Fatty acid composition of light lamb meat from Leccese and Comisana dairy breeds as affected by slaughter age

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ABSTRACT

Fourty lambs of two Italian dairy breeds were used to study the effects of slaughter age and breed on meat fatty acid composition. Lambs were subdivided into four groups (n. 10) according to a factorial scheme of two breeds (Leccese and Comisana) x two slaughter ages (45 and 60 days). The lambs were fed maternal milk supplemented with hay and concentrate from the 30th day to the slaughter. Leccese lambs at 45 days exhibited a FA profile more compatible to nutritional requirements for human health. They displayed a lower SFA proportion, a higher UFA/SFA and MUFA/SFA ratios than Comisana. The delay of slaughtering age at 60 days improved FA

composition in Comisana lambs which had lower SFA content, AI and TI indexes and higher UFA/SFA and MUFA/SFA ratios and n-3 PUFA content than in Leccese. In both the breeds, the slaughter age at 60 days improved the CLA content.

Keywords: Suckling lamb meat, Breed, Slaughter age, Fatty acids

1. Introduction

The importance of meat as a source of high biological value protein and micronutrients is well recognised (Biesalski, 2005; Cabrera and Saadoun, 2014). However, over the last decades these positive features have often been obscured by the emphasis given to different negative attributes (Verbeke et al., 2010). Meat is thought to be a major source of fat in the diet which have been implicated in diseases associated with modern life. An imbalance in dietary cholesterol and fats together with a high fraction of saturated fatty acids (SFAs) are considered the principal cause of atherosclerosis and cardiovascular disease, weight gain and obesity (Martemucci and D'Alessandro, 2013). The type of polyunsaturated fatty acids (PUFAs) and the ratio of PUFA to SFA are important in relation to consumer health, and the balance between n-6 PUFA and n-3 PUFA is considered a risk factor in cancer and coronary heart disease (CHD) (Williams, 2000; Simopoulos, 2008). Among long-chain n-3 PUFA, conjugated linoleic acid (CLA) has drawn significant attention due to its potential health benefits, such as the reduction in body fat, incidence of atherosclerosis, diabetes and cancer (Benjamin and Spener, 2009; Dilzer and Park, 2012). Meat from ruminants has higher levels of CLA than meat from non-ruminants and the highest CLA concentrations were found in lamb's meat (4.32 to 19.0 mg/g fat; Schmid et al., 2006) that is considered to be a highly nutritious with a positive fatty acid profile (Pannier et al., 2010; Nudda et al., 2011).

In the European Mediterranean region, traditional sheep production systems are based on dairy breeds, and devoted to the production of both ewes' milk and meat from suckling lambs. Consumers require more lean meat, with a minimal fat level required to maintain juiciness and flavour, and a consistent quality. Unlike northern Europe where carcasses of 16 to 23 kg from young lambs finished on concentrates are preferred, in the Mediterranean basin the traditional consumer preference is for very light carcasses of 4 to 8 or 8 to 12 kg from milk-fed lambs, (Bernabéu and Tendero, 2005). Light lamb production system is different for each country or region producing a specific weight/age and type of carcass according to the local customs (Cifuni et al., 2000; Sanudo et al., 2007). In Italy, suckling lamb up to 7 kg carcass weight, is the main product as ewe's milk is used for cheese production. Lambs of 7-13 kg carcass weight are also producted and are weaned later or never weaned, and supplemented with concentrate and/or forage as well as left to graze until slaughter.

Typically, in several areas of Southern Italy lambs suckle their mother and receive a supplement of hay/barley straw and/or concentrate from 15-20 days until slaughter, which is performed between 30-45 days or 50-60 days of age. The ewes are managed on pasture or under rationed grazing for several hours daily, without their lambs, and receive a supplement diet (hay/straw/concentrate) indoor.

Leccese and Comisana are the two main dairy breeds reared in Apulia region, Southern Italy; they produce lambs following a production system based on ewes' milk. Leccese is a dairy Apulian autochthonous breed well adapted in rural marginal areas, with an estimated (Castellana et al., 2008) mature weight of 65 and 45 kg for males and females, respectively. Milk production is average 150 kg in 130-180 days of lactation. Leccese breed is to be considered in danger of extinction (around 2000 heads; Castellana et al., 2008), due to its replacement with more productive dairy sheep such as the Comisana breed, expanded in Apulia region in the last years. Recently, European policy has expressed a revival of interest towards native sheep breeds for typical animal production, to preserve the environment, and to their role in exploiting marginal areas. Comisana

78 sheep, originated from Sicilia, is the second dairy ovine breed in Italy, currently estimated at
79 approximately 500,000 heads. Mature weight is estimated of 80 and 50 kg for males and females,
80 respectively. Milk yield is 150–200 kg in 180–210 days of lactation (Casamassima et al. 2008).

81 Meats obtained under traditional production systems are expected to present unique quality
82 and organoleptic characteristics and are worthy of attention in terms of sustainable farming and
83 nutritional characteristics. There is little information to date on the quality of meat from Leccese
84 and Comisana light suckling lambs produced under identical feeding and management conditions.
85 In suckling lambs the carcass weight range is small. However a small increase in the age/ weight at
86 slaughter of lambs may result in higher productivity in meat production system (Santos et al., 2007;
87 D'Alessandro et al., 2013).

