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Effects of maternal nutrition during gestation and lactation on muscles fiber types and fatty acid profile of suckling lambs

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"Agitatevi, perché avremo bisogno di tutto il vostro entusiasmo.

Organizzatevi, perché avremo bisogno di tutta la vostra forza.

Studiate, perché avremo bisogno di tutta la vostra intelligenza."

Antonio Gramsci

Alla mia famiglia

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Table of content

LIST OF TABLE

Introduction

Chapter 3

Chapter 4

Experiment 1

.

Abstract

The aim of this thesis is to evaluate the effect of linseed supplementation in ewe during gestation and lactation, in muscle fatty acid profile and muscle fiber population. For this purpose 3 experiments are included in the thesis. The first experiment studied the effect of maternal omega3 supplementation during gestation in muscle fiber characteristics, chemical composition and fatty acid profile on muscle of newborn lambs. The result showed no effects of maternal omega3 supplementation on fetal growth and number of muscle fiber; the maternal diet influences the content of TFA, total PUFA, PUFA n-3 and PUFAn-6. In the second experiment was investigate the maternal omega3 supplementation both during gestation and lactation on muscles morphometric measurements and fiber population of suckling lambs at weaning. The treatment affected average weights of organs but did not influence the muscles development and in the number of total, type1 and type 2 fibers in the LD muscle. The maternal omega3 supplementation using linseed during gestation showed a depressing effect on lamb weight and daily gain. The continuous linseed supplementation during gestation and lactation restores the growth performances of lambs. The third experiment aimed to evaluate the effect of maternal omega3 supplementation during pregnancy and early lactation on the fatty acid profile of LD and ST muscles and brain tissue. Results showed an increase of the VA, c9t11 CLA and ALA in muscles without change the long chain fatty acid EPA and DHA .The elongase and desaturase indexes evidenced that the efficiency of conversion of ALA into their long metabolites in skeletal muscles of lambs is very low both during fetal growth and during the first month of life.

INTRODUCTION

Lamb meat production in Italy

Sheep farming is an important asset for the economy of many countries in the world. According to data from ISTAT,(2011a), in Italy there are 7.942.641 (Table 1) sheep and 3.444.024 of them are bred in Sardinia (table 1) and represent the 51% of dairy sheep whereas meat sheep are the 4% only. Sardinian sheep bred in Italy are 4.7 million. It means that in Italy only 50% of needs are satisfied (Laore Sardinia VII report, 2006). About 34.4% of sheep meat produced in Italy is represented by suckling lambs (Table 2).

About 350 thousand tons of milk, totally used for cheese production and 30 thousand tons of meat (ISTAT, 2010) are produced in Sardinian farms, the wool value is negligible. Meat produced by dairy breeds, are placed on the market mainly as suckling lambs, slaughtered when they are about one month old and reach a live weight of 9-11 kg. Such type of production is available mainly during the Christmas and, to a lesser extent, Easter period, when the product can reach acceptable prices. About 1.7 million lambs are slaughtered every year (table.2) (Vacca et al., 2005). Except these seasonal windows, lamb meat is not sufficiently appreciated, although it could benefit the PGI "Agnello di Sardegna" (European Union, Commission Regulation No. 138/2001). The sale of meat plays a minor role in the income formation (about 20% of Gross Saleable Product) but it could become more important after the recent increase of sheep meat demand. Some polls indicate that frequent users, as well as occasional ones, appreciate lamb meat, especially of dairy breeds (Bernabeu and Tendero, 2005). This fact could be particularly interesting for the future of sheep farming in Sardinia because it could represent an alternative to better exploit the potential resources of Sardinian livestock. Competitiveness in the market may be developed by exploiting the socio-cultural environment of farms and the quality of products derived from such type of environment.

Table.1. Sheep consistency in different regions of Italy

Regions	Ovine	
	Totals	sheep
Piemonte	86471	75127
Valle d'Aosta	2392	2163
Lombardia	91284	75719
Liguria	21779	20681
Trentino-Alto Adige	59048	47352
Bolzano	37287	26345
Trento	21761	21007
Veneto	30336	27779
Friuli-Venezia Giulia	5387	4133
Emilia-Romagna	89095	80268
Toscana	576127	531375
Umbria	171601	157585
Marche	182451	165591
Lazio	756771	712003
Abruzzo	336167	309103
Molise	158237	140172
Campania	262314	234504
Puglia	237756	218593
Basilicata	379138	346205
Calabria	254850	230278
Sicilia	797413	723122
Sardegna	3444024	3021261
ITALIA	7942641	7123014

(ISTAT, 2011a)

Table.2. Slaughtered lambs in Italy (weight in one hundred kilos). Breakdown by region. Year 2010.

Regions	Lambs				
	Animal number	Total live weight	Average live weight	Average yeld (%)	Dead weight
Piemonte	23536	3128	0.13	54.9	1717
Valle d'Aosta	425	65	0.15	58.5	38
Lombardia	10726	1634	0.15	56.4	922
Liguria	2598	308	0.12	55.2	170
TrentinoAltoAdige	9755	1481	0.15	53.1	786
Bolzano	8767	1330	0.15	52.6	699
Trento	988	151	0.15	57.6	87
Veneto	9074	1300	0.14	58.2	756
Friuli-VeneziGiulia	1484	216	0.15	59.7	129
Emilia-Romagna	4799	671	0.14	56.6	380
Toscana	299925	38108	0.13	60.2	22953
Umbria	99608	15589	0.16	58.4	9105
Marche	130750	18020	0.14	58.6	10556
Lazio	1195702	163038	0.14	55.8	91038
Abruzzo	407246	68684	0.17	58.1	39924
Molise	102574	16445	0.16	58.1	9555
Campania	276840	34275	0.12	59.2	20296
Puglia	498995	58154	0.12	59.4	34563
Basilicata	196851	24882	0.13	58.3	14505
Calabria	89733	9864	0.11	56.8	5602
Sicilia	127787	12836	0.10	61.1	7847
Sardegna	1100371	109323	0.10	59.2	64724
ITALIA	**4588779**	**578021**	**0.13**	**58.1**	**335566**

(ISTAT, 2010)

The Lamb of Sardinia is the only product of the island that enjoys food Protected Geographical Indication (PGI Regulation EC N ° 138/01). This recognition created the opportunity to enhance lamb meat produced in Sardinia, and to find space on the market. The request for recognition of the name has been promoted by a group of farmers who have also contributed to the creation of the consortium for the protection of the Lamb of Sardinia IGP. The consortium currently represents the structure that brings together people involved (farmers, butchers and cutters) in the production chain of the Lamb PGI Sardinia. Farmers, slaughterers and cutters must comply with a specification of production. The obligations which must adhere to the branding and certification of meat Sardinian Lamb PGI are related to the stage of breeding and slaughter of portioning and packaging. Table 3 shows a number of farms that produce PGI lambs with the number of animals raised in Sardinia.

Table.3. *PGI meats Sardinia reports by provinces*

	Farms	breeding	ovine number
Sardegna			
Sassari	661	661	29531
Nuoro	891	891	35423
Cagliari	476	476	16808
Oristano	565	565	20003
Olbia-Tempio	80	80	3361
Ogliastra	30	30	838
Medio Campidano	278	278	11006
Carbonia-Iglesias	161	161	5264
Totale Sardegna	3142	3142	122234

(ISTAT, 2011b)

In this introduction will be dealt the scientific background and the research issue about the muscle fiber composition, the meat chemical composition with specific attention to the fatty acid profile of fat

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CHAPTER 1

MUSCLE FIBER

1. Muscle tissue

The muscle tissue is a parenchyma that forms all types of muscle: smooth, striated, skeletal striated and heart striated. It is responsible for the locomotion and the movement of all body parts thanks to a contractility property that is accompanied with a specific protein like a myosin, actin end regulative proteins.

In vertebrates there are three categories of muscles: striated skeletal, cardiac and striated smooth. The skeletal striated muscle tissue forms muscles of the skeleton, is made by polynuclear syncytial elements called **muscle fiber.** They have a cross-streak and are innervated by the nervous system spinal celebrate.

1.1 Muscle fibers

1.1.2 Istologic organization of muscle

The skeletal muscle tissue is formed by multinucleated elements, called muscle fibers. Striated muscle is formed by bundles of muscle fibers, associated with each other via connective tissue. The muscle is wrapped by a connective tissue called epimysium; it continues with interstitial connective tissue that surrounds bundles of muscle fibers and is called perimysium .From this point, septa of reticular connective that wrap each muscle fiber, called endomisyum,were detached. Fiber is the fundamental unit of the muscle; a dense network of capillaries surrounds it, provides oxygen and nutrients and removes carbon dioxide and catabolites. The striated muscle fiber has a thickness that varies from 10 to 100 um; the length in some muscles can reach few cm. The muscular fibers are made by myofibrils that are composed by sarcomeres. An important fiber characteristic is represented by the presence of many nuclei. It is a syncytium polinucleato that is formed in an embryonic stage after the fusion of several embryonic muscle cells or "myoblasts". Is it surrounded by a membrane called sarcolemma that encloses fluid matrix called sarcoplasm; nuclei, sarcoplasmic organelles, glycogen granules and myofibrils, forming contractile muscle components, are contained inside it. Inside the muscle fiber, branches of the transverse tubules encircle cylindrical structures called myofibrils, between myofibrils there is a membrane system formed by vesicles and tubules that form the sarcoplasmic reticulum. Myofibrils are constituted by myofilaments, protein filaments composed

primarily of actin and myosin. The actin forms the bulk of thin filaments, and the myosin forms thick filaments (figure 1). Their presence determines the alternation of zones with different index of refraction; the I-band zone has only a thin filament, A-Bands formed from both filaments; in the center of the A-bande there is a less dense zone called H-bands, that has in the center a more dark band, called M-band. In the I-band center there is a transversal line called Z-line. The part situated into two Z- lines is a contractile unit in the muscle fiber and it is called Sarcomere (figure 2). The thick filaments are formed by myosin molecules and molecules complex binding myosin as actin tropomyosin, troponin, nebulin, tropomodulin. In the sarcomere there are six thin filaments for each thick filament.

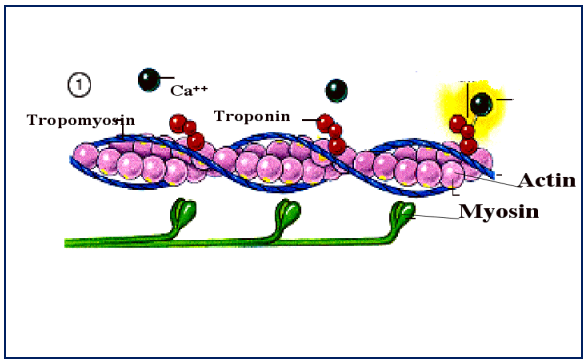

Figure.1: Thick and thin filament

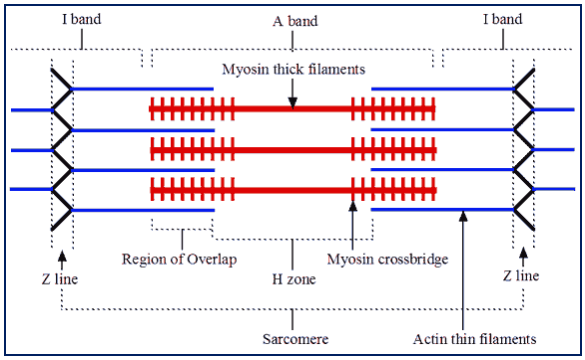

Figure.2: Structure of sarcomere

1.3 Crontractile Proteins of the myofilament

1.3.1 Myosin

Myosin is the predominant protein in skeletal muscle, it is longer 140-150 nm, larger approximately 2nm and its molecular weight is about 500.000 dalton. It forms the thick filaments with approximately 300 unit. Myosin is an hexameric protein that consists in four light chains (MLC), situated in correspondence of the transition region between head and tail (figure 3), and two heavy chains (MHC)formed by an α-elica tail and a globular head that constitutes a motor domain because it has ATPasica activities and it is able to bind to actin monomers of thin filaments (Lefaucheur, 2001). Each one is encoded by a multigenes family, and it includes, therefore, different isoforms (Picard et al., 2002).

Different combinations of these subunits give rise to a large number of isomyosin. In skeletal muscle fiber, the proportion of different isoforms of myosin is the mayor determinant of contractile performance. The difference of these isoforms is due to their specific actin-activated and Ca2+ stimulated. ATPase activities reside in the head region of the heavy chain (Hämäläinen and Pette, 1995). The secret of extraordinary capacity of myosin respond to very different functional requirements is due to the existence of multiple isoform of MHC and MLC. Although it is possible co-expression of two o more MHC genes, according to the general rule, each cell expresses only one MHC genes associated with two MLC genes. This forms a mixed population of muscle fiber, each with a specific combination of MHC and MLC isoform. The existence of hybrid fiber that have different myofibrillar protein isoforms is quite frequent, and it may actually be more the rule than the exception, in fact multiple MHC isoforms are expressed during the growth, during changes in the hormonal state and in the physical activity.

The coexistence of different slow and fast isoforms can be observed in normal muscles, and the proportion of fast MHC on slow may vary independently from changes in MLC isoform (Berchtold et al., 2000).

Isoform heavy chains (MHC):- In mammals 9 different genes that encoded for MHC class II have been found. The MHC genes are distributed in two principal cluster : the first one contains the MHC-β/slow end MHC- α, the second cluster includes MHC-emb, MHC-neo,MHC-eo and adult MHC fast genes (Schiaffino and Reggiani, 1996). Four genes encode for MHC 1 or β/slow, MHC 2 A, MHC 2X and MHC2B Predominant in skeletal

muscle adult in different species of mammals, and expressed in the muscle fibers of the limbs and torso. The relative isoform proportions varies from muscle to muscle, it depends on the its function and position, for example in lower limbs MHC1 is more expressed followed by MHC 2A, whereas in the upper limbs MHC 2A and MHC 2X are more expressed.

Isoforms light chains (MLC)**:-**In the mammalian striated muscle, five light chains isoform (MLC) are identified. Fast skeletal muscle fibers contain two isoforms , MLC 1fast and MLC 3fast; fiber skeletal muscle slow expresses other two isoforms, MLC 1slow/ventricular that is expressed in a ventricular myocardium, and MLC 1slow-a, also expressed in smooth muscle. During the skeletal muscle development a fifth isoform is expressed, MLC 1emb/atrial. These isoforms originate from different genes

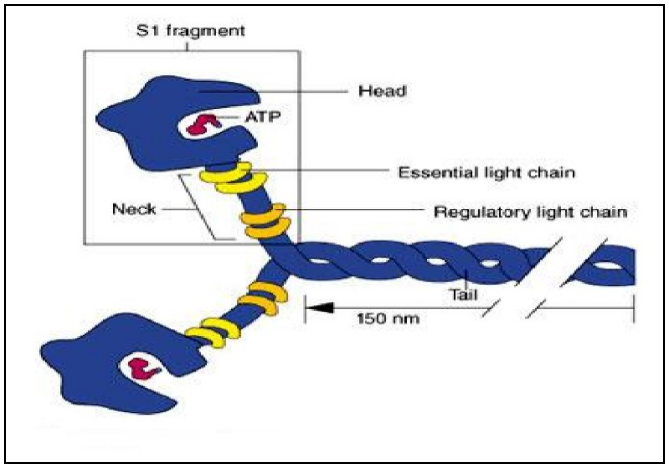

Figure.3: Molecula of myosin

1.3.2 Actin, tropomyosin and troponin

Thin filaments are formed by actin protein, tropomyosin and troponin. Actin protein is the main constituent, and is able to bind to myosin , tropomyosin and troponin are regulative proteins. These three protein classes are strictly related. In vertebrates, six actin isoforms exist and they differ from each other for few amino acids. In a muscle there are 4 actin types (one in the skeletal muscle, one in the cardiac muscle e two in the smooth one) and, two different types are present in not muscular cells. Muscular actins are involved in

contraction mechanisms, whereas the not muscular one are expressed in all cells and participate at few activities such as formation of the cytoskeleton, cell motility, cell division and other. Thin filaments also contain tropomyosin and troponin proteins. Tropomyiosin is a filamentous molecule long about 40 nm, and associated tail-tail to form continuous filaments near two f-actin paired filaments. In a skeletal muscle, tropomyiosin is formed by two subunit alfa and beta with molecular weight of 31.000 and 36.000 respectively, and present in muscle with different ratio (Molinaro et al., 1997).Muscles with quick fibers contain higher concentration of Alfa type whereas beta type predominates in slow fiber type. Troponin is a globular protein, its weight is about 80.000 and it is formed by three subunit. Usually thin filaments of skeletal muscles are long from 0.8 to 1 um , and contain probably 300-400 G-actin and 40-60 tropomyosin molecules .

1.4 *Muscle contraction*

The energy required for muscle contraction is supplied from adenosine triphosphate (ATP) contained in the head region of the heavy chain of myosin. The enzyme adenosine triphosphatase (ATPase), hydrolyzes ATP into adenosine diphosphate (ADP) and inorganic phosphate (PI) providing the necessary energy (Scott et al., 2001). Myosin has ATPasic activity independently of the actin presence but the latter determines strong enzymatic stimulation and activity. The stimulation of myosin by actin allows to remove ADP from the catalytic site of the enzyme for which ADP has the affinity, allowing a new ATP molecule to combine with myosin to produce a further release of energy. Chemical reaction that conduces to ATP hydrolysis and to the movement of flexion of the head myosin molecules on those of actin occurs in three steps:

1) ATP binds with myosin forming a complex myosin-ATP that is hydrolyzed to ADP.

2) Calcium ions are released by sarcotubular system and, after a nervous impulse, combine with tropomyosin. This reaction determines the interaction of myosin-ADP whit a molecula of G-actin.

3) When myosin binds to actin, the stored energy is released, and the head flexes, dragging whit it the actin filament toward the center of the sarcomere. The final

step occurs when a new ATP molecule binds to myosin. Myosin-ATP complex, newly formed, dissociates to actin, and remains available for a new reaction cycle, if the nervous stimulus persists.

