PAPER

Cholesterol and fatty acids oxidation in meat from three muscles of Massese suckling lambs slaughtered at different weights

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Abstract

Eighteen Massese male lambs fed mainly with maternal milk were slaughtered at 11, 14 and 17 kg. Samples of *Longissimus dorsi* (LD), *Triceps brachii* (TB) and *Semimembranosus* (Sm) muscles were collected. Total intramuscular lipids were extracted by means of a mixture of chloroform methanol 2/1. Cholesterol content and its oxidation product (COP) were determined by a gas chromatography apparatus equipped with an apolar 30 m column. Fatty acid oxidation was evaluated by means of thiobarbituric acid reactive substances (TBARS) extracting the sample with aqueous acidic solution. The effect of slaughter weight on oxidation of intramuscular lipids was found only in TB muscles. In this muscle the cholesterol content showed a decreasing trend, while the content of COPs significantly increased with the age of animals. Among the COPs, the 7-ketocholesterol and 7β-hydroxycholesterol were the most abundant, followed by α- and β-epoxy-cholesterol and cholestan-triol. The content of TBARS did not vary owing to a similar fatty acid composition of intramuscular fat across weight of slaughter. In any case, the values of TBARS did not reach the threshold of the detection of off-flavour in meat.

Introduction

The lipid oxidation in meat starts from polyunsaturated fatty acids (PUFA), mainly contained in the phospholipids (PL) of cell membranes, as a consequence of the presence of some pro-oxidant factors such as the iron contained in haemoglobin (Hur *et al.*, 2007). Also the cholesterol contained in the cell membranes, in presence of light, heat and molecular oxygen, may be oxidised, producing hydroperoxides, similarly to the unsaturated fatty acids. The hydroperoxides are chemically unstable compounds that generate more stable products such as chetons, epoxydes and alcohol (Maerker, 1987). More than 70 cholesterol oxidation products (COPs) have been identified (Dutta, 2004). For several COPs, powerful biological negative effects (angiotoxic, citotoxic, atherogenic, thrombogenic, carcinogenic and mutagenic) have been reported (Otaegui-Arrazola *et al.,* 2010). Moreover, unlike the oxidation products of PUFA, COPs are not volatile compounds, so that cholesterol oxidation is not identifiable by bad smelling compounds.

In the literature, many studies have dealt with the oxidation of lipids in different kinds of meat, but most of these studies did not consider COPs content, or, when they did, regarded meat from non ruminant species such as turkey (Boselli *et al.*, 2005), poultry (Concillo *et al.*, 2005; Bonoli *et al.*, 2007) and pork (Thurner *et al.*, 2007; Rudzińska *et al.*, 2007). A few studies have considered cholesterol oxidation of meat from ruminants, mainly beef (Verleyen *et al.,* 2003; Thurner *et al.*, 2007) and very few studies have been done on lambs (Serra *et al.,* 2006; Samouris *et al,.* 2011).

Several studies reported the effects of post-slaughter factors (cooking methods, meat conservation or processing) on the oxidation of intramuscular lipids (Boselli *et al.*, 2005; Rudzińska *et al.*, 2007; Vicente and Torres, 2007; Soto-Rodríguez *et al.*, 2008; Meineri *et al.*, 2010). All these studies were carried out on retailed meat and the effect of pre-slaughter factors such as animal's origin, feeding regimen, rearing system, age at slaughter were not taken into consideration. However, these factors may modify the equilibrium among pro-oxidant and anti-oxidant substances in the muscle, determining a significant effect on lipid oxidation also in raw meat (Lercker and Rodriguez Estrada, 2000).

The aim of this study was to evaluate the cholesterol and fatty acid oxidation in three different types of muscle from lambs of the Massese breed, slaughtered at three different weights, reared by their mothers and fed mainly with maternal milk.