88 The fat content and fatty acid composition of meat are affected by multiple interacting
89 factors (Hopkins and Mortimer, 2014). The effects of breed (De Smet et al., 2004; Marino et al.,
90 2008; Juárez et al., 2009) and age/weight at slaughter (Banskalieva, 1997; Cifuni et al., 2000; Rhee,
91 2000) on lamb lipid profile gave variable results.

92 The aim of this study was to evaluate the effects of breed and slauthering age (45 vs 60 days
93 of age) on fatty acid composition of meat from Leccese and Comisana suckling lambs, raised
94 according to traditional production system based on maternal milk and supplemented with hay and
95 concentrate.

96

97 **2. Materials and methods**

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99 We followed the recommendations of European Union directive 86/609/EEC and Italian law 116/92
100 regarding animal care.

101

102 *2.1. Animals, diets and experimental procedure*

103

104 The study was carried out in the spring on a farm located in southern Italy (Apulia: latitude,
105 40°49'48"72 N; longitude: 16°33'16"20 E) at 500 m above sea level, in which two dairy breeds
106 sheep are reared: Leccese and Comisana. Forty lambs from the two breeds (Leccese, n. 20, and
107 Comisana, n. 20) born as singles from pluriparous dams (3.0 - 3.5 years), were selected for the
108 study. The lambs were divided into four homogeneous groups of 10 animals according to a factorial
109 scheme of 2 x 2 (2 breeds – Leccese and Comisana, and 2 slaughter ages – 45 and 60 days). Lambs
110 were confined in straw-bedded pens and fed with maternal milk from 18:00 to 08:00 of the
111 following day, and received a supplement of alfalfa hay (18% crude protein DM; 31.7% crude fiber
112 DM) and small amounts of commercial concentrate (barley, corn and faba beans; 20% crude protein
113 DM, 10.1% crude fibre DM, 2.5% crude fat DM and 6.9% ash DM), from 30 days of age to
114 slaughter. The concentrate supplement corresponded to 2% of average live weight of the lambs,
115 adjusted weekly. The ewes were fed a basal mixed diet (1,400 g/head/day of unifeed) which
116 consisted of chopped oat hay, clover, vetch and rye grass (800 g), commercial feed (200 g) and
117 water (400 g). In addition, the ewes were allowed to graze (5-6 hours /day) on polyphytic cultivated
118 grassland (40% barley, 40% oats, 10% wheat, 2% rye grass and 8% clover) and received 140
119 g/head/day of a commercial concentrate (barley, corn and faba beans; 15% crude protein DM,
120 10.7% crude fibre DM, 2.5% crude fat DM, and 6.7% ash DM). This system aimed to replicate the
121 common used semi-extensive management in that region.
122
123 *2.2. Sampling and sample treatment*
124
125 A mix of three milk samples from each mother were collected during the trial (at 25, 40 and 55 d)
126 and conserved in oxide chromium and kept refrigerated until analysis, with the aim to evaluate the
127 basic differences in the gross composition of the milk in the two breeds at the study. Milk samples
128 were analyzed for protein, fat, and lactose with an infrared milk analyser (Milkoscan 6000, Foss

129 Italia, Padova). The gross composition of milk from the two breeds was the following: crude fat 7.3
130 *vs*. 6.5, crude protein 5.2 *vs*. 4.8%, lactose 5.4 *vs*. 5. 1% for Leccese and Comisana, respectively.
131 Ten lambs from each breed were slaughtered at 45 days and n. 10 lambs at 60 days of age.
132 After 12 hours of fasting, the lambs were weighed to record slaughter weight, and were slaughtered
133 in a public abattoir according to standard commercial procedures and to welfare codes of practices.
134 After 24 h of refrigeration, longissimus muscles samples from the 6th thoracic to 4th lumbar rib
135 from the right side of each carcass were taken, vacuum packaged and stored at -20 °C until the
136 analytical procedures. Lipid content of meat samples was assessed according to AOAC methods
137 (1995).
138
139 *2.3. Fatty acid analysis*
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141 Fatty acid profile of lipids was analysed after extraction and methylation. Intramuscular fat
142 of longissimus lumborum was extracted according to the method used by Folch et al. (1957).
143 Briefly, a homogenised meat sample (5 g) was blended with extraction solvent
144 chloroform/methanol (2:1, v/v) twice, filtered, placed in separator funnels and mixed with saline
145 solution (0.88% KCl). Lipid were extracted following subsequent separations in two phases,
146 filtration and evaporation by a rotary evaporator at 37 °C. Fatty acid methyl esters were obtained
147 using boron trifluoride (12% v/v methanol solution) according to the method of Morrison and Smith
148 (1964). Methyl esters were then analysed by a gas-chromatography Chrompack CP 9000 equipped
149 with a capillary column in silicate glass (50 m x 0.25 mm internal diameter and 0.2 μm film
150 thickness; Phenomenex, Torrance, CA, USA). The carrier gas was helium at a flow rate of
151 0.7mL/min. The temperature programme was: 135 °C for 7 min, an increase in temperature of 4 °C
152 a minute until 210 °C, where it was held for 10 more min. Identification of the fatty acids was
153 carried out using Sigma–Aldrich reference standards run under the same conditions, and retention

time and the area of each peak were calculated. Fatty acids were expressed as a percentage of total fatty acids.