This model explains also the "*rigor mortis*" process that is observed after slaughter of the animal. In fact the calcium regulation vanishes after death, and as a consequence, many complex myosin-actin are formed. This complex is very stable and can be dissociated only when new ATP molecular binds to myosin. The gradual disappearance of ATP after death prevents the dissociation of myiosin-actin complex, causing muscle rigidity. The nervous signal that reaches to muscular fiber spreads rapidly from the sarcolemma across the tubule T and determines the liberation of calcium ions in intracellular fluide. These ions allow the myosin-actin interaction and the relative myofilaments sliding. Once nervous impulse stopped, calcium is rapidly recaptured in the vesicles of deposit by the calcium pump. Therefore myosin-actin complex is inactivated and the myofibril returns to rest conditions.

1.5 Type of muscle fiber

The muscle fiber can be expressed by one or more MHC combination, as observed in human (Klitgaard et al., 1990) bovines (Picard et al., 1998), horses (Rivero et al., 1999) and rats (Talmadge et al., 1995).

The diversity of muscles fibers involves different structural and functional muscle aspects. Base of this diversity is regulated by two mechanisms:

1) Qualitative mechanism: the majority muscle proteins exist in similar, but not identical, forms called isoforms. They can be derivated from the same gene, whit a splicing alternative mechanism, or from different genes belonging to the same family (isogenes). The isoform existence is the first mechanism which generates diversity among fibers.

2) Quantitative mechanism: many genes may undergo up-regulation and down-regulation processing, on the basis of various factors as a pattern of neuronal discharge, mechanical load and hormonal stimuli (Bortolotto and Reggiani, 2002)

A fiber type is the result of specific gene expression profile (Bottinelli et al., 1997). In some work it has been found that different isoforms in the muscle fiber can be expressed simultaneously in fetal life (Schiaffino and Reggiani, 1996).

Fibers can be distinguished with a functional classification, according to the contractile response that is caused by protein composition and sarcoplasmic reticulum; depending on speed contraction muscle fibers are defined slow or fast. Fibers can be distinguished in MHC composition and also in size. Muscle fibers have different fatigue resistence, depending on the ability to maintain the contractile performance during repeated stimulation. On the basis of the above characteristics the fibers can be distinguished in three groups:

1) The first group is characterized by a slow contraction speed and expresses MHC1 isoform (type 1 fibers or primary fiber).

2) The second group has a fast contraction speed, is fatigue resistant and expresses MCH 2A isoform (type 2 fibers or secondary fiber).

3) The third groups has a fast contraction speed is not fatigue resistance, and express MHC 2X isoform (type 2X fiber or tertiary fiber).

Type 1 fibers have generally a darkest color for the myoglobin resource, a small diameter, low values of ATPase myofibrillar, and a large set of oxidative enzymes, these fibers are responsible for muscle tone, they contract slowly and have high resistance to the fatigue.

Type 2 fibers are light colored and have a fast contraction. Such type of fibers have a developed sarcoplasmatic reticulum which allows the rapid release of calcium ions, they have predominantly aerobic metabolism and generate muscular tension more quickly than fibers of type 1.

The different types of fibers differentiate in different phases of development. During prenatal development, two or three phases of myotubes formation occur: in the first phase comes from embryonic myoblast, the second wave come from fetal myoblasts, these phases form respectively primary and secondary fibers (Feldman and Stockdale, 1992). In sheep(Wilson et al., 1992),pigs (Lefaucheur et al., 1995), bovines (Picard et al., 1998) and

in humans (Draeger et al., 1987) this fibers have a small diameter, and are usually expressed by the development of MHC isoform(Ecob-Prince et al., 1989, Maier et al., 1992). In some mammal the total fiber number is fixed before birth: for example in sheep it has been observed that primary fibers are formed at 32 days of fetal life, the secondary at 38 days and the tertiary between 62 to76 days of fetal life (Wilson et al., 1992) . In bovine the total number of fibers is fixed at the end of the second third of gestation (Gagniere et al., 1999). Some work reported that the primary fibers are under genetic factor control, whereas the number of secondary fiber are under other control factors such as maternal nutrition (Deveaux et al., 2001).

Depending on the metabolic pathways used to generate the ATP required for the contraction, the fibers can be divided into three different types:

1) Oxidative;
2) Oxido-glycolytic;
3) Glycolytic;

In general, muscle at birth is composed of oxidative fibers(Moody et al., 1978). during growth, the proportion of oxidative fibers decreases, while the proportion of glycolytic fibers increases(Solomon et al., 1990).

In muscle biology the criterion to classify fiber types is the difference in the acide and alkaline stability of the myosin ATPase reaction(Brooke and Kaiser, 1970). From a series of pre-incubations with different pH it has been seen that type I fibers had a stable, high ATPase activity after preincubation at either pH 4.3 or 4.6, whereas type II fibers were strongly reactive after preincubation at pH 10.4, and negative after preincubation at pH 4.3.(Lind and Kernell, 1991)

In pigs at birth fibers are mostly oxidative (Bee et al., 2007), and glycolytic metabolism is poorly differentiated. However there are some fibers able to convert during the postnatal growth (Ashmore and Doerr, 1971b, Lefaucheur and Vigneron, 1986). The first post-natal period is a dynamic stage for the metabolic and contractile myofiber maturation. Generally the glycolytic metabolism increases rapidly from the second to the third week after birth,

after which it continues to increase sloewly, while the oxidative metabolism is very fast during the first two or three weeks after which it decreases (Lefaucheur, 2001).

Hormonal, neurological and mechanical factors affect the maturation of myofiber. In many studies, it has been found that there are sex differences relative to muscle fiber characteristics (Klont et al., 1998, Ozawa et al., 2000). The number and the size of fibers is under the control of sex hormones, if differences in androgen hormones are sufficiently high during prenatal periods fiber formation differences in fiber number between males and females can occur (Rehfeldt et al., 2004). In pig similar or larger fiber was reported for females than entire males(Choi and Kim, 2009). In work about pig found that the longissimus dorsi muscle has a large percentage of type IIB fibers (80–90%) and a small percentage of type I fibers (5–15%), while the vastus intermedius muscle has a large percentage of type I fibers (70-80%) (Kiessling and Hansson, 1983). In bovine experiment, brachialis muscles have high percentages of slow-twitch red fibers with lower percentages in the longissimus dorsi and biceps femoris muscles(Kirchofer et al., 2002). Differences in muscle fiber characteristics have been found between breeds (Bee, 2004, Choi and Kim, 2009, Ryu and Kim, 2006, Seideman and Crouse, 1986)

1.6 Growth and development of skeletal muscle

Learning about the growth and muscular development is important for studying the animals and the science of meat. The increase in fiber number (hyperplasia) is completed at birth, growth in postnatal period consists of hypertrophy (Bee, 2004).Most of components that form the muscle are constituents of the muscle fibers, so muscle mass strongly depends on the number and the size of muscle fibers. During myogenesis the extent of muscle cell proliferation depends on how many muscle fibers are formed (Rehfeldt et al., 2000). The number of muscle fibers is mainly determined by genetic and environmental factors that are able to influence the prenatal myogenesis. Postnatal growth is determined by the increase in length and circumference of the muscle fibers, and not, except a few exceptions, by the increase of the number of fibers (Rehfeldt et al., 2008). Hypertrophy of muscle fibers is a principal factor of growth in postnatal, this mechanism is accompanied by proliferative activity of satellite cells that form new nuclei that are

embedded in muscle fibers. In mammals the number of muscle fibers is considered alredy defined at birth, while some studies report that this number increases in fish after birth, although the hypertrophy of the fibers is a very important factor for growth (Stickland, 1983). It is known that the type of fiber composition and the cross section of area (CSA) are factors that influence the biochemical processes post-mortem, and consequently the quality of the meat.

Through the biochemical characteristics of individual types of fibers can understand how important fiber type on meat quality.

Meat quality and muscle mass are influenced by the size and the number of muscle fibers (Rehfeldt and Kuhn, 2006).

Fiber type influences the quality of meat, for example the total amount of type I fiber is negatively correlated with drip loss and lightness (Ryu and Kim, 2006). while a high content of fibers of type II is associated with increasing drip loss, and with differing measurements for water-holding capacity size of fiber influencing also the tenderness in meat(Seideman and Crouse, 1986), In the meat of swine and cattle has been seen that those with a greater number of fibers with a small size, have a greater tenderness, with respect to meat that show fibers of large size (Karlsson et al., 1993, Maltin et al., 1997, Renand et al., 2001)

In cattle a high number of fibers causes muscular hypertrophy with a high growth potential (Picard et al., 2006), In general animals with large size fiber tend to exhibit a meat with worst characteristics (Choi and Kim, 2009).

Intramuscular fat is positively correlated to tenderness (Renand et al., 2001). In cattle intramuscular fat is positively correlated with a type I percentage (Calkins et al., 1981) and a high type I fiber content contributes more to juiciness and flavor

CHAPTER 2

LAMB MEAT QUALITY

2. Introduction

Meat is an important food that becomes part of the diet of many people, especially in the developed world (Delgado, 2003). Then the concept of meat quality is a difficult to define because of the nature of the elements that influence and which are commercially important, in the case of meat for example the color, texture, juiciness and flavor. Defining meat quality is therefore an intricate exercise because the concept is multifaceted. For consumers, quality also includes aspects of edibility of the meat after cooking, nutrition and palatability this includes tenderness, flavor, residue, and succulence. Then you should speak to the quality that the consumer perceives.

For lamb meat, because the presence of many different sheep breeds and production systems, local and international markets are a great variety of carcasses to meet consumer needs. In Northern and Central Europe are preferred heavy carcasses of 16 to 23 kg, in the Mediterranean basin carcasses of lambs suckling very light weighing 4-8 kg, and 8-12 kg from animals finished on concentrates (Beriain, 2000). Western countries are those that consume more this depends on many factors such as wealth, the volume of livestock production, and socio-economic status of consumers (Mann et al., 2006). Other factors influencing meat consumption include sex, age, religion, body mass index (BMI) and total energy intake (Linseisen et al., 2002).

2.1 Meat quality characteristic

2.1.1 *Organoleptic quality*

Organoleptic qualities are determined by different sensory characteristics such as color, texture, smell, taste and juiciness of the meat. Curing of meat in many countries at least 2 to 5 days, increases the tenderness, palatability, as the aging of the meat increases the water holding capacity, and the gradual softening of the muscle. The consumer in the purchase of meat is influenced by other factors such as the method of slaughter, preparation for sales, marketing, cooking traditions of the region and cooking methods (Beriain, 2000).

The organoleptic characteristics of the meat are influenced by several factors such as pH; it is a measure of glycolysis, and in lamb carcasses can range from 6.85 to 6.05 and it start to decrease after 45 post mortem (McGeehin et al., 2001). The lowering of the pH is the result

of the production of lactic acid from glycogen in the anaerobic glycolysis, for the transformation of glycogen into lactic acid, occurs within 24-48 h post-mortem until reaching a final pH of about 5.6 to 5.3 (Immonen and Puolanne, 2000). The pH affects the tenderness of the meat, if in the early hours post-mortem the pH is too high, then cold-shortening can happen when the temperature of the musculature is lowered too rapidly, causing toughening of the meat (McGeehin et al., 2001). Work on lambs indicate that there are no substantial differences in the lowering of the ph post-mortem between males and females, but in a experiment has been found that in females lowering takes place more quickly in lambs.

2.1.2 *Color*

The color of the meat is the first aspect that the consumer perceives,it is linked to the concentration of pigments, in particular to myoglobin. Color of meat depends in particular by physical state of myoglobin on the meat surface, by structure of muscle proteins and the intermuscular fat percentage. Meat color is due to about 90-95% of the myoglobin and about 2-5% of hemoglobin and other proteins that contribute even less(Feiner, 2006). The color and also influenced by various factors such as age of the animal, the intensity increases with increasing age, because it increases the concentration of myoglobin in the meat (Jacobs et al., 1972). Another aspect that affects the color is the animal nutrition, in fact, meat from suckling lamb is paler respect meat from weaned lambs because of the low concentration of iron in ewe milk. While weaned lambs have more dark meat due to the highest amount of iron in forages and concentrates (Lawrie, 1988). The muscles used for walking are the most dark, they have a higher oxygen demand for the release of energy in muscle. Longissimus dorsi presents a lighter colored than the extensor carpi radialis locomotor muscle, for the higher concentration of myoglobin (Cross, 1986).

2.1.3 *Texture*

Texture of meat is an aspect which depends mainly by muscle proteins, can be defined as the ease of cutting and chewing. Texture is perceived by the consumer with different sensations that come from the interaction of the senses with the physical and chemical properties, such as strength, moisture and elasticity. This aspect is also influenced by the

intramuscular fat, the structure of the connective tissue, the size of the muscle bundles and the water retention. In cattle toughness is very important for the consumer that binds to this aspect of meat quality. In the lamb there is a change in toughness after slaughter, if it is followed by properly cooling (Ouali, 1990). The consistency of the meat also depends on the type of muscle fibers present. Breeds muscles with a greater amount of white muscle fibers, or subject to proteolytic degradation during aging of meat produce meat more tender (May, 1976). Meat tenderness is linked to the rate of pH fall. If pH is too high in the early hours postmortem, then cold-shortening can occur when the temperature of the musculature is lowered too quickly causing toughening of the meat (Marsh et al., 1987). With increasing age, increases the number of bonds between the thermo-resistant fibers of collagen, the effect is a reduction of tenderness. From a study done on lambs slaughtered at 12 and 24 kg were found differences in the meat about mastication, swallowing and digestion in the meat of the largest lambs compared to suckling lambs (Kirton, 1970). Tenderness in males decreases with age; this is due to the increase of testosterone, which increases the amount of collagen in the muscles. Among the males and females of the same age, females have more tender meat (Pommier et al., 1989). Food is a very important factor for the tenderness of the meat, in fact we have a balanced diet with an increase in intermuscular fat and a relative decrease in the amount of muscle collagen (Kemp, 1981).

2.1.4 *Juiciness*

The ability to retain moisture from the fresh meat is one of the most important quality characteristics of raw meat products. The largest amount of water in the muscle is found in myofibrils, between them, in the sarcolemma, between the muscle cells and between the bundles of muscles. The amount of water may vary depending on the muscle tissue and administration of the product (Huff-Lonergan and Lonergan, 2005).

Water holding capacity (WHC) is the ability of the meat to retain water in response to a force applied to it, is a very important property because it affects the retention of vitamins and minerals. The muscles lose their water buckets have more and lose more weight during chilling, storage, transportation and marketing. Usually the meat of heavier animals lose water more rapidly than meat from animals more light (Solomon, 1980). The WHC is one of the qualitative criteria that distinguish the meat according to their technological

qualities: those with low WHC , are characterized by an exudation of refrigerated raw meat, and they are considered undesirable for direct consumption and for processing. The meat with a high WHC may be unacceptable to the consumer, but is used in many processes of transformation, because is very important, especially after cooking, retains its water of constitution (Cheng and Sun, 2008).

2.1.5 *Flavor*

The flavor of the meat is an aspect of primary importance for the acceptability of the product by the consumer. depends mainly fatty acids present, in particular from unsaturated ones that are most susceptible to oxidation processes to volatile compounds with low molecular weight such as aldehydes, ketones hydrocarbons, and alcohols, these contribute to the aroma of meat. Phospholipids that which are rich in polyunsaturated fatty acids have a key role in the flavor of the meat. Flavour varies depending on the amount of amino acids and sugars. All these factors vary by race, sex, nutrition, and post-slaughter treatments (Rousset-Akrim et al., 1997). The oxidation of free fatty acids (linoleic, C18:2) and (arachidonic, C20:4) of the fraction of the different lipids of the meat, particularly of the phospholipids in the membranes of the muscles and adipocytes (Christie, 1981), contribute to the formation of volatile compounds responsible for the aroma of the meat (Rhee, 2002). In Mediterranean countries slaughter weight is lower than the other counties and generally there are not large differences between the sexes, in fact, from studies done on lambs, there were no significant differences between the total content of unsaturated fatty acids, and iodine value in total fat. females had a higher percentage of pentadecanoic (C15: 0), palmitic (C16: 0) and palmitoleic (C16: 1) compared to males, but these differences were not large-scale (Horcada et al., 1998). Weight affects the taste of meat, this can increase to undesirable levels generally more lightweight animals get a highest score when implementing the panel test (Gorraiz, 1999, Rousset-Akrim et al., 1997).

CHAPTER 3

NUTRIENT COMPONENT

OF MEAT

3 *Proteins*

The proteins of the food are formed by chains of many amino acids. They are very important and they are necessary for growth, maintenance and repair of body. The raw red meat contains about 20-25 g protein / 100g, while the cooked meat contains 28-36g/100, this is because during cooking you have the loss of water and there is a greater concentration of nutrients (Williams, 2007). Some amino acids can be synthesized in the body, while many others called "essential" must be assimilate through food, so they have to be the diet for a state of good health. Meat provides eight essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine). the digestibility of meat protein hovers around 94% against some vegetables like beans, which is 76% and wheat 86% (Bhutta, 1999). To assess the quality can be utilized Protein Digestibility Corrected Amino Acid Score, the maximum score is 1, the meat usually obtained values of 0.9 compared to values of 0.5-0.7 of some plant foods (Schaafsma, 2000). Meat and meat products (including poultry) contribute 36% of total protein intake. Table 1 showing different protein value off cooking and raw cuts of meat lambs,

Table 1 Protein value off different cooking and raw cuts of meat lambs

Feeding	**Protein (g/100g)**
lambs (Muscle tissue private of the visible fat)	20
Lamb cooked [muscle tissue private of the visible fat]	35.6
Lamb, thigh raw [muscle tissue private of the visible fat]	20
Lamb,thigh , cooked[muscle tissue private of the visible fat, cooked in the oven without the addition of fats and salt]	31.1
Lamb, chop raw [muscle tissue private of the visible fat]	20.4
Lamb, prime rib, baked [muscle tissue private of the visible fat, cooked in the oven without the addition of fats and salt]	28.7
Lamb Offal [heart, lungs, liver, spleen, kidneys]	15.7

Data from www.inran.it

3.1 *Fat*

A very important component of the meat is fat; this is a rich source of energy and provides essential nutrients such as fat-soluble, vitamins and essential fatty acids. For the modern consumer the taste and nutritional value of meat is very important. However, the trend today is to have a product with a higher lean part and a minimum of visible fat (Forrest, 1975), but it must be considered that the fat in meat contributes in its quality(Webb, 2006). Some studies have shown that there is a chemical perception of dietary fat in the oral cavity (Hiraoka et al., 2003). Another aspect to consider is that the quality and quantity of fat influences two important aspects which are the tenderness and flavor(Wood et al., 1999). Fat in the diet is seen by consumers as dangerous as it is associated with risk of colorectal cancer. But from an analysis of different subjects was not found any relationship (Lin et al., 2004), you should always consume a little fat for their properties; the focus has shifted from the quantity of fat to its quality (Laaksonen et al., 2005).

