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Fatty acid composition of meat of Sarda suckling lamb

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Introduction

Scientific research that relate nutrition to human health have increased in recent years, as a result of growing concerns by consumer for food safety, highlighting the ability of food to be both a vehicle for nutrients and prevention tool for some diseases. This led to the emergence of so-called "functional" or "nutraceutical" food, namely foods that contain one or more components that could provide benefits to human health beyond their nutritional role (Hornstra, 1999). For example have been identified peptides with antihypertensive activity, mineral elements such as calcium, which could prevent osteoporosis and polyunsaturated fatty acids (PUFA) with potential for reduce the risk of cardiovascular disease. Research is focusing, in particular, on the role of lipids of animal origin in the induction/prevention of lifestyle diseases related to the dietary change of Western consumer. As a matter of fact, the radical change in diet following the industrial revolution, seems to have prompted the emergence of diseases related to improper eating habits such as metabolic syndrome, colon cancer, cardiovascular disease, atherosclerosis and diabetes. In particular it was highlighted the close link between the intake of high doses for long periods of cholesterol, saturated fatty acids (SFA), and some trans fatty acid, and the onset of these diseases. The relationships between dietary fat and incidence of lifestyle diseases, contributed towards the development of specific guidelines from national and international dietary guidelines in relation to fat in the diet (Table 1). It is recommended that total fat, SFA, PUFA n-3, PUFA n-6 and trans fatty acids should contribute <15–30%, <10%, <5–8%, <1–2% and <1% of total energy intake, respectively. Reducing the intake of SFA and increasing the intake of PUFA n-3 is encouraged.

Table 1 - Nutritional advice for fatty acid in human diet.

Recommendation	Ambit	Reference
Total fat <15–30% SFA <10% *trans* FA <1% PUFA 6 – 10% PUFA n-6 5 – 8 % PUFA n-3 1 – 2 %	International	World Health Organization (2003)
C18:2 n6 /C18:3 n3 between 5:1 and 10:1	International	Food and Agricultural Organization/ World Health Organization (1994)
For adults C18:2 n-6 4.44 g/d C18.3 n-3 2.22 g/d EPA+DHA 0.65 g/d EPA min 0.22 g/d DHA max 0.22 g/d	International	Simopoulos et al. (1999)
For infant formula /diet C18:2 n-6 10% C18.3 n-3 1.50% C20:4 n-6 20% DHA 0.35 % EPA < 0.10	International	Simopoulos et al. (1999)

Meat being the main source of fat in the diet was involved in these issues being accused of containing too much fat, too much saturated fat and too much cholesterol (Chizzolini et al. 1996). However, this should not lead to the exclusion of meat from the diet because, like all products of animal origin, meat is a vehicle for important nutrients. In fact the importance of meat as a source of high biological value protein and micronutrients (including for example vitamins A, B6, B12, D, E, iron, zinc, selenium) is well recognized (Biesalski, 2005; Williamson et al., 2005; Scollan et al., 2006). In addition, numerous scientific evidence can help to redefine the role of lipids of animal origin, in particular to those derived from ruminants, due to the discovery of nutraceutical properties of certain fatty acids (Table 2).

Table 2 - Nutraceutical components of the fat of meat of ruminant animals that showed nutraceutical properties.

Mono Unsaturated Fatty Acid (MUFA)
Poly Unsaturated Fatty Acid (PUFA)
Linolenic and α-Linolenic acid
Vaccenic Acid
Conjugated Linoleic Acid (CLA)
Branched Chain Fatty Acid (BCFA)

- MUFA (monounsaturated fatty acid), reduce the level of blood cholesterol without reducing the level of high density lipoprotein (HDL) (Ulbricht and Southgate, 1991);
- oleic acid (C18:1 *cis*-9) can reduce the incidence of cardiovascular disease (Massaro et al., 1999);
- linoleic acid (LA, C18:2 n-6) and α-linolenic (ALA, C18:3 n-3) acids are essential fatty acids (EFA) as precursors of PUFA that are not synthesized by the human body and should be taken with diet. In addition LA is part of the lipid membrane, and ALA can reduce the risk of neurological disorders, heart disease and cancer in adults and children;
- vaccenic acid (VA, C18:1 *trans*-11) metabolized to CLA play anticarcinogenic activities (Banni et al., 2002);
- the isomers of conjugated linoleic acid (CLA), in particular the *cis*-9, *trans*-11 (Rumenic acid, RA) have anticarcinogenic, antiatherosclerotic, antidiabetic and immunomodulating effects in laboratory animals (Pariza et al., 2001);
- odd and branched chain fatty acid (OBCFA) are important for their anticarcinogenic effects on cancer cells. The highest activity was observed with iso-16:0, and the activity decreased with increase or decrease of the chain-lengths from C16:0 (Wongtangtintharn et al., 2004).

CHAPTER 1

Composition of lamb meat

Composition of lamb meat

Lamb meat, as others red meat, is an important food because of his high nutritional quality that leads to a balanced supply of basic elements (proteins, carbohydrates, lipids) and essential elements that human tissues do not synthesize such as amino acids, essential fatty acids (EFA) and vitamins.

Lamb meat is made up by three main components: water (about 70%), protein (about 20%) and fat (about 6%), minor components make up the remaining percentage, and include carbohydrates (about 1%), soluble nitrogenous substances, minerals and vitamins. The average composition of lamb meat in comparison to those of the other of livestock species is shown in Table 1. Among component of meat protein content and amino acid profiles are little influenced by animal production factors such as nutrition and genetic while micronutrient content, fat content and fatty acid composition may be altered (Scollan et al., 2006).

Table 1 - Composition (for 100 g) of meat of different livestock species.

	Lamb	Beef	Kid	Pork
Moisture (%)	70.1	72.0	74.8	73.0
Protein (%)	20.3	22.5	19.2	21.8
Fat (%)	5.8	3.7	5.0	2.2
Carbohydrates (%)	0.3	0.1-0.5	-	-
Energy (Kcal)	159	362	122	119
Niacin (mg)	6.2	6.2	5.7	6.2
Vit. B12 (µg)	1.7	1.3	-	0.8
P (mg)	190	95	220	176
Fe (mg)	1.9	2.0	1.0	0.9
Zn (mg)	3.3	4.1	-	2.6
Se (mg)	9.2	13.6	-	16.3

Williamson et al.,(2005); INRAN, (2007).

1.1 Proteins

Red meat is a major source of high biological value protein and lamb meat provide about 20 g of protein for 100 g of lamb consumed and its content is less influenced by genetic factors (Table 2).

Table 2 - Protein content (%) in meat of different lamb breeds.

Breed	Protein (%)	Live weight (Kg)	Reference
Sarda	20.37	13.3-11.2	Rassu et al. (2010)
Fabrianese	22.1	33	Polidori et al. (2009)
Comisana	20.86	13.75	Chiofalo et al. (2010)
Massese	21.22	14	D'Agata et al. (2007)
Appenninica	20.62	-	Mazzone et al. (2010)
Barbaresca	20.26	11.84	Lanza et al. (2006)
Italian Merino	22.29	18-21	Morbidini et al. (2001)
Grazalema Merino	20.50	12	Juarez et al. (2009)
Churra Lebrijana	19.78	12	Juarez et al. (2009)
Churra	20.8	9-12	Osorio et al. (2007)
Churra Tensina	19.4	22-24	Joy et al. (2008)
Istrian	20.39	-	Miŏc et al. (2009)

The quality of meat proteins, is linked primarily to their high digestibility around 94% compared to the of 78% in beans and 86% in whole wheat (Williams, 2007) and this is due to their amino-acid sequence that promotes gastric and intestinal proteolytic enzymes. Among intracellular proteins, myosin is the most significant while among extracellular protein collagene is the most important. In meat are also present extractive nitrogenous substances, particularly creatine. An important meat protein is myoglobin that is an iron- and oxygen-binding protein, related to hemoglobin. The myoglobin content is greatest in the red fibers, and forms pigments that are the most responsible for color of meat. Lamb meat proteins, as other red meat proteins, are characterized by an high biological value, because of their complete amino acid profile. Protein from meat in fact, provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) namely those that humans cannot synthesize

despite being essential for its survival (Table 3). Moreover in meat are present in significant amounts branched amino acids (BCAA, Branched Chain Amino Acid) leucine, isoleucine and valine represents approximately 35% of essentials amino acids in the muscle (49g/g) and 40% of those needed of the adult (35mg/Kg/d) (Secchiari, 2008). Moreover, lamb meat is the most abundant dietary source of taurine (110 mg/100g in lamb and 77 mg/100g in beef; Purchas et al., 2004) that should be considered a conditionally essential amino acid during lactation and times of immune challenge, and may offer protection against oxidative stress (Redmond et al., 1998; Bouckenoogh et al., 2006) and is essential in newborn infants who are less able to synthesize this amino acid from cysteine (Higgs, 2000).

Table 3 - Amino acid composition of muscle protein of different livestock species (%).

	Lamb	Kid	Beef	Pork
Essential Amino acid				
Threonine	4.56	4.64	4.66	6.54
Valine	4.87	3.97	5.65	6.09
Isoleucine	4.41	3.93	5.30	5.73
Leucine	7.67	7.03	8.35	8.52
Phenylalanine	4.09	3.63	3.01	1.30
Lysine	7.81	8.36	8.89	9.08
Methionine	3.69	2.22	2.68	-
Tryptophan	1.30	0.99	1.35	-
Non essential Amino acid				
Aspartic acid	10.92	7.73	6.67	10.13
Serine	3.93	3.73	4.66	5.78
Glutamic acid	14.64	13.43	16.16	14.49
Proline	4.78	3.15	3.48	5.10
Glycine	6.29	3.76	3.41	5.71
Arginine	6.45	5.53	5.05	7.50
Cysteine	1.38	0.92	-	-
Alanine	5.67	4.83	6.14	-
Tyrosine	3.27	3.07	2.44	-
Hystidine	2.81	2.26	2.30	-
Glycine	6.29	3.76	3.41	-

Data from FAO, Miguélez et al. (2008); Webb et al. (2005); deSouza et al. (2004); Okrouhlá et al. (2006).

In humans, the protein requirements recommended by FAO / WHO Food and Nutrition Board of the U.S.A., is between 1 and 0.5 g / kg body weight according to age, sex,

physiological status and 100 g of meat covering about half of those needs quantitatively and qualitatively.

1.2 Vitamins

Lamb meat, as other animal foods is a good source of vitamins (Table 4). Generally, concentration of vitamin in meat is more higher in oldest than in youngest animals, so the levels in beef are generally higher than those in veal, and in mutton has more than in lamb (Williams, 2007).

Table 4 - Vitamin content (for 100 g) of raw meat of different ruminant species.

	Mutton	Lamb	Beef	Veal	Goat
Retinol - Vit. A (µg)	7.8	8.6	<5	<5	-
Thiamin – Vit. B1 (mg)	0.16	0.12	0.04	0.06	0.10
Riboflavin – Vit. B2 (mg)	0.25	0.23	0.18	0.14	0.56
Niacin – Vit. B3 (mg)	8.0	5.9	5.0	11.5	3.6
Pantothenic acid – Vit. B5 (mg)	1.33	0.74	0.35	1.50	0.41
Pyridoxine - Vit. B6 (mg)	0.8	0.10	0.52	0.8	0.23
CobalaminVit. B12 (µg)	2.8	0.96	2.5	1.6	1.1
Tocopherol - Vit. E (mg)	0.20	0.44	0.63	0.50	-
β – carotene (µg)	<5	<5	10	<5	-

Data from Williams (2007); Webb et al., (2005); Johnson et al., (1995).

Meat is one of the major dietary source of vitamin B12, indeed the consumption of 100 g /d of meat provide over two thirds of its daily requirement (Cosgrove et al., 2005). Lamb meat contains, besides B12, a number of B vitamins: thiamin, riboflavin, pantothenic acid, niacin, vitamin B6 (Chan et al. 1995). Meat also contribute to dietary intake of Vitamin D that is essential for the development and maintenance of bone (Williamson et al., 2005; Williams, 2007). In addition meat contain small amounts of vitamin E that is a fat-soluble vitamin, for this reason concentration of vitamin E is highest in fattier cuts of meat. Vegetable oils are particularly rich in vitamin E, and therefore the recent trend to

include seed oils in animals diets will have contributed to an increase in the vitamin E content of meat (Williamson et al., 2005). In addition organ meat, such as liver are also particularly rich source of vitamin A and folate compared with muscle (Table 5). However, the amount of vitamins present in liver is affected by age of the animal and the feed composition (Williams, 2007).

Table 5 - Vitamin content of muscle, liver and kidney of mutton.

	Muscle	Liver	Kidney
Retinol – Vit. A (UI)	Tr.	20000	100
Thiamin – Vit. B1 (mg)	0.15	0.27	0.49
Riboflavin – Vit. B2 (mg)	0.23	3.3	1.8
Pantothenic acid – Vit. B5 (mg)	0.5	-	-
Pyridoxine - Vit. B6 (mg)	0.40	0.42	0.30
CobalaminVit. B12 (µg)	2	84	55
Folate (µg)	3	220	31
Ascorbic acid – Vit. C (mg)	0	10	7

Adapted from Casey (1992).

Has been shown that cooking heat treatments decreased vitamins B content (Table 6). Among the vitamins thiamine (B1) was the most susceptible and riboflavin (B2) the more stable to thermal degradation (Lombardi – Boccia et al., 2005; Chan et al., 1995).

Table 6 - Comparison of vitamin content (for 100 g) of raw and cooked meat.

	Raw		Cooked	
	Beef	Lamb	Beef	Lamb
Thiamin (mg)	0.05	0.06	-	-
Riboflavin (mg)	0.12	0.11	0.06	0.05
Niacin – PP (mg)	5.5	6.5	3.4	4.3

Adapted from Lombardi – Boccia et al., 2005.

1.3 Micronutrients

Lamb meat is among the richest sources of bioavailable minerals and trace elements (Table 7). Indeed 100g of meat provide at least one quarter of daily adult requirements (Williams, 2007).

Table 7 - Mineral content (for 100 g) of meat from different ruminant species.

Minerals	Lamb	Veal	Beef	Goat
K (mg)	340	362	363	350
P (mg)	191	260	215	156
Na (mg)	54	51	51	64
Mg (mg)	22	26	25	20
Ca (mg)	2.5	6.5	4.5	11
Zn (mg)	1.8	4.2	4.6	3.5
Fe (mg)	1.6	1.1	1.8	4.3
Cu (mg)	0.12	0.08	0.12	0.3
Mn (mg)	9.5	-	-	-
Se (µg)	4.2	<10	17	-

Data from Miŏc et al. (2009); Williams (2007); Webb et al., (2005).

Lamb meat, as other types of red meat, is an excellent source of iron, having 50-60% in the haem-form that is more readily absorbed and utilized by the human gastrointestinal tract. Indeed, approximately 20–30% of haem iron is absorbed in the intestine, compared with only 7% of non-haem iron (BNF 1999) and meat protein also appears to enhance the absorption of iron from meat. Moreover lamb meat is a good source of zinc and is classified by the British Nutrition Foundation absorption as rich sources of this element (BNF, 2002). Similarly to iron, absorption of zinc from a diet high in animal protein is greater than from plant foods. Lamb meat, as other red meats, is also good sources of selenium, although it is likely that selenium values in meat will be significantly affected by animals feed and the time of the year of sampling (Williamson et al., 2005). Meat is also

a source of copper and its content in raw lean cuts range from 0.05 to 0.19 mg/100g in beef and veal, 0.08 to 0.14 mg/100g in lamb.

1.4 Fat

Lipids are one of the most important nutritional component of meat, are the richest dietary source of energy and supplies essential nutrients such as fat-soluble vitamins and essential fatty acids (EFA) and also provides palatability and flavor to foods (Williamson et al., 2005). Genetic and production system affect markedly fat content in lamb meat. For example has been shown that fat content is lower in grazing lambs compared with concentrate fed ones (Rowe et al., 1999; Priolo et al., 2002; Aurousseau et al., 2007; Popova et al., 2007). In addition differences in fat content are reported between different lamb breed (Table 8). However this marked variability is due besides to genetic factors, to the effect of environmental and dietary factors.

Table 8 - Fat content (%) in meat of different lamb breeds.

Breed	Fat (%)	Live weight (Kg)	Reference
Sarda	2.2	13.3-11.2	Rassu et al. (2010)
Fabrianese	4.5	33	Polidori et al. (2009)
Massese	1.40	13.75	D'Agata et al. (2007)
Comisana	1.78	14	Chiofalo et al. (2010)
Appenine	2.41	-	Mazzone et al. (2010)
Barbaresca	1.39	11.84	Lanza et al. (2006)
Italian Merino	2.06	18-21	Morbidini et al. (2001)
Grazalema Merino	1.75	12	Juarez et al. (2009)
Churra Lebrijana	1.58	12	Juarez et al. (2009)
Churra	4.8	9-12	Osorio et al. (2007)
Churra Tensina	1.70	22-24	Joy et al. (2008)
Istrian	2.45	-	Miŏc et al. (2009)

Fat in carcass is present as visible fat depots, subcutaneous fat, intermuscular fat (between the muscles), and intramuscular or marbling fat (in the muscle fibres). Because the intramuscular fat portion is irreversibly connected with and the muscle fiber it cannot be easily removed before human consumption, in spite of visible fat portions such as

intermuscular, subcutaneous and depots fat. For this reason the intramuscular fat is an important meat quality trait in relation to juiciness, aroma and tenderness and is the fat depot of main dietary interest for human nutrition and health (Scollan et al., 2006). Intramuscular fat is composed of polar lipids, mainly phospholipids, located in the cell membranes, and neutral lipids consisting mainly of triacylglycerols, that serve as a concentrated source of energy for the body, in the adipocytes that are located along the muscle fibres and in the interfascicular area (Gandemer, 1999). The amount of triacylglycerols in intramuscular fat is strongly and positively related with its the total fat content and varies from 0.2 to more than 5% (Sinclair and O'Dea., 1990) and is affected by species, breed, nutrition, and age. Whereas the amount of phospholipid in intramuscular fat is relatively constant, and varies between 0.2 and 1% of muscle weight (DeSmet et al., 2004).

Fat depots in meat is made up of different types of fatty acids in relation of the presence and position of the double bonds: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The largest part of the triacylglycerols fatty acids in intramuscular fat consists of SFA and MUFA, predominantly C16:0 and C18:1 *cis*-9. While the proportions of PUFA, and in particular of linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3), and of long chain PUFA n-3 and n-6 (LC-PUFA) were esterified predominantly in phospholipid fraction (Table 9).

Table 9 - Fatty acid composition (%) of longissimus muscle triacylglycerol and phospholipids sheep, cattle and pigs.

	Triacylglicerol			Phospholipids		
	Sheep	Cattle	Pigs	Sheep	Cattle	Pigs
SFA						
14:0	3.0	2.7	1.6	0.4	0.2	0.3
16:0	25.6	27.4	23.8	15.0	14.6	16.6
18:0	13.6	15.5	15.6	10.4	11.0	12.1
MUFA						
16:1*cis*	2.2	3.5	2.6	1.5	0.8	0.8
18:1*cis*-9	43.8	35.2	36.2	22.1	15.8	9.4
PUFA						
18:2 n-6	1.5	2.3	12.0	12.4	22.0	31.4
18:3 n-3	1.2	0.3	1.0	4.6	0.7	0.6
20:4 n-6	-	-	0.2	5.9	10.0	10.5
20:5 n-3	-	-	-	4.1	0.8	1.0

Wood et al. (2008).

Fatty acid profile of intramuscular fat of meat from different livestock species is shown in Table 10. The most abundant fatty acid are C18:1 *cis*-9 and C16:0 in all species. In comparison with meat of monogastric, ruminant meat is characterized by a higher percentage of SFA due to the biohydrogenation of dietary unsaturated fatty acid that occurs in rumen. On the other hand ruminant meat, particularly of lamb meat, is characterized by a low n-6/n-3 ratio, due to the high presence of PUFA n-3 series, which showed effectiveness in the prevention and treatment of cardiovascular diseases (Marchioli, 1999; Simopoulos, 2002; Siddiqui et al; 2008). Moreover, lamb meat is a interesting source of ALA and its elongation products eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic (DHA, C22:6 n-3) that are particularly important during fetal and early life growth to ensure the development of the nervous and visual system of the newborn (Jensen, 2006; Innis, 2008).

Table 10 - Fatty acid profile of muscle of different livestock species (%).

	Lamb	Suckling Lamb	Beef	Pork
Fatty Acid				
C14:0	4.52	4.11	1.70	1.04
C16:0	22.58	19.33	21.75	22.31
C18:0	15.30	12.02	15.16	12.88
C18:1 *cis* - 9	27.63	29.28	37.69	32.62
C18:2 *n*-6 (LA)	5.90	10.97	9.52	16.97
C18:3 *n*-3 (ALA)	1.70	1.95	0.22	0.47
C20:4 *n*-6 (AA)	2.53	6.89	3.08	4.00
C20:5 *n*-3 (EPA)	1.33	1.65	0.33	0.17
C22:6 *n*-3 (DHA)	0.53	1.25	0.09	0.23
SFA	43.53	37.73	43.97	36.77
MUFA	35.98	34.89	40.00	40.05
PUFA	15.18	27.38	16.22	23.22
PUFA/SFA	0.35	0.73	0.37	0.64
n-6/*n*-3	1.90	2.61	12.23	21.79

Aurousseau (2007a); Lanza et al. (2006); Cifuni et al. (2004); Mas et al. (2010).

In addition lamb meat is one of the main source of conjugated linoleic acid (CLA), in particular of the isomer *cis*-9, *trans*-11 or Rumenic acid (RA) important for its numerous health benefits including actions to reduce carcinogenesis, atherosclerosis, onset of diabetes, and body fat mass (Lee et al., 1994; Parodi 1997; Ip et al., 1999; Belury, 2002). The acronym CLA refers to a family of positional and geometric isomers of linoleic acid with two conjugated unsaturated double bonds (Figure 1).

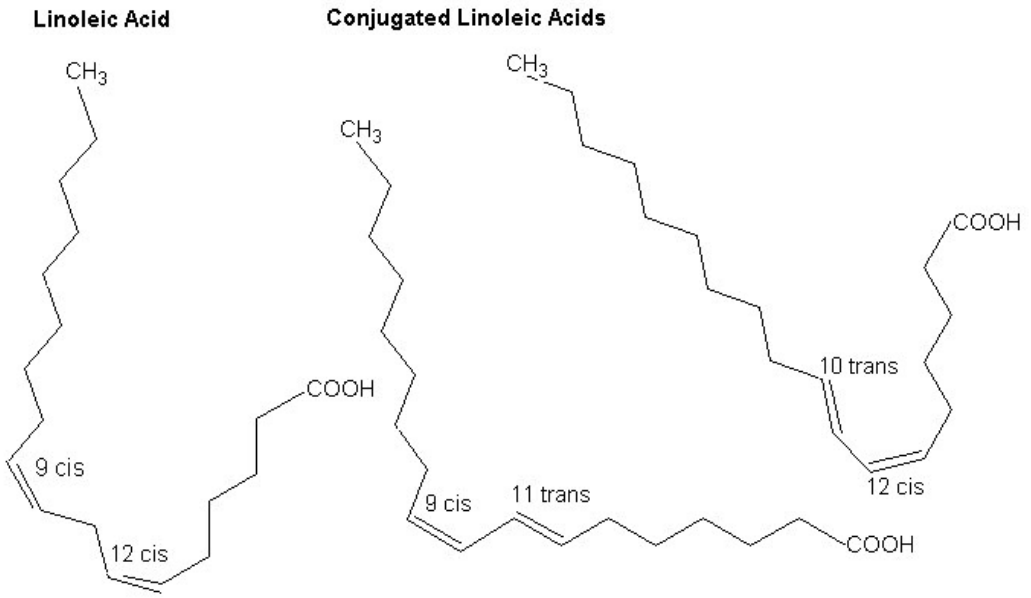

Figure 1 - Structure of C18:2 cis-9, cis-12 and of CLA isomers (cis-9, trans-11-CLA and trans-10, cis-12-CLA)

Many forms of CLA (Sehat et al. 1999; Dhiman et al., 2005) are possible and isomers until now identified are 7-9, 9-11, 10-12, 11-13, 12-14 with a geometry that can be *cis-cis*, *trans-cis*, *trans-cis* and *cis-trans*, for a total of 24 isomers.

In Table 11 is reported the CLA isomeric profile in meat of beef and lamb. The most abundant isomer in meat is the *cis*-9, *trans*-11 CLA (Chin et al., 1992; Yurawecz et al., 1998; Mc Guire et al., 1999; Sehat et al., 1999) which raises up to about 80% in lamb meat, and ruminant products are the richest natural source of this molecule. The second most abundant isomer is the *trans*-7, *cis*-9 CLA that represent about 8% in lamb intramuscular fat. While the isomer *trans*-10, *cis*-12 CLA, which may have detrimental effects on human health (Clément et al., 2002; Wahle et al., 2004), was low in lamb meat and accounted for about 0.2% of total CLA.

Table 11 - Isomers of CLA in ruminant meat (%).

	Beef (intensive)	Beef (extensive)	Lamb
cis-9, *cis*-11	1.08	1.42	-
cis-9, *trans*-11	59.89	78.35	77.31
cis-11, *trans*-13	1.10	0.72	0.15
cis-12, *trans*-14	1.21	1.35	0.64
trans-7, *cis*-9	12.09	9.17	8.04
trans-10, *cis*-12	3.79	2.12	0.22
trans-11, *cis*-13	1.26	1.22	6.86
trans-6, *trans*-8	-	0.23	0.04
trans-7, *trans*-9	15.03	0.81	0.56
trans-8, *trans*-10	0.37	0.38	0.37
trans-9, *trans*-11	1.16	2.14	2.17
trans-10, *trans*-12	1.04	0.59	0.74
trans-11, *trans*-13	0.57	1.03	1.80
trans-12, *trans*-14	0.55	0.48	1.10

Martins et a.l,(2007).

It is now well recognized that different factors can affect fatty acid profile of lamb meat in particular nutritional factors and for this reason more attention is focused by the research to improve the characteristics of such products through appropriate feeding strategies.

CHAPTER 2

Metabolism of fatty acid

Metabolism of fatty acid

2.1 Rumen metabolism of fatty acids

Dietary lipids represented mainly by triglycerides, when consumed by ruminants, undergo two important transformation in the rumen that are lipolysis and biohydrogenation (Harfoot, 1978; Palmquist and Jenkins, 1980; Jenkins, 1993). The first process refers to the release of free fatty acid (FFA) from the esters of the diet lipid fractions. In the second process the FFA are rapidly hydrogenated by microorganisms in the rumen to produce more highly saturated end products.

2.1.1 Lipolysis

After intake, dietary lipids esters are hydrolyzed by rumen bacteria (Figure 1) which causes the constituting fatty acid to be released. The lipolysis is a prerequisite for the biohydrogenation of unsaturated fatty acid (UFA) and can be performed by bacteria producing enzymes having hydrolytic activity such as lipases. Lipases are extra cellular enzyme assembled inside particles equipped with a membrane made up of proteins, lipids and nucleic acids (Jenkins, 1993). Lipase hydrolyzes completely triglycerides to FFA and glycerol leaving small amounts of mono- and di- glycerides. Glycerol is then fermented to propionic acid. 74 bacterial strains capable of hydrolyzing the ester bonds have been identified (Fay et al., 1990), the most important are: *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* (Hespell and O'Bryan-Shah, 1988). *Anaervibrio lipolytica* hydrolyzes triglycerides while *Butyrivibrio fibrisolvens* hydrolyzes phospholipids and glycolipids (Harfoot e Hazlewood, 1997). The extent of hydrolysis is generally high (>85%) and several factors can affect the rate and the extent of this process (Harfoot, 1981; Doreau et al., 1997); for example it is reduced when factors such as a low rumen

pH and the administration of ionophores inhibit the activity and the growth of bacteria (Van Nevel and Demeyer, 1995; Demeyer and Doreau, 1999). In addition to the enzymatic hydrolysis of triglycerides, the fatty acids may also originate from the enzymatic hydrolysis of galactolipids and phospholipids by several galactosidase and phospholipase enzymes (phospholipase A, phospholipase C, lysophospholipase and phosphodiesterase) produced by ruminal bacteria (Jenkins, 1993).

2.1.2 Biohydrogenation

The half-life duration of FFA in the rumen is relatively short because of their rapidly hydrogenation to the saturated form. The percentage of hydrogenated PUFA was estimated to be between 60 and 90% (Antongiovanni et al., 2003).

The major substrates of rumen biohydrogenation are LA and ALA and the rate of rumen biohydrogenation of fatty acids is faster as unsaturation increases. Bacteria are largely responsible for biohydrogenation of UFA in the rumen and this process seems to be useful for ruminal bacteria to protect against the toxic effects of UFA, while protozoa seems to be of only minor importance (Harfoot and Hazlewood, 1988).

Biohydrogenation of UFA involves several biochemical steps and different species of rumen bacteria. In this regard Kemp and Lander (1984) divided bacteria into two groups, A and B, based on the reactions and end products of biohydrogenation. To obtain a complete biohydrogenation of PUFA, bacteria from both groups are generally required. Group A bacteria hydrogenates mainly LA and ALA acid to C18:1 *trans*-11 (Vaccenic acid, VA,) while group B bacteria hydrogenate several octadecenoic acids, including oleic and VA, to stearic acid (C18:0) (Harfoot and Hazlewood, 1988).