To assess the nutritional implications, the sum of SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) as well as the UFA/SFA and the PUFA/SFA ratios were calculated. The atherogenic index (AI) and the thrombogenic index (TI) were also calculated according to the formulas suggested by Ulbricht and Southgate (1991):

$AI = C12:0 + 4 \times C14:0 + C16:0 / \Sigma MUFA + \Sigma PUFA(n-6) \text{ and } (n-3)$

$TI = C14:0 + C16:0 + C18:0 / 0.5 \Sigma MUFA + 0.5 \Sigma PUFA(n-6) + 3 \Sigma PUFA(n-3) + (n-3) / (n-6).$

2.4. Statistical analysis

The data of body weight, carcass weight, total fat content and fatty acid profile were analyzed using the GLM procedure of SAS (2002). The statistical model included the fixed effects of breed (2 levels: Leccese and Comisana) and slaughter age (2 levels: 45 and 60 days), their interaction and residual error. Means were compared using Duncan's multiple range test. All statistical tests were performed for a significance level of $P<0.05$.

3. Results

No significant effects related to age were found between the breeds regarding body weights and carcass weights. The body weights at the slaughter age of 45 and 60 days, were 12.7 ± 0.11 and 16.0 ± 0.23 kg in Leccese, and 13.0 ± 0.13 and 16.6 ± 0.21 kg in Comisana, respectively (mean values ± standard error). Carcass weights were 7.6 ± 0.11 and 9.0 ± 0.15 kg, and 8.2 ± 0.07 and 10.9 ± 0.12 kg for Leccese and Comisana lambs at 45 and 60 days of age, respectively. Total lipid contents in LL muscles were also not influenced by the breed and slaughter age of the lambs,

179 resulting in 2.71 ± 0.27, 3.08 ± 0.32, 2.21 ± 0.23, 3.00 ± 0.30 g/100 g of edible meat, in Leccese
180 and Comisana at 45 and 60 days of age, respectively.

181 Total SFAs were the most abundant intramuscular LL fatty acids followed in descending
182 order by MUFA and PUFA (Table 1). The total SFA content of meat was affected ($P < 0.05$) by the
183 slaughter age of the lambs and by its interaction with breed (Table 1). When the slaughter age
184 increased, meat from Leccese had higher content of total SFAs (Table 1), lauric (C12:0), myristic
185 (C14:0), pentodecanoic (C15:0) and palmitic (C16:0) acids ($P < 0.05$) (Table 2), whereas in meat
186 from Comisana, total SFA and medium-chain SFAs, such as C12:0 and C14:0, decreased ($P < 0.05$)
187 with the increase in slaughter age (Tables 1, 2). Among the SFAs, palmitic acid was quantitatively
188 the most concentrated and showed a significant difference in Leccese lambs in relation to the
189 slaughter age ($P < 0.05$; Table 2). The level of pentadecanoic acid (C15:0) was greater in Leccese
190 lambs slaughtered at 60 days in comparison with the 45-d lambs ($P < 0.01$) and in comparison with
191 the 60-d Comisana lambs ($P < 0.05$) (Table 2). The content of stearic acid was affected by slaughter
192 age ($P < 0.05$) and breed ($P < 0.05$; Table 2). The increase in slaughter age (45 to 60 days)
193 corresponded to a lower ($P < 0.05$) concentration in stearic acid in Leccese lambs. The level of
194 stearic acid was significantly higher ($P < 0.05$) in Comisana than in Leccese lambs when
195 slaughtered at 60 days. No significant differences between groups were observed for capric (C10:0),
196 margaric (C17:0) and arachidic (C20:0) saturated fatty acids (Table 2).

197 The content of total MUFAs and its greatest representative oleic acid (C18:1 cis-9)
198 decreased significantly ($P < 0.05$) with the increase in age in Leccese lambs (Tables 1, 2). In
199 contrast, although without significant evidence, Comisana lambs displayed higher levels of MUFA
200 and oleic acid at the slaughter age of 60 than at 45 days. Differences between the two breeds were
201 observed for C18:1 cis-9 in 45-d lambs, with the higher value ($P < 0.05$) in Leccese than in
202 Comisana. In both the breeds, the intramuscular concentration of palmitoleic (C16:1 cis-9) and
203 heptadecenoic (C17:1) acids increased ($P < 0.05$) with the increase in the slaughter age (Table 2).
204 Among the MUFAs, there were no significant differences between groups for myristoleic (C14:1),

205 pentadecenoic (C15:1), cis-vaccenic (C18:1 n-7), elaidic (C18:1 n-9), eicosenoic (C20:1 n- 9) and
206 erucic (C22:1 n-9) acids (Table 2).