Fat in meat is mainly found in three forms:

- Intermuscular fat, positioned between the muscle,
- Intramuscular fat, positioned within the muscles, (it is also called marbling),
- Subcutaneous fat, located under the skin.

Intramuscular fat is formed by polar lipids and neutral lipids, the first are formed mostly of phospholipids and are found in cell membranes, the second are formed mostly of triglycerides, which are a function of energy for the body (Gandemer 1999). The amount of phospholipids in the intramuscular fat is fairly constant, varies between 0.2 and 1% of muscle weight (De Smet, 2004). some authors studied the composition of fatty acids in muscle lipids and phospholipids in pigs (Wood et al., 2004a), sheep (Demirel et al., 2004) and cattle(Warren et al., 2008) (table.2).

The fat content of red meat varies widely, depending on the type of red meat, the cut and the degree of trimming (Higgs, 2000).

Fat is constituted by several types of fatty acids that we can class to:

- saturated fatty acids (SFA);
- Monounsaturated fatty acids (MUFA);
- Polyunsaturated fatty acids (PUFA).

Fatty acids are recognized different properties, and not as beneficial alteration of cholesterol levels in the blood. For this reason the concentration of acidic foods is a topic much studied.

Table.2 Fatty acid composition (%) of *longissimus* muscle triacylglycerol (neutral lipid) and phospholipid in pigs (Wood et al., 2004a) in sheep (Demirel et al., 2004) and cattle (Warren et al., 2008) concentrate-type diets

	Neutral lipid			**Phospholipid**		
	Pigs	Sheep	Cattle	Pigs	Sheep	Cattle
C14:0	1.6	3.0	2.7	0.3	0.4	0.2
C16:0	23.8	25.6	27.4	16.6	15.0	14.6
C16:1cis	2.6	2.2	3.5	0.8	1.5	0.8
C18:0	15.6	13.6	15.5	12.1	10.4	11.0
C18:1 *cis*9	36.2	43.8	35.2	9.4	22.1	15.8
C18:2 *n*6	12.0	1.5	2.3	31.4	12.4	22.0
C18:3 *n*3	1.0	1.2	0.3	0.6	4.6	0.7
C20:4 *n*6	0.2	ND	ND	10.5	5.9	10.0
C20:5 *n*3	ND	ND	ND	1.0	4.1	0.8

Table from (Wood et al., 2008)

3.2 Micronutrients composition of red meat

Red meat is a food rich in micronutrients, some in high quantities; they are essential to human health and the well-being. The legislation says that a food is classified as a "source" if it contributes to a sixth of the Community consumption. It Is considered "rich source" if a portion of food provides half the recommended daily amount (BNF and Foundation), 2002). Based on this we can say that 100 grams of beef, pork or lamb can be classified as a "rich source" of vitamin B12, niacin, vitamin B6, zinc and phosphorus.

3.2.1 *Vitamin*

Meat with food of animal origin are the only ones able to provide the vitamin B12 naturally, people who do not eat meat are at risk of deficiency of this vitamin. Red meat contains a number of B vitamins: thiamin, riboflavin, pantothenic acid, folate, niacin, B6 and B12 (Chan. W, 1995) in table 3 was show quantity of Thiamine, riboflavin and niacin in different animal (Lombardi-Boccia et al., 2005). Another important vitamin contained in red meat is vitamin D, essential for the development and maintenance of bones. The intake of vitamin D with the meat and food in general is never high because it is derived from the action of sunlight on 7-dehydrocholesterol in the skin. studies have shown that red meat per 100g of beef were 0.10 ug and 0.45 ug of vitamin D3 metabolite 25-hydroxycholecalciferol (25-OH D3), while crane lamb were 0.04 and 0.93 ug/100 g, respectively (Purchase et al., 2007). This means that 100 g of cooked beef could provide 12% of the estimated adequate intake of 10 mg/day for a 51- to 70-year-old individual (Council, 2006). Studies done by the national diet and nutrition survey say that through the flesh meets the 22% of daily requirements of vitamin D (Henderson L, (2003a)). Other vitamins present in meat are vitamin E, which is located most being soluble in the cuts with higher concentration of fat. Recently there is a tendency to administer to animals as vegetable oils rich in this vitamin. The vitamin A is in particular offal, mainly in the liver, in the form of retinol. the amount present in the liver is variable dependent on the age of the animal and the composition of the feed consumed (Williamson et al., 2005)

Table 3: Thiamine, riboflavin and niacin content in raw and cooked meat cuts (mg/100 g)

	Raw			Cooked			
	Thiamine	**Riboflavin**	**Niacin**	**Thiamine**	**Riboflavin**	**Niacin**	**Weight loss (%)**
BEEF							
Sirloin	0.02±0.01[a]	0.12±0.01[a]	5.0±0.35[a]	nd	0.07±0.01[ab]	3.2±0.09[a]	37.7
Fillet	0.08±0.01[b]	0.17±0.01[b]	5.7±0.25[a]	nd	0.07±0.01[ab]	3.3±0.09[a]	38.2
Roast beef	0.05±0.01[b]	0.10±0.01[a]	5.5±0.21[a]	nd	0.10±0.02[a]	3.3±0.08[a]	39.2
Topside	0.08±0.01[b]	0.09±0.01[a]	6.5±0.14[a]	nd	0.05±0.01[ab]	4.2±0,12[a]	43.4
Thick flank	0.01±0:01[a]	0.12±0.01[a]	5.0±0.21[a]	nd	0.04±0.01[b]	3.0±0.13[a]	40.0
VEAL							
Fillet	0.11±0.02	0.08±0.01	6.9±0.35	nd	0.05±0.01	4.3±0.18	41.7
LAMB							
Fillet	0.16±0.04	0.10±0.02	4.5±0.15	nd	0.07±0.01	2.7±0.17	36.2
PORK							
Saddle	0.90±0.12[a]	0.10±0.01[a]	5.2±0.20[a]	nd	0.06±0.01[a]	3.3±0.11[a]	36.9
Loin	0.60±0.10[b]	0.13±0.02[a]	4.2±0.18[a]	0.15±0.01[a]	0.09±0.02[a]	3.2±0.09[a]	33.6
Chump chop	0.90±0.14[a]	0.15±0.03[a]	5.7±0.21[a]	0.21±0.02[a]	0.07±0.01[a]	3.3±0.18[a]	37.6
CHICKEN							
Breast	0.02±0.01	0.06±0.01	7.2±0.28	nd	0.03±0.01	5.3±0.9	30.7
RABBIT	0.05±0.01	0.11±0.01	5.3±0.21	nd	0.06±0.01	3.8±0.10	24.4

Values are the M±SD of 3 replicates. Means with different letters among cuts of the same species are significantly different ($p<0.05$).

Table from (Lombardi-Boccia et al., 2005)

3.2.2 *Minerals*

Meat is also an important source of bioavailable minerals and trace elements, especially iron and zinc. Iron is a carrier of oxygen in the myoglobin in the muscles, and of 'hemoglobin in the blood, is also used for other metabolic processes. The iron in meat is found in two forms, heme iron and non heme iron, approximately 20-30% of heme iron is Absorbed from the gut and is mostly used by the body, compared to 7% of non-heme iron. Total meat contributes 17% of the total iron taken with the diet in UK (Henderson L, (2003a)) and to 22% in new Zealand(Russell D, 1999) . Absorption of iron from meat is typically 15±25%, compared with 1±7% from plant sources (Fairweather-Tait, 1995). Meat allows the absorption of iron made with vegetables during the meal, so it could double the amount of iron absorbed. The mechanism by which this process has not been confirmed but is assumed to be due to the ability of the iron to bind to cysteine inside of the peptides, this is followed by the proteolysis of muscle of the meat. Several studies have shown that iron deficiency is more prevalent in vegetarians than non vegetarians of child-bearing age, (Higgs, 2000). The study on the content and availability of iron is limited by the fact that its amount can vary even within the same meat, because it changes depending on the age of the animal diet and the type of farming. The meat in line with EU rules is considered a source of zinc (BNF and Foundation, 2002). The importance of zinc in the flesh and also due to its high bioavailability, studies done in Ireland has shown that through the flesh meets the needs of 41% zinc (Cosgrove et al., 2005). AnOther study found that zinc intake was lower among female vegetarians compared with meat eaters (Ball and Ackland, 2000). According to animal management, nutrition and age in the flesh can be found in varying amounts, other minerals like magnesium, copper, cobalt, chromium and nickel, most recently in meat is also a good amount of selenium. In particular studies done in New Zealand and Denmark have found that meat contributes to an intake of selenium from 21 to 23%(Russell D, 1999). Table 4 show a content of iron, zinc and copper in raw and cooked meat in different animals (Lombardi-Boccia et al., 2005).

Table 4: Total iron, zinc and copper content in raw and cooked meat cuts (mg/100 g)

	Raw			Cooked			
	Fe*	**Zn**	**Cu**	**Fe***	**Zn**	**Cu**	**Weight loss (%)**
BEEF							
Sirloin	1.93±0.1^{ab}	4.09±0.2^{ab}	0.07±0.1^{a}	3.11±0.4^{a}	5.74±0.3^{a}	0.08±0.1^{a}	37.7
Fillet	2.37±0.1^{ab}	4.01±0.2^{ab}	0.09±0.1^{a}	3.38±0.2^{a}	5.62±0.3^{a}	0.11±0.1^{a}	38.2
Roast beef	1.95±0.1^{ab}	4.75±0.4^{b}	0.05±0.1^{a}	3.46±0.3^{a}	9.44±0.6^{b}	0.08±0.1^{a}	39.2
Top side	1.91±0.1^{ab}	3.94±±0.1^{a}	0.04±0.1^{a}	2.86±0.4^{b}	5.54±0.2^{a}	0.07±0.1^{a}	43.4
Thick flank	1.80±0.2^{a}	4.29±0.2^{ab}	0.05±0.1^{a}	3.50±0.3^{a}	7.23±0.6^{b}	0.10±0.1^{a}	40.0
VEAL							
Fillet	1.20±0.1	5.01±0.3	0.03±0.1	1.93±0.2	9.50±0.6	0.04±0.1	41.7
LAMB							
Chop	1.98±0.3	2.43±0.1	0.10±0.1	2.72±0.4	3.77±0.2	0.15±0.1	40.8
HORSE							
Fillet	2.27±0.2	1.95±0.1	0.12±0.1	2.91±0.2	3.20±0.2	0.19±0.1	42.1
PORK							
Saddle	0.49±0.1^{a}	0.98±0.1^{a}	0.04±0.1^{a}	0.79±0.1^{a}	1.79±0.2^{a}	0.06±0.1^{a}	36.9
Loin	0.42±0.1^{a}	1.55±0.1^{a}	0.05±0.1^{a}	0.68±0.1^{a}	2.54±0.2^{a}	0.07±0.1^{a}	33.6
Chump chop	0.70±0.1^{b}	2.28±0.2^{b}	0.07±0.1^{a}	1.09±0.2^{b}	4.17±0.3^{b}	0.12±0.1^{b}	37.6
CHICKEN							
Breast	0.40±0.1^{*a}	0.65±0.1^{a}	0.05±0.1^{a}	0.58±0.1^{*a}	0.90±0.1^{a}	0.06±0.1^{a}	26.1
leg (lower part)	0.63±0.1^{*b}	1.47±0.1^{b}	0.09±0.2^{a}	1.20±0.2^{*b}	2.40±0.1^{b}	0.13±0.3^{b}	37.9
leg (thigh)	0.70±0.1^{*b}	1.71±0.1^{c}	0.11±0.2^{b}	1.34±0.1^{*b}	3.32±0.1^{b}	0.14±0.2^{b}	39.1
Wing	0.63±0.2^{*b}	1.29±0.1^{b}	0.04±0.3^{a}	0.92±0.2^{*a}	2.41±0.1^{b}	0.09±0.3^{a}	40.4

(°) Values taken from Lombardi-Boccia et al., 2002
(Lombardi-Boccia et al., 2005)

CHAPTER 4

QUALITY OF MEAT FAT

4. Fatty acid in meat

The different fatty acids have different effects on human nutrition and health and therefore is important to know the literature about the content of fatty acid in meat and the effects of the different fatty acids present in meat on human healthiness. In meat, as in others food, there are two important groups of fatty acids, "saturated fatty acid" (SFA) and “unsaturated fatty acids” (UFA). Different studies show that the ratio of polyunsaturated fatty acids/saturated fatty acids (P/S) must have a value > 0.7, while the ratio n-3 /n-6/ must have a value <4 (De Smet, 2004). The fat content and the fatty acid composition of beef, lamb and pork meat samples is reported in Table 1. The lamb and beef has higher content in palmitic (C16: 0) and stearic acid (C18: 0), and a smaller amount of linoleic acid (C18 :2) than pork and poultry meat (Enser et al., 1996). Attention has been focused on the type of PUFA in the diet and in particular the balance between the n-6 PUFA formed from linoleic acid (C18: 2) and n-3 PUFA formed from α-linolenic acid (C18: 3)(Williams, 2000). The amount of C18: 3 in lambs is nearly twice the amount found in beef (Wood et al., 2004b). Meat of ruminants in the ratio P: S is advantageous, especially in animals that graze on pasture and eat grass. The meats of ruminants contain also conjugated linoleic acid (CLA) that has many nutritional benefits (Enser et al., 2001)

Table .1 Fat and fatty acid composition of beef, lamb and pork loin steaks purchased from four supermarkets. (Enser et al., 1996).

	Beef	Lamb	Pork
Whole steak			
Fat[a]	15.6	30.2	21.1
Muscle[b]			
16:0 palmitic	25.0	22.2	23.2
18:0 stearic	13.4	18.1	12.2
18:1 n-9 oleic	36.1	32.5	32.8
18:2 n-6 linoleic	2.4	2.7	14.2
18:3 n-3 α-linolenic	0.70	1.37	0.95
20:4 n-6 arachidonic	0.63	0.64	2.21
20:5 n-3 EPA	0.28	0.45	0.31
22:6 n-3 DHA	0.05	0.15	0.39
Total fatty acids[c]	3.8	4.9	2.2
P:S	0.11	0.15	0.58
n-6:n-3	2.11	1.32	7.22
Fat[b]			
16:0 palmitic	26.1	21.9	23.9
18:0 stearic	12.2	22.6	12.8
18:1 n-9 oleic	35.3	28.7	35.8
18:2 n-6 linoleic	1.1	1.3	14.3
18:3 n-3 α-linolenic	0.48	0.97	1.43
C20–C22 n-3 PUFA[d]	ND	ND	0.36

ND: not detectable. a: % of steak, b; of total fatty acids, C:% of muscle, d: % of fat tissue.

4.1 Saturated and monounsaturated fatty acids

Meat from ruminants contain a high proportion of saturated fatty acid than meat from monograstrics due to the biohydrogenation process for the activity of rumen microorganisms indeed. Nutritionist recommended that the consumption of SFA should be no more than 10% of total daily energy intake (FAO/WHO, 1998). These amounts are based on several studies that have detected the influence of SFA on cardiovascular diseases, because this class of fatty acids is related to the increase of plasma low density lipoprotein (LDL) and increases in the ratio of LDL/HDL (high density lipoprotein) (Daley et al., 2010). In red meat the main saturated fatty acids are palmitic (C16:0) and stearic acid (C18:0). The latter does not seem to lead to increased cholesterol in the blood 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 (Chilliard Y., 2001). While those most responsible for cholesterol increase are lauric (C12:0), myristic (C14:0) and palmitic (C16:0). Beneficial effects of fatty acids were found in the branched chains and the odd chain fatty acids (OBCFA), were found in these anticarcinogenic effects (Wongtangtintharn et al., 2004). The monounsaturated fatty acids (MUFA) have several beneficial properties to human health (Garg, 1998, Ros, 2003), because reduce the metabolic syndrome, promote a good blood lipid profile, blood pressure and blood glucose (Krauss, 2000). Some researchs showed that the Mediterranean diet, rich in MUFA, reduced risk of chronic diseases (Keys et al., 1986) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults). The main MUFA in meat are oleic (C18:1 cis-9) and palmitoleic acid (16:1n-7) representing approximately 92% of cis MUFA (Kris-Etherton, 1999). This is very important to human health because reduces levels of LDL cholesterol, and cardiovascular disease (Givens, 2005), while among those in the trans form there elaidic acid (C18:1trans -9) with hypercholesterolemic properties and present in meat in a very low amount. Mediterranean diet contains naturally a large amount of MUFA that represented by about 40% of fat intake (Willett et al., 1995) (table 2), The type of fat intake is very different in the U.S (Salas-Salvado et al., 2008) becauseMUFA is 13-14% of energy, SFA is in excess 11-12 %, PUFA accounts for about about 7% of energy. Almost 90% of these PUFA is represented by the omega-6, especially linoleic acid (Kris-Etherton et al., 2000, Kris-Etherton, 1999).