Materials and methods

Animals

The study was carried out on 18 male suckling Massese lambs in a dairy farm located in West-Tuscany (Central Italy). No twins were considered. The lambs were born in spring and at birth were randomly divided into three groups of six animals each. The first group was slaughtered at 11 kg (11.29±0.20; mean±standard deviation) when the lambs were nearly 14 days old, while the animals belonging to the other two groups were slaughtered at 14 kg (14.07±0.20; mean±standard deviation) and 17 kg (17.02±0.21; mean±standard deviation), when the lambs were nearly 21 and 28 days old, respectively. During the whole experimental period lambs were kept with their mothers and were fed mainly on maternal milk. The ewes were fed only at pasture which was composed by 50% grass, 25% clovers, and 25% other forage species.

Muscle sampling

When the lambs reached the target slaughter weight, they were electrically stunned and slaughtered by exsanguination in a public abattoir. Carcasses were immediately transferred in a cooler at 4°C and after a chilling period of 24 h, samples of *Longissimus dorsi* (LD), *Triceps brachii* (TB) and *Semimembranosus* (Sm) muscle were collected from the right side of each carcass. The sample of LD muscle was drawn at the level of the 13th thoracic rib, while the whole TB and Sm muscles were removed. Muscle samples included the muscle tissue, epimysium and the adipose tissue. Samples were vacuum packed and stored at -20°C until analysis.

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Analysis

Muscle samples were partially thawed at 4°C and trimmed to remove residual adipose tissue and the epimysium. Total meat lipids (TL) were extracted by means of a chloroform/methanol solution (2:1, v/v) following Rodriguez-Estrada *et al.* (1997). The content of cholesterol and COPs in the muscle samples was determined as follows: 70 mL of a solution of betulin in chloroform (2 mg/mL) and 25 mL of a solution of 19-hydroxycholesterol in hexane/isopropanol (4/1) (1 mg/mL) were added to 250 mg of TL, as internal standards for cholesterol and COPs, respectively. Total lipids were successively saponified according to Sander *et al.* (1989). An aliquot of unsaponifiable matter (*ca* 10 mg) was purified by using SPE-NH_2 cartridge, after Rose-Sallin *et al.* (1995). Finally, COPs and cholesterol were determined after silylation of purified samples and residual unsaponifiable matter. Silylation solution was composed by a pyridine solution of hexamethyldisilazane and trimethylchlorosilane (Sweely *et al.,* 1963).

Trimethysilyl derivatives were identified and quantified by using a gas chromatography equipped with flame ionisation detector and an apolar 30 m×0.25 mm i.d. capillary column by injection of 3 mL of sample. The injection and the detector were set at 325°C; the initial oven temperature was 250°C, then, with a rate of 1°C/min, the temperature was increased at 270°C; a final temperature of 350°C was reached by a rate of 10°C/min and this temperature was held for 5 min; the carrier gas flux was 1 mL/min and the run was carried out in constant pressure mode (Boselli *et al.*, 2001). Cholesterol and COPs meat content were expressed as µg/g of TL and as µg/100 g of muscle.

Fatty acids oxidation was determined by means of thiobarbituric acid reactive substances (TBARS) according to Salih *et al.* (1987); briefly, samples (1 g) were added with a 40 mM solution of thiobarbituric acid in water and heated at 93. Malondialdehyde (MDA) content was determined by a spectrophotometer apparatus at a wavelength of 532 nm. Thiobarbituric acid reactive substances were quantified by comparing the absorbance with a calibration curve using a solution of tetraethoxypropane. Data were expressed as µg MDA/100 g of meat or µg MDA/g of lipid.

Statistical analysis

Data of total cholesterol, COPs, total intramuscular lipids and TBARS were processed, separately for each muscle, by the GLM procedure of SAS (1999), using a linear model that included the live slaughtering weight as only fixed effect and residual error as random effect. Least square means were compared by the predicted difference method with Tukey adjustment test (SAS, 1999). The values of least square means were considered statistically significant when $P<0.05$ and highly significant when $P\leq0.01$.