207 Total content of PUFA was affected ($P < 0.05$) by the interaction of genotype by age (Table
208 1), and increased with the increase in age ($P < 0.05$) in Comisana lambs. The individual PUFAs are
209 reported in Table 3. The amount of linoleic acid (C18:2 n-6, LA) was higher ($P < 0.05$) at the
210 slaughter age of 60 days in comparison with 45-days only in the Comisana breed. The concentration
211 in the octadienoic (C18:2 n-6 cis) acid, which was the most representative isomer of the linoleic
212 acids, followed the same trend but, at the slaughter age of 45-days, was higher ($P < 0.05$) in Leccese
213 than in Comisana. The total content of CLA, due mainly to isomer C18:2 cis-9 trans-11, increased
214 with slaughter age in both Leccese ($P < 0.05$) and Comisana ($P < 0.01$) breed (Table 3). The
215 concentration of the isomer cis-9, trans-11 of CLA was higher ($P < 0.05$) in Leccese than in
216 Comisana lambs when slaughtered at 45 days. Conversely, the trans-10, cis-12 isomer of CLA was
217 higher ($P < 0.05$) in younger Comisana than Leccese lambs. The intramuscular content of linolenic
218 acid (C18:3 n-3, LNA) increased when the slaughter age of the lambs increased and was significant
219 ($P < 0.05$) in the Comisana breed. The higher ($P < 0.05$) proportions of eicosatrienoic (C20:3 n-3),
220 eicosapentanoic (C20:5 n-3, EPA), docosapentanoic (C22:5 n-3, DPA) and docosahesaneoic (C22:6
221 n-3, DHA) acids were observed in older Comisana than in Leccese lambs. Meat from Comisana
222 lambs slaughtered at 60 days also had a higher ($P < 0.05$) content in eicosatrienoic acid (C20:3 n-3)
223 compared with the 45-d lambs (Table 3).

224 Table 4 reports results regarding the dietary properties of lamb's meat as indices of
225 nutritional quality for the fatty acid profiles in the LL muscle. Overall, the amount of n-3 fatty acids
226 was higher in Comisana lambs and reached the highest value at the slaughter age of 60 days in
227 comparison with both the meat from 45-d ($P < 0.05$) and 60-d Leccese lambs ($P < 0.05$). No
228 significant difference was found for the Leccese lambs in relation to the slaughter age. The n-6/n-3
229 ratio, although not significant ($P > 0.05$), was lower in 45-d Leccese lambs (1.52) than in Comisana,
230 whereas in 60-d lambs it was lower (1.38) in Comisana than in Leccese. The PUFA/SFA ratio was

not affected by the age or genotype, and ranged from 0.16 to 0.21. The AI and TI indexes were not significantly affected by age, although, with the increase in slaughter age, they tended ($P < 0.10$) to decrease in the Comisana meat and to increase in the Leccese breed. Thus, the tendentially lower values were observed in the younger lambs (45 days) in the Leccese and in the older lambs (60 days) in the Comisana. Meat from Comisana showed lower ($P < 0.05$) values of AI and TI indexes in relation to the laughter age of 60 days in comparison with the Leccese meat.

4. Discussion

This study aimed to evaluate lipid content and fatty acid composition in the fat of lamb meat by comparing results from two breeds at two different ages (45 and 60 days). Lambs' performance did not exhibite any significant differences between the breeds in body weights, carcass weights, and lipid contents in LL muscles. These findings, as reported by D'Alessandro et al. (2013), are in agreement with other studies (Oriani et al., 2005; Marino et al., 2008). The fat content values found in the present study indicate a lean meat, according to the Food Advisory Committee (1990) which reported that meat containing less than 5% total lipid could be regarded as lean. Pannier et al. (2014) suggested that a level of 3.9% of intramuscular fat is sufficient to ensure the 'good every day' grade.

Total SFA content of meat and medium-chain SFAs, such as C12:0 - C16:0, increased with age in Leccese while decreased in Comisana with the increase in slaughter age. In general, higher amounts of C12:0, C14:0 and C16:0 are considered dangerous for human health (Mensink et al., 2003; Shingfield et al., 2008) because these fatty acids increase the risk of HD with a high atherogenic potentiality (Ulbricht and Southgate, 1991). Moreover, Yu et al. (1995) suggested that C14:0 is 5-6 times more atherogenic or hyper-cholesterolaemic than either C12:0 or C16:0. Thus, meat from older Leccese lambs displayed a less favourable level of SFA, in agreement with other studies (Nürnberg et al., 1998; Juárez et al., 2009). This is probably due to a greater hydrogenation

occurrring in the rumen of older lambs by micro-organisms. However, the values of these FAs were similar to those reported for suckling lambs (Oriani et al., 2005; Juárez et al., 2009; D'Alessandro et al., 2012).