Table 2. Current nutrient intakes in the Mediterranean and United States as compared to the recommended intakes outlined by health professional organizations

	Current intake		**Reccomended intakes**		
	Mediterranean (%)[a]	**U.S (%)[a,b]**	**Dietary guidelines (%)[a]**	**ADA and DC (%)[a]**	**NCEP ATP III (%)[a]**
Total fat	33–40	33	20–35	20–35	25–35
SFA	<8	11–12	<10	<10	<7
MUFA	16–29	13–14	–	≤25	≤20
PUFA	<7	<7	–	≤10	≤10

ADA American Dietetic Association, DC Dietitians of Canada, NCEP ATPIII National Cholesterol Education Program Adult Treatment Panel III. [a]Percent of daily energy. [b]Means of United States male and females (ages 20–59)
data fromGillingham et al., (2011)

4.2 Polyunsaturated fatty acids (PUFA)

The most important PUFA in meat are α-linolenic acid (ALA) (C18:3n-3) for n-3 series and linoleic acid C18:2n-6 for n-6 fatty acids series. Linoleic acid (n-6) (LA) and alpha-linolenic acid (n-3) are essential fatty acids (EFA), because mammals do not have capacity for their de novo biosynthesis due to a natural absence of Δ-15 and delta-12 desaturase enzymes. Fat and oils, meat and poultry, cereals, vegetables and seed were important sources of PUFA n-6, while cereal based products, linseed, fats and oils, fish, meat and milk were source of PUFA-n3 (Benatti et al., 2004, Meyer et al., 2003). Once consumed, these fatty acids are further metabolized throught processes of desaturation (by insertion of double bonds) and elongation (by addition of 2-carbon units) (SanGiovanni and Chew, 2005) as reported in figure 1. Linoleic acid (18:2n-6) is a precursor for arachidonic acid (20:4n-6), whereas α-linolenic acid (C18:3n-3) is the dietary precursor of EPA and DHA (Jump, 2002)(figure 2). 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 (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. Because both essential fatty acids families compete for the same biosynthetic enzymes, dietary lipid balance and composition will affect production and tissue accretion of these nutrients (Su et al., 1999).

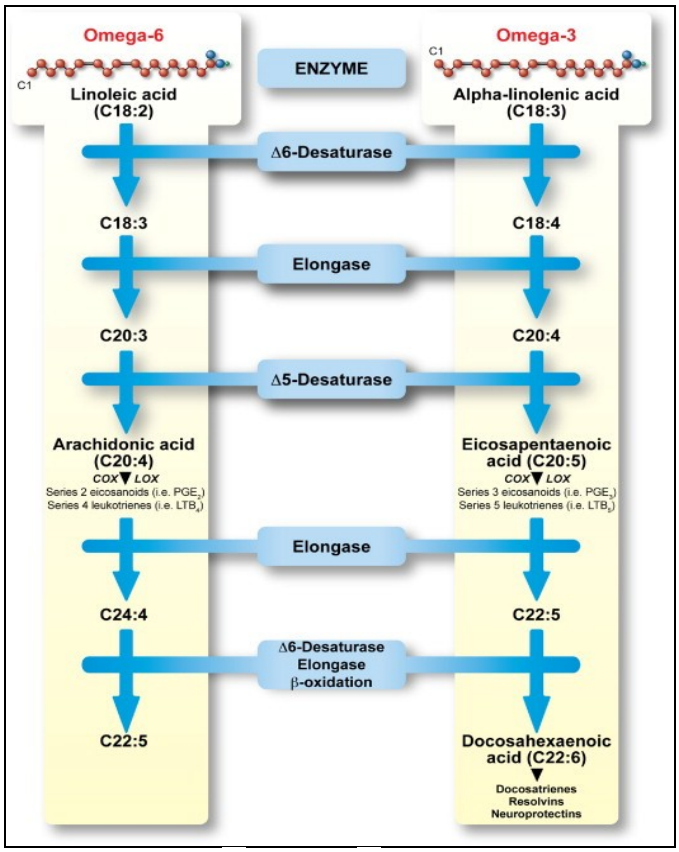

Figure 1 Biosynthetic pathways of **ω** *-3 and* **ω** *-6 long chain fatty acids via a series of elongation and desaturation steps.*

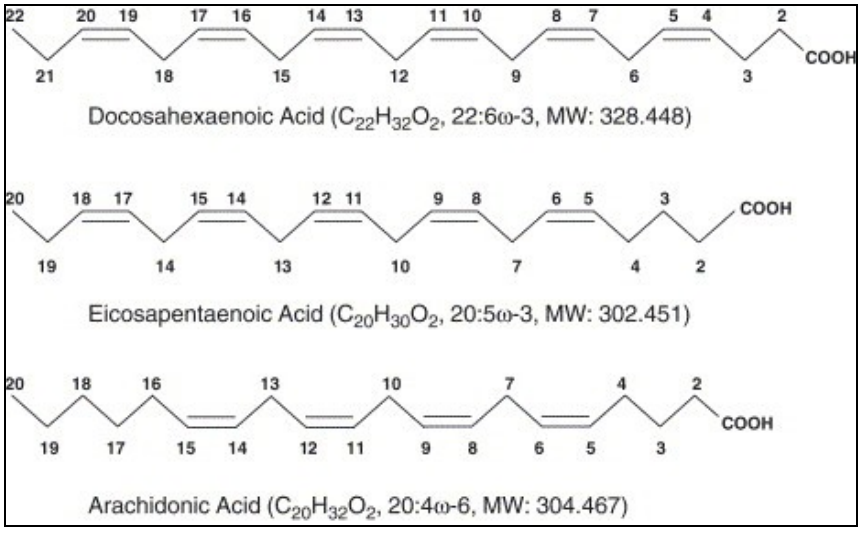

Figure. 2 Chemical structures of DHA (D4,7,10,13,16,19-docosahexaenoic acid), EPA (D5,8,11,14,17-eicosapentaenoic acid), and AA (D5,8,11,14- eicosatetraenoic acid).

Linoleic acid belong to a lipids complex that contribute to form the permeability barrier of the epidermis (De Luca and Valacchi, 2010), while α-linolenic acid has an important role in the prevention of heart disease, cancer and disorders of neurological function in both children and adults, and problems to neurological function of children (Barceló-Coblijn G. and E.J., 2009). PUFA n3 can prevent ventricular arrhythmias and thereby reduce fatal Coronary artery disease (CAD) (Peet, 2002, Willett, 2006). In a case-control study in Costa Rica, where fish intake was extremely low, the risk of myocardial infarction was 80 percent lower in those with the highest alpha-linoleic acid intake (Baylin et al., 2003). PUFA n-3 and n-6 series are involved in mechanism of cellular structure regulation of all body cells and particularly way as those of the brain, retina and reproductive system (Benatti et al., 2004, Yehuda, 2003), and through reactions catalyzed by the enzymes cyclooxygenase and lipoxygenase generate hormone-like substances such as thromboxanes, leukotrienes and prostaglandins in all cells of the organism (Kirkup et al., 2010). Long-chain PUFA n3, including EPA and DHA, are dietary fats with an array of health benefits (Su et al., 2008). They are incorporated in many parts of the body including cell membrane (Lazzarin et al., 2009). Several studies have shown that these fatty acids play a role antiatherogenic, antithrombotic and anti-inflammatory processes (Camuesco et al., 2005; Pischon et al., 2003), and in the viscosity of cell membranes (Conquer et al., 2000; Smith et al., 2011). The mechanisms by which fatty acids exert these important functions are manifold and likely to contribute in a synergistic way to the final effect. The protective action of n-3 fatty acids against cardiovascular diseases of atherosclerotic takes place with regards the interaction between the platelets and the endothelium of blood vessels: this is demonstrated by the fact that the n-3 will extend the bleeding time (Din et al., 2004). The effects of EPA e DHA on the cardiovascular diseases are conducted on the regulation of the production of eicosanoids from arachidonic acid (AA), plasma triglycerides and antihypertensive effects, regulating the flow of ions into cardiac cells, and the regulation of gene expression through the peroxisomal proliferation system (Sinclair et al., 2002). Several studies show that EPA and DHA modify positively serum markers of CVD risk by reducing TGs and increasing HDL-cholesterol (Balk et al., 2006). The majority of investigations assessing CVD and fatty acid intake suggest a beneficial effect of marine-derived PUFA. All four trials reduced secondary cardiac events with between 1.0 and 1.8 g/d fish oil capsules or with 1 serving of fish/d or ALA supplementation

(Anderson and Ma, 2009). In study concerning women, was 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)(Hu et al., 1999). The result of several studies shows that different populations there was a reduction of cardiovascular diseases and cardiac mortality after taking DHA and EPA from fish and fish oil (Balk et al., 2006, Holub and Holub, 2004). Some studies indicate that the Americans who have consumed an amount around the 34 g of fish per day, had a lower risk of 40-50% of the myocardium infarction and risk much lower coronary heart disease (CHD), this amount provides approximately 250-500 mg of DHA and EPA (combined) per day on average (Daviglus et al., 1997, Raper et al., 1992). And has been designated a contribution of DHA and EPA respectively 82 and 35 mg per day, with a DHA:EPA ratio of 2.3/1 (Denomme et al., 2005). The anti-inflammatory and anti-cancer effects of PUFAs n-3 have been widely documented experimentally using in vitro as well as in vivo systems and clinical studies (Chapkin et al., 2008b, Rose and Connolly, 1999). EPA and DHA, may slow the growth of tumor cells and may increased sensitivity to chemo- and radiotherapy and decreased side effects (Pardini, 2006). There is some evidence suggesting a protective role for EPA and DHA in prostate cancer. In vitro studies have identified dose-dependent inhibition of human cancer cell growth (Rose and Connolly, 1991). Experimental studies have shown that DHA functions as a potent suppressor of colon cancer cell proliferation (Ichihara et al., 2011, Jakobsen et al., 2008) inducing cell death in a dose- and time-dependent manner (Ichihara et al., 2011).

4.2.1 *Conjugated linoleic acid (CLA)*

The fatty acid conjugated linoleic acid (CLA), is found in good quantity in the meat of ruminants (Table 3) , with the highest concentration found in lamb (4.3–19.0 mg/g lipid) and with slightly lower concentrations in beef (1.2–10.0 mg/g lipid). Many forms of CLA are possible, the isomers that were found in milk and meat are 7-9, 9-11, 10-12, 11-13, 12-14 in the form cis-cis-trans-cis and trans -trans for a total of 24 isomers. The most attention is focused on isomer cis-9, Trans 11 (rumenic acid, RA) and trans-10, cis-12 (figure 3). CLA content of pork, chicken, and horses meat is usually lower than 1 mg/g lipid. Large variations in the CLA content are not only reported between animal species but also within muscles of the same species (Schmid et al., 2006). In animals reared on pasture were found

the quantities of CLA between 5.4, 6.6, and 10.8 mg CLA/g FAME against 3.7 mg/g FAME in animals fed with concentrated (Schmid et al., 2006). Also in lamb were found higher CLA concentrations in the longissimus muscle of lambs raised on pasture than of lambs fed a concentrate diet (7.1 vs. 3.2 mg/g FAME) (Santos-Silva, 2002). In particular the addition of oil seed of sunflower-rape to the diet increase CLA content in muscle lipid compared to control group (Casutt et al., 2000). The CLA levels in the longissimus dorsi muscle increased by using linseed supplementation in bulls (Stasiniewicz, 2000), and in lambs (Berthelot et al., 2010, Kott, 2003). Another approach to increase CLA is fish oil (Demirel, 2004).

Table 3. Conjugated linoleic acid content of various foods

Food	**mg/g fat**
Meats/fish	
Fresh ground beef	4.3
Veal	2.7
Lamb	5.8
Pork	0.6
Chicken	0.9
Fresh ground turkey	2.6
Salmon	0.3
Egg yolk	0.6
Vegetable Oils	
Safflower oil	0.7
Sunflower oil	0.4

(Lin et al., 2004)

CLA that is found in meat and milk of ruminants is formed in two way: for incomplete PUFA biohydrogenation in the rumen or for conversion of vaccenic acid in animal tissues (Griinari and Bauman, 1999). In milk and meat they are found amounts ranging from 2.9 to 11.3 mg/g (Larque et al., 2001). Most of CLA human tissue is food-borne; there may also be endogenous synthesis through the D-9 desaturation of C18: 1_11t (Adlof et al., 2000).

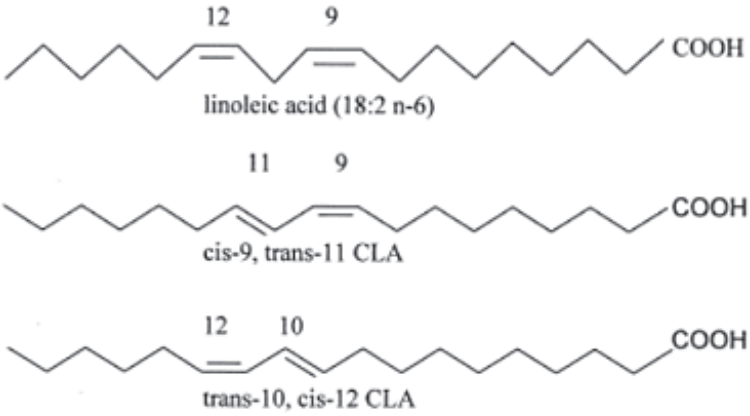

Figure.3. Structure of C18:2 n-6, and of CLA isomers (cis-9, trans-11-CLA and trans10, cis-12-CLA)

Several work has been show that CLA exerts positive effects on cancer, cardiovascular disease, diabetes, body composition, immune system, and bone health(Jahreis, 2000, Khanal, 2004, Pariza, 2004). Studies show that CLA has an antitumor effect, it was demonstrated that is effective in reducing the growth of breast cancer (Wahle et al., 2004), and cancer cells in the colon (Beppu et al., 2006) and prostate (Cesano et al., 1998). The CLA has an anti-atherosclerotic effect (Lee et al., 1994). It is seen that diets enriched with CLA cause a decrease in plasma cholesterol LDL with a mechanism that 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). Studies in rats show that administration of 1.5g/ kg of body weight of CLA for 6 weeks reduces body weight of 10% (West et al., 1998). The effect of reducing the fat has been associated with the trans-10, cis-12 CLA isomer (Khanal, 2004). Effects on weight loss have been documented in several animals such as pigs (Dugan et al., 2004), mice (Hargrave et al., 2004) and rats (Azain et al., 2000).

4.3 The effect of fatty acid profile of fat on meat quality

The fatty acid profile of meat influenced the firmness of the fat tissue, because the number of the double bonds and the length of the chain change the melting point of fat. The firmness of the meat is due to the melting point of the fatty acids, the series of fatty acids C18, stearic acid (C18: 0) has a melting point of 69.9C, oleic acid (c18: 1) to 13.4 C18:2 at

5 C and 18:3 at 11 C to increase of unsaturation, decreases the melting point. The variation of melting point depending on the structure of the molecule, in fact trans-fatty acids melt at a temperature higher than their cis isomers, fatty acids, branched-chain have melting points lower than those with straight chain while having the same number of carbon atoms (Enser, 1984). From a study done on a thousand lambs, it is established that the C18:0 is a good predictor of the melting point of subcutaneous fat and the hardness of the fat when it is eaten. From this study it is seen that the average melting point of subcutaneous fat is of 39.5 °C, the correlation with the melting point of C18:0 was 0.89. It is seen that only 35% of these samples of fat melted in the mouth, the rest was "sticky" (Wood, 2005). Between May and August, the melting point of C18:0 was lower; this period coincided with the grazing of animals. Other researchers have found the soft fat is higher in animals fed with grain-based diet, because has a lower concentration of C18:0, and an increased amount of medium long -chain fatty acids (C10-C17), branched chain fatty acids formed by methylmalonate, a metabolite of propionate (Busboom, 1981).

The color of the fat in meat is another aspect influenced by fatty acids profile; usually fat cells that contain fat solidified with a high melting point, are whiter than those that contain liquid fat with a low melting point. The double-chain unsaturated fatty acids are also implicated in the oxidation stability that is a key aspect for the duration and preservation of meat.

4.4 Strategies for increased the PUFA omega3 and CLA contents in lamb meat

Several strategies have been studied to improve the n-3 PUFA and CLA content in meat. One of the main strategies to increase PUFA and CLA is the use of vegetable oils (Table 4). These studies demonstrated that linseed oil (rich in ALA) can increase the concentration of PUFA n3 and decrease the n-6/ n-3 ratio in lambs tissue. The linseed supplementation during the period of post- weaning increase in muscles the ALA (C18: 3 n-3) (in the range from 1.5 to 3% of the total FA) content and long chain n-3 PUFA, C20: 5 n3 (EPA), C22: 5 n-3 (DPA) or C22: 6 n-3 (DHA) (Bas et al., 2007, Berthelot et al., 2010, Wachira et al., 2002).Conversely, supplementation of sunflower seed or sunflower oil (Manso et al., 2009), soybean oil (Santos-Silva et al., 2004; Bessa et al., 2005) or safflower oil (Boles et al., 2005) that are rich in LA acid can increase the concentration of

cis-9, *trans*-11 CLA and PUFA n6 and n-6/n-3 PUFA ratio. Santos-Silva et al. (2004) showed that the effect of soybean oil inclusion on fatty acid was related to forage particle size. In a trial in which it was administered sunflower seed oil (58 g/kg concentrate diet) in sheep has caused an increase from 2 to 7 g/100 of linoleic acid in perirenal fat (Gibney and L'estrange, 1975). The supplementation of fish oils increased the amount of EPA and DHA incorporated into phospholipids (Ashes et al., 1992). Feeding fish oil also increases the n-3 long chain PUFA concentration in the intramuscular fat due to the high eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) content in fish oil. Ruminal biohydrogenation of EPA and DHA is limited, and therefore considerable amounts of these fatty acids are available for incorporation into the adipose tissue (Raes et al., 2004). Suckling lambs can be considered as "functional monogastric", with the functionality of the groove reticular that avoid the milk passes into the rumen. As a result the milk with high amount of PUFA is transferred efficiently into the tissues, if after weaning lambs are fed with a diet enriched with ALA may have a high amount of n-3 PUFA in muscle tissue and adipose tissue (Lanza et al., 2006). Has also been shown that a combination of soybean oil and marine algal oil in the diet pre-weaning (feeding mothers) increases the content of PUFA in the longissimus dorsi and in adipose tissue but without increasing the content in ALA (Radunz et al., 2009).