The biohydrogenation sequence of linoleic and linolenic acid is presented in Figure 2. The initial step consists in an isomerization of the *cis*-12 double bond of linoleic acid

(LA, C18:2 *cis*-9, *cis*-12). This step is catalyzed by the linoleate isomerase that acts on double bonds in the middle of chain and distant from active functional groups and its kinetic properties have been characterized in a limited number of bacterial species (Kepler and Tove, 1967; Kepler et al., 1970; Kemp et al., 1984) . The enzyme is bound to the bacterial cell membrane and demonstrate an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group. The end product of this step is C18:2 *cis*-9, *trans*-11 (Rumenic acid, RA) that represent a transitory intermediate. The second step is a reduction of the *cis*-9 double bond resulting in VA. In vitro studies demonstrated that the kinetics of this reaction is fast. The final step is a further hydrogenation of the *trans*-11 double bond producing C18:0. The kinetics of this reaction occurred less rapidly and therefore this intermediate is made more available for the absorption and can be subsequently desaturated in tissues.

A similar biohydrogenation in rumen occurs for α-linoleic acid (ALA, *cis*-9, *cis*-12, *cis*-15 C18:3). The first step is an isomerization of *cis*-12 double bond that produce *cis*-9, *trans*-11, *cis*-15 C18:3 as the predominant initial isomerization product. The second step is the reduction of the *cis*- double bonds. As a consequence VA is the end product of this reaction and represent a common intermediate in the biohydrogenation of both LA and ALA. The final step is a further reduction which terminates with C18:0 production.

In addition biohydrogenation of γ-linolenic acid (*cis*-6, *cis*-9, *cis*-12 C18:3) also result in formation of VA (Harfoot and Hazelwood, 1998; Griinari and Bauman, 1999). Changes in rumen bacterial population may alter the normal process of biohydrogenation and consequently the fatty acid profile, for example rumen pH strongly affected isomerization and biohydrogenation (Bessa et al., 2000). In fact decrease rumen pH often results in bacterial population shifts and consequent changes in the of fermentation end products (Van Soest, 1994; Bauman et al., 1999). Griinari and Bauman (1999) propose a putative

pathways in this case leading to C18:1 *trans*-10 instead of VA. This would generate *trans*-10, *cis*-12 C18:2 as the main intermediate product. In addition, rumen biohydrogenation of dietary PUFA is modulated by several factors, such as the amount and type of the lipid supplement (Harfoot and Hazlewood, 1997) and the basal diet (Bessa et al. 2005), resulting in differences in the amount of PUFA that escapes from rumen biohydrogenation and in the type and distribution of biohydrogenation intermediates. Among the dietary factors which depress lypolisis and biohydrogenation may mentioned the use of mature forage and the use of to finely ground feed (Antongiovanni et al., 2003). In the latter case the size of feeds particles affects the adherence of bacteria upon the surface and increase the transit rate through the rumen barrier so reduce the time of exposition to the activity of rumen bacteria (Antongiovanni et al., 2003). Moreover, the type of fat added to the diet can affect the lipids metabolism in the rumen. Generally fats with a high content of LA such as soybean oil inhibit the process of reduction to stearic acid so favoring the accumulation of intermediate compounds like VA.

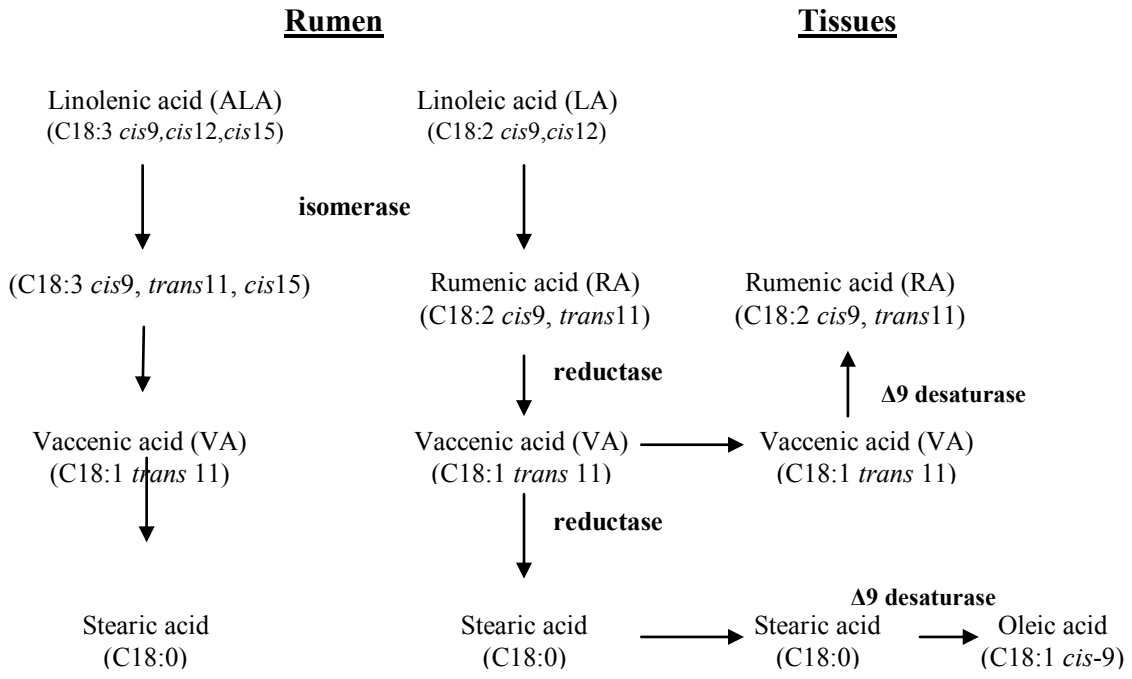

Figure 1 - The pathway of biohydrogenation of linolenic and linoleic acid in the rumen and tissue Δ-9 desaturase activity in tissues.

2.2 Tissue synthesis of CLA

Only a small proportion of *cis*-9, *trans*-11 CLA (Rumenic acid, RA) is directly absorbed from the rumen and small intestine. The major portion of RA in tissue lipids comes from endogenous synthesis via a pathway involving the desaturation of VA by the Δ9-desaturase enzyme (Figure 1) (Griinari and Bauman, 1999; Knight et al., 2003).

The desaturase system is a multienzyme complex that includes NAHD-cytocrome b_5 reductase, cytocrome b_5, acyl-CoA sintetase and the terminal Δ9-desaturase. The Δ9-desaturase, also known as Stearoyl-CoA desaturase-1, is used to synthesize oleic acid, by desaturating stearic acid, a saturated fatty acid either synthesized in the body from palmitic acid or ingested directly, with the enzyme activity increasing as the animal accumulates adipose tissue (Smith et al., 2009; Duckett et al., 1993). Δ9- desaturase

reaction determines the addition of a *cis*- double bond at the 9, 10 position from the carboxyl end of fatty acids. This reaction requires molecular oxygen, NAD(P)H, an electron transport system and a terminal desaturase. Stearoyl-CoA and palmitoyl-CoA are the major substrates for Δ9-desaturase and the fatty acid products of this reaction are components of triglycerides and phospholipids. A wide range of saturated and unsaturated acyl- CoA can serve as a substrate including *trans*-11 octadecenoic acid (Bauman et al., 1999). There are reported species differences in the tissue distribution of Δ9-desaturase. In rodents the concentration of mRNA and enzyme activity are greatest in liver (Ntambi, 1995), however growing ruminants have substantially greater Δ9-desaturase as indicated by mRNA abundance and enzyme activity (Whale, 1974; Chang et al., 1992; Page et al., 1997).

To estimate indirectly the activity of Δ9-desaturase Kelsey et al. (2003) proposed the ratio [product of Δ9-desaturase]/[product of Δ9-desaturase + substrate of Δ9-desaturase] that considers the desaturation product compared to its amount proceeding from the blood and that proceeding from desaturation in tissues.

2.3 Synthesis of PUFA n-3 and PUFA n-6 in tissues

Mammals are unable to synthesize LC-PUFA from acetyl-CoA because of the lack of ω3 desaturase and Δ12-desaturase, hence LA and ALA must be supplied from diets and for this reason are classified as essential fatty acids (EFA). Mammals are able to synthesize LC-PUFA from these precursor (Sprecher, 2000) through a series of Δ5- and Δ6-desaturase steps common to both fatty acid series (Sprecher et al., 1995, Wang et al., 2005; Schmitz and Ecker, 2008). Δ6-desaturase is a membrane bound desaturase and is classified as a front-end desaturase because it introduces a double bond between the pre-existing double bond and the carboxyl end of the fatty acid (Nakamura and Nara, 2004).

Δ5-desaturase is also classified as a front-end desaturase and after desaturation, by Δ6-desaturase, and elongation by elongase, introduce another double bond at the Δ5 position of 20 carbon fatty acid 20:3 n-6 and 20:4 n-3.

The pathways showing the formation of n-3 and n-6 LC-PUFA is reported in Figure 2.

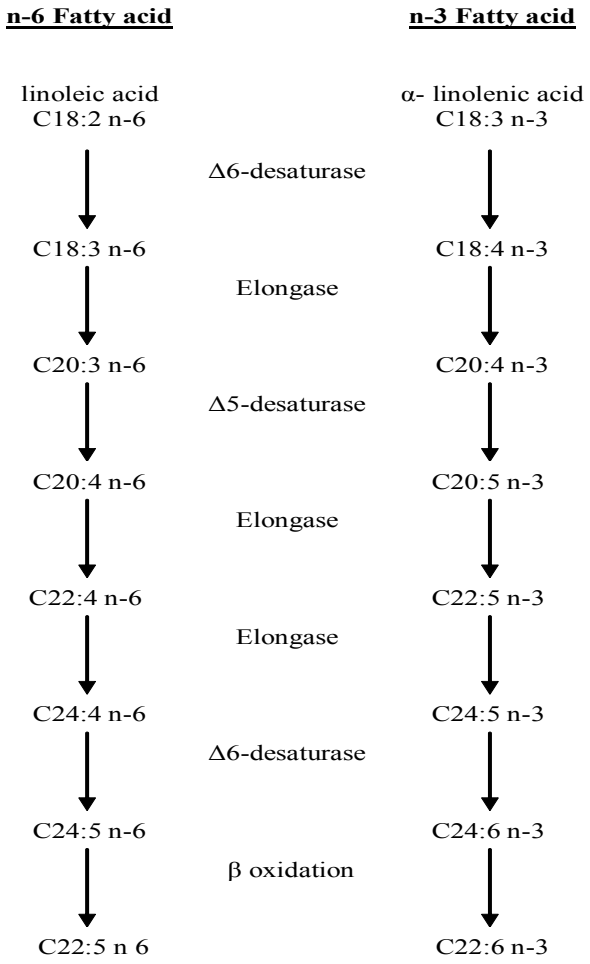

Figure 2 - Pathways showing the formation of n-3 and n-6 LC-PUFA via a series of elongation and desaturation steps.

LA is converted to γ- linolenic acid (C18:3 n-6), and dihomo-γ linolenic acid (C20:3 n-6) to form the key intermediate arachidonic acid (ARA, 20:4 n-6) by Δ5- and Δ6-desaturase and elongase-enzymes. ARA is further metabolized to docosapentaenoic acid (22:5 n-6) or eicosanoids. The n-3 fatty acid α-linolenic acid (C18:3 n-3) is converted to stearidonic acid (C18:4 n-3) and eicosatetraenoic acid (C20:4 n-3) to form eicosapentaenoic acid (EPA, C20:5 n-3) using the same series of enzymes as those used to synthesize ARA. In the final step EPA is further metabolized to docosahexaenoic acid (DPA, C22:6 n-3) or eicosanoids. Thus synthesis of 22:5n-6 and 22:6n-3 requires that the respective 22:4n-6 and 22:5n-3 precursors be elongated to 24:4n-6 and 24:5n-3, followed by Δ6-desaturation, and then β-oxidation to obtain the final products. The β-oxidation step occurs in the peroxisomes via a multifunctional enzyme (Sprecher, 2000; Sprecher et al., 1995). However earlier, it had been assumed, that the final desaturation was mediated by a Δ4-desaturase, but Sprecher (2000) and Sprecher et al. (1995) have shown that this enzyme does not occur in mammalian systems. Recent studies suggest that the final desaturation in C22:6 n-3 may be catalized by the same Δ6-desaturase that catalyzed the first step (Nakamura and Nara, 2004).

As mentioned above, the conversion of n-3 and n-6 fatty acids share the same series of enzymes, thus in LA and ALA levels can influence the metabolic outcome of each other. However, elongase and desaturase appears to have a greater affinity for ALA than for LA (Chapkin, 2008), in fact, 10 times more LA is required to have an equal effect on n-3 metabolism as ALA does on LA elongation (Holman, 1998). In addition evidence from several studies in vivo and in vitro, indicate that these two fatty acid families not only share these enzymes, but they also compete for the same enzymes (Brenner and Peluffo, 1966; Holman, 1968) and an excess of one can cause a significant decrease in the conversion of the other (Hague and Christoffersen, 1986). Although the affinity of Δ6-

desaturase is greater for ALA than for LA, the typically greater concentrations of LA in cellular pools result in greater conversion of the latter to longer chain PUFA and high dietary intakes of PUFA n-6 could be a limiting factor in the conversion of ALA to EPA and DHA (Palmquist, 2009). However, it has been shown that conversion of n-6 fatty acids to biologically active metabolites is often decreased by increasing intake of n-3 fatty acids (Goyens et al., 2006). For these reasons and because mammals cannot interconvert n-3 and n-6 fatty acids, the ALA to LA ratio in the diet is very important (Barcelo-Coblijn et al., 2005, Emken et al., 1999). In addition, fatty acid elongation and desaturation is subjected to feedback regulation because both ARA and DHA suppress endogenous conversion of LA and ALA into longer chain fatty acids, respectively (Emken et al., 1999; Igarashi et a., 2006).

There is limited knowledge available about the protein expression and activity of Δ5- and Δ6-desaturase in ruminant tissues and factors that can modulate their gene expression. However, it has been shown that both Δ5- and Δ6- desaturase are correlated with changes in the muscle intramuscular fat content. Ward et al. (2010) reported that the Δ5- and Δ6- desaturase protein expression increased in relation to fatty acid synthesis and intramuscular fat content.

CHAPTER 3

Factors affecting fatty acid profile in lamb meat

Factors affecting fatty acid profile in lamb meat

Several factors are important in regulating fatty acid composition in lambs. Results of many studies have shown that fatty acid profile of lamb meat is strictly related to slaughtering age and weight, breed, sex and feeding regimen (Cañeque et al., 2005; Diaz et al., 2005; Valvo et al., 2005; Lanza et al., 2006; Juarez et al., 2008; Serra et al., 2009). Among the various factors, the production system, was the main factor to explain variations in fatty acid profiles (Raes et al., 2004; Juarez et al., 2008), for this reason, the nutritional modulation of the fatty acid profile of ruminant edible fats is currently an important research topic.

3.1 Body weight at slaughter

It is known that the fatty acid profile in lamb meat is affected by the slaughter weight (Beriain et al., 2000; Okeudo and Moss, 2007). In lambs, generally, an increase in live weight is correlated with an increase in time from weaning, which results in a progressive decrease in the C14:0, C16:0 and C16:1 content of adipose tissues and an increase in the C18:0 content, moreover the content of odd chain fatty acids (OCFA) increases as a result of de novo synthesis, resulting from the increased activity of rumen micro-organisms (Berian et al., 2000). Effects of live weight at slaughter and carcass weight on fatty acid composition of lamb meat are summarized in Table 1. Juarez et al. (2009) observed a decrease in the content of C14:0, C16:0 and SFA and an increase in PUFA as slaughter body weight increase from 12 to 20 Kg. Diaz et al. (2002) found that lambs slaughtered at the high body weight (28Kg) displayed a lower percentage of C14:0 and higher proportion of C16:0 compared with lambs slaughtered at 24Kg live weight, while the content of C18:0 was similar between groups. An increase of C16:0 and a decrease

of the proportion of PUFA n-3 and PUFA n-6 as body weight increase from 24 to 30Kg was reported by Santos-Silva et al. (2002), probably due to an increase of intramuscular fat deposition (Nürnberg et al., 1998). On the other hand, Dìaz et al. (2003) reported no effect of different body weight at slaughter (10, 12 and 14 kg) on fatty acid profile of intramuscular fat of lambs. In addition, Cañeque et al. (2005) reported few differences on fatty acid composition of lambs with different carcass weight (<5.5 kg, 5.5–6.5 kg, >6.5 kg) obtained from animals slaughtered at an average body weight between 8 and 14 kg. These authors reported a lower proportions of C14:0 and C16:1 acid and higher proportions of C18:0 acid and total UFA in the lightest carcasses.

Table 1 - Fatty acid composition of muscles of lambs slaughtered at different weights.

				Fatty acid (%)					
Kg	C14	C16	*C18*	*LA*	*ALA*	*SFA*	*MUFA*	*PUFA*	*Reference*
24	5.49	26.39	15.58	6.06	1.71	50.02	32.05	12.25	Diaz et al.,
28	4.21	27.31	15.88	5.28	1.41	52.97	35.76	10.62	2002
10	9.35	31.90	12.07	4.41	2.31	61.55	27.87	10.57	Diaz et al.,
12	8.97	31.95	12.01	4.47	2.52	61.15	27.95	10.88	2003
14	8.75	31.44	12.06	4.67	2.47	59.91	28.73	11.35	
24	3.0	18.4	14.5	6.15	1.21	-	-	-	Santos-Silva
30	3.1	19.7	14.2	5.31	1.13	-	-	-	et al. 2002
12	6.36	21.65	15.41	6.88	0.85	46.49	40.39	14.47	Juarez et al.,
20	5.35	20.40	15.48	7.71	0.97	43.84	38.66	15.75	2009
< 5.5	6.47	24.64	13.06	10.54	1.08	47.32	35.55	17.13	Cañeque et
5.5 – 6.5	7.00	24.43	12.90	10.35	1.12	47.54	35.76	16.69	al., 2005
> 6.5	7.29	24.44	11.65	9.99	1.12	47.59	36.46	15.95	
				Fatty acid (g/100 of total lipid)					
11	2.96	13.51	8.60	2.96	1.22	26.49	26.56	7.82	Serra et al.,
14	4.25	14.88	7.87	2.82	1.42	28.75	27.66	7.72	2009
17	3.87	14.78	7.91	2.91	1.34	28.22	26.89	7.34	

Data from Diaz et al. (2002) refer only to Intramuscular fat of Longissimus Dorsi.
Data from Diaz et al. (2003) refer only to Intramuscular fat.
Data from Juarez et a. (2009) refer only to Intramuscular fat of Grazalema Merino lambs.
Data from Serra et al. (2009) refer only to Longissimus Dorsi.

3.2 Age at slaughter

Studies carried out to evaluate the effect of age on fatty acid profile of lamb meat are summarized in Table 2. Even if age did not modify markedly the lipid content and composition, effects of nutritional interest were observed. Medium-chain SFA, such as lauric (C12:0) and myristic (C14:0), predominates in very young suckling lambs probably because of the presence of these fatty acids in maternal milk (Bas and Morand-Fehr, 2000; Velasco et al., 2004; Oriani et al., 2005). In fact very young lambs behave as monogastrics and the lipid profile of their adipose tissues tend to reflect the composition of milk fat. The proportion of PUFA, and in particular the content of LA and ALA, increase with increase age at slaughter (Oriani et al., 2005). Similarly Banskalieva (1997) noted that an increasing age at slaughter caused a slightly higher unsaturation of depot fat in sheep. Conversely, Cifuni et al. (2000) found, an increasing degree of saturation of meat with the increase of age at slaughter, likely as a consequence of progressive rumen development. The age-related increase in the relative proportion of PUFA could be a result of the change from a diet exclusively based on maternal milk to a diet characterized by inclusion of solid feeds (concentrate and pasture), which reduced the intake of milk.

Table 2 - Fatty acid composition of muscles of lambs slaughtered at different days of age.

	Fatty acid (%)									
Age	*C12*	*C14*	*C16*	*C18*	*LA*	*ALA*	*SFA*	*MUFA*	*PUFA*	*Reference*
45	1.59	9.37	23.18	11.85	3.38	1.07	51.98	-	-	Cifuni et al.,
90	1.41	9.45	24.83	12.01	3.34	0.90	53.48	-	-	2000
30	1.55	9.15	28.53	13.73	8.67	2.04	53.82	32.88	12.87	Oriani et al.,
50	1.09	8.88	28.62	12.52	8.23	2.70	52.12	34.88	13.00	2005
70	0.81	7.36	28.46	13.36	11.05	2.52	51.21	33.16	16.09	

3.3 Anatomical fat depot

The fatty acid composition varies between the various lamb fat depots and muscles. Generally internal fat depots (omental, mesenteric and perirenal) and subcutaneous fat are characterized by a higher proportion of SFA in comparison with intramuscular fat; whereas intramuscular fat, that is rich in phospholipids, showed the highest proportion of PUFA and the lowest of SFA (Rhee et al., 2003; Cañeque et al., 2005; Castro et al., 2005; Osorio et al., 2007; Juarez et al., 2009).

The fatty acid composition of different fat depots are summarized in Table 3.

Table 3 - Fatty acid profile of different fat depots of lamb.

	Fatty acid (%)									
Depots	*LA*	*ALA*	*ARA*	*EPA*	*DPA*	*DHA*	*SFA*	*MUFA*	*PUFA*	*Reference*
Intramuscular	10.16	0.56	-	-	-	-	36.92	42.07	15.92	Castro et
Subcutaneous	4.36	0.48	-	-	-	-	42.75	45.98	4.83	al., 2005
Omental	4.96	0.41	-	-	-	-	50.12	40.80	3.37	
Mesenteric	4.44	0.30	-	-	-	-	47.61	41.62	4.69	
Perirenal	4.92	0.56	-	-	-	-	49.52	41.47	5.47	
Subcutaneous	2.91	0.64	0.30	0.11	-	-	48.60	44.82	6.84	Osorio et
Intermuscular	3.06	0.66	0.21	0.04	-	-	50.03	43.29	6.76	al., 2007
Intramuscular	8.44	1.30	4.86	1.44	-	-	40.47	39.90	19.95	
Subcutaneous	3.41	0.91	-	57.15	-	-	39.18	39.18	3.67	Cañeque
Intermuscular	3.18	0.67	-	55.87	-	-	39.77	39.77	3.44	et al.,
Intramuscular	11.48	1.17	5.61	45.56	-	-	36.13	36.13	18.31	2005
Intramuscular	7.16	0.82	3.42	-	-	-	46.40	40.00	13.91	Juarez et
Subcutaneous	3.09	0.60	0.13	-	-	-	53.44	41.31	5.19	al., 2009

Data for Juarez et al. (2009) refer only to suckling lamb.

Cañeque et al. (2005) found higher contents of SFA in intermuscular and subcutaneous fat of Manchego suckling lambs, whereas intramuscular fat was characterized by a lowest proportion of MUFA and by a greater intramuscular levels of PUFA (+415%) due to its higher proportion of LA (+348%) and ALA (+148%). Similar pattern of PUFA have been observed by Castro et al. (2005) comparing intramuscular with omental, perirenal, and mesenteric fat. Juarez et al. (2009)

reported in two Spanish breed a 2.3 fold higher content of LA and 1.4 fold higher content of ALA in intramuscular fat compared with subcutaneous fat and consequently a higher content of PUFA (2.7 fold). By contrast, Osorio et al. (2007) reported the highest proportions PUFA in intermuscular fat compared with intermuscular and subcutaneous fat of suckling lambs. Such non univocal results may be due both to the role of deposit and to the time of fat deposition during growth and development (Terrell et al., 1969).

Differences in fatty acid composition a among type of muscles are summarized in Table 4. Generally leg muscles (*Semitendinosus*, *Semimembranosus*, *Rectus Femoris, Gluteus and Tensor Fascia Latea*) in comparison with *Longissimus Dorsi* showed a higher content of PUFA and a lower content of SFA (Arsenos et al., 2001, Salvatori et al. 2004, Garcia et al., 2008). This result could be explain because the *Longissimus Dorsi* is a glycolytic muscle and contain more than 80% of α fibers lower in PUFA percentage than the redder leg muscles which are described as oxidative muscles exhibiting almost exclusively βR fibers (Alasnier et al., 2000).

Table 4 - Fatty acid profile of different muscles of lamb.

						Fatty acid (%)				
Muscle	*LA*	*ALA*	*ARA*	*EPA*	*DPA*	*DHA*	*SFA*	*MUFA*	*PUFA*	*Reference*
Longiss. Dorsi	7.94	1.20	1.99	-	-	-	45.2	-	6.65	Salvatori et
Semimembranos.	9.71	1.76	3.58	-	-	-	42.6	-	15.0	al., 2004
Gluteobiceps	9.32	1.84	2.30	-	-	-	44.7	-	13.5	
Longiss. Dorsi	9.10	1.04	4.57	-	-	-	49.41	35.72	14.87	Cañeque et
Quadriceps Femoris	11.48	1.17	5.64	-	-	-	45.56	36.13	18.31	al., (2005)
Longiss. Lumb.	16.30	2.00	8.85	1.50	3.03	0.58	34.96	29.80	35.24	Popova et
Semimembanos.	18.98	2.23	7.68	1.20	2.36	0.42	34.87	30.38	34.75	al., 2007
Longiss. Dorsi	6.77	2.64	2.01	1.07	0.90	0.14	39.77	28.09	13.85	Garcia et
Leg muscles	7.37	2.89	2.68	1.37	1.23	0.40	37.08	28.41	16.02	al., 2008
Semitendinos.	7.0	1.1	3.2	1.0	1.4	1.2	42.7	40.0	17.3	Vacca et
Longiss. Dorsi	7.2	1.7	3.1	0.9	0.9	0.7	44.7	38.3	17.0	al., 2008
						Fatty acid (g/100 of total lipid)				
Longiss. Dorsi	2.82	1.42	0.91	0.94	0.84	0.57	28.75	27.66	7.72	Serra et al.,
Triceps Brachii	2.44	1.00	0.86	0.77	0.69	0.45	21.18	21.89	6.49	2009
Semimembranos.	2.48	1.19	0.85	0.86	0.78	0.52	22.81	21.57	6.79	

Data for Popova et al., (2007) refer only to phospholipid fraction.
Data for Serra et al. (2009) refer only to lambs slaughetred at 14 kg live weight.

In the study of Salvatori et al. (2004) the content of PUFA was about 2 fold higher in leg muscles (*Gluteobiceps* and *Semimembranosus*, respectively) compared with *Longissimus Dorsi* due mainly to the higher contents of LA, ALA and ARA. A similar result was reported by Garcia et al. (2008) who reported higher LA and ALA contents in leg muscles (*Semitendinosus*, *Semimembranosus*, *Rectus Femoris, Gluteus and Tensor Fascia Latea)* compared with *Longissimus Dorsi* that accounted for a higher PUFA content. In addition contents of LC-PUFA n-6 and n-3 were higher in leg muscles compared with *Longissimus Dorsi*. Higher content of total PUFA, especially LA and ALA, and lower SFA in *Quadriceps Femoris* compared to *Longissimus Dorsi* were reported also by Cañeque et al. (2005) in suckling lambs. Similar fatty acid profile were observed by Popova et al. (2007) in polar lipid fraction of *Longissimus Lumborum* and *Semimembranosus* muscles of grazing lambs. Conversely Serra et al. (2009) found a slightly higher content of LA, ALA and

PUFA in *Longissimus Dorsi* of suckling lambs compared to *Semimembranosus* and *Triceps Brachii* muscles.

3.4 Breed

The effects of breed on fatty acid composition of intramuscular fat of lamb have been studied widely, showing only slight differences (Fisher et al., 2000; Arsenos et al., 2006; Demirel et al., 2006; Juarez et al., 2009). However, Dìaz et al. (2005), when studied lambs from Spain, Germany, United Kingdom and Uruguay, reported a significant effect of breed-production system on fatty acid profiles, separating clearly Spanish lambs (Rasa Aragonesa breed) from the other breeds. Similarly Zygoyiannis et al. (1985) and Arsenos et al. (2006) reported that breed significantly affected the fatty acid composition of lambs' fat depots. Sañudo et al. (2000) comparing Rasa Aragonesa and Spanish Merino breed lambs, concluded that the production system is more important than the breed to influence the fatty acid profile. Juarez et al. (2009) comparing Grazalema Merino and Churra Lebrijana breeds, observed differences in intramuscular fatty acid composition for several fatty acid and suggest that these differences may be related to genetic differences in the way they synthesize and deposit fat and fatty acids. Moreover, the differences in fatty acid composition between breeds of lambs depends mainly by carcass fatness, and breeds or genetic types with a low concentration of total lipid in muscle, in which phospholipid is a high proportion of the total, will have higher proportions of PUFA in total lipid (Nürnberg et al., 1998; Fisher et al., 2000; Wood et al., 2008).

3.5 Sex

The effects of a different metabolism between sexes caused differences in fatty acid composition of tissues in male and female lambs. The effects of sex on fatty acid composition of lamb's intramuscular fat are reported in Table 5. The results of different studies of the effect of sex on fatty acid composition differ greatly. Generally male lambs had a lower content of SFA, especially C16:0, that accounted for a lower content, in intramuscular fat depots, and a higher content of MUFA, PUFA and LA (Morbidini et al., 2001; Kosulwat et al., 2003; Cividini et al., 2008). This could be ascribed to the fact that females display a greater tendency to accumulate fat from an early age and to the fact that females have a slower growth rate, and consequently are older than males when slaughtered (Díaz et al., 2003). In addition, it has been observed that sex differences were greater for suckling lambs than for heavier lambs (Horcada et al., 1998).