The higher levels of total SFA and C:12-C:14 detected in Comisana lambs slaughtered at 45 days of age than in Leccese, would seem to confirm that breed is a determinant factor affecting the fatty acid profile of lamb's meat (De Smet et al., 2004; Marino et al., 2008, Juárez et al., 2009). Medium-chain SFAs, reflecting their presence in maternal milk (Bas and Morand-Fehr, 2000), are higher in suckling lambs. Higher intake of milk will result in higher degree of SFA (as C12:0 or C14:0) in lamb meat, as probably occurred in 45 day lambs of Comisana breed that have a higher milk production than Leccese. The high level of palmitic acid found in our study is in agreement with the results from different muscles and/or different rearing systems in lambs (Oriani et al., 2005; Popova, 2007; D'Alessandro et al., 2012).

The effect of age on the higher concentration of C15:0 found in Leccese of 60 days could be attributed to the development of ruminal microflora because odd- chain fatty acids are generated from bacterial lipids (Jenkins, 1993). In addition, Leccese and Comisana breeds may have a different development pathway of the ruminal micro-environment.

Stearic acid was affected by slaughter age and breed. The increase in slaughter age corresponded to a lower concentration in stearic acid in Leccese lambs. According to Doreau and Ferlay (1994) this may be related to a greater inhibition of rumen biohydrogenation in the older lambs due to the consumption of concentrate. The amount of stearic acid also seems to be modulated by shorter-chain saturated fatty acids such as myristic and palmitic acids (Bas and Morand-Fehr, 2000) as probably occurred in the younger Leccese lambs. The higher level of stearic acid found in Comisana than in Leccese lambs, when slaughtered at 60 days, may be due to a different genotype activity or the production of hydrogenases by different rumen microflora. Several differences in the rumen fermentation patterns have been reported by Ranilla et al. (2000). Indeed, sheep breed can influence apparent digestibility (Givens and Moss, 1994). With regard the physiological effects on

283 humans, C18:0 is considered as neutral as regard plasma cholesterol content or as having a positive
284 effect in preventing cardiovascular diseases (Martemucci and D'Alessandro, 2013).

285 The content of total MUFAs and its greatest representative oleic acid (C18:1 cis-9) were
286 higher in 45-d Leccese lambs. The highest amount of C18:1 in intramuscular lamb fat of this study
287 agrees with Popova (2007). The enzyme responsible for the conversion of SFA to MUFA is Δ9-
288 desaturase which is candidate for genetic variation in FA composition (Taniguchi et al., 2004).
289 Oleic acid is important for human health because it may reduce both plasma cholesterol and
290 triglycerides and cardiovascular disease risk factors (Martemucci and D'Alessandro, 2013). In
291 addition, together with others FA, it influences the firmness and oxidative stability of muscles thus
292 affecting the juiciness, flavour and colour of the meat.

293 The positive relationship between the increase of intramuscular concentration of palmitoleic (C16:1
294 cis-9) and heptadecenoic (C17:1) acids, and the slaughter age found in the current study is in
295 agreement with the results from other researches (Oriani et al., 2005, Marino et al., 2008). The
296 increase in C17:0 with age might be due to the increasing consumption of concentrated feed, as the
297 rumen synthesizes short-chain volatile fatty acids, such as propionic acid, which is a precursor of
298 odd-chain carbon atom fatty acids (Molenat and Thériez, 1973).

299 Total content of PUFA was affected by the interaction of genotype by age and increased
300 with the increase in age in Comisana lambs. According to Banskalieva (1997), an increasing age at
301 slaughter may cause slightly higher unsaturation of depot fat in sheep. Moreover, it is known that
302 the fatty acid profile in lamb is affected by the slaughter age and breed (Beriain et al., 2000; Oriani
303 et al., 2005; Marino et al., 2008). Among PUFAs, the octadienoic acid, which was the most
304 representative isomer of the linoleic acids, at the slaughter age of 45-days, was higher in Leccese
305 than in Comisana. In humans, LA is an essential n–6 fatty acid which favorably affects the blood
306 lipid profile and is associated with a lower risk of CHD events and reduces the risk of type 2
307 diabetes (Skeaff and Miller, 2009; Aranceta and Pérez- Rodrigo, 2012).