Table 4 PUFA and CLA contents (g/100 g of FATTY ACIDS) in intramuscular fat of lambs supplemented with vegetables oils

Diet	*CLA*	*PUFA n3*	*PUFA n6*	*n6/n3*	*reference*
Control	0.43	0.47	3.3	7.0	Bolte et al., 2002
safflower seed-high oleate	0.65	0.66	2.9	4.4	
safflower seed-high linoleate	0.68	0.5	4.5	9.0	
hay	0.55	4.65	8.42	1.8	Santos-Silva et al., 2004
hay + soybean oil	2.37	2.69	8.66	3.2	
pelleted hay	0.64	2.85	8.03	2.8	
pelleted hay + soybean oil	1.83	1.79	12.18	6.8	
Lucerne	0.85	4.42	9.07	2.1	Bessa et al., 2005
Lucerne + 10% soybean oil	2.39	1.69	10.37	6.1	
Concentrate	0.55	1.04	7.87	7.6	
Concentrate + 10% soybean oil	0.44	1.54	11.33	7.4	
Control	0.07	0.92	4.85	5.3	Bas et al., 2007
3% Linseed	0.08	1.19	4.36	3.7	
6% Linseed	0.12	1.63	5.01	3.1	
9%Linseed	0.08	1.91	4.98	2.6	
Control	0.43	2.17	10.03	4.6	Manso et al., 2009
hydrogenated palm oil 4%	0.59	2.29	9.84	4.3	
sunflower oil 4%	0.56	2.33	9.49	4.1	
linseed oil	1.04	2.04	8.04	3.9	Kitessa et al., 2009
linseed oil 3w	1.13	2.41	9.32	3.9	
linseed oil 6w	1.06	2.97	10.09	3.4	
linseed oil 9w	1.08	3.2	12.11	3.8	
control	0.05	1.58	14.04	8.9	Berthelot et al. , 2010
linseed extruded + wheat	0.11	4.11	14.72	3.6	
linseed extruded + corn	0.12	3.73	12.73	3.4	

The content of ALA and CLA in meat is also increased by pasture because plants are the main source of PUFA n-3. Generally the feeding systems based on pasture increased concentrations of ALA compared with indoor feeding system (Santos-Silva et al., 2002; Fisher et al., 2000). Levels of PUFA n3 in meat are influenced by botanical composition of the forage used in lamb diet: for example Trifolium subterraneum compared to Lolium multiflorum increased the content of PUFA in lamb meat (4.49 and 3.99%, respectively) due to the higher content of ALA in the trifolium than lolium (Chiofalo et al., 2010). The

maturation of forages, that decrease the decrease of more than 50% the ALA content (Nudda et al., 2003) can also influence fatty acid profile of meat reducing the content of ALA and CLA.

CHAPTER 5

CHEMICAL ANALYSIS

5 *Preparation of meat sample*

Sample longissimus dorsi and sample of thigh, not completely thawed, after removal of the external fat were diced using a knife. Meat samples were frozen at -20 °C and stored at -80°C until liophylisation for 3 days in LyoLab 3000 (Heto). Meats were liophylized. Lhiophylized samples were gound by using a domestic grinder machine and stored at –80°C until analysis.

5.1 *Chemical analysis*

Meat was analyzed for moisture, total ash, total nitrogen and total lipids:

- Moisture: was determined by drying samples in an oven for 24 hours at 105°C (Method 950.46, AOAC, 1997).
- Total ash: was quantified by ashing the sample in a muffle furnace at 550°C for 24 h (Method 920.153, AOAC, 1999).
- Total protein: was calculated from Kjeldhal nitrogen using a 6.25 conversion factor.

5.2 *Extraction and analysis of muscle fatty acids*

They were performed for the thigh and longissimus dorsi. Diced meat was weighed in a polypropylene pan, frozen overnight at -80°C, freeze- drying (Lyolab 3000, Heto-Holten, Allerod, Denmark) for 72 H and finely ground in a food processor prior to analysis. Total lipid was extracted from 1 g of sample freeze dried following the method of Folch et al. (1957)(Folch et al., 1957), with chloroform-methanol for extraction. Briefly, 1 g of sample was placed in a 50-ml tube and 30 mL of chloroform: methanols (2:1) were added. The mixture was sonicated for 5 min, shaken vigorously with vortex for 60 s and then centrifuged for 10 min. (1500 x g at room temperatire). The chloroformic phase was filtered through a whatman No.541 filter paper (Whatman Ldt.,Maidstone , U.K.) and 6 ml 0.6%NaCl (wt/w)were added. The upper methanol–water layer was removed using a water aspirator and discarded, the lower organic phase was poured into a round flat- bottom flask (50 ml), chloroform was removed using a vacuum rotator evaporator at 40C (Buchi 461, BUCHI labor AG, Flawil, Switzerland) and sample were evaporate until dryness. The total extracted meat fat was recorder gravimetrically. After 24 h in a desiccator, extracted

fat was redissolved in n-hexane and an aliquot of muscle lipid (20-25 mg) was transferred to a 2mL screw –cap amber –glass vial and used immediately for fatty acid analysis

5.1.2 *Extraction and analysis of brain fatty acids*

Improved method (Hara and Radin, 1978) for lipid extraction to brain consists of the use of a mixture extractant formed by hexane: isopropanol (3:2), which is followed by a washing the extract, with aqueous sodium sulfate to remove non-lipid contaminants. 250-300 mg of Lamb brain lyophilized was weighted into a pyrex test tube of 50 ml and added 18ml of hexane: isopropanol (HIP) (3:2), the mixture was homogenized with a ultra-Turrax for 30 s, the homogenizer were washed three times with 5 ml portion of HIP. Centrifuged for 10 min. (2500x g) and decant the supernatant of filters watman 451, the filtrate is collected in a second tube 50 ml. wash the residue with 5 ml of HIP, vortex for 1 min and centrifuge at 2500 x g for 10 min. Filter and combine the filtrates in the same tube, add 12 ml of anhydrous sodium sulfate (1 g in 15 ml water), vortex 1min and centrifuge 10 min at 2500 x g. Retrieve the hexane phase and place it in a flat-bottomed flask 50 ml previous calibrated. Send to dry the solvent in rotovapor, and keep the lipid extract overnight under vacuum before the subsequent recovery and methylation

5.1.3 *Methylation*

The lipid extract was dried to a constant weight under a stream of N_2, and redissolved in 300ml of toluene for preparation of fatty acid methyl esters (FAME). They Were performed for the thigh of alkaline and acid transesterification. The extracted lipid fractions were initially methylated with $NAOCH_3$, wich was followed a 10%solution of HCL in methanol. Both methylation procedures were carried out at 50°C for 10 min. Tricosanoic acid (C23:0) methyl ester was used as an internal standard (C: 19) for fatty acid quantification. The tubes was centrifuged (2500x g) for 10 min, and the top layer containing FAME in exane was removed and transferred to vials containing 7.5 mol of K_2CO_3, and used immediately for fatty acid analysis

5.1.4 *Fatty acid composition*

Lipid extracted from meat sample was used for fatty acid determination. The lipid sample (about20-25 mg), was esterified by cool base-catalyzed methilation using 0.5 $NaOCH_3$ in methanl (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF 1999); methilation was achieved in 15 min at room temperature. An internal standard was used for quantify the fatty acid methyl esters (FAMEs) nonadecanoic acid (C19:0) methyl ester.

FAMEs were identified by gas chromatography using a Turbo 3400 CX GC (Varian Inc. Palo Alto, Ca) gas cromatograph equipped with a flame ionization detector (FID) and Varian 8200 CX auto sampler. Separation was performed on a CP-select CB for FAME (Varian inc. Palo Alto,Ca) capillary column (100 m x 0.32 mm i.d., 0.25 μm film thickness). The split ratio was 1:100, the injected sample was 1 μl and the total run time was 74 min. Column oven temperature was held at 75°C for 1 min, increased at 8°C/min to 165°C (35-min hold), increased at 5.5°C/min to 210°C (1- min hold), and finally increased at 3°C/min to 240°C and held for 15 min. The injector temperature and the detector temperature were both set at 225°C . High purity helium served as the carrier gas with a pressure psi. High purity hydrogen and chromatographic air were supplied to the FID. Retention time and area of each peak were computed using the Varian Star 3.4.1. Software. Individual FAME was identified by comparing them to a standard mixture of FIM-7FAME Mix (Matreya Inc. Pleasant Gap, Pa., USA)Branched saturated fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap, Pa., USA)as external standard and individual BCFA methyl 14-methylpentadecanoate (*iso*-C16:0), methyl 12-methyltetradecanoate (*anteiso*-C15:0), methyl 12- tridodecanoate (*iso*-C14:0) and methyl 14 –methylhexadodecanoate (*anteiso*-C17:0) (Matreya Inc. Pleasant Gap, Pa., USA).Polyunsaturated fatty acids methyl esters (PUFA) were identified by comparing relative retention times with isomer mixtures PUFA-1, PUFA-2 and PUFA-3 Animal source (Supelco Bellefonte PA) and individual external methyl ester standards, all cis-5,8,11,14,17,C20:5 (EPA), all cis-4,7,10,13,16,19 C22:6 (DHA),all cis-5,8,11,14 C20:4 (ARA), all cis-6,9,12,C18:3, all cis-11,14 C20:2 and all cis-5,8,11 C20:3 (Matreya Inc. Pleasant Gap, Pa., USA). High purity individual *cis*-9, *trans*-11 and *trans*-10, cis-12 CLA (Matreya Inc. Pleasant Gap, Pa., USA), CLA mix standard (Nu-Ceck- Prep. Inc. Elysian,

Minn., USA) and published isomeric profile (Kramer et al., 2004) were also used to help identify the CLA isomers in meat. The identification of C18:1 isomers was based on individual commercial standard (Supelco , Bellafonte, Pa, USA) and on chromatograms published by Kramer et al 2008.(Kramer et al., 2008)

The isomers of C16:1 and C17:1 were identified using published chromatographic reports in ruminant fats (Alves et al., 2006). Fatty acids were expressed as a percentage of total FAME and as absolute concentration (mg/100g of muscle). The artherogenecity (AI) and thrombogenicity (TI) index (Ulbricht and Southgate, 1991) were also calculated.

5.2 *Method for distinguish muscle fiber types*

The method used for fiber is the calcium method for ATPase demonstration, employing solutions of different pH values, have been used primarily to distinguish muscle fiber type. Muscle fibers may be broadly categorized as type 1 ("slow, red muscle oxidative) and type 2 ("Fast, white muscle, glycolytic). The preincubation pH inactivates the myosin-ATPase enzyme of specific fiber types. The remaining active enzyme is attached to a calcium atom which is replaced by a cobalt and finally precipitated as a black insoluble compound by the ammonium sulfide. The technique used is the internal one institute "Agroscope" Posieux Switzerland .Technique: cut 10 micron section in cryostat from snap frozen biopsy. Attach one section to a coverslip.

Pre-incubation solution

A) 10.4 ATP
B) 4.5 ATP

Add the calcium chloride last to prevent precipitation of ATP

Staining procedure

-Place one coverslip for each sample in a separate, labeled Columbia staining jar for each pre-incubation solution.

- Incubate in 4.5 solution for exactly 5 min at room temperature, the 10.4 solution should be added for 15min at room temperature.

- Pour the ATP solution in to a new staining jar: 25 min for the 4.5 ATP stain and 15 min for the 10.4 stain.

- transfer slides in new staining jar contain 1% Coalcium chloride and wash each staining jar with 3 changes of 1% Calcium chloride for a total of approximately 10 min.

- Pour 2% Cobalt Chloride to each jar and transfer slides for 10 min.. Pour out in a container.

-Wash with 4 changes of an approximately 1:20 solution of 0.1 M Sodium Barbital,

- wash with 5 exchanges of deionized H2O

-This step should be done a fume hood, noxious e toxic fumes:

A. Prepare 1% v/v solution of ammonium sulfide

B. For pH 4.5, transfer slides for 20-30 s

Rinse slides with tapwater 5'

Then transfer slides in a new jar contain solution of Erlich's Hematoxyline for 2 min. recover the solution and rinse slides with tapwater.

C. For pH 10.4 tranfer slides for 20-30 seconds. Pour out the solution in a container and rinse in the fume hood for approximately 5 min.

- Tranfer the coverslips with the stained sections to a porcelain rack, cleaning the back side of the coverslip with cotton tipped swab.

-Leave in water until ready to dehydrate

- Dehydrate in ascending alcohols (70%, 80%, 95%) in Columbia staining dish (jar)

-Mount coverslips onto labeled glass slides with Canada balsam.

Calculation

Counting fibers under the microscope

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Experiment 1

Effects of maternal nutrition on muscle fiber differentiation and fatty acid composition of newborn lambs

Abstract

The interest in the nutritional quality of meat has increased recently and this has led to increase the research about that. During the fetal life of animals is already determined the fiber composition of muscles and also has been observed, in human and piglets, a higher efficiency in the conversion of C18:3 n3 on their long chain metabolites. The aim of this study is to evaluate the effects of C18:3 omega3 supplementation by linseed to ewes during pregnancy in the number and type of muscle fibers and muscle fatty acids composition of newborn lambs. Eight ewes were divided in two groups and fed a control concentrate (CON group) and or the experimental concentrate containing linseed as source of C18:3 n3 (LIN group) for approximately the last 8 weeks of gestation. The CON and LIN diets were isoenergetic, isoproteic and isolipidic but differed in the fatty acid composition. Immediately after parturition, 8 lambs were weighted and slaughtered. Three muscles were rapidly removed from the right side of each carcass: two hind limb muscle, semitendinosus (ST) and semimembranosus (SM), and the torso muscle included longissimus dorsi (LD). Muscles sample were analyzed for fat, proteins (CP), moisture and ash. In ST and LD muscle was categorize in two fiber types (I-II type). Results evidenced no effects of maternal omega3 supplementation on fetal growth. The omega 3 supplementation did not influence muscle fiber number. The dietary treatment did not influenced the chemical composition of newborn lambs, whereas influenced significantly almost all fatty acids being the content of TFA, total PUFA, PUFA n-3 and PUFAn-6 higher in muscles of LIN lambs than CON lambs. The omega3 supplementation increased the concentration of EPA, DPA and nervonic acid in muscles and brain tissue, but did not enhance the DHA concentration

1. Introduction

Fat composition of meat has long been studied given its implications for human health (De La Fuente et al., 2009). In this sense, lamb meat could be interesting because of its concentration of long chain PUFA of the omega 3 family (Nudda et al., 2011, Raes et al., 2004, Serra et al., 2009). This aspect is particularly important because of the interest in the nutritional quality of meat has increased recently and this has led to increase the research about that. In particular, low intakes of saturated fat and an increased polyunsaturated to saturated fatty acid ratio are associated with reduced risk of human coronary heart disease (Berthelot et al., 2010, Hooper, 2006, Leon, 2009)**.**

In addition, EPA and DHA, are commonly recognized for their beneficial effects on heart health, proper brain and visual development in the fetus, and maintenance of neural and visual tissues throughout life (Ruxton et al., 2004).

Some studies evidenced that the lambs fed flaxseed or fish oil increase in muscles the amount of polyunsaturated fatty acids of the n3 series (Elmore et al., 2000). Several studies have demonstrated that in the sucking kids (Sauvant et al., 1979, Zygoyiannis et al., 1992) and lambs (Bas, 2000) the fatty acid composition of adipose tissue reflects the milk fatty acid composition of the mother. However, changes in the fatty acid composition of meat are influenced by changes in age and fatness, and the proportion of PUFA in muscle decreases with the increase of the deposition of intramuscular neutral lipids and with age of animal (Link et al., 1970). Generally, in adult animal the concentration of PUFA is lower than in suckling animals, because the high unsaturated PUFA undergoes to rumen biohydrogenation (Doreau, 1993, Enser et al., 1998, Glasser et al., 2008, Nürnberg et al., 1998). However, maternal nutrition can affect strongly the fatty acid composition of newborn and suckling animals. During the fetal life, a higher efficiency in the conversion of C18:3 n3 on their long chain metabolites has been observed in human newborn plasma (Otto et al., 2001) and in the piglet carcass (de Quelen et al. 2010). Therefore, as the concentration of omega 3 is usually greater in young animals than in adults, probably there is a transfer of fatty acids from mother to fetus and greater efficiency of conversion of C18: 3 in into their long-chain metabolites. During fetal life is also determined the fiber composition of muscles (Ashmore et al., 1972); infact maternal nutrition affects the development of myoblasts in the fetus, so a deficient supply of nutrients of the mother can

affect the proliferation of fiber (Brameld et al., 2000). Different studies on laboratory animals has shown that supplementation of animals with omega3 interfere with fetal growth and development, thus we have hypothesized that omega3 supplementation may stimulate fetal myogenesis. This study was performed to investigate if maternal omega3 supplementation by linseed during gestation influenced the fatty acids composition of newborn lambs.

1.2 -Materials and methods

1.2.1 Animals and treatment

The study was carried out in a dairy farm located in the north of Sardinia, in Sassari province. Eight ewes were divided in two groups and fed a control concentrate (CON group) and or the experimental concentrate containing linseed as source of C18:3 n3 (LIN group) for approximately the last 8 weeks of gestation. The ewes received hay ad libitum and 1 kg of the two complete pelleted diets (CPD) formulated specifically for the trial. The ingredients of the concentrate used in the present experiment are reported in Table 1. The CPD was distributed into 3 doses /day for 8 weeks until parturition. In the last 4 weeks of gestation 150g/day of corn flour was added to support the increased energy requirements for fetal growth. The chemical composition of the two CPD is reported in table 2. The CON and LIN diets were isoenergetic, isoproteic and isolipidic but differed in the ingredients (Table 1) to modify the fatty acid profile that is reported in table 3. The predominant FA of the lipid fraction in the CON concentrate were palmitic acid (C16:0), stearic and linoleic acid (C18:2 c9c12, LN). The most abundant FA in LIN concentrate was alpha-linolenic acid (C18:3 c9c12c15, ALA), which accounted for 34% of total FA followed by LN and oleic acid. Immediately after parturition 8 lambs were weighted and slaughtered to remove the tissues for the subsequent analyses.