Morbidini et al. (2001) reported a more SFA profile in female intramuscular fat compared with male and this due to the higher C14:0 and C16:0. On the other hand intramuscular fat of *Longissimus Dorsi* of male lambs was characterized by a higher proportion of PUFA due to the higher content of LA (9.33 and 7.93%, respectively) and ALA (0.93 and 0.85%, respectively). Cividini et al. (2008) reported higher proportion of C16:0, C18:1 *cis*-9 in females than in males and on the other hand lower contents of LA and ALA in female than in males. Due to the higher content of C18:1 *cis*-9 the proportion of MUFA was higher in females and the proportion of PUFA were higher in intramuscular fat of male lambs; while the proportion of total SFA was almost the same in both sexes. The authors concluded that differences in fatty acid composition between males and females is probably due to higher fatness and content of total intramuscular fat in female animals compared with male animals

(3.32 vs. 2.39%). Similar results were reported by Nürnberg et al. (1998). However Tejeda et al. (2008) reported higher contents of LA and consequently of total PUFA in intramuscular fat of *Longissimus Lumborum* of females lambs, while no sex effect were observed in *Semimembranosus* muscle. In concordance with results of Tejeda et al. (2008), Díaz et al. (2003) found higher levels of LA in intramuscular fat of females.

Table 5 - Effects of sex on fatty acid composition of lamb intramuscular fat.

	Fatty acid (%)									
Sex	C14	C16	*C18*	*C18:1 c9*	*LA*	*ALA*	*SFA*	*MUFA*	*PUFA*	*Reference*
Male	4.32	22.90	14.48	43.66	4.10	2.05	-	-	-	Kosulwat et
Female	4.76	23.41	18.05	43.28	3.92	1.71	-	-	-	al., 2003
Male	7.88	28.46	12.62	30.28	9.33	0.93	52.38	33.56	14.03	Morbidini et
Female	8.57	29.84	12.03	30.18	7.93	0.85	53.85	34.09	12.07	al., 2001
Male	-	19.71	19.51	28.37	8.71	3.31	43.27	34.82	21.91	Cividini et
Female	-	21.80	14.93	31.90	6.80	2.83	44.57	38.37	17.05	al., 2008
Male	9.22	31.65	12.24	22.92	4.50	2.51	62.14	26.80	11.05	Diaz et al.,
Female	8.22	31.88	11.92	25.08	4.54	2.36	56.90	29.57	10.82	2003
Male	3.62	22.93	14.71	36.87	9.11	1.16	46.16	40.35	13.47	Tejeda et al.,
Female	3.63	22.40	12.71	34.54	12.62	1.29	43.75	37.97	18.26	2008

Data for Kosulwat et al. (2003) refer only to light weight carcasses.
Data for Tejeda et al., (2008) refer only to Longissimus Lumborum of lambs slaughtered at 24 Kg.

3.6 Nutritional factors

The main dietary factors that influence the fatty acid profile of lamb meat are the use of supplemental lipid sources, such as fish oil and vegetables oil, and the use of forages in the diet of the animals.

3.6.1 Fish and vegetables oils

Fish oil, fish meal and some algae products contain significant amounts of long chain n-3 PUFA, in particular EPA and DHA, that have a wide range of beneficial effects for human health (Riediger et al., 2009). Vegetable oils such as linseed, soybean oil, and rapeseed oil contain significant amounts of ALA (Table 6). Significant amount of ALA is present in lush grass. For this reason the main sources of supplementary fatty acids in ruminant rations for increase the content of PUFA n-3 in meat, can be achieved by supplementing the diet of animals with fish oil or vegetables oils.

Table 6 - Some feed sources of n-3 fatty acids.

Fat Source	Fat g/kg DM	LA	ALA	EPA	DPA	DHA
Canola	400	180	90	-	-	-
Linseed	350	160	530	-	-	-
Soybean	180	530	80	-	-	-
Tuna oil	-	15	4	57	-	224
Marine algae	480	-	27	8	120	250
Rye grass fresh	30 -70	150	680	-	-	-

Adapted by Palmquist (2009).

3.6.1.1 Fish oil supplementation

Fish oils are a rich source of very long chain PUFA n-3 and their use as dietary supplements has been the primary means by which attempts to increase the concentration of PUFA n-3 in meats have been made. The inclusion of fish oil or algae in lambs diet increase the content of total PUFA n-3 content especially by an increase in EPA and DHA concentration in the intramuscular fat (Table 7). However, in ruminant, the incorporation of long chain PUFA n-3 in muscle is lower than in monogastric because of the high amount of biohydrogenation of these fatty acids in the rumen (Doreau and Chilliard, 1997). Supplementation of protected marine oil is able to noticeably increase concentration of EPA and DHA in lamb fatty tissues (Kitessa et al., 2001). In addition has been suggested that the degree of rumen biohydrogenation may be lower for algal oil than fish oil (Cooper, 2002; Cooper et al., 2004) and that increase as length of time on feeding increases (Shingfield et al., 2006). In two trial by Ponnampalam et al. (2001, 2002) lambs were fed a fish meal (168 g DM per day) or a barley (179 g DM per day)/fish meal (84 g DM per day) diet and a fish oil or fish oil/sunflower meal diet. These four treatments resulted in a 2- to 4-fold increase in deposition of EPA and DHA in the *Longissimus Dorsi*, while no effect on ALA deposition was observed compared with the alfalfa and oat based control diet. The ratio n-6/n-3 was better in fish oil supplemented group. Similarly Wachira et al. (2002) found that the inclusion of fish oil in the diet tripled proportions of EPA (from 0.7% to 2.3%) and DHA (from 0.3 to 0.8%) in muscle, although reduced DM intake and lamb live-weight gain. Depression of the intake and performances of lambs with fish oil supplementation was also reported by Kitessa et al. (2001) and this is associated with a reduction in microbial growth and efficiency in the rumen. In this trial fish oil increase fat levels, and both EPA and DHA concentration in muscle tissue samples from tuna oil-fed lambs were thrice those in tallow-fed lambs

(1.81% and 0.61% for EPA; 1.51 and 0.44% for DHA). Similarly Cooper et al. (2004), observed that lambs fed diet containing marine algae had the highest percentage of DHA in the phospholipids and this probably due to the lower level of biohydrogenation of algae meat in comparison to fish oil as shown by Cooper et al. (2002). The percentage of EPA was highest in lambs fed the fish/algae diet. A similar pattern for fatty acid profile was reported by Elmore et al. (2005) who reported a 3.5 fold higher content of EPA and a 12 fold higher content of DHA in lambs fed fish algae than in lambs fed protected linseed. More recently Diaz et al. (2011) found that animals fed fish oil had the highest content of PUFA n-3, particularly reported that 100g of meat from lamb fed the fish oil diet provided 183mg of long-chain PUFA n-3 representing 40% of the daily recommended intake. Similar results as in these lamb experiments were obtained when fish meal (Mandell et al., 1997) or fish oil (Choi et al., 2000; Scollan et al., 2001; Richardson et al., 2004; Scollan et al., 2006; Wistuba et al., 2007) was fed to steers and pig (Nurnberg et al., 1999; Jaturasitha et al. 2002).

Despite the improvement in the fatty acid profile, the meat from lambs fed the fish oil diet had high scores of fish odour and flavour and rancid odour and flavour (Díaz et al., 2011).

Table 7 - Effects of fish oil supplementation on PUFA content in lamb's intramuscular fat.

Fatty acid (g/100 g of FA)									
Treatment	*LA*	*ALA*	*EPA*	*DPA*	*ARA*	*DHA*	*PUFA/SFA*	*n6/n3*	*Reference*
Control diet	4.58	0.79	-	-	1.39	0.44	0.13	-	Kitessa et al.,
Protected tuna oil	8.27	1.06	1.81	-	1.64	1.01	0.23	-	2001
Basal diet	2.23	0.67	0.27	0.34	0.70	0.10	0.17	1.80	Ponnampalam
1.5 % FO	2.55	0.68	0.90	0.56	0.77	0.47	0.16	1.40	et al., 2001
1.5 % FO + 9% SO	2.44	0.57	0.77	0.51	0.77	0.44	0.16	1.40	
Basal diet	3.72	0.91	0.47	0.50	1.19	0.20	-	2.45	Ponnampalam
168g DM FM/d	2.97	0.83	0.93	0.65	0.72	0.47	-	1.29	et al., 2002
179 g DM barley/d + 84g DM FM/day	3.56	0.89	0.90	0.63	0.78	0.48	-	1.50	
Linseed oil	2.11	1.79	0.04	0.11	0.04	0.02	-	1.10	Cooper et al.,
FO	1.51	1.51	0.33	0.41	0.07	0.33	-	0.99	2004
PLS + soybean	1.01	3.83	0.09	0.16	0.12	0.03	-	2.49	
FO/Algae	1.96	0.70	0.77	0.78	0.10	1.88	-	0.51	
PLS/Algae	6.15	2.38	0.32	0.36	0.10	1.50	-	1.38	
Fatty acid (mg/100 of muscle)									
Linseed Protected	157	88	24	23	28	7.7	0.17	1.33	Elmore et al.,
Lipid Protected	545	138	21	24	43	5.2	0.47	3.17	2005
Lipid/Algae	396	98	44	27	26	88	0.33	1.66	
FO/Algae	150	29	85	45	19	94	0.11	0.83	
FO	120	29	48	32	19	23	0.10	1.10	
Control	74.6	7.38	0.96	2.03	3.07	0.74	0.09	7.10	Diaz et al., 2011
Linseed	74.9	32.3	1.61	3.82	2.85	2.55	0.14	1.94	
FO	66.3	6.00	15.5	15.3	10.4	41.4	0.14	0.97	
Linseed +algae	75.3	26.8	1.61	3.35	3.04	2.57	0.12	2.28	

FO = fish oil; FM = fish meal; SO = sunflower oil; PLS = protected linseed and soybean.
Data for Cooper et al., (2004) refer to neutral lipid fraction.
Data for Diaz et al. (2011) refer only to neutral lipid fraction.

3.6.1.2 Vegetables oils supplementation

The dietary inclusion of fatty acids must be restricted (to 60 g/kg dry matter consumed) in ruminant to avoid impairment of rumen function (Harfoot and Hazlewood, 1988). In addition, the capacity to manipulate the fatty acid composition of meat by use of ruminally-available fatty acids is limited due to the extensive rumen biohydrogenation of UFA. For example the extent of ALA biohydrogenation in the rumen is very high between 92% and 96% (Glasser et al., 2008), nevertheless a proportion of dietary PUFA bypasses the rumen intact and is absorbed and deposited in tissues (Wood and Enser, 1997; Glasser et al., 2008). The effects of the use of vegetables oils or seeds in lambs

diet can be summarized in Table 8. Wachira et al. (2000) showed that an increase in ALA lamb diet of 20 g/kg DM results in a 2-fold increase in ALA duodenal flow.

These studies demonstrated that linseed oil (rich in ALA) can increase the concentration of ALA, EPA and DHA in lambs tissue with an associated desirable decrease in the n-6/ n-3 PUFA ratio. Similar results were observed when linseed oil was added to beef (Choi et al. 2000; Scollan et al., 2001; Raes et al. 2003) and kids (Nudda et al., 2008) diet. Incorporating linseed rich in lipid and ALA in the diet has been advocated by Wachira et al. (2002), who reported a 2-fold increase in the proportion of ALA in the *Longissimus Dorsi* and in the subcutaneous adipose tissue of lambs fed linseed diet and a slightly higher levels of DPA and DHA. On the other hand linseed inclusion in the diet lowered ARA muscle content, suggesting a competition between this fatty acids for the Δ6-desaturase. Similarly Elmore et al. (2000) reported a 2.2 fold higher content of ALA in muscle of linseed fed lambs compared with control group and a 1.3 fold lower content of LA. In another study a 3-fold greater concentration of ALA in linseed compared with the Megalac diet and an increase in the concentrations of EPA and DPA with the use of protected linseed were observed (Demirel et al. 2004). In addition has been shown that the increase of PUFA n-3 with linseed inclusion in diet is dependent on the level (Bas et al., 2007; Noci et al., 2006) and the duration of dietary inclusion (Kitessa et al. 2009). For example Bas et al. (2007) reported an increase of PUFA n-3 from 0.6 to 2.2% as increase the dose of linseed in the diet from 0% to 9%, due to the increase on ALA, EPA and DHA concentrations. Whereas Kitessa et al. (2009) found that protected linseed oil in lamb's diet was effective in significantly enriching lamb meat with PUFA n-3 in three weeks, but longer periods were required to increase the levels of the LC-PUFA n-3 (EPA, DPA and DHA) which occurred after 6 or 9 weeks of supplementation with linseed oil.

Conversely, supplementation of sunflower seed or sunflower oil (Mir et al., 2000; Manso et al., 2009), soybean oil (Santos-Silva et al., 2004; Bessa et al., 2005, 2008) and safflower oil (Boles et al., 2005) that are rich in LA acid can increase the concentration of *cis*-9, *trans*-11 CLA and LA in tissue but with an associated increase in the n-6/n-3 PUFA ratio. In addition Santos-Silva et al. (2004) reported that the effect of soybean oil inclusion on fatty acid composition was highly dependent on forage particle size. The content of LA was increased by about 1.6 fold with pellets and 1.2 fold with hay in the diet while the content of ALA decreased about 2 fold with hay and 1.5 fold with pellets. The ratio n-6/n-3 fatty acids increased with oil supplementation both with pellet and hay but with pellets, the value was above the suggested threshold of 4. In another study Bessa et al. (2008) reported that content of LA, ALA and RA increases with a soybean oil diet with the increasing of finishing period. In the same study the authors reported that a short finishing period with a soybean oil diet was highly effective in increasing RA concentration in lamb meat lipids. Moreover, gradually dietary replacement of sunflower oil with linseed oil increased about 3.3-fold the content of ALA, 2.6 fold the content of EPA and about 1.5 fold the content of DHA (Jeronimo et al. 2009). On the other the RA decrease about 1.4 fold and LA decrease about 1.6 fold with linseed diet.

Table 8 - Effects of vegetables oil supplementation on PUFA content in lamb's intramuscular fat.

	Fatty acid (g/100 g of FA)									
Treatment	*LA*	*ALA*	*ARA*	*CLA*	*EPA*	*DPA*	*DHA*	*PUFA/ SFA*	*n6/n3*	*Reference*
0% Linseed	3.9	0.48	0.95	0.07	0.17	0.21	0.06	0.16	5.8	Bas et al.,
3% Linseed	3.7	0.78	0.66	0.08	0.15	0.20	0.06	0.15	3.9	2007
6% Linseed	4.2	1.08	0.81	0.12	0.23	0.25	0.07	0.18	3.2	
9%Linseed	4.2	1.34	0.78	0.08	0.24	0.26	0.07	0.18	2.8	
Control diet	9.61	0.56	4.43	0.05	0.25	0.63	0.14	0.47	9.9	Berthelot
Extruded linseed (L)-Wheat	10.61	2.47	4.11	0.11	0.60	0.83	0.21	0.56	3.9	et al. , 2010
Extruded linseed (L)-corn	9.31	2.21	3.42	0.12	0.58	0.75	0.19	0.49	3.7	
Lucerne	7.2	2.69	1.87	0.85	0.62	0.88	0.23	-	2.0	Bessa et
Lucerne+10% SO	9.3	1.16	1.07	2.39	0.14	0.35	0.04	-	6.1	al., 2005
Concentrate	6.4	0.36	1.47	0.55	0.15	0.36	0.17	-	7.7	
Concentrate+10% SO	9.5	0.61	1.83	0.44	0.23	0.53	0.17	-	7.5	
Control diet	5.99	0.44	4.04	0.43	0.51	0.80	0.42	0.31	-	Manso et
4% hydrogenated palm oil	5.87	0.40	3.97	0.59	0.58	0.96	0.35	0.30	-	al., 2009
4% sunflower oil	5.75	0.30	3.74	0.56	0.63	0.93	0.47	0.32	-	
0 W protected LO	5.78	0.92	2.26	1.04	0.69	0.35	0.08	-	3.94	Kitessa et
3 W protected LO	7.51	1.32	1.81	1.13	0.48	0.41	0.20	-	3.87	al., 2009
6 W protected LO	8.22	1.67	1.87	1.06	0.72	0.43	0.15	-	3.40	
9 W protected LO	9.85	1.67	2.26	1.08	0.79	0.48	0.26	-	3.78	
Control	3.3	0.31	-	0.43	0.10	-	0.06	0.13	-	Bolte et
High oleate safflower seed	2.9	0.48	-	0.65	0.11	-	0.07	0.12	-	al., 2002
High linoleate safflower seed	4.5	0.37	-	0.68	0.08	-	0.05	0.17	-	
lucerne hay (H)	5.91	2.50	2.51	0.55	0.980	1.36	0.46	-	1.62	Santos-
H +soybean oil (SO)	7.16	1.27	1.50	2.37	.33	0.73	0.25	-	3.38	Silva et al.,
P	6.42	1.62	1.61	0.64	0.44	0.75	0.35	-	2.56	2004
P+SO	10.81	1.11	1.37	1.83	0.13	0.50	0.18	-	6.58	
CCO	9.44	0.82	2.582	0.75	0.36	0.66	0.24	0.29	5.97	Bessa et
COO	13.3	1.01	.96	1.21	0.36	0.69	0.21	0.43	7.21	al., 2008
LLO	13.9	1.17	3.92	1.28	0.61	1.03	0.29	0.45	5.81	
LOO	14.8	1.14	3.60	1.47	0.52	0.90	0.26	0.49	6.56	
100% SFO	9.36	0.93	2.45	2.13	0.19	0.46	0.14	0.30	7.04	Jeronimo
66% SFO+ 33.3%LO	7.52	1.57	1.91	2.06	0.29	0.54	0.15	0.25	3.78	et al., 2009
33.3% + 66% LO	6.74	2.62	1.87	1.84	0.51	0.62	0.19	0.27	2.26	
100 % LO	5.09	3.05	1.52	1.56	0.50	0.54	0.20	0.24	1.60	
	Fatty acid (mg/100 of muscle)									
Control	143	43.7	29.1	-	23.3	20.3	8.5	-	-	Elmore et
Linseed	109	96.3	19.8	-	26.5	21.5	10.2	-	-	al., 2000

SO = soybean oil; P = ground and pelletted lucern; LO = linseed oil; SFO = sunflower oil, W=week. CCO=concentrate+lucerne meal+10%SO/2W; COO=concentrate+lucerne meal+10%SO/4W; LLO= lucerne+lucerne meal+10%SO/2W LOO=lucerne+lucerne meal+10%SO/4W

3.6.2 Pasture

Plants are the primary source of PUFA n-3 and have the ability to synthesise de novo ALA which is the building block of the n-3 series of essential fatty acids and elongation and desaturation of this fatty acid results in the synthesis of EPA and DHA. In Table 9 is reported fatty acid composition of different forage species (Claphman et al., 2005; Pulina et al., 2003).

Table 9 - Fatty acid composition in different forages (means of 3 harvests.)

	Fatty acid (% of total lipid)							
Forages	*C12*	*C14*	*C16*	*C16:1*	*C18*	*C18:1*	*LA*	*ALA*
Triticale	0.05	0.40	4.10	0.69	0.19	0.69	3.76	20.73
Perennial ryegrass	0.05	0.62	6.42	0.75	0.30	1.06	5.99	31.00
White clover	0.05	0.45	5.66	0.78	0.48	1.17	6.80	21.60
Chicory	0.02	0.41	6.02	0.90	0.23	0.66	7.58	28.83
Borage	0.01	0.30	5.42	0.50	0.58	1.40	5.07	24.80
Plantain	0.00	0.43	5.21	0.70	0.36	0.47	6.45	21.30
Natural pasture	-	-	12.92	-	1.03	2.05	10.57	60.36

Data from Clapham et al. (2005); Pulina et al., (2003).

The lipid fraction of forages is characterized by a large proportion of glycolipids (80%) and phospholipids and is rich in unsaturated fatty acid especially LA and ALA with ALA the most abundant (Cabiddu et al., 2005; Clapham et al., 2005). Because of its presence in glycolipids within the cell structure, ALA is more resistant to rumen hydrolysis and less susceptible to biohydrogenation than lipid in oilseeds that are predominantly triglycerides (Givens et al., 2006). The effects of pasture on CLA and PUFA n-3 contents in meat depend by the seasonal variation of quality forages, by the species whose pasture is composed and their fatty acid composition (Dewhurst et al., 2001); for example legumes are richer in ALA compared to grasses (Table 10).

Table 10 - Content of LA and ALA in grasses and legumes (% of total fatty acid).

	Specie	*LA*	*ALA*
Grasses	*Lolium rigidum*	11.57	62.45
	Lolium multiflorum	13.18	61.33
Legumes	*Medicago polymorpha*	14.88	63.92
	Hedysarum coronarium	9.04	63.52
	Trifolium subterraneum	14.16	72.30

Data from Cabiddu et al. (2005); Chiofalo et al. (2010).

The transfer of ALA from forage to meat is dependent on two processes (Palmquist et al., 2005): 1) increasing the level of ALA in the forage and 2) reducing the extent of ruminal biohydrogenation.

Generally grass-based systems increased concentrations of ALA and EPA compared with indoor feeding system, conversely concentrates, rich in LA, lead to higher concentrations of LA and its longer chain derivatives such as ARA (Table 11).

For example Santos-Silva et al. (2002) found a 3.3-fold higher content of ALA, a 2.8 fold higher content of RA and a 2.4 fold-higher content of EPA in lambs fed on pasture compared with concentrate fed lambs. Similarly, content of ALA, EPA and DHA was increased by about 3.3, 3.3 and 2 fold respectively in grass fed lambs compared with indoor fed ones (Fisher et al., 2000). The increase of the dried grass percentage in the ration increased the proportion of ALA, EPA and DHA in lamb meat (Demirel et al. (2006). A marked increase in ALA in meat of grazing compared to indoor lambs was found by Velasco et al. (2001) and by Nuernberg et al. (2008) and was about1.7 and 1.5 fold, respectively. Nuernberg et al. (2008) observed also that the RA content was 2 fold higher in grazing lambs than in concentrate fed ones. Conversely Joy et al. (2008) found no significant differences in fatty acid composition between pasture and indoor fed lamb probably due to the short raising period and by the similar amount of intramuscular fat in both treatments. The main effects of grazing on fatty acid profile, were not removed by a short period of finishing indoor with concentrate. However, as increase period of finish

indoor the contents of ALA, RA, EPA and DHA were brought to the levels observed in the group reared indoor during both growing and finishing period (Aurousseuu et al., 2007). Those results are in agreement with those reported in beef (Nuernberg et al., 2005; Scollan et al., 2006; Leheska et al., 2008; Garcia et al., 2008; Alfaia et al., 2009; Daley et al., 2010), and goat (Bas et al., 2005) reared under different feeding system.

Table 11 - PUFA and CLA content in muscle of lambs fed pasture.

	Fatty acid (g/100 g of FA)									
Treatment	*LA*	*ALA*	*CLA*	*EPA*	*DPA*	*ARA*	*DHA*	*PUFA /SFA*	*n6/n3*	*Reference*
Drylot (maize soybean – wheat)	2.63	1.14	-	-	-	0.32	-	0.10	-	Rowe et al., 1999
Grazing	3.85	0.20	-	-	-	0.21	-	0.10	-	
Grass	6.8	2.3	-	1.3	1.5	2.6	0.6	-	-	Fischer et al., 2000
Concentate	9.7	0.7	-	0.4	0.8	3.3	0.3	-	-	
Pasture	5.89	4.30	-	-	-	2.33	-	0.35	1.39	Velasco et al., 2001
Drylot	5.76	2.60	-	-	-	1.09	-	0.27	2.01	
Pasture	4.98	1.69	0.79	0.97	1.11	2.63	0.48	-	1.85	Santos-Silva et al. 2002
Pasture+concentrate	5.94	1.30	0.65	0.74	0.97	2.62	0.42	-	2.59	
Concentrate (C)	6.28	0.51	0.28	0.40	0.66	2.67	0.29	-	5.47	
Indoor	7.18	1.95	-	-	-	-	-	0.21	-	Joy et al., 2008
Grazing with dams	8.16	1.72	-	-	-	-	-	0.23	-	
Grass hay	4.65	2.27	-	1.23	0.77	1.26	0.38	0.16	1.28	Demirel et al., 2006
Concentrate	10.7	0.72	-	0.60	0.31	2.17	0.17	0.26	7.11	
Grass (G)	5.8	2.6	1.1	1.8	2.4	2.7	0.6	-	1.3	Aurousseau et al., 2007
G + finishing indoor (22 d)	5.7	1.7	1.0	1.3	1.8	2.3	0.5	-	1.7	
G + finishing indoor (41 d)	5.7	1.2	0.9	1.2	1.5	2.5	0.5	-	2.2	
Indoor	6.4	1.3	0.7	1.0	1.4	2.6	0.5	-	2.4	
Mountain pasture	9.23	3.27	-	1.48	-	4.01	-	0.55	2.1	Cividini et al., 2008
Hay + C *ad libitum*	6.28	2.88	-	1.08	-	1.92	-	0.34	1.6	
Pasture + C	16.29	3.09	-	1.84	3.03	7.72	0.66	0.99	3.01	Popova, 2007
C + Lucerne hay	19.02	1.15	-	0.87	2.37	8.82	0.35	1.01	6.61	
	Fatty acid (mg/100 of muscle)									
Concentate	110.9	18.6	11.3	12.7	14.5	46.5	6.8	0.30	3.2	Nuernberg et al., 2008
Pasture	97.7	27.2	21.3	15.3	15.1	48.2	5.5	0.28	2.4	

Data for Popova et al., (2007) refer only to phospholipid fraction.

Pasture composition – Levels of PUFA in meat are o markedly affected by botanical composition of the diet in lambs fed different pasture mixtures. For example, Chiofalo et al. (2010) reported a higher PUFA n-3 content in *Longissimus Dorsi* of lamb grazing on *Trifolium subterraneum* compared to lamb grazing on *Lolium multiflorum* (4.49 and 3.99%, respectively) due to the higher content of ALA (1.97 and 1.52%, respectively). This is due to the different fatty acid profile of grasses and legumes as a matter of fact legumes are richer in ALA than grasses. Adnoy et al. (2005) compared the quality of meat from Norwegian Crossbred lambs grazing on mountain (1000 m above sea level) with meat from lambs grazing on cultivated lowland pastures and found the a significant increase of PUFA content as altitude increases. A similar pattern was reported by Lind et al. (2009) who found small but significant increase in PUFA content in mountain pasture compared to cultivated pasture (3.2% and 2.9%) and suggests that pre-slaughter fattening on cultivated pastures alters meat characteristics. Seasonal variation of quality of forages, can also influence fatty acid profile of meat as reported by Mazzone et al. (2010) who found a 2-fold higher content in ALA and RA in autumn's lambs muscle compared to winter's lamb due to the differences in diet through different rearing season. The fact can be explained by a seasonal variation in ewe's milk fatty acid profile that reflect seasonal variation in fatty acid composition of forage (Nudda et al., 2005; Mel'uchová et al., 2008). In fact in Mediterranean area the decrease in forage quality normally occurs as pasture plants mature from the vegetative to the reproductive stage, as indicated by the decrease of more than 50% in ALA content (Nudda et al., 2003).

3.6.3 Milk composition

In Mediterranean areas milk-fed lamb (slaughtered at approximately 20 kg body weight) is popular and differences in their meat fatty acid profile tended to reflect the fatty acid profile of the of the mother's milk (Scerra et al., 2007; Valvo et al., 2005; Velasco et al., 2001; Serra et al., 2009). Therefore, changing the fatty acid profile of the dam's milk by dietary means could also change the fatty acid composition of suckling lamb's meat as reported also in suckling kids by Nudda et al. (2008). In general PUFA n-3 and CLA concentration are higher in milk from ewes fed pasture versus those fed dry diets (Nudda et al., 2003; Valvo et al., 2005; Atti et al., 2006; Scerra et al., 2007). Scerra et al., (2007) evaluate the effects of ewe dietary treatments on the intramuscular fatty acid composition of suckling lambs. The author reported that intramuscular fat of grazing lambs shown a greatest content of ALA, EPA and DPA in comparison with stall fed lambs, reflecting the higher content of ALA in milk of grazing sheep in comparison with stall fed sheep (4.5 and 2.6%, respectively). Moreover, the intramuscular fat from pasture-fed lambs displayed a higher PUFA/SFA ratio and a lower n-6/n-3 ratio. Similarly Valvo et al. (2005) found a 3-fold higher ALA in fat of suckling lambs from the grass ewes compared with lambs suckled by ewes fed concentrates (13.23 and 4.24%, respectively). On the other hand LA and ARA content were at higher concentration in the fat from lambs raised by ewes fed concentrates compared to the grass group.

As mentioned above, in Mediterranean areas the typical dairy sheep production system is based on breeds specialized to produce milk and ewe milking commences soon after weaning or slaughtering of lambs. To increase milk availability for cheese production and to allow the survival of lambs, the artificial rearing of lambs has been developed even though the fatty acid profile of these two milk sources have shown substantial differences (Table 12).

Table 12 - Fatty acid composition of ewes' milk and milk-replacer.

	Ewes' Milk	Milk – replacer
Fatty acid (g/100 of FA)		
C16:0	24.4	19.7
C18:0	11.2	11.9
C18:1 *trans*-11 (VA)	2.54	1.56
C18:1 *cis* – 9	24.7	29.0
C18:2 n-6 (LA)	2.85	7.46
C18:3 n-3 (ALA)	1.72	0.58
SFA	62.90	56.47
MUFA	29.05	33.36
PUFA	7.66	10.30
n6/n3	1.97	13.9

Data from Napolitano et al. (2002); Lanza et al. (2006), Osorio et al. (2007).

In general the milk substitute showed a lower content of SFA and a higher content of MUFA and PUFA compared with sheep milk, and this is because most of the fat components of milk substitutes are derived from vegetable oils, which are characterized by a lower level of saturation compared to animal fats. Substantial differences between milk and replacer are for odd and branched chain fatty acid (OBCFA) which are 3-10 fold higher in milk (Osorio et al., 2007).