308 The total content of CLA increased with slaughter age in both Leccese and Comisana breed. The
309 concentration of the isomer cis-9, trans-11 of CLA was higher in Leccese than in Comisana lambs
310 when slaughtered at 45 days. Conversely, the trans-10, cis-12 isomer of CLA, which in humans is
311 considered to reduce body fat and to be the most effective in affecting blood lipids (Benjamin and
312 Spener, 2009), was higher in younger Comisana than Leccese lambs. In agreement with
313 Banskalieva (1997), an increase in unsaturation of fat depots was noted with increasing age at
314 slaughter. This could be associated with the change in diet from maternal milk to the increased
315 supplementation with solid feed (hay, for C18:3, and concentrate for C18:2) given to the lambs
316 from 30 days after birth, which reduced the consumption of milk. Thus, the differences in C18:2
317 and cis-9, trans-11 CLA contents between the two breeds could be associated with a different
318 consumption of concentrate (Juárez et al., 2009) and /or milk, being milk rich in CLA (Martemucci
319 and D'Alessandro, 2013). It should be emphasized that a high content of intramuscular C18:2 has
320 been related to the flavour of lamb's meat (Juárez et al., 2009). Nutritionists state that CLA fatty
321 acids are very beneficial for human health (Benjamin and Spener, 2009; Dilzer and Park, 2012).
322 The isomer C18:2 cis-9 trans-11 is reported as the most biologically active CLA and as effective in
323 preventing cancer, cardiovascular disease and diabetes, and in protecting the immune system. The
324 C18:2 trans-10, cis-12 isomer of CLA has also been reported as a biologically active isomer with
325 positive anti-obesity effects, a marked decrease in insulin sensitivity, and as regulating the lipid
326 metabolism.
327 Linolenic acid (C18:3 n-3) consumption has been suggested as reducing the CHD risk (Aranceta
328 and Pérez-Rodrigo, 2012). In our study, the intramuscular content of LNA increased when the
329 slaughter age of the lambs increased and was significant in the Comisana breed. The positive effect
330 of the increase of age in the LNA content in lamb's meat is in agreement with the results of Marino
331 et al. (2008).
332 Through elongase and desaturase activities, LNA is a precursor of long-chain n-3 FA.

333 The high percentage of the 3 PUFA (EPA + DPA + DHA) recorded in 60-d Comisana lambs is in
334 accordance with the levels observed in Merino Branco lambs (Bessa et al., 2005) while the
335 concentration of the long chain n-3 PUFAs (34.8 mg/100 g meat, data no shown) is lower if
336 compared to those found in studies from older and heavier lambs of Merino cross-breed (49.7
337 mg/100 g meat) (Ponnampalam et al., 2010). According to Nudda et al. (2011), the high content of
338 LNA, EPA and DHA in suckling lamb's meat, as observed in the present study in the older
339 Comisana lambs, suggests that it could be profitably used in commercial baby food based on lamb's
340 meat, because is thought to be optimal for neonatal growth and development. With particular
341 reference to the nutritional effects, EPA and DHA consumption have demonstrated physiological
342 benefits on blood pressure, triglycerides, and heart rate (Aranceta and Pérez-Rodrigo, 2012). A
343 dietary supplementation with EPA and DHA has been suggested as a potential way to compensate
344 and/or replace SFA, MUFA and n-6 PUFA in foods (Jiménez-Colmenero et al., 2006; Aranceta and
345 Pérez-Rodrigo, 2012). Long-chain FA are substrates for the formation of further converted to
346 eicosanoids such as prostaglandines (PGE), prostacyclines (PGI), tromboxanes (TXA) and
347 leucotrienes (LT) (Williams, 2000). PGE2, PGI2, TXA2 and LT4 are synthesised from n-6 fatty
348 acids, whereas PGE3, PGI3, TXA3 and LT5 are synthesised from n-3 fatty acids. The 2-and 4-
349 series PGE/ PGI/ TXA/ LT may help to stimulate proliferation and promote anti inflammatory
350 properties; conversely, 3- and 5-series PGE/ PGI/ TXA/ LT can inhibit these processes (Das, 2006;
351 Calder, 2010). It is therefore highly desirable to decrease the n-6/n-3 rate in the human diet.
352 Under a nutritional point of view the ratio of n-6 to n-3 FA in diets in the West is estimated
353 to be 15-20:1, and a more ideal ratio may be 1:1 (Simopoulos, 2008). In our study the n-6/n-3 ratio
354 was lower in Leccese at the slaughter age of 45 days and in Comisana at at the slaughter age of 60
355 days. De Smet et al. (2006) reported that the n-6/n-3 PUFA ratio is affected much more by the
356 feeding than the breed. However, rather than the n-6/n-3 PUFA ratio it is probably more important
357 to consider the absolute amount of n-3 PUFAs ingested daily by consumers (Aranceta and Pérez-
358 Rodrigo, 2012).

359 The reduction of SFA intake from ruminant meat and the increasing in n-3 PUFA is strongly
360 encouraged. The PUFA/SFA ratio fixed for human nutrition should be around 0.7 or lower (Raes et
361 al., 2003). In our study, the PUFA/SFA ratio was not affected by the age or genotype, and ranged
362 from 0.16 to 0.21, in agreement with Oriani et al. (2005).
363 The AI and TI indexes were affected by genotype showing lower values in the younger lambs (45
364 days) in the Leccese and in the older lambs (60 days) in the Comisana. The effect of genotype on
365 different values of AI and TI in relation to the age of the lambs has also been observed by Marino et
366 al. (2008).
367

368 **5. Conclusions**

369

370 Fatty acid profile of lamb meat was influenced by the interactions breed and slaughter age.
371 The slaughtering age at 45 days improved fatty acid composition in Leccese lambs which showed
372 lower SFA proportion, higher UFA/SFA and MUFA/SFA ratios. The delay of slaughtering age
373 improved fatty acid composition in Comisana. At the slaughering age of 60 days, Comisana lamb
374 meat resulted in lower SFA content and AI and TI indexes, and higher UFA/SFA ratio, MUFA/SFA
375 ratio and n-3 PUFA content than Leccese. In both Leccese and Comisana breeds, the increase in
376 slaughter age to 60 days resulted in an increase in conjugated linoleic acid. These findings might be
377 useful when planning lamb production systems.
378