Results and discussion

Slaughter weight influenced the content of intramuscular fat (TL) in LD and TB (Tables 1 and 2), but only in tendency, probably due to the large variability within groups. Similarly, the content of cholesterol in meat did not vary across weight of slaughter within each muscle. Nevertheless, on TL basis, the cholesterol content tended to decrease in Sm and TB with the increase of the weight of lambs (Tables 3 and 4). Also in this case, the lack of statistical significance was probably due to the high variability of data within groups. It is well known that cholesterol content in meat is not affected by the fatness status of the animal, but by the type of muscular fibre (Arsenos *et al.*, 2000). Red fibres are smaller than white ones; therefore, the cell membrane perimeter of the former is greater than the latter (Klont *et al.*, 1998). As a consequence, the content of cholesterol is higher in red fibers than in white ones (Alasnier *et al.*, 1996). Although in the present work the muscle fibres were not characterised, an effect of the muscle fibre composition on the decreasing trend of cholesterol content could be supposed. At birth, in fact, all muscular fibres are red, but some of them (namely αR) become white within 15 days after birth, mainly in inactive or partially active muscles

Table 1. Intramuscular total lipids, cholesterol and cholesterol oxidation products in *Longissimus dorsi* muscle from lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
TL, g	1.96	2.00	2.20	0.13	0.38
Cholesterol	57,123.69	55,512.74	59,484.24	4300.10	0.82
7β-hydroxycholesterol	20.50	34.56	23.48	10.73	0.60
7-ketocholesterol	37.01	29.94	35.49	7.00	0.74
α-epoxycholesterol	9.29	13.83	22.31	4.61	0.19
β-epoxycholesterol	18.44	22.08	21.36	7.58	0.92
Triol-cholesterol	7.36	3.14	11.87	4.54	0.48
Total COPs	92.61	103.55	114.51	25.24	0.82
COPs/cholesterol, %	0.12	0.14	0.15	0.03	0.76
TBARS (MDA)	20.73	14.30	17.73	5.31	0.70

TL, total lipids; COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as µg/100 g muscle, except for COPs/cholesterol and TL.

Table 2. Intramuscular total lipids, cholesterol and cholesterol oxidation products in *Triceps brachii* muscle from lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
TL, g	1.98	2.02	2.64	0.33	0.30
Cholesterol	50,624.80	45,099.92	47,382.57	3984.04	0.62
7β-hydroxycholesterol	18.28	22.06	36.22	5.39	0.14
7-ketocholesterol	21.03^{b}	20.05^{b}	55.47^{a}	7.52	0.02
α-epoxycholesterol	13.11^{a}	19.82^{ab}	25.96^{b}	2.05	<0.01
β-epoxycholesterol	9.07^{a}	12.79^{b}	24.63^{b}	2.51	<0.01
Triol-cholesterol	6.66	11.08	8.94	3.10	0.61
Total COPs	70.96^{a}	85.81^{ab}	151.21^{b}	16.75	0.02
COPs/cholesterol, %	0.15^{a}	0.20^{ab}	0.32^{b}	0.04	0.04
TBARS (MDA)	17.06	13.77	16.84	2.96	0.69

TL, total lipids; COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as µg/100 g muscle, except for COPs/cholesterol and TL. ^{a,b}Least square means in the same row with different superscript letters are significantly different ($P<0.05$).

(Ashmore *et al.,* 1972). The decreasing trend of cholesterol content of intramuscular lipids, therefore, could be due to changes in the muscular fibre composition.