The impact of a milk feeding regime on lamb meat quality is important because in this phase, lambs are "functional monogastric", so there is no ruminal biohydrogenation of the milk fatty acids before they are absorbed from the intestine. In suckling lambs (Napolitano et al., 2002; Valvo et al., 2005; Scerra et al., 2007; Lanza et al., 2006; Osorio et al., 2007) and kids (Bañon et al., 2006; Sanz Sampelayo et al., 2006) reared with artificial or natural milk, differences in their meat fatty acid profile tended to reflect the fatty acid profile of the suckled milk (Table 13). Napolitano et al. (2002) showed that animals fed with mother milk had significantly more fat and a higher content of SFA, ALA and a two- to three fold higher content of EPA and DHA compared to artificially reared lamb meat. A similar pattern was observed in Barbaresca suckling lambs by Lanza

et al. (2006) and in Churra suckling lambs by Osorio et al. (2007). The authors concluded that a feeding regime exclusively based on artificial milk adversely affected the dietetic value of lamb meat. compared to a natural rearing system, reducing the level of desirable fatty acids such as n-3 series and CLA.

Table 13 - Fatty acid profile of muscle of lamb reared with different milk sources (g/100 of total fatty acid).

	Fatty acid (g/100 of total fatty acid).									
Milk source	*LA*	*ARA*	*ALA*	*CLA*	*EPA*	*DPA*	*DHA*	*PUFA/SFA*	*n6/n3*	*Reference*
Ewes's milk	4.05	1.09	1.37	-	0.62	0.91	0.45	0.25	1.95	Napolitano et al., 2005
Milk repalcer	8.48	2.07	0.42	-	0.18	0.49	0.17	0.31	9.54	
Natural milk	10.97	6.89	1.95	1.13	1.65	2.34	1.25	0.73	2.61	Lanza et al., 2006
Artificial milk	18.47	10.00	0.37	0.47	0.80	1.18	0.53	0.96	9.70	
Ewes'milk	8.44	4.86	1.30	0.51	1.44	-	-	0.49	5.23	Osorio et al., 2007
Milk replacer	8.01	2.36	0.12	0.67	0.30	-	-	0.48	16.32	

Data for Osorio et al., (2007) refer only to intramuscular fat.

CHAPTER 4

Fatty acid and human health

Fatty acid and human health

Fatty acids have different effects on human health, some beneficial and some adverse, and for this reason is important to determine the potential benefits or adverse effects of fat on health. In this chapter, is summarized the literature about the the effects of the different fatty acids present in animal foods on human health.

4.1 Saturated fatty acid (SFA)

Since decades, lipids from animal foods are a target of dietician's criticism due to the negative effects of excessive consumption of SFA on human health and, for this reason in most developed countries, dietary guidelines produced have proposed reductions in total fat and in SFA intake as a means of reducing the prevalence of coronary heart disease (CHD). The content of SFA of the lipid of meat and, especially, in ruminants is due to the regulating mechanisms implemented by rumen microorganisms. Indeed, in the rumen diverse populations of microorganisms with their metabolic activity, alter the molecular form of dietary food to ensure its survival. National and international dietary guidelines have recommended that saturated fatty acids should contribute no more than 10% dietary energy (COMA, 1991, COMA, 1994; FAO/WHO,1998). These recommendations have been based on clinical and epidemiological old studies that demonstrated that this lipid class contribute to the increase of plasma low density lipoprotein (LDL) and raising the ratio LDL/HDL (high density lipoprotein) cholesterol (Keys et al., 1965; Hegsted et al., 1965) and, therefore, be considered as predisposing factors for cardiovascular disease (CVD). The main SFA in red meat are palmitic (C16:0) and stearic acid (C18:0). Not all SFA were considered to have the same effect

on blood cholesterol: mainly lauric (C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids which are responsible for increasing plasma total and LDL cholesterol concentrations while stearic acid (C18:0) not appear to affect blood concentrations of cholesterol (Keys 1965; Bauman at al., 1999). Not all saturated fatty acids have the same cholesterol elevating effect and myristic acid is thought to be the most atherogenic and has four times the cholesterol raising potential of palmitic acid (Ulbricht and Southgate, 1991). Whilst the other major SFA, stearic acid has been shown not to increase total cholesterol or LDL-cholesterol concentrations (Yu et al., 1995; Grundy, 1994; Mensink, 2005), this is because in human tissues is active the enzyme Δ9 desaturase which is able to convert about 40% of stearic acid in oleic acid (Bauman et al., 1999; Chilliard et al., 2001).

Increasing interest by scientists is for the branched chain and the odd chain fatty acid (OBCFA) because of their anticarcinogenic effects on cancer cells (Wongtangtintharnet al., 2004). The highest biological activity was observed with iso-16:0, and the activity decreased with increase or decrease of the chain-lengths from C16:0. Anteiso-BCFA, as well as iso-series, was cytotoxic to the breast cancer cells. Cytotoxicity of BCFA was comparable to that of conjugated linoleic acid (CLA) known as anti-tumoral fatty acid. BCFA has been reported to suppress the proliferation or development of tumor cells (Wongtangtintharnet al., 2004). A previous study reported that iso-C15 saturated FA purified from a soy-fermentation product inhibited cell proliferation and induced apoptotic cell death in many cancer cells such as prostate carcinoma, leukemia and mammary adenocarcinoma (Yang et al., 2000). The lowering of the fatty acid biosynthesis by reducing the precursors, in addition to a direct inhibitory effect on FA synthetase enzyme has been suggested as putative mechanism for the anti-tumoral activity of BCFA (Wongtangtintharn et al., 2004).

These fatty acid are detectable in appreciable amount only in milk and meat from ruminants, especially small ruminants because of their presence in membrane lipids of rumen bacteria (Vlaemink et al., 2005).

4.2 Monounsaturated fatty acids (MUFA)

In meat, MUFA are composed mainly by oleic acid (C18:1 *cis*-9). Several studies reported that MUFA are able to reduce the level of serum LDL cholesterol, but, contrary to PUFA n-6, without effects on HDL cholesterol (Ulbricht and Southgate, 1991). The protective role MUFA against various diseases is related to the maintenance of the functional integrity of cell membranes (Ackman, 1999). In light of this beneficial effect on serum cholesterol, foods that contain high amounts of oleic acid can be defined as a functional food in relation to the reduction of cardiovascular disease (Hornstra, 1999). Oleic acid can come either from dietary sources or endogenous synthesis by the action of the enzyme complex Δ9- desaturase (stearoyl-CoA desaturasi) which converts about 50% of stearic acid in oleic acid. For this reason has increased in recent years the potential for reducing SFA through substitution with MUFA because improvements in animal feeding technology has made it possible to alter the fatty acid composition of milk and meat fat by altering the fatty acid composition of animal diets. Dairy products produced using this approach have been shown to successfully reduce blood cholesterol levels in human volunteers (Noakes et al., 1996).

4.3 Trans fatty acid (TFA)

Various epidemiological studies have shown a positive correlation between dietary intake of TFA and the development of cardiovascular diseases (Mensink et al., 1990; Sanders, 1998; Williams, 2000; Combe et al., 2007). Several clinical studies have in fact shown that TFA induce an increase in LDL and a decrease of HDL, affecting the ratio LDL/HDL cholesterol (Nestel et al., 1992, Mensink et al. 2003; Mozzafarian et al., 2006). However, there are more scientific evidence that show that increases in LDL cholesterol in the blood are dependent on the isomer that is considered (Chadigny et al., 2008), for that reason, is important to know if the origin of trans fatty acids is natural or industrial.

Trans fatty acid are found in ruminant fats (dairy products, beef, lamb) as intermediates during the biohydrogenation of dietary PUFA by rumen bacteria *Butyrivibrio fibrisolvens*, and in partially hydrogenated vegetable oils poduced during industrial hydrogenation process. The distribution of trans positional isomers differs somewhat in fats originating from industrial hydrogenation, where predominantly isomer is elaidic acid (C18:1 *trans*-9), whereas ruminant biohydrogenation results in a predominance of vaccenic acid (VA, C18:1 *trans*-11) that varied from 14 to 72% of total TFA (Chilliard , et al., 2001; Ledoux et al., 2007; Steijns, 2008). Only few studies have investigated the effect of natural sources of trans fatty acid on human health, but preliminary results seem to indicate that TFA from natural sources could not contribute to increased CVD risk (Chardigny et al., 2008). Moreover, humans can utilize VA in the endogenous synthesis of RA (Adlof et al., 2000; Steijns, 2008).

4.4 Conjugated linoleic acid (CLA)

Conjugated Linoleic Acid (CLA) is a collective term for different positional and geometric isomers of octadecadienoic acid which contain a pair of double bonds in a conjugated configuration (Sehat et al., 1999; Dhiman et al., 2005). Many forms are possible, and the isomers until now identified in ruminant milk and meat are: 7-9, 9-11, 10-12, 11-13, 12-14 with a geometry that can be *cis*-*cis*, *cis*-*trans*, *trans*-*cis* and *trans*-*trans*. Among the different isomers, the more attention is focused on *cis*-9, *trans*-11 (Rumenic acid, RA) and *trans*-10, *cis*-12 CLA. The CLA found in milk and meat of ruminants is obtained by two ways (Griinari and Bauman, 1999): the manner is an incomplete biohydrogenation of PUFA in the rumen and the latter is the conversion of VA in animal tissues.

Studies on laboratory animals demonstrate that CLA has potential anticarcinogenic, immunomodulating and antiatherosclerotic effects (Pariza, 2001).

4.4.1 Anticarcinogenic effect of CLA

CLA has been shown to be effective in animal models of mouse skin and forestomach carrcinogenesis and tumorigenesis (Ha et al., 1987; Ha et al., 1990) and of mammary gland in rat (Belury, 1995). CLA seems to have potential effects at many stages of cancer development, including tumorigenesis, promotion, mitogenesis, mutagenesis, carcinogen activation, detoxification and signal transduction (Scimeca et al., 1994). CLA was found to be effective in reducing the growth of human breast cancer (Wahle and Heys, 2002; Wahle et al., 2004); prostate cancer (Cesano et al., 1998; Whale et al., 2004) and colon cancer cells (Beppu et al., 2006). Ip et al. (1991) found that administration of butter with different doses of CLA (0.5%, 1.0% e 1.5%) during

mammary gland maturation period induced an inhibition of mammary tumors induced with 7,12-dimethykbenz(a)anthracene (DMBA) of 32%, 56% e 60%, respectively, in a dose dependent way until concentrations of 1%; while higher concentrations did not result in a further effect. However, continuous intake of CLA is required for inhibition of tumorigenesis (Ip et al., 1995). Incubation of MCF-7 breast cancer cells with a mixture of CLA isomers or the isomer *cis*-9, *trans*-11 CLA resulted in a reduction of cell proliferation by 60%. (Durgam et al., 1997). However, the mechanism of CLA action in the prevention of cancer is still under study

4.4.2 Anti-atherosclerotic effects of CLA

Atherosclerosis is a cardiovascular disease which is the main cause of mortality and morbidity in developed countries. Anomalies of plasma lipoproteins and of lipid metabolism are the better known risk factors for atherosclerosis. The incorporation of CLA in the diet has been shown to exert different responses on lipid profiles, and antiatherogenic activity of the most important isomers (*cis*-9, *trans*-11 and *trans*-10 *cis*-12) has been well documented in various animals models on which, administration of diets enriched with CLA, caused a decrease of plasma LDL cholesterol and thus decrease the formation of atheromatous plaques. Although the complete mechanism is not yet known, probably the two isomers of CLA, through competition with ARA which is responsible for the synthesis of factors promoting aggregation of atheromatous plaques (TXA2; thromboxane A2), inhibits cyclooxygenase, an enzyme that is very active in the ARA cascade (Pariza et al., 2001). Lee et al. (1994) providing rabbits an atherogenic diet enriched with CLA (0.5g CLA/rabbit/d) showed a reduction of atherogenesis and of lipid deposition compared with control group. Administration of CLA for 12 weeks resulted

in a substantial plaque regression and in a decrease of LDL cholesterol and triglycerides. Similarly, hamsters fed a hypercholesterolemic diet enriched with CLA (1% of diet) showed a reduction of aortic plaque (Nicolosi et al., 1997). Subsequently, a study in mice fed CLA reported an increase in the serum HDL-cholesterol/total cholesterol ratio and lower serum triglycerides but increased development of the aortic fatty streaks (Munday et al., 1999). One of the possible mechanisms for the action of CLA in animal models is due to the fact that it can reduced secretion of apolipoprotein-B. Studies that have evaluated the effects of CLA on cardiovascular risk factors in humans are relatively few and result are controversial. Benito et al. (2001) found no changes in plasma levels of lipids or lipoproteins after consumption of 3.9 g CLA/d containing 11. 4% of *cis*-9, *trans*-11. No effect on blood triglycerides, total cholesterol and HDL-cholesterol was observed after the administration of 2.1 g CLA/d for 45 days in sedentary non-obese young women (Petridou et al., 2003). By contrast, Mougios et al. (2001) observed that supplementation of 0.7 g/d of CLA for 4 weeks resulted in the reduction of total cholesterol, triglycerides and HDL cholesterol. Noone et al. (2002) reported that the administration of mixtures of CLA isomers containing 50% of *cis*-9, *trans*-11 and 50% *trans*-10, *cis*-12 resulted in the reduction of plasma triglycerides in normolipidemic subjects, in addition the use of a mixture containing 80% of *cis*-9, *trans*-11 and 20% *trans*-10, *cis*-12 also reduced the levels of low density lipoprotein (VLDL). Another study showed that levels of triglycerides and LDL/HDL ratio increased by administration of *trans*-10, *cis*-12 and decreased by administration of *cis*-9, *trans*-11 CLA (Tricon et al., 2004). Animal studies have shown that consumption of CLA prevents or reduces the development of atherosclerotic lesions preformed. However results from human studies showed that the beneficial effects of CLA on cardiovascular risk factors, such as plasma

lipids and lipoproteins are strongly related to the type of isomer administered. Further studies are needed to determine clearly the anti-atherosclerotic effects of CLA.

4.4.3 Effects of CLA on adipose tissue

CLA seems to have anti obesity properties. Park et al., (1997) observed that administration of 0.5% of CLA in rats (50% *cis*-9, *trans*-11 and 50% *trans*-10, *cis*-12) caused the reduction of body fat and increasing lean body mass. West et al. (1998) administered to mice 1.5g CLA/kg of body weight (equal to 1% of the diet) for 6 weeks and observed a reduction of body weight of 10% and a reduction of body fat of 70% compared to placebo. The fat reducing effect was associated with the n-10, *cis*-12 CLA isomer. The effects of CLA on body composition have been documented in studies on pigs (Dugan et al. 1997; Ostrowska et al., 1999), mice (Hargrave et al., 2004); and rats (Azain et al., 2000).

Effects of CLA on human body composition are controversial. Administration of 0; 1.7; 3.4; 5.1 or 6.8 g/d of CLA for 12 weeks showed that 3.4 g/d of CLA seems to be sufficient to reduce body fat mass significantly in overweight and obese humans (Blankson et al., 2000). On the contrary Malpuech-Brugère et al. (2004) did not observed differences between the groups treated for 18 weeks with the dose of 1.5 and 3 g/d of *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA.

Regarding the CLA effects on body composition, the observations in human trials markedly differ by those reported for laboratory animals. These differences may probably due to the higher metabolic rate of mice compared with humans (Terpstra, 2001). Mechanism through which CLA reduces body weight and fat deposition remain to be fully understood. The effects of *trans*-10, *cis*-12 seems to be specie dependent. In mice

the effects of CLA are generally of greater magnitude (40-80% reduction in fat mass) compared with rat. The activity of *trans*-10, *cis*-12 CLA seems to be under the control of several enzymes that play a central role in lipid metabolism, such as stearoyl-Coenzyme A desaturase (SCD1) and lipoprotein lipase (LPL) involved in fat deposition and mobilization and oxidation of fat reserves. *Trans*-10, *cis*-12 CLA seems to inhibit SCD1 mRNA expression in epatic cells in mice (Lee et al., 1988) and in adipose tissue in pigs (Smith et al., 2002). A similar effects was observed in adipocytes, where trans-10, cis-12 CLA leads to production of smaller fat globules (Choi et al., 2000). In other experiments on mice, the CLA has been shown to reduce both the number and size of adipocytes (Bouthegourd et al., 2002; Xu et al., 2002). While the mechanism by which the *trans*-10, *cis*-12 CLA reduces the activity of LPL, however, is unknown.

4.4.4 Effects of CLA on immunity system

Immunological studies indicate that CLA may have implications in immunity system.. Key mediators of the inflammatory response are represented by pro-inflammatory cytokines (TNF-α, IL-6, IL-1, etc.) and by anti-inflammatory cytokines (IL-10, INF-γ, etc.). In vitro studies have shown that CLA can induce a significant inhibition of T cell proliferation and increased expression of IL-2 and INF-γ (Luongo et al., 2003). In another study, a mixture of CLA isomers and the isomer *cis*-9, *trans*-11 were able to inhibit production of TNF-α, while the isomer *trans*-10, *cis*-12 showed no effect (Yu et al., 2002). In some animal studies, CLA was able to inhibit pro-inflammatory cytokines. Male mice fed 1% of CLA for 8 weeks showed a reduced blood concentrations of TNF-α and leptin levels compared to mice fed a diet enriched in linoleic acid (Akahoshi et al., 2002). By contrast, another study conducted on the same group of male rats fed different

diets and 1% CLA for 3-4 weeks showed no effect on serum leptin and TNF-α, although epididymal perirenal fat declined significantly (Sugano et al., 2001). Only few studies in humans have been conducted. However the dietary CLA supplementation (3 g/d, 50:50 mix of the two major isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12) affected beneficially the immune function in healthy human volunteers as expressed by decreased the levels of proinflammatory cytochines TNF-α and IL-1β and increased levels of the antinflammatory cytokine IL-10 (Song et al., 2005). A similar trial that used a mix of CLA enriched in *cis*-9, *trans*-11 CLA (80%) reported that CLA supplementation had minimal effect on the markers of human immune function and had no immunological benefit compared with linoleic acid (Nugent et al., 2005).

4.4.5 CLA effects on haematic insulin

The effects of CLA on blood concentration of insulin are contrasting for different species. The administration of CLA in mice showed antidiabetic effects (Belury and Vanden Heuvel, 1999; Ryder et al., 2001). By contrast, other researchers demonstrated that a mixture containing 36% of *trans*-10, *cis*-12 CLA (Tsuboyama-Kasaoka et al., 2000) and only the isomer *trans*-10, *cis*-12 CLA (Clément et al., 2002; Roche et al., 2002) induced lipodistrophy and insuline resistance in mice. Glucose serum concentration was higher in hamster fed a CLA mixture (Bouthegourd et al., 2002) than hamster fed only the isomer *trans*-10, *cis*-12 CLA (Simon et al., 2006). These results were confirmed in humans. A trial that involved women in good health status showed that administration of a mixture containing 23% *trans*-10, *cis*-12 CLA resulted in an increase in blood levels of insulin (Medina et al., 2000). The administration of the isomer

trans-10, *cis*-12 CLA resulted in an increase in insulin resistance and dyslipidemia in obese subjects (DeLany et al., 1999; Tsuboyama-Kasaoka et al., 2000).

The mechanisms by which this isomer induced insulin resistance are not yet known.

4.5 Polyunsaturated fatty acid (PUFA)

Polyunsaturated fatty acids (PUFA) are classified as n-3 and n-6 and both types of fatty acids are obtained through diet. In westernized diets LA is the most abundant PUFA followed by ALA. Major sources of n-6 fatty acids are vegetable oils whereas n-3 fatty acid sources are fish and meat (Given and Gibbs, 2006; Schmitz and Ecker, 2008). Because these two fatty acids cannot be synthesized in mammals, they are defined as essential fatty acids (EFA). Moreover these fatty acid play important functions. LA is important for the impermeability of the epidermis (Downing, 1992), while ALA has an important role in the prevention of heart disease, cancer and disorders of neurological function in both children and adults (Horrobin and Bennet, 1999; Barcelò – Coblijin and Murphy, 2009). PUFAs are also precursors of eicosanoids (prostaglandins, prostacyclin, thromboxanes, leukotrienes) involved in many physiological processes such as blood clotting and inflammatory response. The difference between n-3 and n-6 fatty acid-derived eicosanoids is that most of the mediators formed from EPA and DHA are anti-inflammatory, whereas those formed from ARA are pro-inflammatory or show other disease- propagating effects (Bagga et al., 2003; Schmitz and Ecker, 2008; Siddiqui et al., 2008). For these reasons is important to maintain a balance between n-6 and n-3 fatty acids. The optimal ratio between n-6/n-3 fatty acid may vary with the disease under consideration. In the prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer and suppressed inflammation in patients with rheumatoid arthritis (Simopoulos, 2008).

4.5.1 Biological effect of PUFA n-3

The interest in the beneficial effects of n-3 fatty acids on human health, especially in relation to cardiovascular disease, was born in the late 70s, following the observation that in the populations of Greenland, who eat a large amount of fish particularly rich in these fatty acids, the incidence of cardiovascular disease was much lower than that recorded in other populations (Kanta, 1987). Meat, especially of animal fed grass diet (Aurousseau et al., 2004; Enser et al., 1998; French et al., 2000; Ponnampalam et al., 2006), is an important source of PUFA n-3 (ALA, EPA, DPA and DHA), because ALA, represent over 50% of total fatty acids in grass and grass products (Bauchart et al., 1984). ALA, has been associated with a reduced risk of CVD by epidemiological studies (Ascherio et al., 1996; Hu et al., 1999; Roth and Harris, 2010). Its elongation products, EPA and DHA, are widely recognized for their numerous effects on heart health: improve platelet aggregation, vasodilatation and thrombotic tendency (Mann et al., 2006; Siddiqui et al., 2008), are critical for proper brain and visual development in the foetus, and for the maintenance of neural and visual tissues throughout life (Leaf et al., 2003; Calder, 2004); and may have roles in reducing cancer and obesity/type-2 diabetes (WHO, 2003). Red meat is, also, an important dietary source of DPA, which accumulates in mammals but not in fish oil (Givens et al, 2006). Little research exists on the clinical significance of DPA, but it has been suggested to be inversely related to atherosclerotic risk and risk of acute coronary events in middle-aged men from Finland (Hino et al., 2004).

4.5.1.1 PUFA n-3 and cardiovascular diseases

Cardiovascular disease (CVD) is one of the most common in the Western world. there is a variety of risk factors associated to the development of CVD, such as total cholesterol, levels of homocysteine, elevated triacylglycerols and hypertension and, many of these are modifiable or preventable by a healthy diet. There have been several clinical trials investigating the effects of fish consumption and PUFA n-3 supplementation for the prevention of CVD. Epidemiological and clinical studies suggests a significant inverse relationship between intake of PUFA n-3, especially EPA and DHA and mortality associated with CVD (Hu et al., 2002; Lemaitre et al., 2003; Yokoyama et al., 2007; von Schacky and Harris., 2007). The American Hearth Association (AHA) recommends daily consumption of about 1 mg of EPA + DHA for patients with known coronary artery disease and to eat fish a minimum of twice per week, which would provide 400 to 500 mg of EPA + DHA daily, for individuals without know coronary hearth disease (Roth and Harris, 2010). Singh et al. (1997) in 240 myocardial infarction patients supplemented with 1.8 g/d of EPA + DHA or 2.9 g/d of ALA, observed that, after one year, total cardiac event were 25% and 28%, respectively, versus 35% in the placebo group; von Schacky et al. (2001) observed that the progression of atherosclerosis decreased with low doses of PUFA n-3 (1.65 g/d). Hu et al. (1999) in a study involving women, observed that the group with the highest ALA intake (1.36 g/d) was associated with 45% fewer cardiac deaths compared with the group with the lowest intake (0.71 g/d). The same author in the "Nurses' Health Study" (2002) observed that deaths related to CVD were 50 % lower in women who consumed fish five times per week; Marchioli et al. (1999) in the "GISSI - prevention study", that involved patients with a recent myocardial infarction, reported that 850 mg of EPA + DHA/d compared with a control group, significantly reduced the relative risk of all cause mortality, cardiac death and sudden

death by 21%, 35% and 45% respectively. Another study that involved hypercolesterolaemic patients (cholesterol ≥ 250 mg/dL), showed that 1800 mg/d of EPA was associated with a reduction of 19% 21%, and 25% of the major coronary events, fatal myocardial infarction and non fatal myocardial infarction respectively; EPA also, reduced recurrent stroke rates from 10.5% to 6.8% (Yokoyama et al., 2007).

Based on the results from cellular and molecular studies, the cardioprotective effects of PUFA n-3 appear to be due to a synergism between multiple mechanisms that involve triglycerides lowering, antiinflammatory, inflammation-resolving, membrane fluidity and antiarrhythmic and antithrombotic effects (Adkins and Kelley, 2010). Inflammation of the vascular wall is a key factor in the dynamic process of atherosclerosis (Libby, 2008). PUFA n-3 have the ability to respond to inflammation in atherogenesis through direct and indirect mechanisms. A direct mechanism through which PUFA n-3 decrease inflammation includes its rapid effect on the regulation of transcription factors (Jump, 2002; Teran-Garcia et al., 2002), and indirect modes of actions include the production of eicosanoids (von Schacky et al., 1985; Wanten and Galder, 2007) and inflammation-resolving lipid mediators (Lu et al., 2005; Arita et al., 2005; Serhan et al., 2008). The antiinflammatory action of n-3 PUFA eicosanoids and their involvement in signaling pathways are mechanisms for their cardioprotective effects (Calder, 2004). Abe et al (1998) reported a 9% reduction in soluble ICAM-1 and a 16% reduction in soluble E-selectin but not in soluble VCAM-1 in hypertriglyceridemic subjects receiving 3.4 g/d of PUFA n-3 for 7 to 12 months. There is also in vitro evidence that DHA reduces the expression of interleukin (IL)-6, and IL-8 in stimulated cells (De Caterina et al., 2000). Another potential antiatherogenic mechanism of PUFA n-3 is their interference with the ARA cascade that generates a wide variety of eicosanoids (Uauy et al., 1999). EPA not only can replace ARA in membrane phospholipid bilayers, but it is also a competitive

inhibitor of cyclooxygenase, reducing the production of the 2-series prostaglandins, thromboxanes, and prostacyclins and the 4-series leukotrienes. DHA, although not a direct inhibitor of ARA metabolism, nevertheless can inhibit platelet aggregation by reducing the affinity of platelet TxA2/PGH2 receptor for its ligand (Bayon, 1995; Wanten and Calder, 2007).

Another mechanism through which PUFA n-3 play an important role on CVD is their hypotrigliyceridemic effect (Kris-Etherton, 2002; Adkins and Kelley, 2010), as a matter of fact exists a dose-response relationship between PUFA n-3 intake and trygliceride (TG) lowering (Kris-Etherton et al., 2002). Harris (1997) reported that 4 g/d of PUFA n-3 from fish oil decreased serum TG concentrations by 25% to 30%, with accompanying increases in LDL cholesterol of 5% to 10% and in HDL cholesterol of 1% to 3%, while in hypertriglyceridemic men Kelley et al., (2007) observed that DHA supplementation reduced both the fasting and postprandial TG by more than 25%.

4.5.1.2 PUFA n-3 in growth and development

Dietary essential fatty acids mediate brain and visual functions and structures from infancy to aging. In particular, DHA is an important component of neural membranes, where constitutes about 30% of the ethanolamine and serine phosphoglycerides (Svennerholm, 1968), and retinal membranes of mammalian species, where represent about 45% of the same phosphoglycerides (Anderson et al., 1975). In brain, DHA is especially concentrated in membranes surrounding synapses (Martin et al., 1992), while in retina, both synaptic regions and the disk membranes of the photoreceptors are highly enriched in DHA (Anderson et al., 1975).

Animal studies provided the first evidence that changes in retinal and brain DHA could alter neural function. Monkey infant exposed to PUFA n-3 deficient diet both in utero and after birth and demonstrated poor visual acuity and increases in stereotypical behavior suggesting that brain development had been impeded (Reisbick et al., 1997). Descriptive studies in the late 1970 and early 1980 of infants fed diets with (human milk) or without (infant formula) DHA provided the first evidence that early diet could alter DHA status of infants (Sanders and Naismith, 1979) and that the addition of DHA to formula could enhance DHA status of infants (Carlson et al., 1986; Liu et al., 1987). Carlson et al. (1996) have been shown higher visual acuity, in preterm human infants fed formulas with DHA, and presumed to have higher brain DHA accumulation compared to infants fed diets without DHA. A meta-analysis of 12 studies on the role of PUFA n-3 supplemented infant formula for infants born at term reported that the combined visual resolution acuity measured with behaviorally based methods shows an improvement of 0.32 octaves at two months compared to infants fed standard non-supplemented infant formula (San Giovanni et al., 2000), while breastfed infants show an average improvement of 0.49 octaves at two months and 0.18 octaves at four months compared to those fed standard infant formula. In another study with preterm infants during the initial hospitalization after birth fed infant formula supplemented with 0.3% DHA and 0.6% ARA, Innis et al. (2002) reported no improvement in visual resolution acuity at weeks 8 and 17 compared to preterm infants fed the same infant formula but without long chain PUFA supplementation.

DHA accumulate at a rapid rate (14.5 mg/week) in fetal central nervous system, during the last intrauterine trimester (Clandinin, 1980; Martinez, 1992; McNamara and Carlson, 2006) and the first 18 month of postnatal life. Prior to birth DHA is provided by placental transfer of n-3 fatty acids, a total of approximately 600 g of essential fatty acids

are transferred from mother to foetus during a full term gestation (Dangour and Uauy, 2008). After birth, the fatty acid status of the mother continues to impact on her newborn via the delivery of breast milk; a naturally rich source of DHA (Crawford et al., 1981; Brenna et al., 2007). Breast milk reflects the habitual fatty acid intake of the mother (Helland et al., 2003; Jensen et al., 2005). Sanders et al. (1978) found that women following vegan and vegetarian diets usually have 0.1% or less DHA in their milk fat. For this reason an adequate supply of PUFA n-3 during pregnancy and lactation is important. An expert panel recommended a DHA intake of 300 mg/d during pregnancy and lactation (Simopoulos, 1999), other recommendations for 200–300 mg/d during this period have been made (AOCS, 2003).