Table 1. Ingredients of the control and treatment concentrates

CONTR	LIN
Wheat bran	Soybean hulls
Alfalfa meal	Extruded linseed
Distilled from wheat	Wheat bran
Wheat bran,	Flour of extraction of sunflower
Sunflower extraction meal	Alfalfa meal
Corn germ cake	Sugar beet pulp dried
Dried sugar beet pulp	Corn gluten feed
Calcium carbonate	Calcium carbonate
Soya Meal	Soya meal
Maize	Maize
Chloride Sodium	Sodium chloride
Sodium Bicarbonato	Sodium bicarbonate
Magnesium Oxide	Magnesium oxide

Table 2. Chemical composition of the experimental diets (% of DM)

	CONTR DIET **%**	**LIN DIET** **%**
DM	89.40	89.41
Ashes	8.86	8.56
Crude protein	18.42	18.37
NDF	42.94	41.98
ADF	26.02	28.39
ADL	6.00	5.48
EE	7.34	6.99

Table 3. Fatty acid composition of the the control (CON) and experimental (LIN) completed pelleted diets

Fatty acid	CON	LIN
C12:0	1.033	0.147
C14:0	1.130	0.183
C16:0	31.500	10.346
C18:0	27.352	6.071
C18:1 c9	10.731	20.627
C18:2 n6	22.780	26.280
C18:3 n3	2.026	34.217

1.2.2 Tissue samples preparation

At slaughter, three muscles were rapidly removed from the right side of each carcass: two hind limb muscle, semitendinosus (ST) and semimembranosus (SM), and the torso muscle longissimus dorsi (LD). The sampling is conducted using a scalpel ten minutes after death. Fresh weight of the 3 muscles was also recorded. Each muscle was rapidly photographed to determined geometric measure (Figure1) of length for LD and ST and girt for ST. Samples were then be pinned in a rubber stick, slightly stretched, frozen in isopentane cooled with liquid nitrogen. After the samples were well frozen, were immediately wrap in aluminum foil and put in liquid nitrogen. After this procedure the muscles sample are packaged under vacuum and stored at -80°C.

After this, the carcass was skinned, refrigerated for 24 hours at 4°C and the gastrointestinal tract, offal, spleen, heart, liver, kidney and brain weight were recorded for the analysis.

1.2.3 Sample analysis

Muscles sample were analyzed for fat (Folch et al., 1957), proteins (CP) (Kjeldahl), moisture and ash (method AOAC 920.153. 1999). Composition of fatty acids from 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 5.2. The chromatographic conditions and fatty acid identification were carried out as reported in chapter 5 paragraph 5.2.2.

The amount of each fatty acid is reported as mg AG/100gTQ. 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.

For fiber determination, a section of frozen muscle samples at -80°C were equilibrated to −4°C and then a section was cut from the stick and trimmed to facilitate the transverse sectioning. It was mounted on a cryostat chuck with a few drops of tissue-freezing medium (TissueTek; Sakura Finetek Europe, Zoeterwoude, The Netherlands), and 10-µm-thick sections were cut using a Cryotome (Shandon Inc., Pittsburgh, PA). The sample was mounted on glass microscopic slides and allowed to dry for one night. Sections were stained for the determination of myofibrillar ATPase (mATPase) activity after alkaline (pH 10.3) or acid (pH 4.5) preincubation according to the multiple staining procedure (Solomon and Dunn, 1988) followed by incubation. Stained sections was observed at 125× with a BX50 microscope in transmitted light mode (Olympus Optical Co., Hamburg, Germany) equipped with a high-resolution digital camera (ColorView12, Soft Imaging System GmbH, Munster, Germany). In ST and LD muscle was categorize two type ,type I (figure 1) myofiber stain light and Type II (figure 2) myofiber dark, using the basic preincubation condition, whereas the opposite occurs after acid preincubation (tab. 4). Fiber type distribution was calculated as the percentage of fiber type to the total of all fibers (fiber number percentage; FNP).

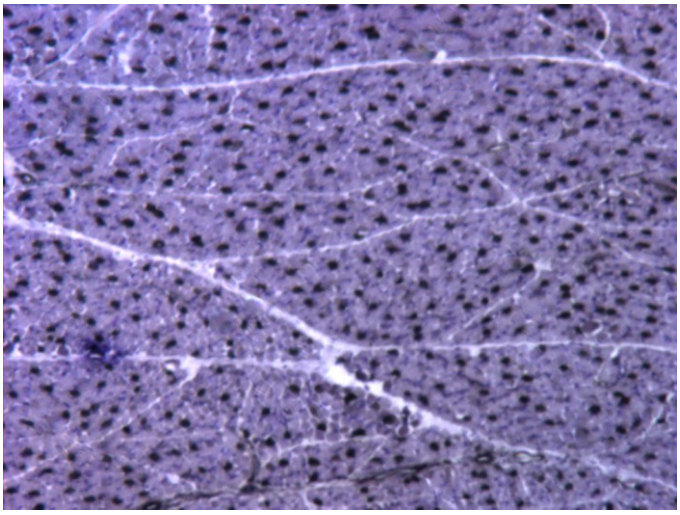

Figure 1.Cross-section of the newborn lambs muscle stained after preincubation at pH 4.5 (obj. 2) (the type I fiber are darkly stained)

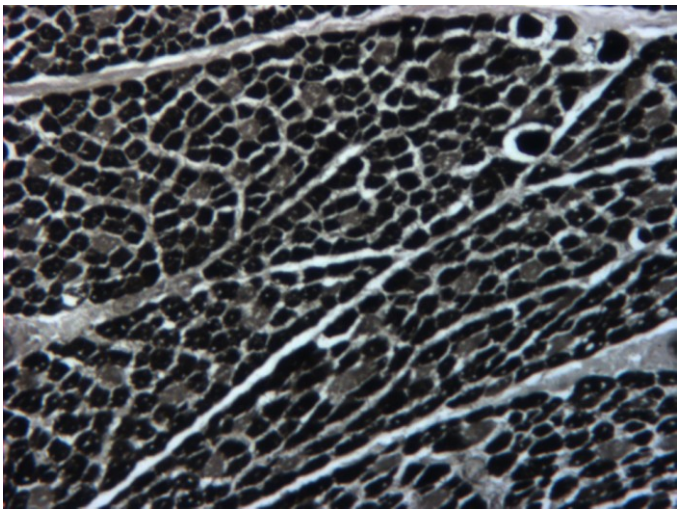

Figure 2. Cross-section of the newborn lambs muscle stained after preincubation at pH 10.3 (obj. 10) (the type II fiber are darkly stained)

1.3. Statistical analyses

The effect of maternal diet on newborn lambs traits, morphometric measures, muscles fiber number and fatty acid profile were analyzed with the following linear model:

$$\mathbf{Y_{ijk} = \mu + T_i + S_j + (T \times S)_{ij} + (T \times P)_{ik} + \varepsilon_{ijk}}$$

Where Y_{ijk} = observation; μ= overall mean; RS_i = fixed effect of treatment (i = ….); S_j = fixed effect of muscle j; and ε_{ijk} = residual error. Statistical analysis was performed using Minitab 16 software package (MINITAB®, State College, PA, USA) and significant differences were declared for $P \leq 0.05$ and a tendency for $P \leq 0.10$.

1.4. Results and discussion

1.4.1 Effects of maternal supplementation on birth weight and muscle weight

The effects of maternal treatment on birth weight and muscle weight at birth are reported in table 4. Birth weight tended to be higher in CON than LIN group. The weight of muscles in LIN lambs did not differ from the control lambs. These results evidenced no effects of maternal omega3 supplementation on fetal growth. No comparative data are available on newborns lambs but studies on laboratory animals showed that maternal supplementation with linseed in gestating rats caused a lower body weight of offspring at birth and after 7 weeks of age compared to control (Fernandes et al., 2011a).

Table 4. Effects of maternal diets on birth and muscle weights in newborn lambs

	Groups		P-value
	CON	LIN	
Birth body weight, Kg	2.79	2.43	†
LD	0.019	0.020	ns
ST	0.005	0.005	ns
SM	0.013	0.018	ns

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

The percentages of the organs on birth weight are reported in Table 5. Liver weight was significantly higher in LIN group compared to CON group ($p< 0.05$). The relative weight of kidney tended to be higher in LIN than CON group ($p<0.10$) in accordance with results of Greenwood et al., (2004) where the weight of kidney was highest in lambs with low birth weight. The perirenal fat and the heart relative weight tended to be higher in LIN group than CON group ($p<0.10$). No differences in brain and head relative weights were observed between the two groups. The weight of digestive system was ($p<0.05$) higher in LIN group, compared with CON group. In many species, including humans, the

gastrointestinal tract undergoes toward a noticeable marked structural and functional maturation in the periods immediately before and after birth (Grand et al., 1976, Jensen et al., 2001, Montgomery et al., 1999, Schwarz and Heird, 1994, Shulman et al., 1998, Widdowson et al., 1976).

Table 5 Effects of maternal diets on relative organ weight (as % on BW at birth)

	Groups (G)		p. value
As % of birth weight	Contr.	lin	G
Offal	9.45	9.34	ns
Spleen	0.20	0.27	ns
Heart	0.88	1.08	†
Liver	1.55	2.42	*
Kidney	0.59	0.95	†
Perirenal fat	0.29	0.41	†
Head	9.45	9.60	ns
Brain	2.00	2.04	ns
weight skin + arts	18.40	20.54	†
weight of the digestive system	8.23	13.41	**

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

The results of the correlation analysis are reported in table 6. The correlation evidenced a close negative relationship among BW and the weight of offal and spleen. This contrasts with the observation of Alexander (1974) in Merino ewes where a linear correlation between BW at birth and spleen weight were reported. However, in accordance with this author (Alexander, 1974) ,the correlation was positive and significantly different from zero for BW with heart, head and brain. No relationship between BW and liver, kidney or perirenal fat was observed. This contrast with observation of Alexander (1974) in Merino ewes, where a linear correlation between BW at birth and liver and kidney weight was

reported. Nevertheless, the weight of the organ usually is reported to be disproportionate heavy in small lambs, especially when the mother is underfed (Alexander, 1974).

Table 6. Correlation matrix between body and organ weight in newborn lambs

	BW	Offal	Spleen	Heart	Liver	Kidney	Head	Perirenal fat
Offal	-0.689 0.006							
Spleen	-0.773 0.001	0.512 0.061						
Heart	0.531 0.051	-0.585 0.028	-0.338 0.237					
Liver	0.081 0.784	-0.548 0.043	0.394 0.163	0.468 0.092				
Kidney	0.055 0.852	-0.16 0.585	-0.042 0.887	0.793 0.001	0.312 0.278			
Head	0.893 0.000	-0.577 0.031	-0.464 0.094	0.587 0.027	0.286 0.321	0.162 0.58		
Perirenal fat	0.024 0.936	-0.083 0.777	0.005 0.988	0.785 0.001	0.235 0.419	0.912 0.000	0.201 0.492	
Brain	0.904 0.000	-0.511 0.062	-0.629 0.016	0.585 0.028	0.112 0.702	0.174 0.552	0.87 0.000	0.111 0.705

1.4.2 Effects of maternal nutrition on muscle fiber number

The muscle fiber number for LIN and CON lambs is reported in Table 7. The omega 3 supplementation did not influence this parameter. Muscle fibers number in mammals is almost completed during gestation and fixed at birth and usually, postnatal nutrition did not affect muscle fiber number; in fact maternal nutrition during gestation can modify myoblast proliferation and the onset of fibers differentiation (Brameld et al., 2000). In this experiment the maternal nutrition during gestation did not affected the muscle fibers distribution likely due to the stage of gestation esamined, or to the type of muscles or to a

general lack of effects of omega 3 fatty acids on fiber differentiation. Greenwood et al., (2000) reported that not all muscles are equally affected by variation in prenatal nutrition, presumably because they develop at different times of gestation.The interaction treatment x muscle tended to a statistical significance (P <0.10) (figure 3); in the CON group there was the percentage of T1 and T2 fibers tended to differ, whereas in the LIN group the T1 and T2 percentage on total myofiber did not differ.

Table 7.Effects of feeding system in muscle fiber number

	Groups (G)		Muscle (M)		p-values		
%	contr	lin	ld	st	G	M	G*M
Primary myofibers	9.91	10.10	9.25	11.05	ns	ns	†
Secondary myofibers	90.09	89.90	90.75	88.95	ns	ns	†
Secondary/primary ratio	10.23	10.02	10.75	9.26	ns	ns	†

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

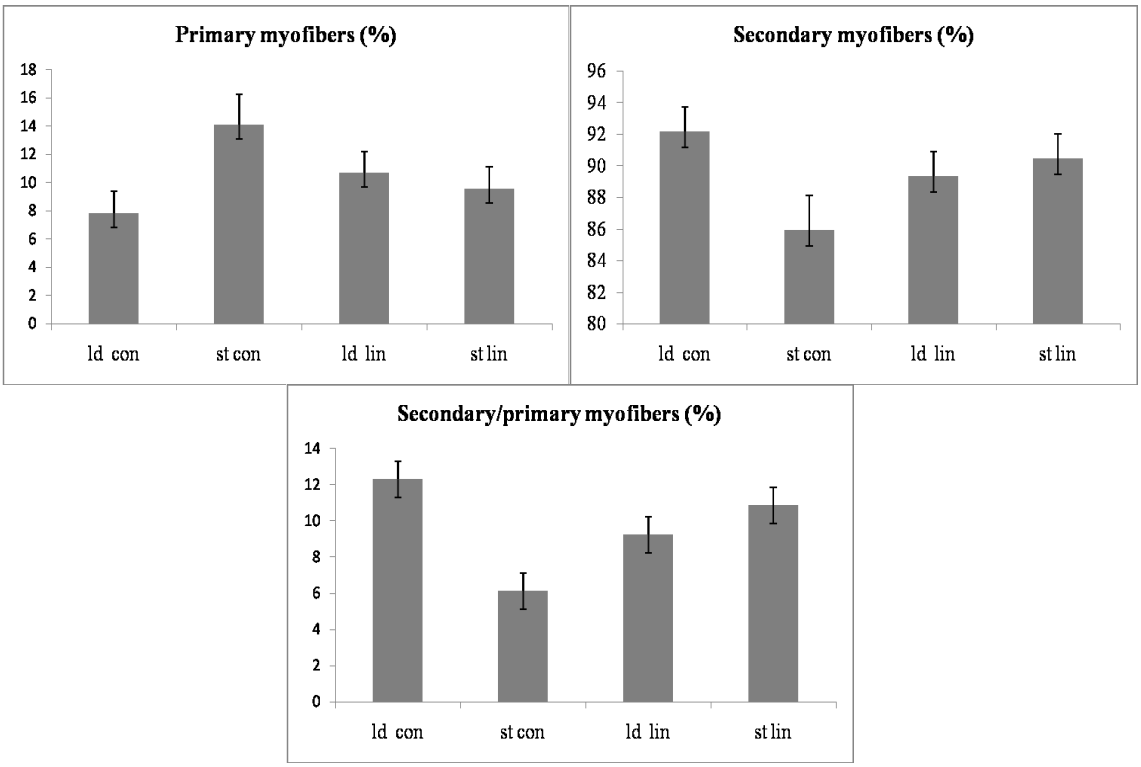

Figure 3. *Effects of feeding system in the interaction treatment x muscle in muscle fiber number*

1.4.3 Effects of maternal diet on chemical composition of muscles

The effects of maternal feeding treatment on chemical composition of the thigh and LD muscles of newborn lambs are showed in table 8. The chemical composition of newborn lambs was not influenced by the dietary treatment of the ewes. The chemical composition did not differ between the two muscles except for the content of ash that was significantly higher in thigh than longissimus dorsi muscle. The percentage of fat is numerically highest in animal from lin groups, and in longissimus dorsi muscle. It was observed that supplementation of PUFA in ruminant animals change the composition of fatty acids in adipose tissues (Bolte et al., 2002) and increases the concentration of lipids in the muscle (Ashmore and Doerr, 1971a). Protein content, was not affected by treatment. Its concentration was lower than value of suckling lambs of the same breed or others breeds

(Chiofalo et al., 2010, Osorio et al., 2007, Rassu et al., 2010) due to the higher moisture content in the fetus muscles compared to lambs after one month of age.

Table 8. Effects of feeding system on chemical composition of thigh and longissimus dorsi muscle from newborn lambs.

	Feeding system (Fs)		Muscle		Fs	Muscle	Fs* M
selected nutrient							
%	contr	lin	Thigh	L.dorsi	p-value	p-value	p-value
fat	1.19	1.27	1.15	1.35	ns	ns	ns
proteins	16.90	16.74	16.79	16.82	ns	ns	ns
moisture	79.01	79.26	79.39	78.83	ns	ns	ns
ashes	1.15	1.14	1.18	1.09	ns	**	ns

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

1.4.4 Effects of maternal diet on fatty acids profile of muscles and brain

The effects of maternal treatment on fatty acid profile of the thigh and L.D muscles of newborn are reported in Table 9. It has been observed that SFA (P<0.10) and MUFA (P<0.05) were higher in L.D than tight muscles. The maternal diet influences significantly almost all groups of FA being the content of TFA, total PUFA, PUFA n-3 and PUFAn-6 higher in muscles of LIN lambs than CON lambs. The odd-branched chain FA was numerically higher (p<0.10) in LIN group, generally fatty acids from the lipids of carcass and internal organs of the lambs contained 0.2% of branched-chain acids, compared with 3% of branched chain FA in the depot fat of adult sheep (Downing, 1964).The fatty acid profile of newborn muscles from lambs of the ewes fed CON or LIN diet is presented in Table 10. The lambs from ewes that received linseed showed a higher concentration of vaccenic acid (C18:1 t11; VA), alpha-linolenic acid (ALA C18.3 n3), CLA c9t11, steridonic acid (C18:4 n3; STA) and CLA t10 c12 and a lower value of C18:1 c11 (P<0.05). The higher content of VA, CLA and C18:3 in muscles of newborn lambs were expected because an effect of maternal linseed supplementation on muscles fatty acid profile of the weaned lambs (Berthelot et al., 2012, Vieira et al., 2012).However the concentration of those fatty acids was reported to be very low compared the concentration of the mother tissue (Nudda et al., 2007).