In the present study five different COPs were detected (Figure 1). Two products were the most abundant: 7β-hydroxycholesterol (7β-OH) and 7-ketocholesterol (7-keto), followed by α and β epoxy-cholesterol (α-EP and β-EP) and cholestan-triol (Triol). 7β-OH ranged from 16.21 μg/100 g of muscle in Sm-11 (Table 5) to 36.22 μg/100 g of muscle in TB-17 (Table 2), while 7-keto ranged from 15.28 μg/100 g of muscle in Sm-11 (Table 5) to 55.47 μg/100 g of muscle in TB-17 (Table 2); the sum of 7β-OH and 7-keto was more than 50 to 55% of total COPs. The values of α-EP ranged from 9.29 μg/100 g of muscle in TB muscle from lightest animals to 25.96 μg/100 g of muscle in TB muscle from heaviest ones (Table 2); β-EP ranged from 9.07 μg/100 g of muscle in TB muscle from animals slaughtered at 11 kg to 24.63 μg/100 g of muscle in TB muscle from animals slaughtered at 17 kg (Table 2); the sum of α- and β-epoxy epimers was nearly 30 to 35% of total COPs. The maximum content of Triol was reached in LD from animals slaughtered at 17 kg (Table 1).

7β-OH and 7-keto are primary products of cholesterol oxidation, while α-EP and β-EP are secondary oxidation products, being the products of the reaction between hydroperoxides and the 5,6-double bond of cholesterol. Triol results from the hydration of α-EP and β-EP (Maerker, 1987). Hence, Triol is the last product of cholesterol oxidation. Since in our study the primary oxidation products (7β-OH and 7-keto) accounted for more than 50% of COPs and the content of triol was very poor in all muscles, the oxidation process of cholesterol may be evaluated at an early status.

Nevertheless, the total amount of COPs detected in this study was not negligible. On the basis of TL, in fact, total COPs ranged from 29.46 μg/g of TL to 60.12 μg/g of TL in TB muscle from lambs slaughtered at 11 and at 17 kg, respectively (Table 4). These values are higher than those reported by Boselli *et al.* (2005) and Concillo *et al.* (2005) in raw turkey and chicken meat (23.7 and 7.4 μg of COPs/g of TL, respectively), but lower that those found in a previous study of ours (Serra *et al.,* 2006) in lamb slaughtered at 15 kg (81.8 μg/g).

In LD and Sm muscles the slaughter weight did not affect cholesterol oxidation either on muscle or on TL basis (Tables 1, 3, 5 and 6). On the contrary, in TB muscle the content of 7-keto, expressed on TL basis, significantly increased (P<0.05) with the weight at slaughter (Table 4). Since cholesterol content in intramuscular lipid of TB muscle tended to decrease with slaughter weight, the COPs/cholesterol ratio, used as a proxy of cholesterol oxidation, significantly increased (P<0.05) with weight of at slaughter (Table 4). In TB muscle, a similar pattern was found also when data were expressed on the basis of muscle weight. Slaughter weight, in fact, significantly affected the content of 7-keto (P<0.05), α and β cholesterol epoxy epimers (P<0.01), total COPs and also the COPs/cholesterol ratio (P<0.05) (Table 2).

These findings seemed to indicate that cholesterol oxidation was not related to its content

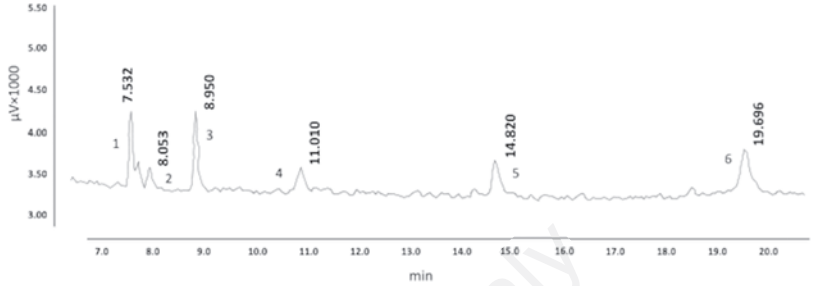

Figure 1. Chromatogram of the run for cholesterol oxidation products identification. 1=7β-hydroxycholesterol; 2=19-hydroxycholesterol (internal standard); 3=α-epoxycholesterol; 4=β-epoxycholesterol; 5=triol-cholesterol; 6=7-ketocholesterol.