Some authors have examined the relationship between PUFA n-3 intake by mother and infants later development. Jorgensen et al. (2001) found a correlation between maternal fish consumption and better infant visual acuity, whereas Daniels et al. (2004) found that mothers who ate fish four times a week during pregnancy had babies with higher developmental scores at 18 months compared with those who ate no fish. In another observational study, Williams et al. (2001) reported that children whose mothers ate oily fish during pregnancy were more likely to develop high-grade stereoacuity at 3.5 years of age than were children whose mothers did not eat oily fish. Innis (2003) observed that infant fed breast milk with 0. 17 % DHA had lower erythrocyte DHA at 60 days of age and lower visual acuity and language development during the first 14 months of life than infants fed breast milk with 0.34% DHA. In a study by Jensen et al. (2005), infants whose mothers received 200 mg/d algal DHA versus placebo, with resultant breast milk DHA contents of 0.35 compared with 0.2 mol % of total fatty acids, respectively, during the first 4 months postpartum performed significantly better neurodevelopment at 30 months of age. Helland et al. (2003) observed that maternal intake of cod liver oil (10

ml/d), that supply 1.18 g of DHA and 0.8 g of EPA, from 18 weeks of pregnancy until three months after delivery result in an average increase of 4.1 points in cognitive function of the offspring at four years of age.

It is believed that PUFA n-3 enable fluidity in neuronal membranes and help regulate neurotransmitters (Yehuda et al., 1999), both crucial for optimal brain function. Others studies also suggest that DHA is important in neurogenesis and also influences phospholipid synthesis and turnover (Coti Bertrand et al., 2006; Kawakita et al., 2006). Studies with rodents and piglets have shown that PUFA n-3 alter the metabolism of several neurotransmitters, including dopamine and serotonin, membrane-associated enzyme and receptor activities in the brain (de la Pressa Owens and Innis, 1999; Zimmer et al., 2002; Innis, 2007). Others studies have shown that n-3 fatty acid deficiency decreases the mean cell body size of neurons in the hippocampus, hypothalamus and parietal cortex, and decreases the complexity of cortical dendritic arborization (Ahmad et al., 2002; Wainwright et al., 1998).

4.5.1.3 PUFA n-3 in mental health

Mental health disorders are an important cause of dysfunction throughout the world (Ramakrishnam et al., 2009). Researchers have begun to focus on the role of nutrition in mental health and the evidences suggested that essential fatty acid, particularly DHA, are involved in many brain related disorders. Evidence for the role of PUFA n-3 and, in the etiology and treatment of mental disorders, such as depression and dementia has been reviewed (Freeman, 2000; Bourre, 2005; Dangour and Uauy, 2008; Jicha and Markesbery, 2010). The first documented case of human PUFA n-3 deficiency was reported by Holman et al. in 1982. This case report describes a 6 year old female patient

maintained for 5 months on an ALA deficient parenteral nutritional preparation following intestinal surgery. The patient exhibited a 17% reduction in plasma DHA concentrations, presented dermatitis and neurological symptoms, including neuropathy, blurred vision, and psychological disturbances. When the parenteral nutritional preparation was replaced with an ALA-fortified preparation, plasma n-3 fatty acid levels normalized and neurological symptoms disappeared (Holman et al., 1982, 1998). Hibbeln (1998) demonstrated a significant correlation between high annual fish consumption and a low prevalence of major depression. Tanskanen et al. (2001) studied a sample of Finnish adults and found a significant correlation between low fish consumption and depressive symptoms. A survey of New Zealand adults found that fish consumption was significantly associated with higher self-reported mental health status (Silvers and Scott, 2002). Others studies have found reduced PUFA n-3 levels in the tissues of depressed patients (Maes et al., 1999) and a relationship between low n-3 status and severity of symptoms (Adams et al., 1996). Evidence from supplementation trials, suggests that PUFA n-3 are useful in the treatment of depression (Ruxton et al., 2004). Elderly patients treated with DHA supplemented phosphatidylserine demonstrated significant reductions in depressive symptoms compared with a placebo group (Cenacchi et al., 1993). Nemets et al., (2002) in patients with unipolar depressive disorder reported significant improvements in symptoms after 4 weeks of treatment with 2g/d of EPA. Stoll et al. (1999) in a trial with 30 patients, aged 18 to 65 years, with bipolar disorder, showed that episodes of severe mania and depression were significantly reduced in the PUFA n-3 (EPA+DHA) supplementation group (9.6g/day) compared with the placebo group. However, Marangell et al. (2003), in an intervention trial on severely depressed patients reported not effect although it could be that the dose of 2 g/d of DHA was too low.

Postnatal depression has also been linked with a low PUFA n-3 status, which fits with the evidence for high fetal requirements for DHA in the third trimester. An epidemiological study covering 23 countries showed that the risk of postpartum depression was inversely proportional to fish consumption and the DHA in the mother's milk (Hibbeln, 2002) thus pregnant women are invariably likely to benefit from a prophylactic treatment based on DHA and EPA (De Vriese et al., 2003). Hibbeln (2002) suggests that mothers selectively transfer DHA to their fetus to support optimal neurological development leaving themselves at risk of depletion if dietary PUFA n-3 if intake is low. Llorente et al. (2003) supplemented mothers for 4 months after delivery with 0.2 g/d DHA finding no significant effect on self-rated depression despite increases in plasma DHA status. In a prospective study, Kalmijn et al. (1997a), observed than people with a fish consumption of more than 20 g/d had a reduced risk of cognitive impairment, cognitive decline, dementia and Alzheimer's disease. The same author in another study of men aged 69 – 89 years reported that a high LA intake was associated with cognitive impairment, whereas high fish consumption was inversely associated (Kalmijn et al., 1997b). A prospective study found a strong inverse relationship between fish intake and Alzheimer disease, elderly people who ate fish at least once a week had a 60% lower risk of developing the disease over a 4 year period (Morris et al., 2003). Van Gelder et al. (2007) reported that older men who consumed approximately 400 mg/d of PUFA n-3 had less cognitive decline than men who consumed only 20 mg/d of PUFA n-3. Plasma studies support the evidence that low PUFA n-3 levels are associated with dementia. Conquer et al. (2000) found lower levels of plasma phospholipid DHA in patients with Alzheimer's disease and other dementias. Heude et al. (2003) measured erythrocyte membrane fatty acid composition and cognitive function in elderly people and followed them for 4 years, the author reported that those with high PUFA n-6 and

low PUFA n-3 in their erythrocytes were most likely to experience cognitive decline. Schaeffer et al. (2006) in a trial that involved men and women with a median age of 76 years, reported that the group with the highest DHA intake (200 mg/d), has the highest plasma phosphatidylcoline DHA concentration and was 47% less likely to developed all cause dementia and 39% less likely to develop Alzheimer's disease.

4.5.1.4 PUFA n-3 and cancer

The results of animal and in vitro studies indicated that the consumption of PUFA n-3, especially the EPA and DHA, can slow the growth of cancer cells, increase the efficacy of chemotherapy and reduce the side effects of the chemotherapy or of the cancer (Hardman, 2002). Epidemiologic studies indicate that populations that consume high amounts of n-3 fatty acids have lower incidences of breast, prostate and colon cancers than those that consume less n-3 fatty acids (Hardman, 2002; Riediger et al., 2009). From a review of literature, Colomer et al. (2007) concluded that administration of EPA and DHA in doses at least of 1.5 g/d for a prolonged period to patients with advanced cancer is associated with an improvement in clinical and biological parameters. Studies revealed that diets rich in monounsaturated fats or high n-3 fatty acid content in erythrocyte membranes were inversely correlated with the development of colorectal cancer (Caygill et al., 1996; Kuriki et al., 2006) and breast cancer (Kuriki et al., 2007). Norat et al. (2005) reported that people who ate 80 g or more fish per day had a 40% less chance of developing colorectal cancer than did those who ate less than 10 g per day. The mechanisms by which dietary n-3 fatty acids contribute to the prevention of cancers have not been fully established and the n-6/n-3 ratio of eicosanoid production seems to play the major role. Other mechanisms may include modifications in the

hormonal status, cell membrane structure and function, cell signaling transduction pathways and gene expression, and immune function (Escrich et al., 2006). Courtney et al., (2007) reported that daily consumption of EPA (2 g/day) by patients with colorectal adenomas led to the production of 3-series prostaglandins such as PGE3, the suppression of crypt cell proliferation, and increased apoptosis in colonic mucosa. In addition, PUFA n-3 fatty acid supplementation of 0.2 g/kg body weight through total parenteral nutrition in patients who had undergone colorectal cancer resection was associated with a significant reduction in interleukin-6 levels and a trend toward reduced postoperative hospitalization period compared to controls (Liang et al., 2008).

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CHAPTER 5

Lipid analysis

Lipid analysis

5.1 Fat extraction

Milk

Fat extraction of milk was performed according to the Röse-Gottlieb method (AOAC, 1990) modified as described by Secchiari et al. (2003). Briefly, ammonia 25 % (o.4 ml), ethyl alcohol 95% (2 mL), and hexane (5 mL) were added to 1 g of milk. Samples were centrifuged at 4 °C at 3000 rpm and the upper layer was collected. Extraction was repeated a second time using ethyl alcohol 95% (2 mL) and hexane (5 mL); samples were centrifuged at 4°C at 3000 rpm and the upper layer was collected. A third extraction was repeated using only 5 mL of hexane; samples were centrifuged and the upper layer was collected. The hexanic phase containing lipids was evaporated under vacuum in a rotary evaporator.

Meat and baby food

Meat and baby food samples were frozen at -20 °C and stored at -80°C until liophylisation for 3 days in LyoLab 3000 (Heto). Only meat samples were liophylized. Lhiophylized samples were gound by using a domestic grinder machine and stored at –80°C until analysis. Lipids were extracted from 1g of ground muscle and baby food with chloroform-methanol (2:1 v/v) according to the method of Folch et al. (1957) modified. Briefly, 1 g of sample was placed in a 50-ml tube and 30 mL of chloroform:methanol (2:1) were added. The tube was capped, shaken by hand for 30 s, sonicated for 5 min in ultrasonic bath (BRANSON 2510, BRANSONIC® and then centrifuged at 1500 rpm × g for 10min at room temperature. The supernatant was collected and filtered under vacuum pump. Filtered samples were washed with 6 mL of 1% NaCl (wt/vol) were added. The tube was then centrifuged at approximately 1500 rpm × g for 10 min to

separate the two phases. The upper phase was removed using a water aspirator and discarded while the chloroform extract layer was evaporated under vacuum in a rotary evaporator.

Extracted fat samples were recovered with different amounts of hexane in order to obtain samples ranging the fat concentration around 25 mg/ml.

5.2 Esterification procedure

The fatty acid methyl esters (FAME) were prepared with a base-catalyzed trans-esterification according the FIL-IDF standard procedure (1999).

1 ml of sample was dried under nitrogen flow and approximately 25 mg of lipid extract were mixed with 500 µl of sodium methoxide in methanol, and vortexed for 2 min, 1ml of hexane containing the internal standard (0.5 mg/mL), and vortexed for 2 min. After separation of the two phases the upper layer was collected and used for gas chromatography.

5.3 Two step methylation procedure

Lipids were extracted from 1g of animal feed (forage, concentrate and milk replacer) and added 1 mL exhane and 2 mL of sodium methoxide (0.5 M in methanol) solution. Samples were vortexed lightly, incubated in a 50°C water bath and then removed from the water bath in order to allow cooling for 5 min. Samples were added 3 mL of methanolic HCl, vortexed, incubated in the water bath at 60°C for 30 min. After water bath cooling samples for 7 min is needed. Cool samples were added 3 mL of exhane and 7.5 mL of K_2CO_3 (6%), vortexed and finally centrifuged to allow the separation of the two phases.

5.4 Gas – cromatograph conditions

The FAME was separated in a capillary column (CP-select CB for Fame; 100 m×0.32 mm i.d., 0.25-μm film thickness, Varian Inc., Palo Alto, CA) and quantified using nonadecanoic acid (C19:0) methyl ester (Sigma Chemical Co., St. Louis, MO). The injector and FID temperatures were 255 °C. For all samples the temperature program was as follows: 75 °C for 1 min, increased at 8 °C/min to 165 °C, held for 35 min, increased at 5.5 °C/min to 210 °C, held for 1 min, and finally increased at 15 °C/min to 240 °C held for 15 min. The split ratio was 1:40 and He was the carrier gas with a pressure of 37 psi.

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Objectives

The overall objective of the present thesis was to study the fatty acid profile of meat of suckling lamb from Sarda dairy ewes, with particular attention on PUFA n-3 and CLA by deepening the effect of nutritional factors and different farming system in order to improve meat fat quality in relation to human health.

The specific experimental contribution were:

1. First experimental contribution: Effects of farming system on fatty acid profile of Sarda breed suckling lambs.

2. Second experimental contribution: Relationships between the contents of Vaccenic acid, CLA and highly unsaturated fatty acids of sheep milk and the muscle of their suckling lambs.

3. Third experimental contribution: The effect of natural or artificial milk on fatty acid profile of Sarda breed lambs.

4. Fourth experimental contribution: Comparison of fatty acid profile in lamb meat and baby food based on lamb meat.

CHAPTER 6

The effects of farming system on fatty acid profile of Sarda breed suckling lambs

6.1 Introduction

Increasing interest to enhance the nutritional quality of animal products has stimulated research on nutritional manipulation of their fatty acid profile. Great attention is focused on conjugated linoleic acid and PUFA n-3 which have several benefits to human health. Biomedical studies with animal models have demonstrated that CLA, in particular the isomer *cis*-9, *trans*-11 CLA (Rumenic acid, RA), have many biological effects including actions to reduce carcinogenesis, atherosclerosis, onset of diabetes, and body fat mass (Lee et al. 1994; Ip et al. 1999; Parodi 1997; Belury 2002). While C18:3 n-3 (α-linolenic acid, ALA), has been associated with a reduced risk of cardiovascular disease by epidemiological studies (Roth and Harris, 2010). Its elongation products, eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic (DHA, C22:6 n-3), are widely recognized for their numerous effects on heart health and moreover are critical for proper brain and visual development in the foetus, the maintenance of neural and visual tissues throughout life (Ruxton et al., 2009). The relationships between dietary fatty acid intake and human health contributed towards the development of specific guidelines from the World Health Organisation in relation to fat in the diet (WHO, 2003).

In Mediterranean countries lamb meat originates from dairy sheep breeds and animals are slaughtered very young, after a suckling period of 20–30 days, in order to reduce the milk loss for cheese production and also because consumers prefer this kind of product. Traditionally, these animals are raised with their dams and fed almost exclusively with maternal milk but small amounts of roughage may be ingested, when lambs follow their mothers at pasture. Meat from suckling lamb meets consumer demand especially in specific periods (Christmas, Easter), commanding high market prices (Sañudo et al., 2000).

The effect of production system particularly of pasture vs. indoor feeding on intramuscular fatty acids of lambs has been previously considered (Diaz et al., 2002; Santos Silva et al., 2002; Velasco et al., 2001; Nuernberg et al., 2008). Has been observed that lambs fed on pasture had a better fatty acid profile in regard to human health. In particular meat of animal fed grass, rich in ALA, is an important source of RA and PUFA n-3 that accounted to a lower n-6/n-3 ratio. Nuernberg et al. (2008) reported that ALA was 1.5 fold higher and RA 1.8 fold higher in pasture fed lambs than in indoor fed ones. Similarly Santos-Silva et al. (2002) found that pasture raised lambs showed higher proportion of PUFA n-3, RA and lower n-6/n-3 ratio, than concentrate fed lambs.

The aim of the experiment was to compare the effect of different lamb management systems, indoor vs. outdoor, on fatty acid profile of fresh meat of Sarda breed suckling lamb focusing on RA, PUFA n-3 and total PUFA.

6.2 Materials and methods

6.2.1 Animals and diets

Twenty four Sarda breed suckling lambs were selected from 18 Sarda dairy ewers grazing on natural pasture. At birth the lambs were divided in two groups (12 lambs each): lambs kept indoor during the grazing time of the ewes (group I) and lambs which followed the mother on pasture (group O).

Feed samples were collected at the beginning and at the end of the trial for subsequent chemical analysis. Individual milk samples were taken before the morning suckling (weekly) and stored at −20 °C for subsequent fatty acid analysis. The lambs were weighed and then slaughtered at 28 days of age. The cold carcass weight (CCW, body weight minus blood, skin, viscera, feet, tail) and pH was measured in after 24 h of storage at 4 °C. After 24 h of refrigeration the tight muscles (*Semitendinosus*, *Semimembranosus* and *Femoral Biceps*) were dissected from each right half-carcass, vacuum packaged and stored at – 80°C until the fatty acid could be analysed.

6.2.2 Feed and muscle analysis

The DM content of muscle was determined by oven-drying at 105 °C for 24 h. Were also determined: ash (AOAC 920.153. 1999), crude protein (CP) (Kjeldahl), and fat (Folch et al., 1957). Composition of fatty acids from milk and muscles was determined by gas chromatography using a Varian 3400 GC. Fat extraction and esterification procedure for fatty acid analysis were performed as reported in chapter 5 paragraph 1 and 2. The chromatographic conditions and fatty acid identification were carried out as reported in chapter 5 paragraph 4. The relative amount of each fatty acid (% of total FAME) is reported as a percentage of total peak area for all fatty acids. The sum of n-3 PUFA

(C18:3 n-3, C20:5 n-3, and C22:6 n-3), the sum of n-6 PUFA (C18:2 n-6, C20:3 n-6, C20:4 n-6, and C22:4 n-6), and the ratio n-6/n-3 were calculated. The desaturase activities were estimated indirectly as [product]/[precursor + product] ratio as suggested by Kelsey et al. (2003). Thus, in muscle the Δ5-desaturase activity was calculated as [C20:4 n-6]/[C20:3 n-6 + C20:4 n-6] ratio, Δ6-desaturase activity was calculated as [C20:3 n-6]/[C18:2 n-6 + C20:3 n-6] ratio and Δ9-desaturase activity as [C18:1 n-9]/[C18:0 + C18:1 n-9] ratio (Andersson et al., 2000). Atherogenic index (AI), as a dietary risk indicator for cardiovascular disease, and Thrombogenic index (TI), as a sign of the potential aggregation of blood platelets, were calculated according to the formulas suggested by Ulbricht and Southgate (1991).

$$AI = \frac{C12:0 + 4 * C14:0 + C16:0}{UFA + \sum JFA\omega6 + \sum JFA\omega3}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5\sum UFA + 0.5\sum JFA\omega6 + 3\sum JFA\omega3 + \omega}$$

6.2.3 Statistical analysis

Differences in meat composition and fatty acid in intramuscular fat of suckling lambs were assessed with ANOVA one-way using management system as the main factor. Differences between males and females were assessed by *t*-test.

Statistical analysis was performed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA).

6.3 Results and discussion

The lambs body weight (mean±SD) at slaughtering was 10.07±2.41 Kg for O lambs and 9.14±1.32 Kg for I lambs. The CCW was 4.54±1.16 Kg for O lambs and 4.38±0.80 for I lambs.

6.3.1 Fatty acid composition of ewes milk

The fatty acidprofile of milk suckled by lambs is reported in Table 1. The C16:0 and C18:1 *cis*-9 were the most abundant fatty acid. The C18:1 *trans*-11 (Vaccenic acid, VA) and RA contents of milk were similar to the mean of those fatty acid in milk of animals grazing on natural pasture. The content of *trans*-10, *cis*-12 CLA, which is thought to be detrimental to human health (Wahle et al., 2004), was low (0.06 mg/100 mg of FAME) in accordance with other observations in sheep (Antongiovanni et al., 2004). The content of ALA was in line with values usually observed in dairy Sarda breed sheep in the presence of lush natural pasture (Nudda et al., 2005).

Table 1 - Least square means of fatty acid profile (% of total FAME) of sheep milk.

	Milk	SD
Fatty acid (g/100g of FAME)		
C4:0	1.60	0.47
C6:0	1.73	0.46
C8:0	2.06	0.43
C10:0	6.29	2.11
C12:0	4.44	3.34
C14:0	9.96	1.73
C14:1	0.26	0.10
C15:0	1.09	0.32
C15:1 *n*−5	0.29	0.11
C16:0	21.95	2.20
C16:1 *n*−7	0.26	0.09
C17:0	0.86	0.19
C17:1	0.28	0.23
C18:0	10.07	4.14
C18:1 *cis* – 9	22.03	5.01
C18:1 *trans* – 11 (VA)	3.44	1.20
CLA *cis* - 9, *trans* – 11 (RA)	1.52	0.53
CLA *trans*-9, *cis*-11+C20:0	0.17	0.07
CLA *trans* - 10, *cis* – 12	0.06	0.05
CLA *trans*-9, *trans*-11+C20:1	0.07	0.10
C18:2 n-6 (LA)	3.40	2.06
C18:3 n-3 (ALA)	1.05	0.21
C20:4 n-6 (ARA)	0.17	0.08
C20:5 n-3 (EPA)	0.08	0.04
C22:5 n-3 (DPA)	0.17	0.08
C22:6 n-3 (DHA)	0.05	0.05
Sums and ratio		
SFA	60.43	3.70
MUFA	34.59	3.68
PUFA	4.98	2.23
Σn−3 PUFA[a]	1.35	0.30
Σn−6 PUFA[b]	3.63	2.06
PUFA/SFA	0.08	0.04
Σn−6 / Σn−3	2.68	1.36
AI[c]	1.70	0.36
TI[d]	1.80	0.29
CLA desaturase index[e]	0.31	0.21

a [C18:3 n−3+C20:5 n−3+C22:5 n-3+C22:6 n−3].
b [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].
c Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].
dThombogenic index=[C14:0+C16:0+C18:0]/[(0.5* ΣMUFA)+ 0.5*PUFA n-6+3*PUFA n-3+n-3/n-6)].
e CLA desaturase index= [RA]/[RA+VA].

6.3.2 Chemical composition of lamb meat

Chemical characteristics of muscle of Sarda suckling lambs (mean±SD) is reported in Table 2. Lamb management did not affect chemical composition of suckling lamb meat except for fat content and ash that were higher (P<0.05 and P<0.01, respectively) in I lambs compared with O lambs.

Table 2 - Chemical composition of intramuscular fat of Sarda suckling lamb produced with different management systems (g/100 g).

	Lamb management				P value
	Indoor	SD	Outdoor	SD	
Dry Matter DM (%)	26.26	2.06	24.49	0.66	ns
Protein (%)	19.96	1.55	20.51	0.18	ns
Fat (%)	3.12	0.65	2.47	0.13	*
Ash (%)	1.31	0.06	1.13	0.02	**
pH	5.70	0.07	5.73	0.10	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

Meat of Sarda breed suckling lamb showed a good protein content (20%) that is in agreement with those reported by Mazzone et al. (2010) in *Longissimus lumborum* of Apennine suckling-lambs slaughtered at 60 days of age and by Osorio et al. (2007) in muscle from Churra suckling lambs slaughtered at an average age of 30 days.

Fat content was higher (P<0.05) in I lambs compared with O lambs. Lower fat content in muscle of grazing lambs compared with indoor fed lambs were previously observed (Rowe et al., 1999; Priolo et al., 2002; Aurousseau et al., 2007; Popova et al., 2007). The mean fat content range between 2.47 and 3.12%; the values agrees with previously observation in Sarda suckling lamb (Vacca et al. 2008; Nudda et al., 2009), whereas is higher than reported by Lanza et al. (2006) in Barbaresca suckling lamb slaughtered at 40 days of age and fed only maternal milk (1.93%) and lower than observed by Polidori

et al. (2009) in Fabrianese heavy lambs slaughtered at four months of age (4.5%), maybe due to the higher slaughter weight (33 Kg).

6.3.3 Fatty acid composition of lamb meat

The fatty acid profile of intramuscular fat of Sarda suckling lambs produced with different management system is reported in Table 3. There was no significant differences between males and females in all fatty acid of interest in this study (VA 2.34 g/100 g of FA vs. 2.38, P=0.78; RA 1.53 vs. 1.59 g/100 g of FA, P=0.58; PUFA n-3 3.16 g/100 g of FA vs. 3.59, P=0.51; total PUFA 9.95 vs. 11.77 g/100 g of FA, P=0.41, in males and females, respectively). Therefore, all data for males and females were pooled.

Management system had relevant effect on several fatty acids. Lamb meat from O lamb presented lower contents of C14:0; C16:0, C17:0, total SFA and high contents of LA, DPA, DHA, PUFA n-6, PUFA n-3 an total PUFA in comparison with I lambs. In addition PUFA/SFA was significantly higher and AI and TI significantly lower in O lambs than I lambs.

Table 3 - Least square means of fatty acid profile (% of total FAME) of intramuscular fat of Sarda suckling lamb produced with different management systems.

	Lamb management				P value
Fatty acid (g/100g of FAME)	Indoor	SD	Pasture	SD	
C14:0	6.51	1.30	5.55	1.58	†
C14:1	0.14	0.06	0.17	0.09	ns
C15:0	0.55	0.07	0.51	0.13	ns
C15:1 *n* – 5	0.19	0.03	0.17	0.04	ns
C16:0	23.32	1.91	21.71	2.46	†
C16:1 *n* – 7	0.59	0.08	0.51	0.11	ns
C17:0	1.04	0.11	0.96	0.10	†
C17:1	0.54	0.07	0.49	0.07	ns
C18:0	13.65	0.81	13.06	1.46	ns
C18:1 *cis* – 9	36.57	2.80	34.17	3.06	†
C18:1 *trans* – 11 (VA)	2.33	0.37	2.39	0.31	ns
CLA *cis* - 9, *trans* – 11 (RA)	1.48	0.24	1.64	0.26	ns
CLA *trans*-9, *cis*-11+C20:0	0.11	0.04	0.12	0.03	ns
CLA *trans* - 10, *cis* – 12	0.03	0.02	0.03	0.02	ns
CLA *trans*-9, *trans*-11+C20:1	0.12	0.02	0.13	0.05	ns
C18:2 *n* - 6 (LA)	4.29	0.71	6.63	2.55	**
C18:3 *n* - 3 (ALA)	1.21	0.19	1.39	0.34	ns
C20:4 *n* - 6 (ARA)	1.12	0.25	2.01	1.81	ns
C20:5 *n* - 3 (EPA)	0.53	0.18	0.79	0.63	ns
C22:5 *n* - 3 (DPA)	0.67	0.24	1.16	0.78	*
C22:6 *n* – 3 (DHA)	0.37	0.15	0.63	0.33	*
Sums and ratio					
SFA	45.80	3.07	42.62	3.90	*
MUFA	45.68	3.17	44.19	3.33	ns
PUFA	8.52	1.25	13.19	6.77	*
Σn−3 PUFA[a]	2.77	0.68	3.97	2.01	†
Σn−6 PUFA[b]	5.75	0.79	9.22	4.81	*
PUFA/SFA	0.19	0.03	0.33	0.23	*
Σn−6 / Σn−3	2.18	0.66	2.36	0.33	ns
AI[c]	0.93	0.19	0.79	0.20	†
TI[d]	1.27	0.17	1.07	0.23	*
Δ5 desaturase[e]	0.77	0.03	0.77	0.04	ns
Δ6 desaturase[f]	0.08	0.02	0.07	0.02	ns
Δ9 desaturase[g]	0.73	0.02	0.72	0.03	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

[a] [C18:3 n−3+C20:5 n−3+C22:5 n-3+C22:6 n−3].

[b] [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].

[c] Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].

[d]Thombogenic index=[C14:0+C16:0+C18:0]/[(0.5* ΣMUFA)+ 0.5*PUFA n-6+3*PUFA n-3+n-6/n-3)].

[e] Δ5 desaturase activity=[C20:4 n−6]/[C20:3 n−6+C20:4 n−6].

[f] Δ6 desaturase activity = [C20:3 n−6]/[C18:2 n−6+C20:3 n−6].

[g] Δ9 desaturase activity = [C18:1n−9]/[C18:0+C18:1 n−9].

The percentages of C14:0 ($P<0.10$) and C16:0 ($P<0.05$), were lower in O lambs compared with I lambs while no significant differences were observed for C18:0 content. This result is important from a nutritional point of view because of the high atherogenic potential which characterized these fatty acids (Moloney et al., 2001). The average content of C14:0 and C16:0 agrees to those reported in Merino grazing lambs slaughtered at 30 Kg live weight (Garcia et al., 2008) while were slightly lower than those found in the intramuscular fat of grazing Talaverana suckling lambs slaughtered at 14 Kg live weight (Vealsco et al., 2001). On the other hand Dìaz et al. (2002) found a higher proportion of C14:0 and C16:0 in muscle of Talaverana grazing lambs slaughtered at 28 Kg live weight (8.51 and 25.51%, respectively) and this is probably related to the great intake of forages that probably increase the production of substrates for the synthesis ex novo of fatty acid.

Among the odd chain fatty acid (OCFA) significant differences were observed only for C17:0 which was higher in I lambs compared with O lambs. This result is in contrast with Velasco et al. (2001) that found a higher C17:0 in Talaverana suckling lambs raised on pasture in comparison with indoor fed lambs. Since these fatty acids are useful indicators of an incipient rumen activity, the result found by Velasco et al. (2001) can indicate a start of rumen activity in pasture fed lambs than in indoor fed ones. However values of C17:0 observed in Sarda lamb meat were similar to those reported Joy et al. (2008) in Churra Tensina grazing lambs slaughtered at 22 or 24 Kg and lower than reported by Velasco et al. (2001) (1.67%).