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380

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386

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518

519 **Table 1**

520 Fatty acid classes (%) of intramuscular fat in longissimus lumborum muscle of Leccese and
521 Comisana lambs slaughtered at 45 and 60 days of age

	Leccese breed		Comisana breed		SEM [f]	Level of significance [e]		
	45 d	60 d	45 d	60 d		Breed (B)	Age (A)	B x A
SFA	51.85 [ac]	54.58 [ad]	54.82 [bc]	51.66 [bd]	0.65	ns	*	*
MUFA	39.19 [ac]	36.55 [d]	36.16 [b]	37.50	0.74	ns	ns	*
PUFA	9.09	8.91 [a]	8.94 [c]	10.94 [bd]	0.52	ns	ns	*
UFA	48.28 [ac]	45.46 [ad]	45.10 [bc]	48.44 [bd]	0.59	ns	*	*

522

523 SFA, saturated fatty acids = (C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0); MUFA,
524 monounsaturated fatty acids = (C14:1+C15:1+C16:1+C17:1+C18:1n-7+C18:1n-
525 9+C18:1t9+C20:1n-9+C22:1n-9); PUFA, polyunsaturated fatty acids =
526 (C18:2+C18:3+C20:3+C20:4+C20:5+C22:5+C22:6+
527 C18:2c9,t11); UFA, unsaturated fatty acids = (MUFA+PUFA).

528 [a, b] significant difference between breeds within the same age ($P < 0.05$).

529 [c, d] significant difference between ages within the same breed ($P < 0.05$).

530 [e] ns: not-significant; *: $P < 0.05$.

531 [f] SEM: standard error of the means

532

533 **Table 2**

534 Saturated (SFA) and monounsaturated (MUFA) fatty acids (% of total fatty acids) of intramuscular fat in longissimus lumborum muscle of

535 Leccese and Comisana lambs slaughtered at 45 and 60 days of age

Fatty acids	Leccese breed		Comisana breed		SEM [f]	Level of significance [e]		
	45 d	60 d	45 d	60 d		Breed (B)	Age (A)	B x A
SFA								
C10:0, capric	0.41	0.54	0.55	0.31	0.12	ns	ns	ns
C12:0, lauric	1.10 [c]	1.76 [Ad]	1.31 [c]	0.85 [Bd]	0.18	ns	*	**
C14:0, myristic	9.32 [c]	11.08 [ad]	9.95 [c]	8.61 [bd]	0.38	ns	*	*
C15:0, pentadecanoic	0.71 [c]	0.90 [ad]	0.77	0.74 [b]	0.11	ns	ns	*
C16:0, palmitic	25.78 [c]	27.55 [d]	26.97	26.66	0.76	ns	ns	ns
C17:0, margaric	0.99	1.06	1.01	1.01	0.15	ns	ns	ns
C18:0, stearic	13.41 [c]	11.57 [ad]	14.11	13.34 [b]	0.52	*	*	ns
C20:0, arachidic	0.13	0.11	0.15	0.14	0.05	ns	ns	ns

MUFA								
C14:1, myristoleic	0.19	0.21	0.22	0.21	0.06	ns	ns	ns
C15:1, pentadecenoic	0.24	0.24	0.22	0.27	0.07	ns	ns	ns
C16:1, palmitoleic	1.61 ^{ac}	1.92 ^{ad}	1.25 ^{bc}	1.61 ^{bd}	0.12	ns	*	ns
C17:1, heptadecenoic	0.47 ^{c}	0.58 ^{d}	0.47 ^{c}	0.56 ^{d}	0.06	ns	*	ns
C18:1, oleic	36.53 ^{ac}	33.49 ^{d}	33.87 ^{b}	34.70	0.78	*	ns	ns
C18:1 n-7, cis-vaccenic	0.79	0.74	0.71	0.77	0.18	ns	ns	ns
C18:1 n-9 trans, elaidic	0.44	0.39	0.38	0.46	0.10	ns	ns	ns
C18:1 n-9 cis, oleic	35.30 ^{ac}	32.36 ^{d}	32.78 ^{b}	33.47	0.58	*	ns	ns
C20:1 n-9, eicosenoic	0.10	0.08	0.11	0.10	0.06	ns	ns	ns
C22:1 n-9, erucic	0.04	0.04	0.009	0.07	0.05	ns	ns	ns

536

537 $^{a, b; A, B}$ Significant difference between breeds within the same age (^{a,b}: $P < 0.05$; $^{A. B}$: $P<0.01$).

538 $^{c, d}$ Significant difference between ages within the same breed ($^{c, d}$: $P <0.05$).

[e] ns: not-significant; *: $P < 0.05$; **: $P < 0.01$.

[f] SEM: standard error of the means.

.