Table 3. Cholesterol, cholesterol oxidation products and thiobarbituric acid reactive substances in intramuscular lipid from *Semimembranosus* muscle of lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
Cholesterol	28,206.80	21,143.80	23.937.55	1939.00	0.11
7β-hydroxycholesterol	9.38	11.02	14.31	3.91	0.67
7-ketocholesterol	8.55	15.79	13.45	2.60	0.21
α-epoxycholesterol	8.52	7.66	7.71	2.06	0.95
β-epoxycholesterol	7.37	6.41	9.05	2.36	0.77
Triol-cholesterol	2.40	5.39	1.71	1.25	0.20
Total COPs	36.23	46.27	46.24	9.14	0.69
COPs/cholesterol, %	0.13	0.22	0.20	0.04	0.40
TBARS (MDA)	8.18	7.43	10.68	1.97	0.49

COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as μg/g of total lipids, except for COPs/cholesterol.

Table 4. Cholesterol, cholesterol oxidation products and thiobarbituric acid reactive substances in intramuscular lipid from *Triceps brachii* muscle of lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
Cholesterol	26,344.42	22,321.26	18,592.92	0.26	0.19
7β-hydroxycholesterol	7.32	11.01	14.94	3.27	0.33
7-ketocholesterol	7.98^{a}	9.83a	21.46^{b}	3.64	0.04
α-epoxycholesterol	6.54	9.92	10.52	1.65	0.23
β-epoxycholesterol	3.62	6.35	10.33	1.79	0.08
Triol-cholesterol	2.63	2.23	1.86	0.26	0.19
Total COPs	29.46	42.81	60.12	10.01	0.17
COPs/cholesterol, %	0.15^{a}	0.20^{ab}	0.33^{b}	0.04	0.04
TBARS (MDA)	8.43	6.86	7.09	1.38	0.69

COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as μg/g of total lipids, except for COPs/cholesterol. ^{a,b}Least square means in the same row with different superscript letters are significantly different (P<0.05).

in the meat. The oxidation of cholesterol in muscle, in fact, is the result of the interaction between pro-oxidant and anti-oxidant factors that act before and/or after the animal's slaughter. More studies are needed in order to assess whether the higher content of COPs in meat from older and heavier lambs could also be due to changes in feeding regimen. Indeed, as reported in a previous work (Serra *et al.*, 2009), lambs slaughtered at 17 kg showed an incipient rumen activity due to the intake of a small amount of feed, as pasture or other roughage, in addition to maternal milk. Moreover, the content of some antioxidant substances like carotenoids and tocopherols could be enhanced by the grazing activity in heavier lambs.

The content of TBARS ranged from 13.18 μg MDA/100 g of meat in Sm muscle of lambs slaughtered at 14 kg (Table 5), to 20.73 μg of MDA/100 g of meat in LD muscle of lambs slaughtered at 11 kg (Table 1). These values did not reach the threshold of detection for off-flavour (50 μg MDA/100 g of meat; Lanari *et al.*, 1995). These findings are in agreement with those of Linares *et al.* (2007) and Vieira *et al.* (2012) who reported, in suckling lambs, values of MDA ranging from 10 to 19 μg/100 g of raw meat, and with those of Santé-Lhoutellier *et al.* (2008), who found <50 μg MDA/100 g of raw meat in muscle from weaned lambs. On the contrary, in a previous work (Serra *et al.* 2006) we found a higher value of TBARS (35 μg/100 g of raw meat from meat slaughtered at 15 kg). Linares *et al.* (2007) reported values of TBARS higher in meat from weaned lambs than from suckling lambs. In the present study any effect of weight at slaughter was revealed on fatty acid oxidation. On the other hand, as reported in Serra *et al.* (2009) fatty acid composition of meat from suckling lambs did not differ across weight at slaughter, under the same feeding regimen.