The C18:1 *cis*-9, that is the most abundant fatty acid in suckling lamb muscle, tended to be higher in I lambs compared with O lambs. Lower proportions of C18:1 *cis*-9 in intramuscular fat of grazing lambs compared with indoor fed lambs were previously reported (Diaz et al., 2002; Santos-Silva et al., 2002; Aurousseau et al., 2007; Joy et al.,

2008). The mean C18:1 *cis*-9 content was similar to previously observation in intramuscular fat of Churra suckling lambs slaughtered at 30 days of age (Osorio et al. 2007) whereas were lower than those reported in Apennine suckling-lambs slaughtered at 60 days of age and higher body weight (29.52%, Mazzone et al., 2010).

The VA that represent 70% of total trans monoenic fatty acid did not differ significantly between groups. VA is formed in the rumen during biohydrogenation of LA and ALA presents in feed fat and is usually higher in meat of animals fed pasture (Santos-Silva et al., 2002; Aurousseuau et al., 2004; Aurousseau et al., 2007, Nuernberg et al., 2008). The lack of differences in VA content between the I and O lambs can be explained by the fact that the O lambs, that usually start to assume grass after the third week of life, have not yet acquired rumen functionality and therefore the VA accumulated in meat is that presents in suckled milk. The mean VA values in Sarda meat lamb were lower than reported by Aurousseau et al. (2007) in *Longissimus thoracis* muscle of Ile de France grazing lambs slaughtered at 34 Kg live weight (4.4%) and by Santos-Silva et al. (2002) in Merino Branco grazing lambs slaughtered at 24 or 30 Kg live weight (3.34%). Lower values of VA were reported in Massese suckling lambs slaughtered at 11 Kg live weight (Serra et al., 2009). The content of RA, which represent 80% of total CLA, did not differ significantly between groups. Several studies (Santos-Silva et al., 2002; Aurousseau et al., 2004; Nuernberg et al., 2008) lambs fed pasture has shown a significant increase in VA and RA. Since VA and RA originates mainly as intermediates during rumen biohydrogenation process of dietary unsaturated fatty acid, our result can be explained because of in this phase lambs are "functional monogastrics" so there is no ruminal biohydrogenation of dietary LA and ALA fatty acid before they are absorbed from the intestine. Therefore, the content of RA accumulated in meat of suckling lambs comes partly from RA present in milk and partly by the activity of Δ9- desaturase

enzyme on VA (Bauman et al., 1999). Anyway, the mean content of RA in our experiment agrees to that reported in Comisana lambs suckled from ewes fed on pasture and slaughtered at 38 days of age (1.35%; Valvo et al., 2005), and higher than observed in Castellana and Assaf lambs (0.60%; Lurueña-Martinez et al., 2010) slaughtered at similar live weight (11 kg) but suckling milk from dams fed indoor with concentrate.

The proportion of LA, which is the predominant PUFA n-6, in lamb meat, was higher ($P<0.01$) in O lambs compared with I lambs. A higher content of LA was found in stall fed lambs compared with grass fed lamb by Aurousseau et al. (2007), but this is related to the inclusion of concentrate, rich in LA, in stall fed lambs. The mean content of LA was similar to those reported in the literature in grass fed Ile de France lambs slaughtered at 34 Kg live weight (Aurousseau et al., 2007) and two-fold higher than reported in Talaverana lambs reared on pasture and slaughtered at 28 Kg live weight (Diaz et al., 2002); whereas a higher LA content was reported in Comisana grazing lambs slaughtered at 90 days of age (Chiofalo et al. 2010) and in 30 day-old Italian Merino suckling lambs (Oriani et al., 2005).

Proportion of ALA, which is the most important PUFA n-3 tended to be higher in O lambs compared with I lambs although the limit of significance was not reached ($P=0.12$). This result can be related to the fact that lambs kept on pasture with their own dams usually start to ingest a small amount of herbage, rich in ALA (Chilliard et al., 2001) from the second or third week of life. Higher content of ALA in intramuscular fat of lambs reared on pasture compared with indoor fed lambs were previously reported (Diaz et al. 2002; Santos-Silva et al., 2002; Popova et al., 2007). The average ALA content are similar with previous observation in intramuscular fat of Sarda suckling lambs (Nudda et al., 2009) and Merino lambs slaughtered at 24 Kg live weight (Tejeda et

al., 2008) whereas are lower than reported by Velasco et al. (2001) in grass-fed Talaverana suckling lambs slaughtered at 14 Kg live weight (4.30% of total FAME).

Among LC-PUFA n-3, only DPA and DHA were higher ($P<0.05$) in O lamb than in I lambs. Higher proportion of DPA and DHA in pasture fed lambs were previously reported (Demirel et al., 2006; Santos-Silva et al., 2002; Popova, 2007). The mean content of DHA was higher than observed in Apulian lambs slaughtered at 45 or 90 days of age (0.39 and 0.26%, respectively, Cifuni et al., 2000) whereas the mean content of DPA was higher than observed in Turkish breed grazing lambs (0.77%, Demirel et al., 2006) while similar values of DPA and DHA were previously reported in Sarda suckling lamb and in Comisana lambs (Nudda et al., 2009; Chiofalo et al., 2010). The DPA and DHA originates in tissues from ALA via the desaturation-elongation pathways. No information are available about the extent of DPA and DHA synthesis from ALA in ruminant tissue after birth. However DHA is already present in fetal tissue of the lambs at 145 days of gestation (0.83%; Nudda et al., 2007) suggesting that it is mainly accumulated in lamb tissue during intrauterine life. However, the higher content of this fatty acid in O than in I lambs suggest, even if small, a synthesis of DHA from ALA during this part of life.

The increase in ALA and LC-PUFA n-3 levels is considered beneficial from a nutritional point of view due to the importance of these fatty acid for heart health and for a proper visual and nervous system development in the foetus (Ruxton et al., 2009).

The groups of FAs were significantly affected by the farming system of the lambs (Table 3). Total SFA percentage was lower in O lambs compared with I lambs ($P<0.05$). The average contents of SFA are in agreement with previously observation in Talaverana and Comisana unweaned lambs (Velasco et al. 2004, Napolitano et al., 2002); whereas higher SFA content were reported in Talaverana and Comisana weaned and grazing lambs (Diaz

et al., 2002; Chiofalo et al., 2010). The high content of SFA in suckling lambs meat is related to fatty acid composition of maternal milk that is rich in these fatty acids than milk of non-ruminats.

The proportion of PUFA in meat fat were higher ($P<0.05$) in O lambs compared with I lambs and this is mainly due to the higher level of both PUFA n-6 and PUFA n-3 ($P<0.05$ and $P<0.10$, respectively). The higher PUFA content in O lambs is beneficial from a nutritional point of view because determined a higher PUFA/SFA ratio in O lambs compared with I lambs ($P<0.05$). Coinciding with our own results Velasco et al. (2001) and Diaz et al. (2002) observed a higher PUFA/SFA ratio in lambs fed on pasture compared with indoor fed lambs. However, the PUFA/SFA ratios were low according to the recommended value of 0.45 (Wood et al., 2003) and comparable with previously observation in meat from Massese and Italian Merino suckling lamb (Serra et al., 2009; Oriani et al., 2005). Whereas lower values of PUFA/SFA ratio were observed in Barbaresca suckling lambs fed only maternal milk and slaughtered at 40 days of age (Lanza et al., 2006).

The n-6/n-3 ratio did not differ among groups. In human diet values of n-6/n-3 ratio lower than 4 are indicated as the most favorable by COMA in order to prevent some cardiovascular diseases (Department of Health, 1994); an increased consumption of PUFA n-3 has been recommended to overcome the perceived imbalance in the ratio of PUFA n-6/n-3 in human diets (Dervishi et al., 2010). The values of n-6/n-3 ratio found in our study were lower than recommended values and comparable with previously observation in ovine meat from Sarda and Barbaresca suckling lambs (Nudda et al., 2009; Lanza et al., 2006). Whereas lower values were found in Massese and Talaverana breed suckling lambs slaughtered at 14 Kg live weight (1.03 and 1.39, respectively; Serra et al., 2009; Velasco et al., 2001).

Atherogenic index (AI), as a dietary risk indicator for cardiovascular disease, and Thrombogenic index (TI), as a sign of the potential aggregation of blood platelets, were significantly lower ($P<0.10$ and $P<0.05$, respectively) in O lambs compared to I lambs. This result, beneficial from a nutritional point of view, is probably due to the lower content of SFA and to the higher content of PUFA in O lambs compared with I lambs. Values of AI were lower than found by Oriani et al. (2005) in Italian Merino lambs slaughtered at different ages, and similar to those found by Salvatori et al. (2004) for the *Semimembranosus* muscle of two crossbred genotypes (Ile de France x Pagliarola and Gentile di Puglia x Sopravissana), slaughtered at 64 days of age and reared under extensive conditions. TI value are lower than found by Oriani et al. (2005) probably due to the higher overall SFA content in comparison with our experiment.

Based on the indirect measurement of desaturase activity indoor lamb management don't cause any changes in the desaturase activity in muscle compared with traditional management of Sarda suckling lambs.

6.3.4 Contribution of Sarda lamb meat to human nutrition

Lamb meat is also interesting from a nutritional point of view for its composition and its fatty acid profile. In Italy, lamb meat is the first meat recommended by many pediatricians at weaning because is presumed to have a low allergenicity (Martino et al., 1998). In the diet of adult, 100 g of meat from Sarda suckling lamb can satisfy about 40% of protein recommended daily allowance (RDA) of adult (IOM, 2002). Regarding the importance of fatty acid in nutrition, the adequate daily intake (ADI) of PUFA n-3 is about 1.4 g/d. Considering that pasture feeding determined a PUFA n-3 content of 3.97% of the total fatty acids and a lipid content of 3 g/100g of leg meat, it can be estimated that 100 g of meat can satisfy about 10% of ADI for PUFA n-3.

Scientific evidences showed that consumption of PUFA n-3 has important role in the prevention of cardiovascular diseases, in the improvement of vision and learning in young and in the retardation of mental deterioration in elderly (Koletzo et al., 2008).

In human diet values of n-6/n-3 ratio lower than 4 are indicated as the most favorable by COMA in order to prevent some cardiovascular diseases (Department of Health, 1994); an increased consumption of PUFA n-3 has been recommended to overcome the perceived imbalance in the ratio of PUFA n-6/n-3 in human diets (Dervishi et al., 2010). The values of n-6/n-3 ratio found in our study were lower than recommended values as observed in different breeds of lambs (Lanza et al., 2006; Serra et al., 2009; Velasco et al., 2001; Rassu et al., 2010).

The meat of suckling lamb, especial on pasture, is an interesting source of RA compared with ruminant fed concentrate diet (Santos-Silva et al., 2002) or non-ruminant meat (Schmid et al., 2006; Corino et al., 2007). In this trial, 100 g of meat provide about 44 mg of CLA. Schmid et al. (2006) reported that meat and meat products contribute about 25-30% of the total human CLA intake in western population.

*Table 4 - Estimation of percentage of recommended daily allowance (% of RDA) satisfy by 100 g of raw meat from suckling Sarda lambs following the RDA of IOM (2002)**

	RDI/ADI g/d – males (IOM, 2002)	RDI/ADI g/d – females (IOM, 2002)	% of RDA or AI (males)	% of RDA or AI (females)
Protein	56	46	36	43.5
PUFA n-3	1.6	1.1	7.7	11.2
PUFA n-6	14	11	0.88	1.2

6.4 Conclusions

In conclusion lambs management system influence fatty acid composition of intramuscular fat. Generally lambs which followed their mother on pasture showed a qualitatively better fat, from a nutritional perspective, than lambs raised indoor due in particular to lower C14:0 and C16:0 and to the higher ALA, DPA and DHA content. Nutritional index such as PUFA/SFA ratio, AI and TI were also beneficial in regard to human health in lambs reared on pasture with their dams. Moreover Sarda suckling lamb meat contains a good level of fat and protein and of valuable health-promoting PUFA n-3.

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CHAPTER 7

Relationships between the contents of Vaccenic acid, CLA and highly unsaturated fatty acids of sheep milk and the muscle of their suckling lamb

7.1 Introduction

In several Mediterranean areas sheep meat is mainly produced from sucking lambs, raised exclusively on maternal milk and slaughtered between 20 and 30 days of age. This product, typical in Sardinia and known under the name of "agnello da latte", is very popular with consumers especially in specific periods (Christmas and Easter).

The characteristics of milk feeding regime could play a central role for lamb meat quality because in this phase, the lambs are "functional monogastrics", so there is no ruminal biohydrogenation of the milk fatty acids before they are absorbed from the intestine. Therefore milk fat of lactating sheep is the main source of fatty acid for the development of the fat depots of suckling lambs. Recently Nudda et al. (2008) reported a strong relationships between the concentration of some fatty acids (C18:1 *trans*-11; *cis*-9, *trans*-11 CLA and C18:3 n-3) in the muscle of suckling kids and ant those in their mother's milk and demonstrated that is possible to modify the fatty acid profile of meat from suckling kids by manipulating the diet of the dams.

The aim of the present work is to determine the degree of variability of fatty acid profiles of grazing sheep milk caused by supplementation with two doses of concentrate and to quantify the relationship between fatty acids profiles of lamb's meat and their mother's milk. Focus was given to changes in the content of C18:1 *trans*-11 (Vaccenic acid, VA), *cis*-9, *trans*-11 CLA (Rumenic acid, RA) and PUFA n-3 in milk and meat.

7.2 Materials and methods

7.2.1 Animals and diets

Twenty-four lambs were selected from 18 Sarda dairy ewers (average body weight 47.0±4.06 kg). The ewes grazed on natural pasture (8 hours/d) and supplemented with concentrate. After lambing the ewes were divided in two groups, balanced for single e twins lambs and supplemented with low (200 g/d; group L) or high (600 g/d; group H) amount of concentrate in order to modify the fatty acid profile of the milk. Lambs were kept into pens when their mothers were on pasture, and therefore were fed exclusively with mother milk.

Feed samples of the mother were collected at the beginning and at the end of the trial for subsequent chemical analysis. Ewe milk was collected the day before the lambs were slaughtered. One aliquot of milk was analysed for fat content and another was stored at – 20°C for subsequent fatty acid analysis.

The lambs were weighed and then slaughtered at 28 days of age. The cold carcass weight (CCW, body weight minus blood, skin, viscera, feet, tail) and pH was measured after 24 h of storage at 4 °C. After 24 h of refrigeration the tight muscles (*Semitendinosus*, *Semimembranosus* and *Femoral Biceps*) were dissected from each right half-carcass, vacuum packaged and stored at – 80°C until the fatty acid could be analysed.

7.2.2 Feed and muscle analysis

The DM content of muscle was determined by oven-drying at 105 °C for 24 h. Were also determined: ash (AOAC 920.153.1999), crude protein (CP) (Kjeldahl), and fat (Folch et al., 1957). Composition of fatty acids from milk and muscles was determined by gas chromatography using a Varian 3400 GC. Fat extraction and esterification procedure for fatty acid analysis were performed as reported in chapter 5 paragraph 1

and 2. The chromatographic conditions and fatty acid identification were carried out as reported in chapter 5 paragraph 4. The relative amount of each fatty acid (% of total FAME) is reported as a percentage of total peak area for all fatty acids. The sum of n-3 PUFA (C18:3 n-3, C20:5 n-3, and C22:6 n-3), the sum of n-6 PUFA (C18:2 n-6, C20:3 n-6, C20:4 n-6, and C22:4 n-6), and the ratio n-6/n-3 were calculated. The desaturase activities were estimated indirectly as [product]/[precursor + product] ratio as suggested by Kelsey et al. (2003). Thus, in muscle the Δ5-desaturase activity was calculated as [C20:4 n-6]/[C20:3 n-6 + C20:4 n-6] ratio, Δ6-desaturase activity was calculated as [C20:3 n-6]/[C18:2 n-6 + C20:3 n-6] ratio and Δ9-desaturase activity as [C18:1 n-9]/[C18:0 + C18:1 n-9] ratio (Andersson et al., 2000) . For milk fatty acids, an indirect index of Δ9-desaturase activity was calculated as the [RA]/[VA + RA] ratio.

Atherogenic index (AI), as a dietary risk indicator for cardiovascular disease, and Thrombogenic index (TI), as a sign of the potential aggregation of blood platelets, were calculated according to the formulas suggested by Ulbricht and Southgate (1991).

$$AI = \sum \frac{C12:0 + 4 * C14:0 + C16:0}{UFA + \sum JFA\omega6 + \sum JFA\omega3}$$

$$TI = \frac{}{0.5\sum UFA + 0.5\sum JFA\omega6 + 3\sum JFA\omega3 + /\omega} \quad C14:0 + C16:0 + C18:0$$

7.2.3 Statistical analysis

Differences in meat fatty acids among treatments (H and L concentrate of the ewes) and between males and females were assessed by *t*-test. Linear regression was used to examine relationships between fatty acid concentrations in meat and milk (mean of all weeks), and relationship between some FA in muscle. Significant differences are at $P < 0.05$ unless indicated otherwise.

Statistical analysis were performed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA).

7.3 Results and discussion

The chemical composition and fatty acid profiles of diet ingredients are shown in Table 1.

Table 1 - Fatty acid profile of the ingredients used in the diet.

	Food	
Fatty acid (g/100g of FAME)	Concentrate	Grass
C14:0	0.00	0.46
C16:0	15.73	1.82
C18:0	2.46	0.89
C18:1 *cis* 9	29.15	1.35
C18:2 n-6 (LA)	50.30	10.79
C18:3 n-3 (ALA)	2.36	69.12

Grass was richer than concentrate in PUFA, in particular, it had a higher proportion of ALA. On the contrary, the concentrate was richer in C16:0, C18:1 *cis*-9 and LA. Sheep consumed the whole daily amount of concentrates supplied.

Body weight of lambs (mean±SD) at slaughter was 9.54±1.43 Kg for male lambs and 9.07±1.43 Kg for female lambs. The average CCW was 4.57±0.84 Kg for male lambs and 4.17± 0.82 for female lambs.

7.3.1 Fatty acid composition of milk

The fatty acid profile of milk fat of ewes is reported in Table 2. The largest proportion of fatty acid in ewe milk was made up by C16:0, C18:0 and C18:1 *cis*-9.

Table 2 - Least square means of fatty acid profile (% of total FAME) of milk of grazing ewes fed different dose of concentrate.

	Concentrate				P value
Fatty acid (g/100g of FAME)	High	SD	Low	SD	
C4:0	1.81	0.14	1.64	0.20	†
C6:0	1.73	0.19	1.82	0.32	ns
C8:0	1.84	0.31	2.16	0.50	†
C10:0	5.01	1.27	7.06	1.93	**
C12:0	3.19	0.58	4.06	1.54	†
C14:0	8.70	0.80	10.20	1.43	**
C14:1	0.27	0.05	0.24	0.09	ns
C15:0	0.97	0.08	1.38	0.52	*
C15:1 *n*−5	0.28	0.04	0.30	0.05	ns
C16:0	21.20	1.70	23.17	1.93	*
C16:1 *n*−7	0.29	0.10	0.19	0.09	*
C17:0	0.84	0.05	0.84	0.13	ns
C17:1	0.21	0.11	0.31	0.08	*
C18:0	13.54	1.53	7.96	4.92	**
C18:1 *cis* – 9	24.34	2.86	17.76	7.48	*
C18:1 *trans* – 11 (VA)	3.82	0.56	7.22	6.31	†
CLA *cis* - 9, *trans* – 11 (RA)	1.52	0.28	1.28	0.65	ns
CLA *trans*-9, *cis*-11+C20:0	0.15	0.08	0.16	0.05	ns
CLA *trans* - 10, *cis* – 12	0.06	0.03	0.02	0.03	**
CLA *trans*-9, *trans*-11+C20:1	0.01	0.02	0.09	0.06	**
C18:2 n-6 (LA)	2.39	0.34	3.41	1.36	*
C18:3 n-3 (ALA)	1.05	0.13	1.03	0.49	ns
C20:4 n-6 (ARA)	0.10	0.06	0.18	0.03	**
C20:5 n-3 (EPA)	0.05	0.04	0.10	0.07	†
C22:5 n-3 (DPA)	0.10	0.06	0.13	0.06	ns
C22:6 n-3 (DHA)	0.03	0.03	0.04	0.03	ns
Sums and ratio					
SFA	59.00	3.04	62.46	2.73	**
MUFA	37.23	2.86	32.40	4.29	**
PUFA	3.77	0.57	5.15	2.17	*
Σn−3 PUFA[a]	1.24	0.21	1.29	0.58	ns
Σn−6 PUFA[b]	2.54	0.40	3.65	1.37	*
PUFA/SFA	0.06	0.01	0.08	0.03	†
Σn−6 / Σn−3	2.06	0.20	3.01	1.13	*
AI[c]	1.46	0.24	1.83	0.33	**
TI[d]	1.82	0.19	1.87	0.28	ns
CLA deaturase index[e]	0.28	0.03	0.24	0.14	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.
a [C18:3 n−3+C20:5 n−3+C22:5n-3+C22:6 n−3].
b [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].
c Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].
dThombogenic index=[C14:0+C16:0+C18:0]/[(0.5* ΣMUFA)+ 0.5*PUFA n-6+3*PUFA n-3+n-3/n-6)].
e CLA desaturase index= [RA]/[RA+VA].

The dose of concentrate influenced significantly several fatty acid. The content of VA tended to be higher ($P<0.10$) in L milk compared to H milk, whereas CLA and ALA in milk fat were not modify by the dose of concentrate in the diet. Those fatty acid are usually increased by the presence of pasture in the diet or by the higher forage to concentrate ratio. In this case both group of animals were on pasture for 8 hours a day and a higher grass intake from animals with lower amount of concentrate was expected. This hypothesis is supported by high content of VA in milk of the ewes with the low dose of concentrate that suggest an extensive ruminal biohydrogenation of ALA into VA and a great transfer of VA from rumen to mammary gland. The correspondent increased of in CLA content in L milk did not occurs. In fact the relationship between RA and VA was high and positive in H milk (Figure 1) and unexpectedly negative in the L group (Figure 2) due to the presence of 3 outlier that correspond to a very high and unusual content of VA in the milk of dairy ewes. The removal of the outliers resulted in the expected positive relationship between these two parameters ($r^2 = 0.83$).

The amount of *trans*-10, *cis*-12 CLA was about 1% of total CLA and was higher in H than L milk.

Among the highly unsaturated fatty acids (HUFA), the contents in milk were extremely low and showed differences between L and H ewes only for ARA ($P<0.01$) and EPA ($P<0.10$).

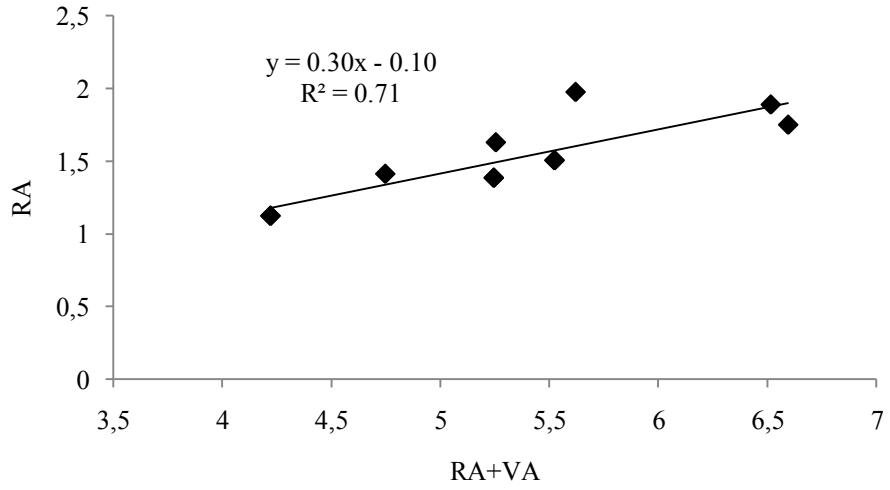


Figure 1 - Relationship between RA and RA+VA in milk of high group.

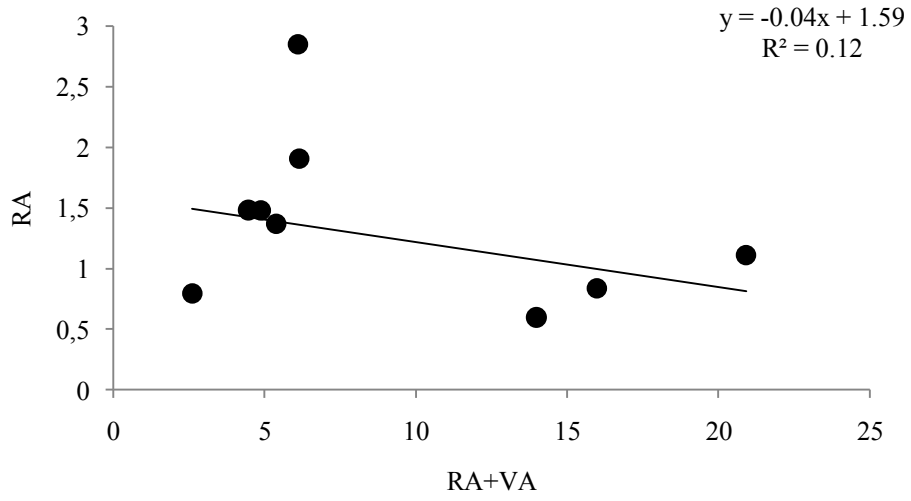


Figure 2 - Relationship between RA and RA+VA in milk of low group.

7.3.2 Chemical composition and fatty acid profile of lamb meat

Table 3 reports chemical characteristics of muscle of lambs with two different breeding system; only ash is influenced by ewe's diet ($P<0.01$).

Table 3 - Chemical composition of intramuscular fat of Sarda suckling lambs of Sarda ewes fed different dose of concentrate.

	Concentrate				P value
	High	SD	Low	SD	
Dry Matter DM (%)	26.26	2.06	25.19	0.90	ns
Fat (%)	3.16	0.67	2.93	1.70	ns
Protein (%)	19.96	1.55	21.03	0.85	ns
Ash (%)	1.31	0.06	1.18	0.03	**
pH	5.70	0.07	5.64	0.06	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

The mean fatty acid profiles of muscle fat of the experimental groups are reported in Table 4. There was no significant differences between males and females in all fatty acid of interest in this study (VA 2.73 g/100 g of FA vs. 2.41, P=0.15; RA 1.63 vs. 1.41 g/100 g of FA, P=0.13; PUFA n-3 3.28 vs. 2.96 g/100 g of FA, P=0.45, in males and females, respectively). Therefore, all data for males and females were pooled.

The fatty acid patterns of lamb's meat were similar to those of milk from the suckled ewes only for some fatty acids; in addition, several fatty acids that differed by treatment in milk did not differ significantly in muscle (Table 4).

Table 4 - Least square means of fatty acid profile (% of total FAME) of intramuscular fat of Sarda suckling lambs of Sarda ewes fed different dose of concentrate.

	Concentrate				P value
Fatty acid (g/100g of FAME)	High	SD	Low	SD	
C14:0	6.51	1.30	6.15	2.03	ns
C14:1	0.14	0.06	0.14	0.10	ns
C15:0	0.55	0.07	0.57	0.18	ns
C15:1 *n*−5	0.19	0.03	0.19	0.08	ns
C16:0	23.32	1.91	22.47	3.03	ns
C16:1 *n*−7	0.59	0.08	0.52	0.07	*
C17:0	1.04	0.11	0.96	0.10	†
C17:1	0.54	0.07	0.54	0.08	ns
C18:0	13.65	0.81	12.19	2.00	*
C18:1 *cis* – 9	36.57	2.80	34.36	4.96	ns
C18:1 *trans* – 11 (VA)	2.33	0.37	2.65	1.11	ns
CLA *cis* - 9, *trans* – 11 (RA)	1.48	0.24	1.56	0.42	ns
CLA *trans*-9, *cis*-11+C20:0	0.08	0.06	0.10	0.06	ns
CLA *trans* - 10, *cis* – 12	0.03	0.02	0.04	0.03	ns
CLA *trans*-9, *trans*-11+C20:1	0.08	0.06	0.15	0.08	*
C18:2 n-6 (LA)	4.29	0.71	6.21	2.09	**
C18:3 n-3 (ALA)	1.21	0.19	1.18	0.32	ns
C20:4 n-6 (ARA)	1.12	0.25	1.90	0.77	**
C20:5 n-3 (EPA)	0.53	0.18	0.69	0.31	ns
C22:5 n-3 (DPA)	0.67	0.24	0.99	0.35	*
C22:6 n-3 (DHA)	0.37	0.15	0.60	0.29	*
Sums and ratios					
SFA	45.80	3.07	43.22	4.72	ns
MUFA	45.68	3.17	44.63	3.72	ns
PUFA	8.52	1.25	12.14	3.81	**
Σn−3 PUFA[a]	2.77	0.68	3.47	1.17	†
Σn−6 PUFA[b]	5.75	0.79	8.67	2.76	**
PUFA/SFA	0.19	0.03	0.29	0.11	**
Σn−6 / Σn−3	2.18	0.66	2.52	0.36	ns
AI[c]	0.45	0.19	0.42	0.30	ns
TI[d]	1.27	0.17	1.12	0.26	ns
Δ5 desaturase[e]	0.77	0.03	0.77	0.03	ns
Δ6 desaturase[f]	0.08	0.02	0.09	0.03	ns
Δ9 desaturase[g]	0.73	0.02	0.74	0.02	ns
CLA desaturase index[f]	0.39	0.02	0.38	0.07	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

[a] [C18:3 n−3+C20:5 n−3+C22:5 n-3+C22:6 n−3].

[b] [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].