542 **Table 3**

543 Polyunsaturated fatty acid compositions (% of total fatty acids) of longissimus lumborum muscle of Leccese and Comisana lambs slaughtered

544 at 45 and 60 days of age

Fatty acids	Leccese breed		Comisana breed		MSE [f]	Level of significance [e]		
	45 d	60 d	45 d	60 d		Breed (B)	Age (A)	B x A
C18:2 linoleic	4.65	4.60	4.39 [c]	5.10 [d]	0.38	ns	ns	ns
C 18:2 n-6 trans, linoelaidic	0.14	0.18	0.66	0.12	0.26	ns	ns	ns
C 18:2 n-6 cis,octadienoic	4.51 [a]	4.42	3.73 [bc]	4.98 [d]	0.28	*	*	ns
Total CLA	1.07 [c]	1.36 [d]	0.95 [C]	1.52 [D]	0.12	ns	**	ns
C 18:2 trans-10, cis-12	0.05 [a]	0.05	0.16 [b]	0.07	0.02	ns	ns	ns
C 18:2 cis-9, trans-11	1.02 [ac]	1.31 [d]	0.79 [bc]	1.44 [d]	0.04	*	**	ns
C18:3 n-3, linolenic	0.85	0.91	0.84 [c]	1.00 [d]	0.08	ns	ns	ns
C18:3 n-6, γ-linolenic	0.15	0.16	0.24	0.17	0.10	ns	ns	ns

C20:2 n-6, eicosadienoic	0.10 [A]	0.12 [a]	0.36 [B]	0.23 [b]	0.04	**	ns	ns
C20:3 n-3, eicosatrienoic	1.27	0.99 [a]	1.19 [c]	1.74 [bd]	0.08	ns	ns	*
C20:3 n-6, dihomo-γ-linolenic	0.16	0.08	0.10	0.14	0.09	ns	ns	ns
C20:4 n-6, arachidonic	0.01	0.01	0.04	0.01	0.02	ns	ns	ns
C20:5 n-3, eicosapentaenoic (EPA)	0.17	0.17 [a]	0.25	0.29 [b]	0.04	*	ns	ns
C21:5 n-3, heneicosapentaenoic	0.05	0.07	0.10	0.02	0.04	ns	ns	ns
C22:5 n-3, docosapentaenoic	0.48	0.38 [a]	0.54	0.62 [b]	0.08	*	ns	ns
C22:5 n-6, docosapentaenoic	0.03	0.02	0.05	0.08	0.04	ns	ns	ns
C22:6 n-3, docosahesaenoic (DHA)	0.16 [a]	0.14 [A]	0.23 [b]	0.25 [B]	0.04	**	ns	ns

545

546 [A, B; a, b] Significant difference between breeds within the same age ([A, B]: P<0.01; [a,b]: P < 0.05).

547 [C, D; c, d] Significant difference between ages within the same breed ([C, D]: P<0.01; [c, d]: P <0.05).

548 [e] ns: not-significant; *: P < 0.05; **: P < 0.01.

549 [f] SEM: standard error of the means.

550

551

552 **Table 4**

553 Indices of nutritional quality for fatty acid profiles of intramuscular fat in longissimus lumborum
554 muscle of Leccese and Comisana lambs slaughtered at 45 and 60 days of age

	Leccese breed		Comisana breed		SEM [f]	Level of significance [e]		
Fatty acid	45 d	60 d	45 d	60 d		Breed (B)	Age (A)	B x A
UFA/SFA	0.93 [ac]	0.83 [ad]	0.82 [bc]	0.94 [bc]	0.05	ns	*	*
MUFA/SFA	0.75 [ac]	0.67 [ad]	0.66 [bc]	0.73 [bd]	0.04	ns	ns	*
PUFA/SFA	0.17	0.16	0.16	0.21	0.10	ns	ns	ns
n-6 PUFA	5.09	5.00	5.18	5.73	0.23	ns	ns	ns
n-3 PUFA	3.50	3.06 [a]	3.70 [c]	4.55[bd]	0.14	ns	ns	*
n-6/n-3 PUFA	1.52	1.66	1.61	1.38	0.12	ns	ns	ns
AI [g]	1.39	1.73 [a]	1.53	1.31 [b]	0.10	ns	ns	ns
TI [h]	1.50	1.71 [a]	1.60	1.40 [b]	0.05	ns	ns	ns

555

556 UFA/SFA = unsaturated /saturated fatty acids ratio; MUFA/SFA: monounsaturated/ saturated fatty
557 acids ratio; PUFA/SFA: polyunsaturated/ saturated fatty acids ratio; n-6 PUFA= (C18:2+C18:3+
558 C20:2+C20:4+C22:5+C22:6); n-3 PUFA = (C18:3+C20:3+C20:5+C21:5+C22:5+C22:6); n-6/n-3
559 PUFA= n-6 / n-3 PUFA ratio

560 [a, b] Significant difference between breeds within the same age ($P < 0.05$)

561 [c, d] Significant difference between ages within the same breed ($P < 0.05$)

562 [e] ns: not-significant; *: $P < 0.05$

563 [f] SEM: standard error of the means

564 [g] AI, atherogenic index (Ulbricht & Southgate, 1991)

565 [h]TI, thrombogenic index (Ulbricht & Southgate, 1991)

566

567