Polyunsaturated fatty acids are preferentially esterified in PL and they are the main substrate for the onset of fatty acid oxidation. Although in the present study we did not determine the phospholipids PL content in the muscles, an estimation of PL content may be obtained by means of the ratio between total fatty acid and total lipid (TFA/TL). In fact, since PL molecule contains two fatty acid molecules and triglyceride (TG) three, if PL do constitute the total intramuscular fat, this ratio should accounted for no more than 0.66. On the contrary, if TG do constitute the total intramuscular fat, the ratio should approximate to the unity. In a previous paper based on the same experiment (Serra *et al.*, 2009) we reported that the ratio TFA/TL was 0.62 in LD muscle and 0.51 in TB and Sm muscles; so, one can speculate that the fatty acid composition of intramuscular fat in the samples considered in the present experiment was mainly composed by the PL. Moreover, in each muscle, TFA/TL ratio was similar across the weight of slaughtering (Serra *et al.*, 2009), helping to explain why fatty acid oxidation was not affected by weight of slaughter.

Table 5. Intramuscular total lipids, cholesterol and cholesterol oxidation products in *Semimembranosus* muscle from lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
TL, g	1.80	1.84	1.81	0.09	0.95
Cholesterol	50,029.32	39,149.66	46,146.29	3713.75	0.17
7β-hydroxycholesterol	16.21	20.42	27.85	7.74	0.58
7-ketocholesterol	15.28	29.12	25.52	4.62	0.16
β-epoxycholesterol	14.36	14.52	14.42	3.35	0.99
α-epoxycholesterol	12.53	12.47	17.34	4.41	0.69
Triol-cholesterol	4.54	9.41	3.36	2.27	0.26
Total COPs	62.95	85.95	88.49	16.97	0.53
COPs/cholesterol, %	0.13	0.22	0.20	0.04	0.40
TBARS (MDA)	14.48	13.18	18.92	3.21	0.44

TL, total lipids; COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as μg/100 g muscle, except for COPs/cholesterol and TL.

Table 6. Cholesterol, cholesterol oxidation products and thiobarbituric acid reactive substances in intramuscular lipid from *Longissimus dorsi* muscle of lambs slaughtered at 11, 14 and 17 kg of live weight.

	Weight of slaughtering, kg			SEM	P
	11 (n=6)	14 (n=6)	17 (n=6)		
Cholesterol	35,140.63	30,082.59	27,738.49	5321.47	0.68
7β-hydroxycholesterol	10.31	16.22	10.95	4.82	0.64
7-ketocholesterol	17.91	15.16	17.04	3.21	0.82
α-epoxycholesterol	4.97	6.83	10.37	2.33	0.33
β-epoxycholesterol	8.27	10.31	9.98	3.09	0.87
Triol-cholesterol	1.78	1.98	5.86	1.85	0.32
Total COPs	43.25	50.51	54.21	10.48	0.76
COPs/cholesterol, %	0.11	0.14	0.15	0.03	0.76
TBARS (MDA)	10.65	7.34	8.35	2.80	0.70

COPs, cholesterol oxidation products; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde. Values are expressed as μg/g of total lipids, except for COPs/cholesterol.

Conclusions

In the present study the effect of slaughter weight of suckling lambs on the oxidation of intramuscular lipids was revealed only in TB muscles. In this muscle, the cholesterol content showed a decreasing trend, while the content of COPs significantly increased with the age of animals. The higher susceptibility to cholesterol oxidation showed by older and heavier animals could be due to incipient change of feeding regimen in animals at 20 days old. The content of TBARS did not vary as a consequence of a similar fatty acid composition of intramuscular fat across weight of slaughter.

In conclusion, the effect of slaughter weight on cholesterol oxidation differed according to muscle type. Further studies are needed in order to assess whether this difference in lipid oxidation may be due to the fibre composition of muscles or to other pre-slaughter factors.

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