[c] Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].

[d] Thombogenic index=[C14:0+C16:0+C18:0]/[(0.5* ΣMUFA)+ 0.5*PUFA n-6+3*PUFA n-3+n-3/n-6)].

[e] Δ5 desaturase activity=[C20:4 n−6]/[C20:3 n−6+C20:4 n−6].

[f] Δ6 desaturase activity = [C20:3 n−6]/[C18:2 n−6+C20:3 n−6].

[g] Δ9 desaturase activity = [C18:1n−9]/[C18:0+C18:1 n−9].

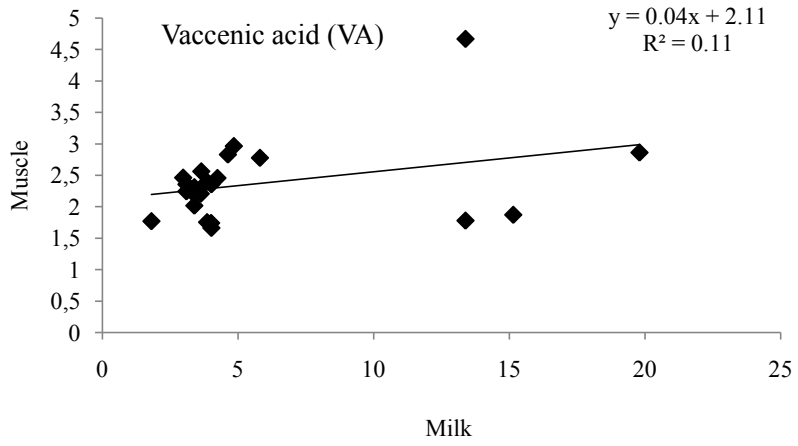
[f] CLA desaturase index =[RA]/[RA+VA].

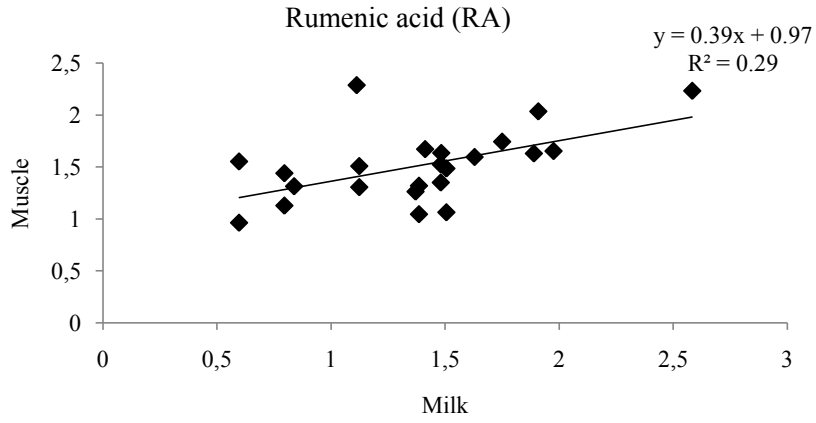
7.3.3 Relationships among various fatty acid between milk and muscle

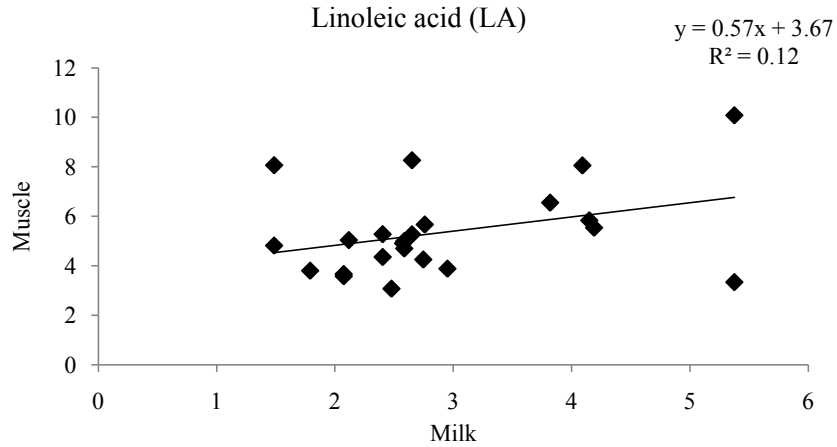
Regression analysis showed that the relationship between the percentage of C4 – C12 in muscle (Y) and in milk (X) was very low or absent (r^2=0.08) as would be expected because of the low accumulation of this fatty acid in tissues as reported also by Nudda et al. (2008) in suckling kids.

The linear regression (Figure 3) between percentages of VA in muscle and in milk showed a positive (but low) transfer of this fatty acid from milk to meat. The relationship between milk and meat for RA is positive and stronger (Figure 3) than that of VA in contrast with previous observation in suckling kids (Nudda et al., 2008). However, the positive intercepts for both VA ($P < 0.01$) and RA ($P<0.01$) suggest that these fatty acids may be present at birth. This is supported by recent observations that showed the incorporation of CLA and VA into lipids of the foetus (Nudda et al., 2007). Anyway, while the presence of VA in muscle is only exogenous, the RA in tissues has a dual origin because it derives partly from the milk and partly from endogenous synthesis from VA via Δ9-desaturase activity (Griinari et al., 2000).

Positive intercepts for LA and ALA suggest in *utero* incorporation for these fatty acids also, as shown by the presence of these fatty acids in phospholipids of maternal uterine endometrium of ewes in late gestation (Elmes et al., 2004). However, previous analysis of sheep foetal tissues at 145 days of gestation showed accumulation of ALA (Nudda et al., 2007). A low positive relationship was observed between milk and muscle for LA (r^2=0.12) and ALA (r^2=0.13) (Figure 3). This result indicates similar rates of incorporation for those essential fatty acids.


Vaccenic acid (VA)
y = 0.04x + 2.11
R² = 0.11
Muscle
Milk
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
5
0
5
10
15
20
25


Rumenic acid (RA)
y = 0.39x + 0.97
R² = 0.29
Muscle
Milk
0
0,5
1
1,5
2
2,5
0
0,5
1
1,5
2
2,5
3


Linoleic acid (LA)
y = 0.57x + 3.67
R² = 0.12
Muscle
Milk
0
2
4
6
8
10
12
0
1
2
3
4
5
6

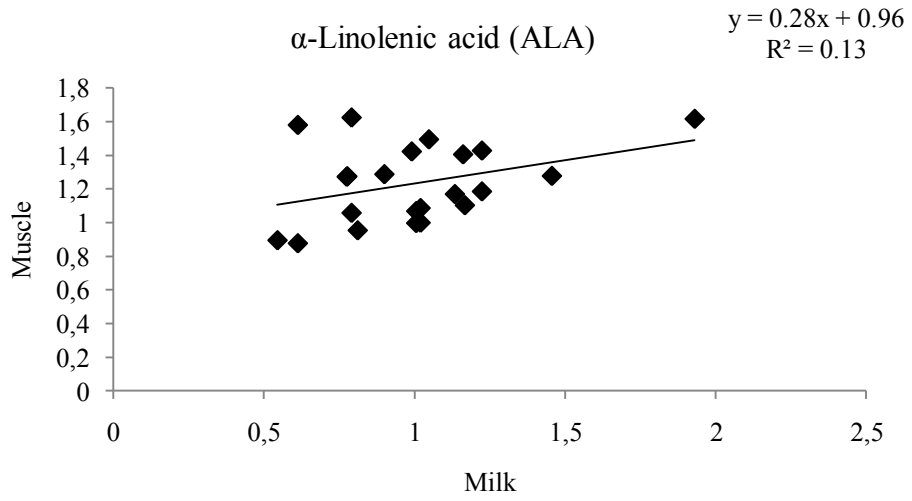
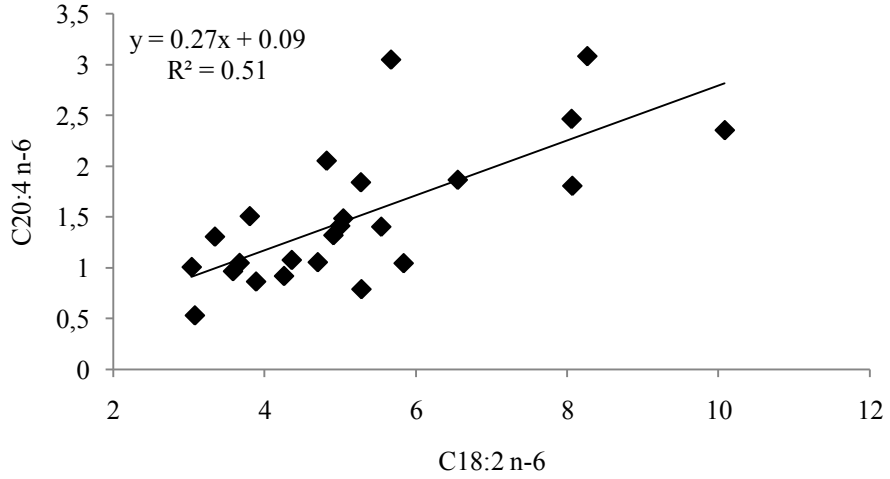


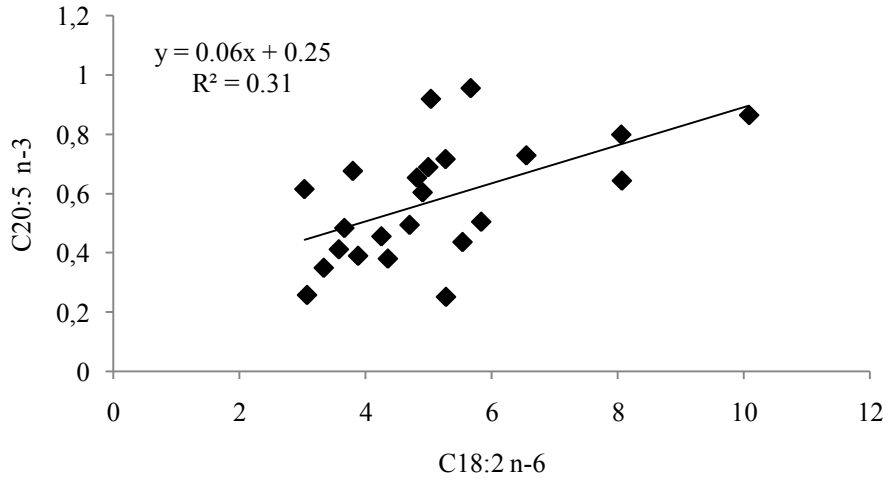
Figure 3 - Relationships between muscle of suckling lambs and milk of Sarda ewes fed different dose of concentrate for contents (g/100 g of FAME) of Vaccenic acid, Rumenic acid, Linoleic acid and α-Linolenic acid.

The eicosanoic fatty acids (ARA, EPA and DHA) in muscle were all positively correlated with increasing concentrations of both LA and ALA (Figure 4).

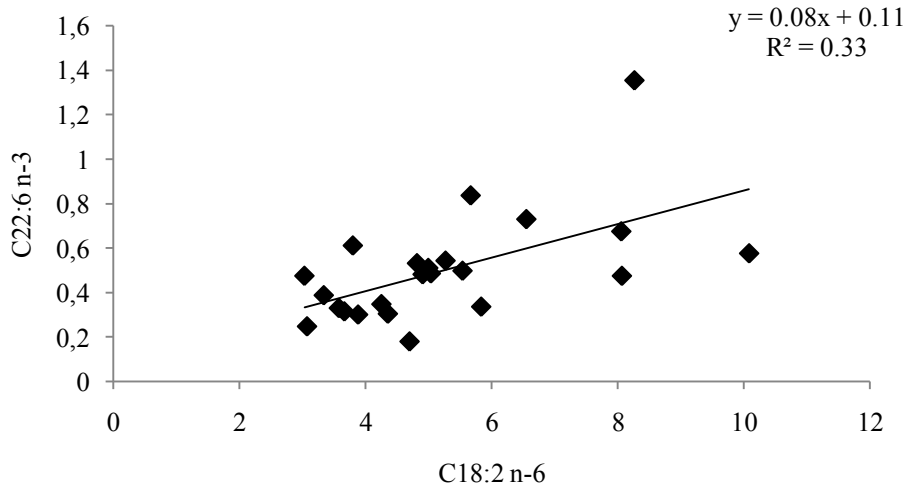
A)



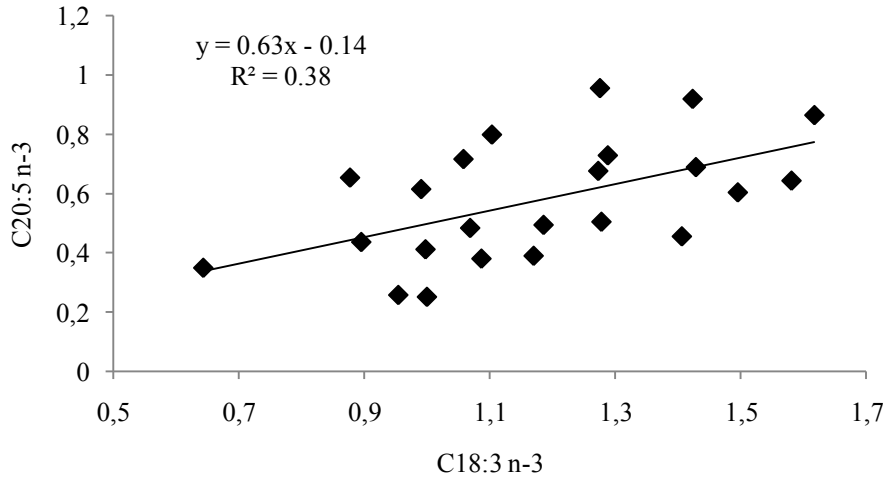
B)


y = 0.06x + 0.25
R² = 0.31
C20:5 n-3
C18:2 n-6
1,2
1
0,8
0,6
0,4
0,2
0
0
2
4
6
8
10
12

C)


y = 0.08x + 0.11
R² = 0.33
C22:6 n-3
C18:2 n-6
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
2
4
6
8
10
12

D)



E)

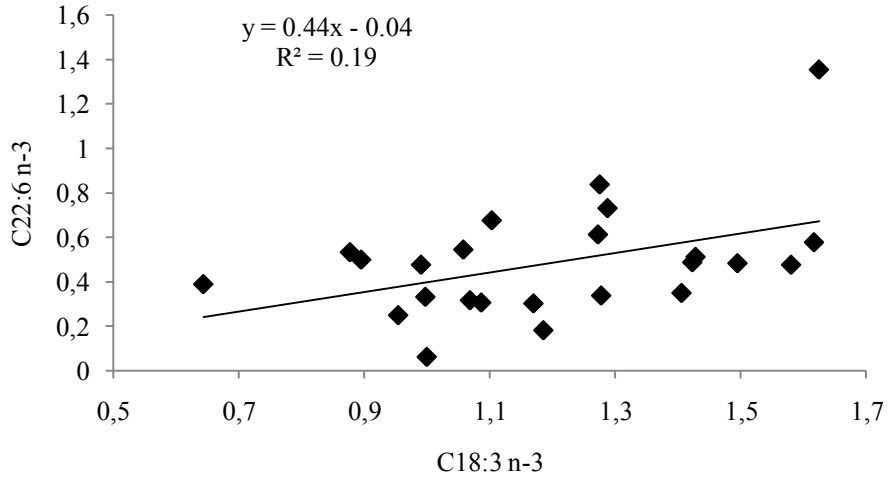


Figure 4 - Relationships between C18:2 n-6 and C20:4 n-6 (A), C20:5 n-3 (B), or C22:6 n-3 (C), and between C18:3 n-3 and C20:5 n-3 (D) or C22:6 n-3 (E) (g/100g of FAME) in muscle of suckling lambs of Sarda ewes fed different dose of concentrate.

It is interesting that although increasing concentrations of n-6 and n-3 fatty acids usually competitively inhibit chain lengthening and desaturation (Sprecher, 2000), this was not apparent in the leg muscle of young lambs and confirm previous observation in kids (Nudda et al., 2008). Most likely the concentrations of LA and ALA in milk were not

high enough to achieve inhibitory concentrations in muscles. Those fatty acids are already present before lambing (Nudda et al., 2008), by the tissue rate of conversion of ALA to DHA after birth is not know. In human and laboratory animals have a very limited metabolic activity to convert ALA to DHA (Brenna et al., 2009). In our trial the positive, even if low, relationship between LA and ALA with very long chain UFA suggest that a conversion of essential fatty acid to their long derivatives occur in this specie after birth.

7.4 Conclusions

The relationships between the concentrations of VA, RA and linolenic acid in the muscle of suckling lambs and those of their mother's milk is low but seen influenced by the diet of the mother. The relationship of LA and ALA with very long chain unsaturated FA in the muscle tissue support that a conversion of essential fatty acid to their long derivatives occur in this specie after birth.

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CHAPTER 8

The effects of natural or artificial milk on fatty acid profile of Sarda breed suckling lambs

8.1 Introduction

In Mediterranean areas, the typical dairy sheep production system is based on milk specialized breeds and ewe milking commences soon after weaning or slaughtering of lambs, traditionally 25–35 days after birth. These young animals are traditionally raised with their dams and fed only with maternal milk, but breeders are increasingly applying early weaning programmes followed by artificial suckling with milk replacers, in order to increase milk availability for cheese production and to allow the survival of lambs. However, composition of these two milk sources have shown substantial differences especially on fatty acid profile (Vincenti et al., 2004; Lanza et al., 2006; Osorio et al., 2007).

The characteristics of milk feeding regime could play a central role for suckling lamb meat quality because in this phase lambs are "functional monogastric", so there is no ruminal biohydrogenation of the milk fatty acids before they are absorbed from the intestine. As a consequence, intramuscular fatty acid composition could be modified by the different fatty acid profile of milk source (Napolitano et al., 2002; Vincenti et al., 2004; Lanza et al., 2006; Osorio et al., 2007). Napolitano et al. (2002) showed that intramuscular fat of lamb reared with maternal milk had significantly more fat and a higher content of SFA, ALA and a two- to three fold higher content of EPA and DHA than artificially reared lambs. A similar pattern was observed in Barbaresca suckling lambs by Lanza et al. (2006) and in Churra suckling lambs by Osorio et al. (2007).

The aim of this work was to evaluate meat quality of Sarda suckling lambs fed exclusively a milk replacer as compared to lambs fed exclusively maternal milk, with emphasis on intramuscular fatty acid composition.

8.2 Materials and methods

8.2.1 Animals and diets

A total of 24 lambs of the Sarda breed were involved in the experiment. After delivery lambs from each ewe were divided into two groups. One group of 12 lambs was given maternal milk as their sole feed (M group). The second group of 12 lambs was separated from their mothers and housed in a straw-bedded pen. The lambs from this group were given a milk replacer (R group) as their sole feed (crude protein 24%, crude fat 24%, crude fibre 0%, ash 7%) for the whole experimental period. The milk replacer used in our trial included, as reported in commercial label, milk products, cereal products, a mixture of vegetable oils, mineral and vitamin. Individual milk samples were taken before the morning suckling and stored at −20 °C for subsequent fatty acid analysis.

The lambs were weighed and then slaughtered at 28 days of age. The cold carcass weight (CCW, body weight minus blood, skin, viscera, feet, tail) and pH was measured after 24 h of storage at 4 °C. After 24 h of refrigeration the tight muscles (*Semitendinosus*, *Semimembranosus* and *Femoral Biceps*) were dissected from each right half-carcass, vacuum packaged and stored at – 80°C until the fatty acid could be analysed.

8.2.2Milk and muscle analysis

The DM content of muscle was determined by oven-drying at 105 °C for 24 h. Were also determined: ash (AOAC 920.153. 1999), crude protein (CP) (Kjeldahl), and fat (Folch et al., 1957). Composition of fatty acids from milk and muscles was determined by gas chromatography using a Varian 3400 GC. Fat extraction and esterification procedure for fatty acid analysis were performed as reported in chapter 5 paragraph 1 and 2. The chromatographic conditions and fatty acid identification were carried out as reported in chapter 5 paragraph 4. The content of each fatty acid was expressed as a

percentage of total fatty acid. The sum of n-3 PUFA (C18:3 n-3, C20:5 n-3, and C22:6 n-3), the sum of n-6 PUFA (C18:2 n-6, C20:3 n-6, C20:4 n-6, and C22:4 n-6), and the ratio n-6/n-3 were calculated. The desaturase activities were estimated indirectly as [product]/[precursor + product] ratio as suggested by Kelsey et al. (2003). Thus, in muscle the Δ5-desaturase activity was calculated as [C20:4 n-6]/[C20:3 n-6 + C20:4 n-6] ratio, Δ6-desaturase activity was calculated as [C20:3 n-6]/[C18:2 n-6 + C20:3 n-6] ratio and Δ9- desaturase activity as [C18:1 n-9]/[C18:0 + C18:1 n-9] ratio (Andersson et al., 2000). Atherogenic index (AI), as a dietary risk indicator for cardiovascular disease, and Thrombogenic index (TI), as a sign of the potential aggregation of blood platelets, were calculated according to the formulas suggested by Ulbricht and Southgate (1991).

$$AI = \frac{C12:0 + 4 * C14:0 + C16:0}{UFA + \Sigma JFA\omega6 + \Sigma JFA\omega3}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5\sum UFA + 0.5\sum JFA\omega6 + 3\sum JFA\omega3 + \omega/\omega}$$

8.2.3 Statistical analysis

All data were analyzed with ANOVA one-way to asses differences in FAME of milk source and muscle, using milk source as the main factor. Differences between males and females were assessed by *t*-test.

Statistical analysis was performed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA).

8.3 Results and discussion

The lambs body weight (mean ± SD) at slaughtering was 9.30±1.47 Kg for M reared lambs and 8.09±1.92 Kg for R reared lambs. The CCW was 4.37±0.83 Kg for M reared lambs and 3.57±0.50 for R group. Higher body and carcass weights in Comisana and Barbaresca suckling lambs reared with replacer were previously observed compared with animals reared with milk (Napolitano et al., 2002; Lanza et al., 2006). The better welfare condition in lambs fed with maternal milk in compared to lambs fed milk replacer (Napolitano et al., 2002) may have partially contributed to the higher body and carcass weight found in our trial.

8.3.1 Fatty acid profile of ewe milk and milk replacer

Fatty acid contents of ewe milk and milk replacer used for the lambs are shown in table 1. Significant differences between the feeds were observed for almost all fatty acid analyzed. Strong differences between M and R were observed for odd chain fatty acid (OCFA) and branched chain fatty acid (BCFA) that were undetectable in milk replacer compared with ewe's milk, probably related to the high presence of vegetable products in the replacer. The milk replacer had a lower amounts of short and medium chain fatty acid compared to milk replacer due to the addition of vegetable oils, which are characterized by the lack of fatty acid with a carbon length <C16:0.

The VA, that represents 75% of total trans monoenic fatty acid in ewe milk, was completely absent in milk replacer. Similarly, RA, that represent about 70% of total CLA was almost undetectable in the replacer compared to ewe's milk.

The content of LA, was three times higher in milk replacer compared to maternal milk and this accounted for a higher PUFA n-6 proportion. The content of PUFA n-3 did not differ significantly between the two milk sources due to the similar content of ALA. This

is easily explained by the facts that in the most common vegetable oils (sunflower, soybean, rape, etc.), the LA is the most abundant PUFA.

Due to the higher content of PUFA n-3 and the lower content of PUFA n-6 the n-6/n-3 ratio was lower in ewe milk compared with replacer. Moreover ewe milk showed a higher content of SFA and a lower of PUFA that accounted for a lower PUFA/SFA ratio.

The milk fatty acid profile of commercial milk-replacers used for rearing lambs or kids were previously analyzed and, then, compared to the fatty acid contents of ewes' (Napolitano et al., 2002; Lanza et al., 2006; Osorio et al., 2007) or goat's milk (Bañon et al., 2006). In all studies, the comparison the two milk sources showed the higher percentages of SFA and lower of PUFA in ewe milk fat, explained by the fact that milk replacer usually include in its formulation different sources of vegetable oils that are characterized by a high content of LA.

Table 1 - Least square means of fatty acid profile (% of total FAME) of ewes' milk and milk-replacer.

	Milk replacer	Ewe's milk	P value
Fatty acid (g/100g of FAME)			
C4:0	0.10	1.68	**
C6:0	0.31	1.80	**
C8:0	2.37	2.09	ns
C10:0	2.14	6.46	†
C12:0	14.90	3.98	**
C14:0	6.79	9.63	†
C14:1	0.00	0.28	*
C15:0	0.09	1.18	*
C15:1 *n*−5	0.00	0.29	**
C16:0	22.14	22.54	ns
C16:1 *n*−7	0.00	0.26	**
C17:0	0.09	0.85	**
C17:1	0.00	0.28	**
C18:0	4.21	10.25	ns
C18:1 *cis* – 9	35.12	21.25	*
C18:1 *trans* – 11 (VA)	0.00	4.31	**
CLA *cis* - 9, *trans* – 11 (RA)	0.07	1.58	**
CLA *trans*-9, *cis*-11+C20:0	0.28	0.16	*
CLA *trans* - 10, *cis* – 12	0.00	0.05	ns
CLA *trans*-9, *trans*-11+C20:1	0.00	0.04	ns
C18:2 n-6 (LN)	8.77	2.90	**
C18:3 n-3 (ALA)	1.19	0.95	ns
C20:4 n-6 (ARA)	0.00	0.15	**
C20:5 n-3 (EPA)	0.05	0.07	ns
C22:5 n-3 (DPA)	0.00	0.12	*
C22:6 n-3 (DHA)	0.00	0.03	ns
Sums and ratios			
SFA	53.26	60.81	†
MUFA	36.64	34.92	ns
PUFA	10.01	4.27	**
Σn−3 PUFA[a]	1.24	1.17	ns
Σn−6 PUFA[b]	8.77	3.10	**
PUFA/SFA	0.19	0.07	**
Σn−6 / Σn−3	7.06	2.81	*
AI[c]	1.38	1.68	ns
TI[d]	1.25	1.86	*

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

[a] [C18:3 n−3+C20:5 n−3+C22:5 n-3+ C22:6 n−3].

[b] [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].

[c] Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].

[d]Thombogenic index=[C14:0+C16:0+C18:0]/[0.5* ΣMUFA+ 0.5*PUFA n-6+3*PUFA n-3+n-3/n-6)].

8.3.2 Chemical composition of lamb meat

Chemical characteristics of intramuscular fat of Sarda suckling lambs reared with different milk sources were reported in Table 2.

Table 2 - Chemical composition of intramuscular fat of Sarda suckling lamb reared with different milk source (g/100 g).

	R	SD	M	SD	P value
Dry matter (DM) %	23.92	0.30	25.72	1.61	*
Fat (%)	1.80	0.37	3.04	1.24	*
Protein (%)	19.66	0.36	20.49	1.32	ns
Ash	1.25	0.03	1.24	0.08	ns
pH	5.79	0.11	5.67	0.07	ns

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

Meat from the R group showed a lower DM (P<0.05) and fat content (P<0.05). The lower fat in R group could be attributed to the different carcass weight. Similar results were reported by Lanza et al. (2006) when compared meat of lambs milk reared with different milk source. This author reported higher values of crude fat and protein in *Longissimus Dorsi* of naturally milk reared lambs compared to artificially reared lambs (1.92 vs. 1.63%, respectively and 20.41 vs. 20.26%, respectively). Similarly in suckling kids reared with milk replacer Bañon et al. (2006), reported lower fat and protein content in comparison to goat's milk suckled kids (1.02 vs. 1.19% and 22.2 vs. 21.0, respectively).

8.3.3 Fatty acid composition of lamb meat

Fatty acid contents of intramuscular fat of Sarda suckling lambs reared with different milk sources were reported in Table 3. There was no significant differences between males and females in all fatty acid of interest in this study (VA 1.33 g/100 g of FA vs.

0.93, P=0.66; RA 0.92 vs. 0.63 g/100 g of FA, P=0.63; PUFA n-3 2.8 g/100 g of FA vs. 3.24, P=0.35, total PUFA 15.26 vs. 20.68 g/100 g of FA, P=0.32, in males and females, respectively). Therefore, all data for males and females were pooled.

Milk source had relevant effect on almost all fatty acid analyzed.

Table 3 - Least square mean of fatty acid profile (% of total FAME) of intramuscular fat of Sarda suckling lamb reared with different milk source.

	Milk replacer	SD	Ewe's milk	SD	P value
Fatty acid (g/100g of FAME)					
C10:0	0.00	0.00	0.11	0.10	*
C12:0	1.46	0.72	0.58	0.26	**
C14:0	4.92	0.95	6.29	2.08	†
C14:1	0.19	0.05	0.16	0.09	ns
C15:0	0.08	0.05	0.53	0.15	**
C15:1 *n*−5	0.00	0.00	0.19	0.08	**
C16:0	19.90	1.66	23.03	2.95	*
C16:1 *n*−7	0.33	0.09	0.44	0.19	ns
C17:0	0.17	0.01	0.97	0.12	**
C17:1	0.01	0.03	0.56	0.07	**
C18:0	8.47	0.83	13.07	1.62	**
C18:1 *cis* – 9	37.91	2.10	35.35	4.22	ns
C18:1 *trans* – 11 (VA)	0.00	0.00	2.34	0.34	**
CLA *cis* - 9, *trans* – 11 (RA)	0.08	0.04	1.63	0.29	**
CLA *trans*-9, *cis*-11+C20:0	0.11	0.06	0.10	0.05	ns
CLA *trans* - 10, *cis* – 12	0.00	0.00	0.04	0.02	**
CLA *trans*-9, *trans*-11+C20:1	0.28	0.06	0.11	0.06	**
C18:2 n-6 (LN)	15.56	1.08	5.08	1.47	**
C18:3 n-3 (ALA)	0.82	0.04	1.26	0.22	**
C20:4 n-6 (ARA)	5.15	1.21	1.58	0.77	**
C20:5 -3 (EPA)	0.45	0.09	0.66	0.30	ns
C22:5 n-3 (DPA)	0.89	0.25	0.95	0.35	ns
C22:6 n-3 (DHA)	0.52	0.11	0.59	0.29	ns
Sums and ratios					
SFA	35.32	2.39	44.63	4.61	**
MUFA	40.81	1.88	44.77	3.77	*
PUFA	23.87	2.60	10.60	3.32	**
Σn−3 PUFA[a]	2.68	0.43	3.46	1.10	ns
Σn−6 PUFA[b]	21.19	2.21	7.15	2.30	**
PUFA/SFA	0.68	0.11	0.24	0.10	**
Σn−6 / Σn−3	7.97	0.65	2.08	0.34	**
AI[c]	0.64	0.19	0.90	0.30	*
TI[d]	0.85	0.23	1.18	0.24	**
Δ5 desaturase[e]	0.91	0.11	0.76	0.04	**
Δ6 desaturase[f]	0.03	0.04	0.09	0.03	**
Δ9 desaturase[g]	0.82	0.06	0.73	0.02	**

**P≤0.01; *P≤0.05; †P≤0.10; ns = not significant.

[a] [C18:3 n−3+C20:5 n−3+C22:5 n-3 + C22:6 n−3].