Among the long chain fatty acids of the omega9 family the LIN lambs evidenced a higher content of eicosatrienoic acid (C20:3 n9; ETA) ($p<0.05$) and erucic acid (C22:1 n-9; ERA) ($p<0.001$). Among the long chain fatty acid of the omega6 family, C20:3 n-6 and C20:4 n6 (ARA) no differences was observed between the maternal diets. In the omega3 family only the EPA and DPA ($p<0.05$) were increased in lambs from LIN ewes. The DHA was not influenced by the ALA supplementation to the mothers in accordance with other work with lambs (Bas et al., 2007), beef (Raes et al., 2003a, Raes et al., 2003b) and pig (Ahn et al., 1996, Riley, 2000, Romans et al., 1995a, Romans et al., 1995b). These results, evidenced that the omega 3 supplementation to the mother during gestation is ineffective in increasing the concentration of DHA in muscles tissues.

Table 9. Partial sum of fatty acid (mg/100g FAME) of thigh (semitendinosus, semimembranosus and femoral biceps) and L. dorsi muscles of newborn lambs from ewes supplemented (LIN) or not (CON) with omega 3 in the last 8 weeks of gestation.

	Feeding system (Fs)		Muscle		Feeding system	MUSCLE	Fs X M
	controllo	lino	Thigh	L.dorsi	p-value	p-value	p-value
SFA	151.04	175.50	144.67	181.86	ns	†	ns
MUFA	290.63	282.71	251.76	321.58	ns	*	ns
TFA	10.97	29.38	18.83	21.52	**	ns	ns
PUFA	100.13	128.51	114.35	114.29	*	ns	ns
PUFA-n3[a]	16.91	29.07	23.47	22.51	**	ns	ns
PUFAn-6[b]	52.32	66.24	60.37	58.19	*	ns	ns
HP-PUFA	81.31	89.70	85.46	85.55	ns	ns	ns
OBCFA	8.59	10.46	9.24	9.82	†	ns	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].

Table10. Fatty acid profile (g/100g FAME) of thigh (semitendinosus, semimembranosus and femoral biceps) and L. dorsi muscles of newborn lambs from ewes supplemented (LIN) or not (CON) with omega 3 in the last 8 weeks of gestation.

	feeding system (fd)		muscle (m)		p-value	p-value	p-value
Mg/AG100g TQ	CONTR	LIN	THIGH	L.DORSI	DIET	MUSCLE	D X M
C16:0	82.88	90.31	79.78	94.41	ns	ns	ns
C16:1 t6-t7	0.22	0.35	0.18	0.40	ns	*	ns
C17:0	2.52	2.99	2.67	2.84	ns	ns	ns
C18:0	67.50	68.83	60.94	75.39	ns	ns	ns
C18:1 t11 (VA)	3.12	7.99	5.46	5.65	†	ns	ns
C18:1 c9	244.83	216.70	204.58	256.95	ns	ns	ns
C18:1 c11	26.59	22.67	23.70	25.56	*	ns	ns
C18:2 n6 (LA)	12.12	26.03	21.07	17.05	ns	ns	ns
C18:3 n3 (ALA)	1.17	5.74	3.54	3.37	*	ns	ns
CLA c9 t11	0.80	5.86	3.26	3.55	*	ns	ns
C18:4 n3	0.05	0.22	0.15	0.12	*	ns	ns
CLA t10 c12	0.01	0.31	0.18	0.14	*	ns	ns
C20:3 n-9	28.00	23.19	24.61	26.58	*	ns	ns
C20:3 n-6	1.86	2.12	2.10	1.88	ns	ns	ns
C20:4 n6 (ARA)	27.90	24.01	26.86	25.05	ns	ns	ns
C20:3 n-3	0.28	0.41	0.36	0.33	†	ns	ns
C20:4 n-3	0.27	0.33	0.30	0.30	ns	ns	ns
C22:1 n-11	0.54	0.58	0.59	0.54	ns	ns	ns
C22:1 n-9	0.02	0.15	0.09	0.9	***	ns	ns
C20:5 n-3 (EPA)	1.77	4.00	3.03	2.74	*	ns	ns
C22:5 n-3 (DPA)	9.41	12.62	11.28	10.75	*	ns	ns
C22:6 n-3(DHA)	4.97	5.72	5.82	4.87	ns	†	ns

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

1.4.5 Effects of maternal supplementation on fatty acids in brain

The fatty acids group and the individual fatty acids of brain fat in newborn lambs are reported in tables 11 and 12.

The sums of odd branched chain fatty acid (OBCFA), was higher in linseed group ($p<0.10$) than CON. The concentration of branched-chain acids in the human nervous system included the brain was at least 0.3-0.9%, while there were only trace in the other tissues as liver, kidney, muscle, and skin(Kishimoto et al., 1973). This is confirmed in lamb tissues were the concentration of OBCFA was higher in brain (Table 11) than muscles (Table 9). However the high concentration ($p<0.10$) of OBCFA in muscles and brain of the LIN group lambs suggest a higher blood concentration of those fatty acids in the LIN mothers or a higher delivery from the placenta to the fetus. The other fatty acid groups did not differ in brain of the 2 lambs groups.

The pattern of the individual fatty acid in both groups (Table 6) is very interesting. The most abundant FAs in brain were C16:0, C18:0, C18:1 n9, ARA and DHA. The brain of lambs from the mothers of the CON group did not contained C18:3 n3. The maternal C18:3 supplementation increase the content of C18:1 n9 ($p<0.10$) C22:1 n11, C22:1 n9, C23:0. The C18:3 supplementation increase also the concentration of nervonic acid (C24:1 c15; NA) in the brain fat. Among the long chain fatty acid of omega 3, the linseed increased the content of EPA and DPA but not DHA. The high proportions of DHA in brain are a characteristic feature of the mammalian brain, even among herbivores, and regardless of low concentrations of DHA in plasma and hepatic lipids (Innis, 2007). Plasma lipid levels of DHA, on the other hand, are low in most terrestrial animals, including humans, suggesting the brain has particular mechanisms to concentrate DHA. Before birth, DHA is transported across the placenta via pathways involving fatty acid binding, and transport proteins are then released to the fetal circulation. Observational and intervention studies concur that higher dietary intakes of DHA during pregnancy result in maternal to fetal transfer of DHA (Capper et al., 2006). The supplementation of C18:3 did not affect the brain DHA concentration. However the fish oil supplementation to ewes increased the EPA and DHA concentration in brain of their lambs compared to the control that received megalac (Capper et al., 2006).

Table 11. Effects of feeding system in partials sums and nutritional ratios of brain

Mg/AG100g TQ	Feeding system		
Partial sums	CONTR	LIN	p-value
SFA	723.19	835.74	ns
MUFA	440.61	518.24	ns
PUFA	429.35	515.32	ns
PUFA3	207.09	263.9	ns
PUFA6	173.54	192.13	ns
HP-PUFA	386.49	462.42	ns
OBCFA	58.8	68.93	†

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

Table 12.effects of feeding system in brain

	Feeding system		
Mg/AG100g TQ	**CONTROLLO**	**LINO**	**P VALUE**
C16:0	377.033	426.407	NS
C16:1 t6-t7	0.89	1.94	*
C16:1 t8	1.55	0.16	*
C16:1 c9	10.79	11.59	NS
C17:0	4.05	4.92	†
C16:4 n1	22.75	26.64	NS
C18:0	262.63	305.38	NS
C18:1 t6-8	0.23	0.27	NS
C18:1 t10	0.45	0.45	NS
C18:1 t11 (VA)	6.64	8.4	NS
C18:1 c9	296.7	353.3	†
C18:1 c11	73.98	84.7	NS
C18:1 c13	1.39	1.81	†
C18:2 n6	1.63	3.77	NS
C18:3 n4	1.15	1.63	†
C18:3 n3	0.00	0.81	***
CLA c9 t11	0.25	1.42	*
CLAt9+C20	7.27	9.22	†
C20:3 n9	13.25	17.76	NS
C20:3 n6	3.74	4.71	†
C20:4 n6	98.76	109.33	NS
C20:3 n3	0.36	0.33	NS
C22:1 n11	0.67	0.95	*
C22:1 n9	0.37	0.59	*
EPA	7.59	10.28	**
C23:0	0.03	0.9	***
C22:4 n6	63.06	65.06	NS
C24:0	14.41	14.57	NS
C24:1 c15	0.75	1.27	**
DPA	18.42	28.01	**
DHA	189.41	224.45	NS
TFA	13.20	16.36	†

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

1.6 CONCLUSION

In this study the linseed supplementation to pregnant ewes for 8 weeks before parturition reduced the lambs live BW and increased the weight of their livers compared to CON group. The FA profile of muscle tissues was markedly influenced by the maternal fat supplementation as evidenced by an increase in the concentration of FA of the omega 3 and omega 9 family. However among the LCFA n3 only EPA and DPA were increased in lambs from LIN group whereas DHA did not change. Interestingly, the FA composition of the brain evidenced the presence of C18:3 only in supplemented group. The linseed supplementation increased the concentration of EPA, DPA and nervonic acid in brain but any effect was observed on DHA concentration.

In the next experiments will be treated the effect of maternal nutrition during gestation and lactation on the body weight, carcass characteristics and fiber differentiation of lambs at weaning. However, the prenatal nutrition which reduced the birth body weight of about 33% also has been considered to retard growth in lambs (Alexander 1974). In this trial the BW of newborn lambs was reduced by 11% from LIN supplementation of ewes and therefore a positive effect of omega 3 growing of lambs is not expected. Anyway, the effect of maternal linseed supplementation on the growth performance of suckling lambs from birth to weaning will be presented in the experiment 2.

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Experiment 2

Effects of maternal nutrition on lambs trait, carcass characteristics and morphometric muscle measurements

Abstract

Skeletal muscle is a heterogeneous tissue because it is formed by different fibers type. Primary myofibers are considered to be resistant to environmental factors, whereas the secondary myofibers are considered susceptible to several factors, including nutrition treatments. This experiment aims to investigate if maternal omega3 supplementation by using linseed during gestation, lactation or both, can affect the muscles morphometric measurements and the fiber population in the suckling lambs since weaning. We hypotized that maternal omega3 supplementation results in a positive effect in the carcass parameters and in the number of muscle fibers in the resulting lambs. Forty-eight selected sheep were divided in two groups fed different diets from approximately the last 8 weeks of gestation, to the first 4 weeks of lactation. After parturition half of the ewes were maintained in their initial treatment received during gestation whereas the other half ewes were exchanged from their treatment. At birth the lambs were kept with their mothers and they were fed exclusively with maternal milk. All lambs were slaughtered at 28 days of age. Three muscles were rapidly removed from the right side of each carcass: muscle semitendinosus (ST) and semimembranosus (SM), and the longissimus dorsi (LD). The gastrointestinal tract, offal, spleen, heart, liver, kidney and brain weight were recorded. The maternal omega 3 supplementation during gestation showed a depressing effect on lamb weight and daily gain. On the other hand, the continuous linseed supplementation during lactation restores the growth performances of lambs. No defined effect of linseed supplementation on the organ weight of lambs has been evidenced. Linseed supplementation to ewes did not show a defined effect on muscle fiber population of muscles.

2.1. Introduction

Skeletal muscle is a heterogeneous tissue because it is formed by different fibers type. Primary fibers develop first and this is followed by the development of the secondary fibers on the surface of primary ones (Suzuki and Cassens, 1980). Primary myofibers are considered to be resistant to environmental factors, whereas the secondary myofibers are considered susceptible to several factors, including nutrition treatments. Some works suggest that the fibers type proportions are associated with different meat and eating quality traits (Wood et al., 2004a). Maternal nutrient intake during pregnancy can influence the muscle fiber number in the offspring (Wu et al., 2006). In sheep muscle differentiation and fibers formation occur at 80 days of gestation (Fahey et al., 2005) and they are influenced by genetic factors and by nutrition. In particular, the primary fiber development is mainly related to genetic factors; the secondary muscle fiber development is more influenced by maternal and environmental stimuli. A low maternal nutrition can reduce muscle fiber differentiation (Brameld et al., 2000), whereas higher maternal nutrition results in a higher number of muscle fibers in specific muscles. Applying maternal nutrition restriction between 30-70 days of gestation observed an increased proportion of primary myofiber and a decrease proportion of secondary myofibers in 2 weeks old lambs. The dietary restriction of ewes during gestation between 55-95 days and between 85-115 days of gestation did not influence the muscle fiber proportion of 2 weeks old lambs (Fahey et al., 2005). The effect of maternal undernutrition during gestation on muscle differentiation of lambs has been largely investigated not only in sheep (Daniel et al., 2007, Nordby et al., 1987) but also in pig (Handel and Stickland, 1987) and chicken (Deaton, 1995, Li et al., 2007). Some researches have been focused on how maternal lipid nutrition can influence the growth and survival of offspring. In pigs the omega-3 PUFA supplementation to the sow diet reduced pre-weaning mortality (Rooke et al., 2001) and increased piglet serum IgG concentrations at weaning (Rooke et al., 2003). However there is no a research available on the role of maternal omega3 supplementation during gestation and lactation on the muscle differentiation of the lambs.

2.1.1Objective

The study aims to investigate if maternal lipid supplementation with omega3 during gestation, lactation or both (gestation and lactation) can affect the muscles morphometric measurements and the fiber population in the suckling lambs at weaning. We hypnotized that maternal omega3 supplementation results in a positive effect in the carcass parameters and in the number of muscle fibers in the resulting lambs. The effects of sex were also investigated to find possible differences in carcass and muscles morphometric measurements between suckling male versus female lambs.

2.2. MATERIAL AND METHODS

2.2.1 Animals and treatments

This experiment was done in a dairy farm located in Osilo (Sardinia). Forty-eight selected sheep were divided in two groups fed with different diets from approximately the last 8 weeks of gestation, to the first 4 weeks of lactation.

During gestation sheep were divided in two groups, one of them received the control concentrate (CON group) and the second group received the experimental concentrate containing linseed as source of C18:3 n3 (LIN group). The ingredients of the concentrate formulated for the present experiment are reported in table 1 and the chemical composition of the concentrate is reported in table 2. The ewes received hay ad libitum and 1 kg of the two complete pelleted diet (CPD) formulated specifically for the trial. The CPD was distributed into 3 doses /day for 8 weeks until parturition. In the last 4 weeks of gestation 150g/day of corn flour was added and divided in 3 doses/d. The CON and LIN diets were isoenergetic, isoproteic and isolipidic but differed in the fatty acid composition. The fatty acid composition of the concentrate is reported in table 3. After parturition the sheep were divided in 4 groups.

1) Group CON-CON were fed the control diet during gestation and lactating,

2) Group LIN-CON that received linseed only diet during gestation,and control diet during lactation

3) Group CON-LIN that receive linseed diet only during lactation,

4) Group LIN-LIN that receive linseed diet during both gestation and lactation.

Table 1 - Ingredients of the control and treatment concentrate.

CONTR	LIN
Wheat bran	Soybean hulls
Alfalfa meal	Extruded linseed
Distilled from wheat	Wheat bran
Wheat bran,	Flour of extraction of sunflower
Sunflower extraction meal	Alfalfa meal
Corn germ cake	Sugar beet pulp dried
Dried sugar beet pulp	Corn gluten feed
Calcium carbonate	Calcium carbonate
Soya Meal	Soya meal
Maize	Maize
Chloride Sodium	Sodium chloride
Sodium Bicarbonato	Sodium bicarbonate
Magnesium Oxide	Magnesium oxide

Table 2 - Analytic component of the experimental concentrates %

	CONTR DIET %	LIN DIET %
DM	89.40	89.41
Ashes	8.86	8.56
Crude protein	18.42	18.37
NDF	42.94	41.98
ADF	26.02	28.39
ADL	6.00	5.48
EE	7.34	6.99

The fatty acid composition of the concentrate is reported in table 3.

Table 3 – fatty acid composition of the concentrate

Fatty acid	CON	LIN
C12:0	1.033	0.147
C14:0	1.130	0.183
C16:0	31.500	10.346
C18:0	27.352	6.071
C18:1 c9	10.731	20.627
C18:2 n6	22.780	26.280
C18:3 n3	2.026	34.217

The predominant FA of the lipid fraction in the CON concentrate were palmitic acid (C16:0), stearic and Linoleic acid (C18:2 c9c12, LN). The most abundant FA in LIN concentrate was alfa-linolenic acid (C18:3 c9c12c15, ALA), which accounted for 34% of total FA followed by LN and oleic acid.

At birth the lambs were kept with their mothers and they were fed exclusively with maternal milk. All lambs were slaughtered at 28 days of age.

Feed samples of the ewes' diet were collected at the beginning and at the end of the trial for subsequent chemical analysis.

2.2.2 Tissue samples preparation

At slaughter three muscles were rapidly removed from the right side of each carcass: two hind limb muscle semitendinosus (ST) and semimembranosus (SM), and the torso muscle included longissimus dorsi (LD). The sampling was conducted using a scalpel ten minutes after death. Fresh weight of the 3 muscles was also recorded. Each muscle was rapidly photographed to determined geometric measure (figure 1) of length for LD and ST and girt for ST. Samples were then pinned in a rubber stick, slightly stretched and frozen in isopentane cooled with liquid nitrogen. After the samples were well frozen, they were immediately wrap in aluminum foil and put into liquid nitrogen. After this procedure the muscles sample were packaged under vacuum and stored at -80°C.

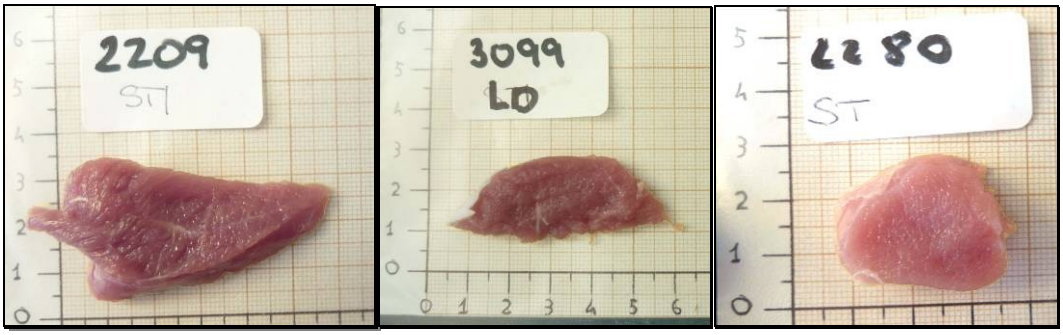

Figure 1. Example photo for geometric measure determination. Delimitation in yellow is used by the program to perform all other measurements (Area, Rect. Mean, perimeter, diameter and convex perimeter).

