peas, faba bean and chickpeas legume seeds (3.8 - 9.3 % total fatty acids for peas, Yoshida *et al.*, 2007, Ryan *et al.*, 2007; 3.8 - 5.1 % total fatty acids for faba bean, Yoshida *et al.*, 2008; 2.4-3.2 % total fatty acids for chickpeas, Priolo *et al.*, 2003, Ryan *et al.*, 2007). At similar level of dietary legume seeds inclusion (42 g/kg on as feed basis of peas, in our trial vs 40 g/kg on as feed basis of chickpeas, in the experiment of Priolo *et al.*, 2003) the concentrate including peas showed nearly 4-fold more C18:3 linolenic acid content (around 11, if expressed as g/100 g FAME vs

2.92 g/100 g FAME) compared to concentrate including 42 % of chickpeas, thus a higher C18:3 *n*-3 level in meat would be expected. Probably a more intense C18:3 *n*-3 ruminal biohydrogenation in lambs fed peas-included diet compared to lambs fed a chickpeas-based concentrate led to a lower C18:3 *n*-3 meat content. Scerra *et al.* (2011), with a lower inclusion of peas (24% on as feed basis) found a similar C18:3 *n*-3 content in concentrate including peas (11.79 if expressed as g/100 g FAME) but with a higher proportion of this beneficial fatty acid in meat compared to our values (1.39 vs 0.36 g/100 g FAME). This enforced the hypothesis that in our trial dietary C18:3 *n*-3 probably underwent an intense ruminal biohydrogenation. The differences among treatments in linolenic acid content did not affect significantly the proportions of *n*-3 derivatives much involved in human healthiness (C20:5 - EPA, C22:5 - DPA and C22:6 - DHA) apart from eicosapentaenoic acid (EPA) content, which was slightly higher in PEA meat compared with the other groups. This finding could suggest a substantially similar $\Delta 5$ and $\Delta 6$ desaturase and elongase enzyme activity in muscle tissues of both groups.

Due to the higher amount of C18:3 *n*-3, the level of total *n*-3 fatty acids was higher in meat from PEA lambs than in meat from SBM lambs. In meat from FB lambs, despite the significantly lower C18:3 *n*-3 content compared to the PEA group, the total *n*-3 fatty acids proportion was comparable either with levels found in PEA meat or in SBM meat.

As a consequence of higher levels of polyunsaturated fatty acids *n*-3 in lamb meat from the PEA group, the *n*-6/*n*-3 ratio was significantly lower and more favourable in the latter group than in the others, approaching the recommended value COMA (< 4) (Committee on Medical Aspects of Food Policy) (Department of Health, 1994).

The *n*-6/*n*-3 ratio in meat from the PEA group also resulted more favourable compared to the value (around 9) reported by Scerra *et al.* (2011) and to the value (around 7) found in a trial (Priolo *et al.*, 2003) where the lambs received a diet with 42% of chickpeas, another local alternative legume seed, in replacement of soybean meal.

Literature on nutritional strategies to improve linolenic acid and their *n*-3 derivative content in beef and sheep meat recommended the use of feedstuffs such as linseed products, fish oil, marine algae or a pasture-based feeding regime (Cooper *et al.*, 2004; Enser *et al.*, 1998; Scollan *et al.*, 2006; Raes *et al.*, 2004). The use of legume seeds such as chickpeas (Priolo *et al.*, 2003) or lupins associated to hay

(Ponnampalam *et al.*, 2002) together with our findings on the use of peas and faba bean could be consistent with the latter nutritional strategies to improve the dietary quality of lamb meat to some extent.

Certainly the *n*-3 series PUFA levels (21-24 mg total *n*-3/100 g fresh tissue) measured in our experiment in meat from lambs fed legume seeds based - diets correspond to 4.6-5.3 % of the average recommended daily intake for human diet (450 mg/person/day, Scientific Advisory Committee on Nutrition, Committee on Toxicity, 2004) and are not comparable with levels reported (130-260 mg total *n*-3/100 g fresh tissue) in meat from lambs fed higher linolenic acid content-diets (pasture, linseed products, fish oil, algae based-diets) (Cooper *et al.*, 2004; Enser *et al.*, 1998).

Conclusions

Based on the results of this trial, it may be concluded that a concentrate including 40% of peas given to finishing lambs significantly affected the intramuscular fatty acid composition compared to concentrates including a similar proportion of faba bean or 18% of soybean meal, respectively, as main protein sources. Briefly, peas based-concentrate increased oleic, linolenic, eicosapentaenoic acids compared to the soybean meal-including concentrate while only linolenic acid was found in higher proportion in peas group compared to faba bean one. In contrast faba bean and soybean meal concentrates improved *trans*-vaccenic acid levels compared to peas diet. Despite of the differences in *trans*-vaccenic acid proportions no effects on CLA rumenic acid contents were found among treatments.

Linoleic acid was in tendency higher in meat from soybean meal diet compared to peas one while intermediate levels were found in faba bean concentrate.

As a consequence of previous results peas based-concentrate improved the total *n*-3 fatty acids content compared to soybean meal-diet while intermediate proportions were found in meat from lambs fed faba bean-concentrate, thus leading to a lower *n*-6/*n*-3 ratio in meat from lambs fed peas-diet compared to those fed on faba bean and soybean meal concentrates. Overall only meat from lambs given peas concentrate showed a *n*-6/*n*-3 ratio close to recommended values for human health (< 4).

To summarize, the acidic profile of meat from lambs fed concentrates including legume seeds such as peas or faba bean has not to be considered a pivotal strategy to improve the

dietetic acidic profile of lamb meat. The levels of C18:3 *n*-3 and their derivatives found in meat from lambs given legume seed diets were clearly lower than those obtained from pasture-fed ruminants as well as from linseed products and fish oil fed animals. Nevertheless, considering also the results findings by other authors on the same topic even if with different percentages of inclusion of the alternative protein source in the concentrate, this experiment confirmed that local alternative legume seeds in concentrate-based lamb diets could be a favourable option to improve to a certain extent the acidic profile of lamb meat in Mediterranean areas where they are local cheap protein sources.

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