[b] [C18:2 n−6+C20:3 n−6+C20:4 n−6+C22:4 n−6].

[c] Atherogenic index=[(C12:0+ 4*(C14:0)+(C16:0)]/[ΣMUFA+ΣPUFA n-6+ΣPUFAn-3].

[d]Thombogenic index=[C14:0+C16:0+C18:0]/[(0.5* ΣMUFA)+ 0.5*PUFA n-6+3*PUFA n-3+n-3/n-6)].

[e] Δ5 desaturase activity=[C20:4 n−6]/[C20:3 n−6+C20:4 n−6].

[f] Δ6 desaturase activity = [C20:3 n−6]/[C18:2 n−6+C20:3 n−6].

[g] Δ9 desaturase activity = [C18:1n−9]/[C18:0+C18:1 n−9].

The percentages of saturated fatty acid (SFA), were higher in meat from the M lambs than in meat from the R lambs due to a higher content of C14:0 ($P<0.10$), C15:0 ($P<0.01$), C16:0 ($P<0.05$), C17:0 ($P<0.01$) and C18:0 ($P<0.01$), Since the fatty acid profile of meat may reflect the composition of feeds ingested by lambs, this result could be attributed to the higher presence of these SFA in maternal milk compared to milk replacer (60.81 vs. 53.26 g/100g of FAME respectively). Higher percentages of SFA was observed in Barbaresca (Lanza et al. 2006), Comisana (Napolitano et al., 2002) and Churra (Osorio et al. 2007) suckling lambs and in perirenal fat of Murciano - Granadina suckling kids (Bañon et al., 2006) reared with different milk source.

The content of VA which represents about 75% of total trans monoenic fatty acid in intramuscular fat was not detectable in R lambs and this is probably due to its lack in milk replacer as shown by Lanza et al. (2005). VA is an important source of conjugated linoleic acid in animal tissues, through Δ9-desaturase action (Bauman et al., 1999) and consequently, the content of RA, that accounted for about 60% of total CLA in muscle, was twenty-fold higher in M than in R group intramuscular fat. The difference between the groups in terms of the RA proportion in meat could be attributed to the different RA concentrations in natural milk and milk replacer (1.59 and 0.07 g/100g of FAME, respectively). Similar results were observed by Lanza et al. (2006) that reported a 2.4 fold higher content of RA in natural milk reared lambs compared with artificially reared lambs. On the other hand a contrasting result was found by Osorio et al. (2007), who observed a higher content of RA in intramuscular fat of Churra lambs reared with milk replacer compared with maternal milk reared lambs (0.67 and 0.57%, respectively). The author explained this discrepancies by differences in *trans*-C18:1 fatty acid contents of milk sources.

The amount of LA (P<0.01) and ARA (P<0.01) were significantly higher in R group compared to M group. This result is in agreement with Osorio et al. (2007) who found in intramuscular fat of natural suckled lambs lower proportion of LA, ARA and consequently of PUFA n-6 compared of replacer suckled lambs (4.05 vs. 8.48%, 1.09 vs. 2.07%, 11.13 vs. 14.36%, respectively). These differences in fatty acid profile of meat are likely to be associated to the different fatty acid composition of milk sources. In fact the fatty acid profile of milk replacer, rich in LA due to a large vegetable oils inclusion, justifies this result as previously reported (Napolitano et al., 2002, Lanza et al., 2006; Osorio et al., 2009). The higher content of ARA in R meat compared to M meat should be related to the origin of this fatty acid from LA in tissues via the desaturation-elongation pathways.

The content of ALA (P<0.01) showed a higher proportions in M lambs than in R lambs although (P<0.05). Its derivatives, such as EPA, DPA and DHA are numerically higher in M lambs compared to R lambs even if the differences did not reach the level of significance. Therefore, the total PUFA n-3 was higher in meat of M than R lambs. Our results are in agreement with Osorio et al. (2007) that found higher content of ALA and consequently of total PUFA n-3 in ewe's milk group compared with milk replacer group (1.30 vs. 0.12%, and 3.09 vs. 0.70% respectively). The opposite result was reported by Vincenti et al. (2004) in lambs (0.93 vs. 0.73%) and by Bañon et al. (2006) in kids (0.40 and 2.04%, respectively) which found higher proportion of ALA in meat of lambs reared with replacer than in naturally reared lambs due to the supplementation of replacer with PUFA.

The nutritional ratios were significantly affected by the different milk source. The higher content of PUFA and the lower of SFA in R lambs accounted for a higher (P<0.01) PUFA/SFA ratio in comparison to M lambs, according to previous observation

(Napolitano et al., 2002; Lanza et al., 2006). The value of the n-6/n-3 ratio in meat from the M lambs was lower than in meat from R lambs ($P<0.01$) and in the recommended range for human health. Whereas the ratio in the R group was much higher (around 8) than that of 4.0 normally recommended in the diet of humans (Simopoulos et al., 2008). This could be related to the higher content of PUFA n-6 and in particular of LA in this group due to the different fatty acid composition of replacer and maternal milk. Our result agrees with previously observation in intramuscular fat of lambs reared with different milk sources (Osorio et al., 2007, Lanza et al., 2006, Napolitano et al., 2002). The mean values for n-6/n-3 ratio were in agreement with those reported in Barbaresca and Comisana suckling lambs reared with maternal or artificial milk (2.61 and 9.70; 1.95 and 9.53, respectively for Barbaresca and Comisana) (Lanza et al., 2006; Napolitano et al., 2002).

Atherogenic index (AI), as a dietary risk indicator for cardiovascular disease, and Thrombogenic index (TI), as a sign of the potential aggregation of blood platelets, were significantly lower ($P<0.05$ and $P<0.01$, respectively) in M lambs compared to R lambs. This result is probably due to the lower content of SFA and to the higher content of PUFA in R lambs compared with M lambs. Contrasting with our own results Vincenti et al. (2004) found similar values of AI and TI in raw meat of milk replacer fed lambs compared to maternal milk fed lambs (1.32 vs. 1.34 and 1.70 vs. 1.68 respectively). Whereas a similar result were observed when milk replacer was supplemented with PUFA (Vincenti et al., 2004).

Based on the indirect measurement of desaturase activity, milk replacer source caused significant changes in the Δ5-, Δ6- and Δ9-desaturase activity ($P<0.01$) in muscle of lambs. The Δ9-desaturase, also known as Stearoyl-CoA desaturase-1, is used to synthesize oleic acid, by desaturating stearic acid, a saturated fatty acid either

synthesized in the body from palmitic acid or ingested directly. Its activity, measured indirectly, was higher ($P<0.05$) in R lambs compared to M lambs. The Δ6- and Δ5-desaturases are required for the synthesis of highly unsaturated fatty acids (HUFA) such as EPA and DHA (synthesized from ALA), and ARA (synthesized from LA) which are mainly esterified into phospholipids and contribute to maintaining membrane fluidity (Nakamura and Nara, 2004). The value of Δ5- was higher in R lambs compared to M lambs that evidenced a higher conversion of LA in ARA. On the contrary, the value of Δ6- was higher in M lambs compared to R lambs suggesting a higher efficiency in the conversion of ALA to HUFA n-3.

8.4 Conclusions

In conclusion the results of this experiment found that milk source can affect the nutritional characteristics of meat of unweaned lambs. In fact, in unweaned young lambs rumen is not yet functional and fatty acid profile of meat seems to reflect the composition of ingested milk. Suckling lamb feeding regime, based on a milk replacer as the exclusive feed, tended to reduce growth performance and carcass weight as compared to a natural milk-feeding regime. Intramuscular fatty acid composition showed a less favorable profile in meat from lambs artificially reared than in lambs naturally milk-suckled. Feeding a milk replacer may improve the PUFA/SFA ratio toward the value of 0.45 required in the human diet. Nevertheless, this technique had a negative effect on the n-6/n-3 ratio due to higher linoleic acid and its derivatives, and showed a lower CLA content than meat from lambs naturally reared.

Therefore the fatty acid composition of commercial milk-replacers should be designed in order to make fat composition of milk-replacer reared suckling lamb meat more appropriate for human nutrition.

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CHAPTER 9

Comparison of fatty acid profile in lamb meat and baby food based on lamb meat

9.1 Introduction

The importance of fatty acids (FA) in human nutrition for optimal foetal and neonatal development is well known (Cetin and Koletzko, 2008). At weaning the first meat recommended by Italian pediatricians to be introduced in the baby's new feeding regimen is lamb meat. This is related to the lower allergenicity of this meat to infants with atopic dermatitis compared to other red meats (Martino et al., 1998; Fiocchi et al., 2000). Available information on fat in meat baby foods refers mainly to the total amount of saturated, monounsaturated and polyunsaturated fat, whereas that on FA composition of these products is not commonly given. Moreover, the origin of the ingredients used in baby foods is normally not available for consumers. For example, labels do not indicate neither the kind of lamb meat used nor the amount of lamb meat included in lyophilized baby food. Since the FA composition of lamb meat is strictly related to breed, feeding regimen and slaughtering age and weight (Diaz et al., 2005; Valvo et al., 2005; Lanza et al., 2006; Serra et al., 2009), the origin of lamb meat may influence the final nutritional characteristics of lamb-based baby food. Sarda sheep is the main ovine breed in Italy; thus, meat from suckling lambs of this breed could be a reliable source of meat for the baby food industry. Therefore, the aim of this preliminary study was to compare the FA profile of fresh lamb meat with those of infant foods.

9.2 Material and methods

In the year 2008, 12 samples of homogenized lamb meat baby food (80-g jars; HO) produced by three companies, 12 samples of lyophilized (freeze–dried) lamb meat baby food (30-g jars; LIO), produced by two companies, and 12 samples of fresh lamb meat (FM) were collected and analyzed. The label of the HO reported a content of 40% of meat, whereas LIO samples contained 85% of lamb meat. Suckling lambs born from Sarda ewes were slaughtered, and samples of leg muscle (*Longissimum Dorsi*, *Semitendinosus*, *Semimembranosus* and *Femoral Biceps)* were obtained. After removal of the intermuscular residual adipose tissue from these muscles, the FM samples were freeze-dried and finely ground in a food processor. Fat extraction was performed using chloroform:methanol (2:1). Fatty acid methyl ester (FAME) from the triglyceride fraction was obtained using the standard FIL-IDF methylation procedure (1999). The chromatographic conditions were the same as those described by Nudda et al. (2008). The FA were identified by comparing the retention times of peaks with those of methyl ester standards. The content of each FAME was expressed as a percentage of total FAME.

9.2.1 Statistical analysis

Data were analyzed with one-way ANOVA using type of product as the main effect. Differences among brands for each baby food were tested with ANOVA. Differences were considered significant at $P<0.05$. Statistical analysis was performed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA).

9.3 Results

The average fat content of LIO and HO products determined in the laboratory were in accordance with the medium lipid content reported in their nutritional labels; the values of fat content of LIO and HO products showed a high variability between the brands both for LIO and HO (Figure 1 and 2).

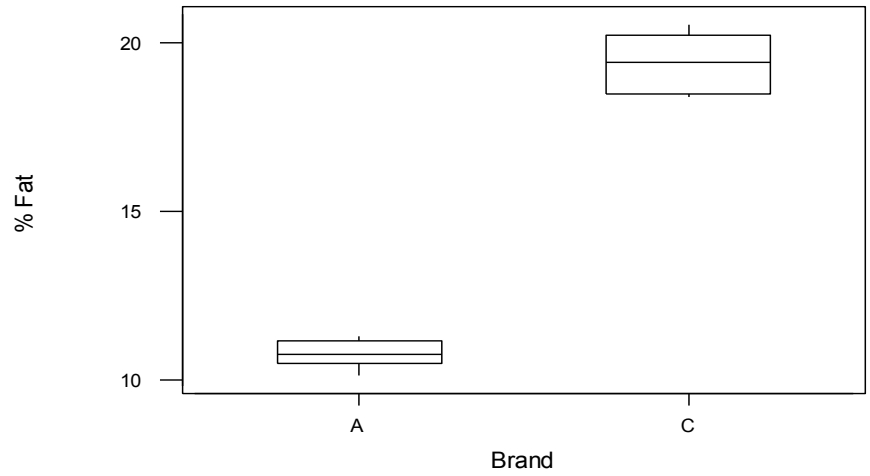

Figure 1 - Box plot of fat content (%) in the different brands of LIO analyzed.

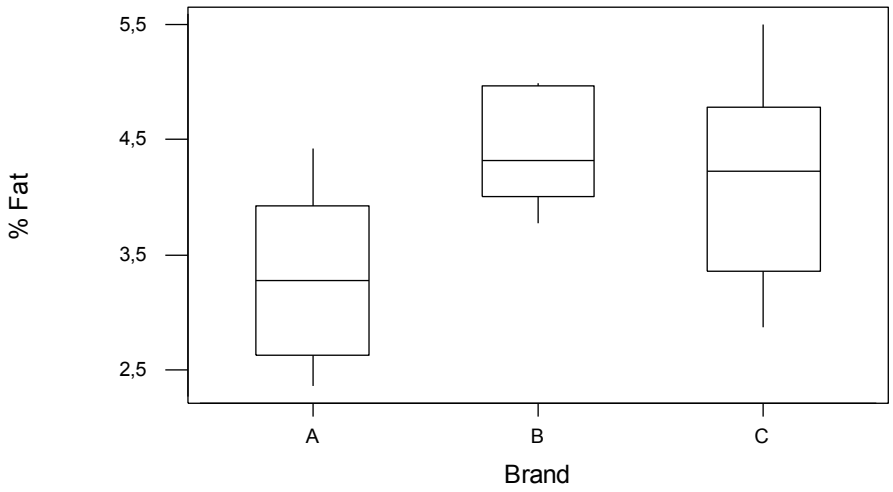

Figure 2 – Box plot of fat content (%) in the different brands of HO analyzed.

Values of fat content ranged from 15.0 and 23.0% in LIO and from 4.0 to 5.4% in HO. However differences between fat content reported in nutritional label and fat content determined in the laboratory are probably due to the different method of analysis. In fact the method used in our laboratory is the method of Folch et al. (1957), while the official method for the determination of fat in meat products is the method Soxhlet.

The FA profile of the 3 products analyzed are reported in Table 1. Significant differences were observed for fat content and for all fatty acid analyzed except for C18:1 *cis*-9, *cis*-9, *trans*-11 CLA (Rumenic acid, RA) and total CLA.

Table 1 - Fat content and fatty acid profile of fresh lamb meat (FM) and homogenized (HO) and lyophilized (LIO) baby food based on lamb meat.

	FM	LIO	HO	P value
Fat content, % as fed	3.4	15.1	4.0	**
Fatty acid (g/100g of FAME)				
<C14	0.92[a]	0.48[b]	0.36[b]	**
C14:0	5.68[a]	3.78[b]	2.37[b]	**
C14:1	0.17[a]	0.11[b]	0.10[b]	**
C16:0	20.65[a]	22.62[a]	17.42[b]	**
C16:1	1.44[a]	1.45[a]	0.92[b]	**
C18:0	14.43[b]	20.69[a]	15.72[b]	**
C18:1 *trans*-11 (VA)	2.17[b]	3.70[a]	2.96[ab]	*
C18:1 *cis*- 9	34.27	33.95	32.47	ns
C18:2 c9, c12 (LA)	6.36[b]	3.11[b]	19.51[a]	**
C18:3 n-3 (ALA)	1.22[a]	0.91[b]	0.85[b]	**
CLA c9, t11(RA)	1.28	1.30	0.97	ns
C20:4 n-6 (ARA	2.09[a]	0.29[b]	0.36[b]	**
C20:5 n-3 (EPA)	0.57[a]	0.08[b]	0.11[b]	**
C22:5 n-3 (DPA)	0.87[a]	0.22[b]	0.21[b]	**
C22:6 n-3 (DHA)	0.56[a]	0.05[b]	0.05[b]	**
CLA total	1.70	1.81	1.49	ns
PUFA n-6	3.22[a]	1.25[b]	1.22[b]	**
PUFA n-3	8.67[b]	3.42[b]	19.91[a]	**
n-6/n-3	2.77[b]	2.82[b]	19.13[a]	**
SFA	43.83[b]	50.42[a]	38.24[c]	**
MUFA	44.28[a]	44.90[a]	40.63[b]	**
PUFA	11.89[b]	4.68[c]	21.13[a]	**
SFA/UFA	0.80[b]	1.02[a]	0.65[c]	**
AI	0.81[a]	0.77[a]	0.46[b]	**
TI	1.18[b]	1.66[a]	1.07[b]	**

**, P≤0.01; *, P≤0.05; ns, not significant
a,b,c values within the same row with different superscript differ significantly (P<0.05)
AI, Atherogenic Index; TI, Thrombogenic Index

The LIO fat showed the highest content of short-chain FA (SFA), due to its highest C18:0 content compared with FM and HO. The content of RA did not differ among the 3 types of products.

The content of vaccenic acid (VA; C18:1 *trans*-11), which represents more than 75% of the total C18:1 trans FA in all products, was higher in LIO and HO compared FM samples. The VA content in the samples ranged from 1.1 to 3.1 in FM, from 1.9 to 5.5 in LIO, and from 0 to 5.3% in HO; with only one sample of HO not having any detectable VA. The FM samples showed the highest content of C18:3 n-3 (α-linolenic acid, ALA),

EPA, DPA and DHA. The content of C20:4 n-6 (arachidonic acid, ARA), which has an important role in infant nutrition, was more than 6-fold higher in FM compared to LIO and HO samples. Since PUFA are mainly esterified in the phospholipid fraction of meat fat, the lowest levels of PUFA in LIO samples could be related to the high fat content of the lamb meat used for the LIO products. The content of C18:2 c9, c12 (linoleic acid, LA) in HO products was almost 3-fold higher than LIO and 5-fold higher than FM samples, probably related to the presence of vegetable oil in HO, usually sunflower oil which is particularly rich in LA. This is the reason of the highest content of PUFA n-6 and ratio of omega-6 to omega-3 essential fatty acids (n-6/n-3) in HO samples compared to LIO and FM samples. The ratio n6/n3 was 19/1 in HO, which is much higher than that of 2-3/1 normally recommended in the diet of humans (Simopoulos et al., 2008). In contrast, the fresh and powered lamb meat had a more appropriate balance between n-3 and n-6 fatty acids, both having a ratio n-6/n-3 of 2.8.

Tables 2 report the fat content and the fatty acid profile in the three brands of homogenized (HO) baby foods based on lamb meat on sale in Italy. Brands influenced significantly fat content and the content of several FA.

Table 2 - Fat content and fatty acid profile of different brands of homogenized (HO) baby food based on lamb meat.

	Brand			P value
	(A)	(B)	(C)	
Fat content, % as fed	3.28[b]	4.40[a]	4.10[ab]	†
Fatty acid g/100 g of FAME				
<C14	0.09[b]	0.33[ab]	0.53[a]	†
C14:0	1.30	2.43	2.92	ns
C14:1	0.06	0.09	0.12	ns
C16:0	15.51	17.16	18.65	ns
C16:1	0.75	0.87	1.05	ns
C18:0	15.85	17.52	14.44	ns
C18:1 *trans*-11 (VA)	1.25[b]	4.13[a]	3.14[ab]	*
C18:1 *cis*- 9	37.23[a]	29.99[b]	31.48[b]	**
C18:2 c9, c12 (LA)	21.24	18.52	19.20	ns
C18:3 n-3 (ALA)	0.68	0.91	0.90	ns
CLA c9, t11(RA)	0.34[b]	1.14[ab]	1.20[a]	*
C20:4 n-6 (ARA	0.40	0.32	0.36	ns
C20:5 n-3 (EPA)	0.10[b]	0.12[ab]	0.12[a]	*
C22:5 n-3 (DPA)	0.19	0.22	0.23	ns
C22:6 n-3 (DHA)	0.03	0.04	0.06	ns
CLA total	0.82[b]	1.72[a]	1.70[a]	†
PUFA n-6	21.68	18.90	19.60	ns
PUFA n-3	1.00	1.29	1.30	ns
n-6/n-3	26.08	15.36	17.80	ns
SFA	35.00	40.01	38.85	ns
MUFA	42.32	39.80	40.25	ns
PUFA	22.67	20.18	20.90	ns
SFA/UFA	0.57	0.67	0.67	ns
AI	0.34	0.46	0.54	ns
TI	0.97	1.12	1.10	ns

**, P≤0.01; *, P≤0.05; †, P≤0.10; ns, not significant
a,b,c values within the same row with different superscript differ significantly (P<0.05)
AI, Atherogenic Index; TI, Thrombogenic Index

Fat content tended to be lower (P<0.10) in brand A compared with the other two brands. The proportion of short-chain FA tended to be higher in C brand while the lowest content was found in A brand. The content of VA, which represents a proportion variable between 85% and 100% of total trans FA in HO was higher in B brand while the lowest value was observed in A brand. The VA content in HO ranged from 0.5 to 3.0% in A brand, from 3.1 to 5.3% in B brand and from 0.9 to 5.1% in C brand; only one sample of HO of the A brand not having any detectable VA. The content of C18:1 *cis*-9

was higher in A brand than in B and C brands and this account for a higher MUFA content although the limit of significance was not reached (P=0.3). The proportion of RA was higher in C brand while the lowest content was observed in A brand. Similarly to the content of VA, the content of RA varied widely among the three brands; ranged from 0.2 to 0.8% in A brand, from 0.9 and 1.3 in B brand and from 0.3 to 1.8 in brand C. Due to the higher content of RA, which represent about 60% of total CLA in HO, the content of total CLA tended to be higher in C brand than in A and B brands. Among LC-PUFA n-3 only EPA was higher in C and B brands than in A brand. The ratio n-6/n-3 did not differ among the brands and the values of this nutritional index was much higher than that recommended for human diet (Simopoulos et al., 2008).

Tables 3 report the fat content and the fatty acid profile in LIO baby food of the two of brands on sale in Italy. As for HO brands influenced significantly the fat content and the fatty acid profile of LIO, except for the content of C16:0 and DPA.

Fat content was higher (P<0.01), in C brand compared with A brand however there is a discrepancy between fat content reported in the nutritional label and our result and this is likely due to the different method of analysis.

The content of short and medium chain FA was 5.4-fold higher in C brand compared with A brand. Similarly the content of C14:0 was 2.6 fold higher in C brand compared with A brand. By contrast the content of C18:0 and C18:1 *cis*-9 were higher in A brand compared with C brand. The content of VA was 2.7 fold higher in C brand than in A brand. The VA content ranged from 1.90 to 2.08% in A brand and from 5.38 and 5.45% and represent about 65% and 87% of total C18:1 trans FA respectively in A and C brands. The content of RA ranged from 0.55 to 1.00% and from in A brand and 1.91 to 1.99% in B brand and was 3 fold higher in samples of B brand.

Table 3 - Fat content and fatty acid profile of different brands of lyophilized (LIO) baby food based on lamb meat.

	Brand		
	(A)	(C)	P value
Fat content, % as fed	10.75	19.42	**
Fatty acid g/100 g of FAME			
<C14	0.15	0.81	**
C14:0	2.09	5.48	**
C14:1	0.07	0.14	**
C16:0	22.28	22.97	ns
C16:1	1.48	1.42	**
C18:0	21.85	19.54	**
C18:1 *trans*-11 (VA)	1.98	5.42	**
C18:1 *cis*- 9	36.93	30.96	**
C18:2 c9, c12 (LA)	3.75	2.46	**
C18:3 n-3 (ALA)	0.80	1.03	**
CLA c9, t11(RA)	0.65	1.95	**
C20:4 n-6 (ARA	0.39	0.20	**
C20:5 n-3 (EPA)	0.07	0.08	*
C22:5 n-3 (DPA)	0.22	0.22	ns
C22:6 n-3 (DHA)	0.04	0.06	**
CLA total	1.13	2.49	**
PUFA n-6	4.16	2.68	**
PUFA n-3	1.13	1.38	**
n-6/n-3	3.70	1.95	**
SFA	49.09	51.76	**
MUFA	45.62	44.18	**
PUFA	5.29	4.07	**
SFA/UFA	0.96	1.07	**
AI	0.60	0.94	**
TI	1.62	1.71	**

**, P≤0.01; *, P≤0.05; †, P≤0.10; ns, not significant
AI, Atherogenic Index; TI, Thrombogenic Index

The content of LA was 1.5 fold higher in A brand and its content ranged from 3.31 and 4.03 in A brand and from 2.42 and 2.48 in C brand. The proportion of ARA, which originates in tissues from LA via the desaturation-elongation pathways, was significantly higher in A brand than in C brand. The content of ARA ranged from 0.36 to 0.41% in A brand and from 0.19 to 0.21% in C brand. The higher content of LA and of ARA in A brand accounted for a higher PUFA n-6 in A brand compared to C brand. On the other hand the content of ALA and its elongation products (EPA and DHA) were higher in C

brand compared with A brand. This is the reason of the highest content of PUFA n-3 in samples of C brand compared with samples of A brand. The content of PUFA n-3 ranged from 1.08 and 1.18% in A brand and from 1.33 and 1.52 in C brand.

The higher content of PUFA n-3 and the lower of PUFA n-6 accounted for a lower n-6/n-3 ratio in LIO of the C brand compared with samples of A brand. However in both brands the values of n-6/n-3 ratio were in the range of the recommended values for human health (Simopoulos et al., 2008).

9.4 Conclusions

In conclusion, the composition of LIO and HO samples showed a high variability in terms of fat content and fatty acid composition between the brands sampled. Fatty acid composition was more similar between LIO and FM samples than between HO and FM samples, probably as a consequence of the high level of vegetable oil added to HO products. On the other hand, meat used for LIO products probably originates from lambs heavier than suckling lambs, as reflected by its lowest levels of PUFA. In conclusion, the use of meat from suckling lambs for baby foods may be a reliable way to improve essential and long-chain PUFA content of LIO products. On the other hand, the large use of vegetable oils as ingredients of HO products causes a deep modification of FA composition and, as a consequence, leads to a great difference between the FA profile of HO based on lamb meat and that of fresh lamb meat.

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Conclusions

Scientific research that relate nutrition to human health have proliferated in recent years, as a result of growing concerns by consumer for food security, highlighting the ability of food to be both a vehicle for nutrients and prevention tool for some diseases. The fatty acid composition of dietary fat has an important role in human nutrition because can help to prevent or reduce the risk of appearance of some diseases.

Meat is the main source of fat in the diet and is a vehicle for important nutrients. Lamb meat, as others red meat, has an high nutritional quality that leads to a balanced supply of basic elements (proteins, carbohydrates, lipids) and essential elements that our body does not synthesize such as amino acids, essential fatty acids (linoleic and α-linoleinic acid) and vitamins. In particular, lamb meat has an interesting fatty acid profile due to significant levels of PUFA n-3 and conjugated linoleic acid (CLA). Several factors are important in regulating fatty acid composition in lambs meat such as slaughtering age and weight, breed, sex and feeding regimen. The experimental activity reported in this thesis showed that:

1. lambs management system influence fatty acid composition of intramuscular fat. Lambs which followed their mother on pasture showed a qualitatively better fat, from a nutritional perspective, than lambs raised indoor due to lower proportions of C14:0 and C16:0 and to the higher C18:3 n-3 (α-linolenic acid, ALA), and its elongation products (DPA and DHA) content. In addition 100 g of lamb meat can satisfy about 10% of ADI for PUFA n-3.

2. there is a low relationships between the concentrations of VA, RA and ALA in the muscle of suckling lambs and those of their mother's milk. The relationship of LA and

ALA acid with very long chain unsaturated fatty acid in the muscle tissue support that a conversion of essential fatty acid to their long derivatives occur in this specie after birth.

3. milk source can affect the nutritional characteristics of meat of unweaned lambs. In fact intramuscular fatty acid of lambs feed only a milk replacer compared to lambs fed exclusively with maternal milk showed a fatty acid profile less favorable from a nutritional point of view. In fact feeding a milk replacer increased the n-6/n-3 ratio due to higher linoleic acid and its derivatives, and showed a lower CLA content than meat from lambs naturally reared.

4. the fresh lamb meat showed a fatty acid profile nutritionally more balanced compared to baby food based on lamb (homogenized and lyophilized meat). The baby foods based on lamb meat showed a high variability in terms of fat content and fatty acid composition between the brands sampled and among lots of the same brand. Fatty acid composition was more similar between lyophilized and fresh meat samples than between homogenized and fresh meat samples. So this thesis suggest that the use of meat from suckling lambs for baby foods may be a reliable way to improve essential and long-chain PUFA content of baby food products.