After this the carcass was skinned, refrigerated for 24 hours at 4°C and the gastrointestinal tract, offal, spleen, heart, liver, kidney and brain weight were recorded.

The pH was measured at 24 hours postmortem on each muscle portion using pH meter (Orion Research inc., model 250 A, Boston ,MA) equipped with thermometer and penetrating glass-electrode (Hamilton Company USA, model 238405,Reno,NV).

2.3. Sample measurement and analysis

2.3.1 Geometric measure determination in muscles

On the photo (figure 1) of each sample were measured the area, perimeter, and diameter, using the analySIS software (Soft Imaging System GmbH).

2.3.2. Histochemical analysis

For fiber determination, a section of frozen muscle samples at -80°C were equilibrated to −4°C and then a section were cut from the stick and were trimmed to facilitate the transverse sectioning. It were mounted on a cryostat chuck with a few drops of tissue-freezing medium (TissueTek; Sakura Finetek Europe, Zoeterwoude, The Netherlands), and 10-µm-thick sections were cut using a Cryotome (Shandon Inc., Pittsburgh, PA). The sample was mounted on glass microscopic slides and allowed to dry for one night. Sections were stained for the determination of myofibrillar ATPase (mATPase) activity after alkaline (pH 10.3) or acid (pH4.5) preincubation according to the multiple staining procedure (Solomon and Dunn, 1988) followed by incubation. Stained sections were observed at 125× with a BX50 microscope in transmitted light mode (Olympus Optical Co., Hamburg, Germany) equipped with a high-resolution digital camera (ColorView12, Soft Imaging System GmbH, Munster, Germany). In ST and LD muscle two types of fiber were categorize, primary myofiber stain light and secondary myofiber dark, using the basic preincubation condition for the first one, whereas the opposite occurs after acid preincubation. Fiber type distribution was calculated as the percentage of fiber type to the total of all fibers (fiber number percentage; FNP).

2.4. Statistical analysis

Lambs traits, carcass morphometric measures and muscles fiber number data were analyzed separately for each muscle with a linear model that included the fixed effects of dietary treatment of ewes, sex and prolificacy and the first order of interaction by using Minitab software (Minitab inc., State College, PA). Statistical analysis was performed using Minitab 16 software package (MINITAB®, State College, PA, USA) and significant differences were declared for $P \leq 0.05$ and a tendency for $P \leq 0.10$. Some animals did not reach the optimal body weight for slaughter owing to an extremely low growth and therefore were excluded from the trial.

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2.5. RESULTS AND DISCUSSION

2.5.1. Effect of diet

2.5.2 The lambs traits and carcass measures

The lambs traits and carcass measures in the experimental group are summarized in table 4.

Table 4 – Effects of maternal diets on growth performances and carcass measurements

	GROUPS				
	contr-contr	lin-contr	contr-lin	lino-lino	p-value
Birth body weight, kg	3.65	3.46	3.59	3.59	†
Pre-slaughtered-weight, kg	7.37^{a}	5.65^{b}	7.15^{a}	7.51^{a}	***
Daily gain, g	132.77^{a}	78.32^{b}	138.13^{a}	140.06^{a}	***
Cold carcass weight, kg	4.69^{a}	3.51^{b}	4.52^{a}	4.78^{a}	***
Dressing, %	63.62	62.29	62.79	63.56	ns
pH	5.43^{b}	5.57^{ab}	5.62^{a}	5.52^{ab}	†

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

At birth, the average body weight (BW) of LIN group did not differ from CONT group (P>0.05) suggesting no substantial effect of the omega3 supplementation on fetal growth. In our trial, BW at birth was numerically lower (-9.5%) in lambs from LIN mother compared to CON ones. Some observations on humans and rats (Haggarty et al., 2002) advocate that it is possible to affect the growth of the fetus by the composition of fatty acids delivered by the placenta. For example, maternal supplementation of flaxseed in rats during gestation showed lower body weight at birth and after 7 weeks of age in offspring compared to control (Fernandes et al., (2011b)., Daleprane et al., (2010)) supplementing weaned rats with 25% of flaxseed up to 180 days observed a lower BW in animals that received linseed, although the growth rate did not differ from the control group.

In lambs, no differences in the daily gain were observed between LIN/LIN and CON/CON groups. Instead, the lambs from mothers feeding linseed only during gestation (LIN/CON) showed a lower average daily gain compared to the others groups causing significant lower weight at slaughter (P<0,001). Although in animal models the weight loss could be positive as a strategy to reduce the risk of cardiovascular disease, in this case could be a

negative aspect because the reduction of weight at slaughter. The use of linseed determined also a higher pH value in the carcass of the CON/LIN lambs.

2.5.2. Morphometric measurements in lambs

The weight of organs of lambs from different experimental groups are summarized in Table 5. The average weights of almost all organs were affected by treatments. However a no defined effect of linseed supplementation was observed. In particular, the lambs from ewes that received linseed only during gestation(LIN/CON) showed the lowest weight of the offal, spleen, heart, liver ($p<0.001$), kidney ($p<0.05$), perirenal fat and head ($p<0.05$) compared to the others lambs. The continuous linseed supplementation (LIN/LIN) caused lower spleen and perirenal fat compared to the CONTR/CONTR group ($P<0.001$ and $P<0.01$).

Table.5 Effects of maternal diets on carcass measurements

	GROUPS				
	contr-contr	lin-contr	contr-lin	lin-lin	p-value
Offal g	679.11^{a}	445.77^{b}	637.37^{a}	643.19^{a}	***
Spleen g	41.70^{a}	17.79^{c}	40.58^{a}	31.28^{b}	***
Heart g	69.72^{a}	51.27^{b}	62.63^{ab}	68.87^{a}	***
Liver g	184.45^{a}	118.55^{b}	195.41^{a}	168.86^{a}	***
Kidney g	54.02^{a}	42.52^{b}	50.21^{ab}	53.28^{a}	*
Perirenal fat g	84.10^{a}	38.02^{b}	54.61^{b}	60.57^{ab}	**
Head g	606.01^{a}	520.35^{b}	592.78^{ab}	630.59^{a}	*
Brain g	82.98^{a}	82.03^{ab}	76.51^{b}	83.38^{a}	**

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

However, the comparison of organ weights between groups could be misleading because differences in body weights between lambs in different groups. Therefore, better information can be obtained by the ratio of the organ weight to body weight (to account for differences in body weight) and the ratio of the organ weight to the brain weight.

The relative organs weight on the body weight measured before the slaughter is reported in table 6

Table 6. Effects of maternal diets on relative organ weights (g/100 g of BW)

	GROUPS				
	contr-contr	lin-contr	contr-lin	lin-lin	P-value
Offal /BW	9.12[a]	7.97[b]	8.97[a]	8.50[ab]	*
Spleen/BW	0.55[a]	0.34[b]	0.57[a]	0.42[b]	***
Heart/BW	0.94	0.91	0.87	0.92	ns
Liver/BW	2.47[ab]	2.14[b]	2.77[a]	2.25[b]	*
Kidney/BW	0.73	0.74	0.70	0.70	ns
Perirenal Fat/BW	1.09[a]	0.65[b]	0.76[b]	0.78[ab]	*
Head/BW	8.34[b]	9.14[a]	8.35[b]	8.44[b]	*
Brain/BW	1.15[b]	1.42[a]	1.09[b]	1.12[b]	*

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

The CONT/CONT treatment resulted in a significantly higher (P<0, 05) perirenal fat deposition compared to the lambs from ewes that received linseed during gestation, during lactation or continuously for all the experimental period. The perirenal and visceral fat in human and laboratory animals are found to be associated to disease (Lamacchia et al., 2011, Okhunov et al., 2012) and insulin resistance (Gastaldelli et al., 2007, Indulekha et al., 2011). In addition, a higher liver weight (p<0.05) has been observed in lambs from ewes that received CON diet during gestation. No fat content has been determined in the liver, but also liver fat accumulation has been associated with insulin resistance (Girard and Lafontan, 2008).

A growing number of studies have shown that incorporating n-3 PUFA into diet of laboratory animals reduces body fat accumulation (Ruzickova et al., 2004) and led to a fat distribution away from the visceral compartment (Rokling-Andersen et al., 2009). These hypothesis is supported by recent data on laboratory animals where linseed supplementation showed reduction of body weight and fat accumulation, the lipid profile improvement, and blood pressure control (Park and Velasquez, 2012) The results of our

trial also suggest the positive effects of omega3 from linseed supplementation to contrast the body fat accumulation in lambs.

The spleen showed the lower relative weight in groups that received linseed during gestation or continuously from gestation to lactation compared to the others groups ($p<0.001$).

The liver showed the lower relative weight in animals that received LIN during gestation and the highest relative weight in animals that received LIN during lactation($p<0.05$).

The LIN supplementation during gestation (LIN/CON) showed higher relative brain weight compared to CON/CON and LIN/LIN groups ($p<0.05$).

2.5.3. Muscle measurements

The morphometric muscle measurements are reported in tables 7 and 8. No significant effects were observed on the weight and the length of the muscle semitendinosus (ST) of lambs, of linseed supplemented the ewes, suggesting no effect of LIN on muscle development. The LD muscle from the LIN-CON animals showed the lower weight compared to the others group. Usually, the maternal diet during gestation and lactation influences fetal and postnatal growth and development (Dwyer et al., 1994, Wu et al., 2004). In the present experiment the two diets were isoenergetic, isoproteic and isolipidic in order to verify the effect of the type of fat. However, the maternal intake of n3 fatty acids provided during pregnancy and/or lactation did not have a significant impact on the muscle size of lambs. Likewise, no effects of the treatments were observed on geometric measures. To our knowledge, little information is available about the effect of omega3 supplementation on muscle growth of animal. In pig the effect of omega3 supplementation on muscle mass are not univocal, probably because of the different source of oil used and the specific growth and development period of animals. For example,(Nuernberg et al., 2005) reported no effect of linseed supplementation on LD muscle area. On the other hand, dietary linseed promotes muscle mass of growing–finishing barrows (Huang et al., 2008).

Table 7- Effect of maternal diet on size of muscles LD and ST

	GROUPS				
	contr-contr	lin-contr	contr-lin	lin-lin	P-value
Lenght					
LD	36. 35	31.87	33.99	34.12	ns
ST	9.68	9.25	9.50	9.62	ns
Weight					
LD	136.85^{a}	82.80^{b}	126.87^{ab}	124.68^{a}	*
ST	24.50	21.93	22.83	26.88	ns

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

Table 8 –Effect of maternal diet on geometric measures in muscles LD and ST

	GROUPS				
	contr-contr	**lin-contr**	**contr-lin**	**lin-lin**	**P-value**
LD					
Area	871.99	1045.96	994.60	1040.96	ns
Diameter	35.32	35.98	34.22	35.95	ns
Perimeter	143.01	166.57	147.83	157.66	ns
ST					
Area	655.31	912.54	1126.56	1399.20	ns
Diameter	26.96	32.22	34.92	37.26	ns
Perimeter	97.76	124.93	142.71	149.23	ns
SM					
Area	1197.84	1268.23	1304.14	1218.91	ns
Diameter	37.67	38.28	39.37	37.23	ns
Perimeter	169.94	167.68	171.80	175.94	ns

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

2.5.4. *Muscle Fiber Distribution*

The effects of LIN diet on myofiber characteristics of LD and ST muscles are summarized in Table 9 and in figure 1 . The results evidenced that maternal linseed supplementation did not modify the number of total, type1 and type 2 fibers compared to control group in the LD muscle. The lack of effects in type I fiber was expected because the primary myiofiber is hypotized to be genetically determined and their development depends on the conditions that occur in uterus (Dwyer et al., 1993). In ST muscle the number of primary fibers tended to be higher in the groups that received LIN continuously or only during lactation ($P<0.10$). This suggests that an effect of maternal nutrition can be exerted also on primary fibers.

In ST muscle of lambs from ewes that were supplemented with linseed during gestation (LIN/CON) showed the highest secondary/primary ratio compared to the lambs from ewes that received LIN only during lactation. The others two group were intermediate ($p<0.05$).

Some studies have suggested that in sheep, myogenesis is completed between 80 and 125 d of gestation (Ashmore et al., 1972, Fahey et al., 2005, Maier et al., 1992). However, to our knowledge, studies on the effect of maternal omega3 supplementation on fiber population in sheep muscle have not been published yet. We can suppose that if maternal nutrition treatments influence the fiber population, this must occur within this period of gestation. In our trial, the different nutrition treatment between groups has been applied between 90 and 150 days of gestation and therefore an effect of maternal nutrition have been expected on fiber population.

Overall, the linseed supplementation to ewes did not show a define effect on muscle fiber population of the lambs. Therefore, the effect of maternal omega3 supplementation on fiber population of offspring needs further investigation.

Table 9. Effect of maternal diet on myofiber composition of LD and ST muscles

	GROUPS				
	contr-contr	**lin-contr**	**contr-lin**	**lin-lin**	**P-value**
LD					
Primary myofibers	73.19	127.24	126.99	85.86	ns
Secondary myofibers	1000.16	1566.17	1401.29	1287.02	ns
% Primary myofibers	7.51	7.45	8.35	5.91	ns
% Secondary myofibers	92.49	92.55	91.65	94.09	ns
Secondary/primary ratio	12.88	13.28	11.59	16.07	ns
Tot myofibers	1073.35	1693.42	1528.28	1372.89	ns
ST					
Primary myofibers	78.77	84.16	156.83	106.31	†
Secondary myofibers	1159.36	1776.46	1579.27	1549.19	ns
Primary myofibers, %	6.38	5.02	10.58	8.35	†
Secondary myofibers, **%**	93.62	94.98	89.42	91.65	†
Secondary/primary ratio	14.32^{ab}	21.37^{a}	10.10^{b}	13.61^{ab}	*
Tot myofibers	1238.13	1860.62	1736.11	1655.50	ns

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

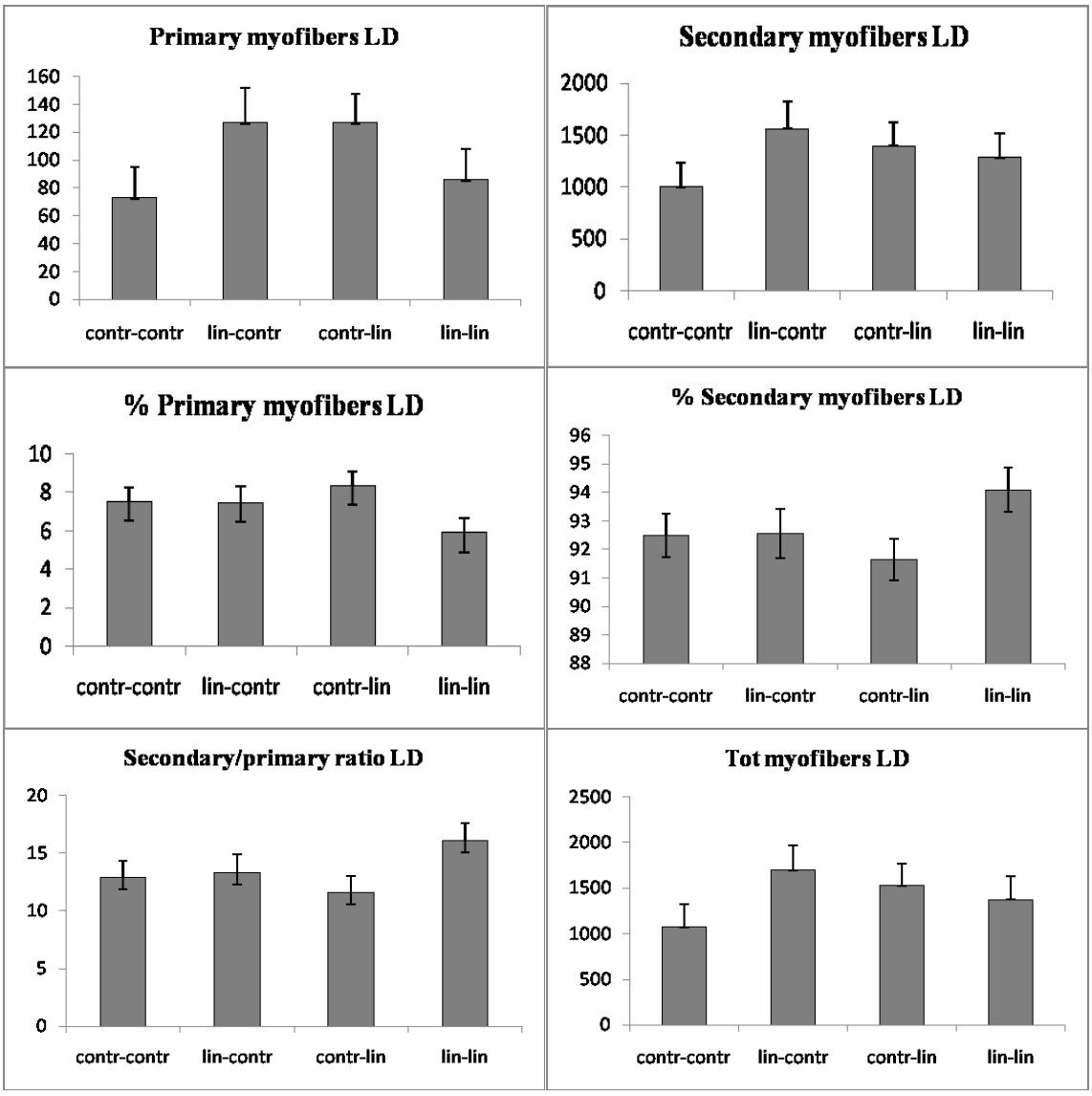

Figure 1 Effect of maternal diet on myofiber composition of LD and ST muscles

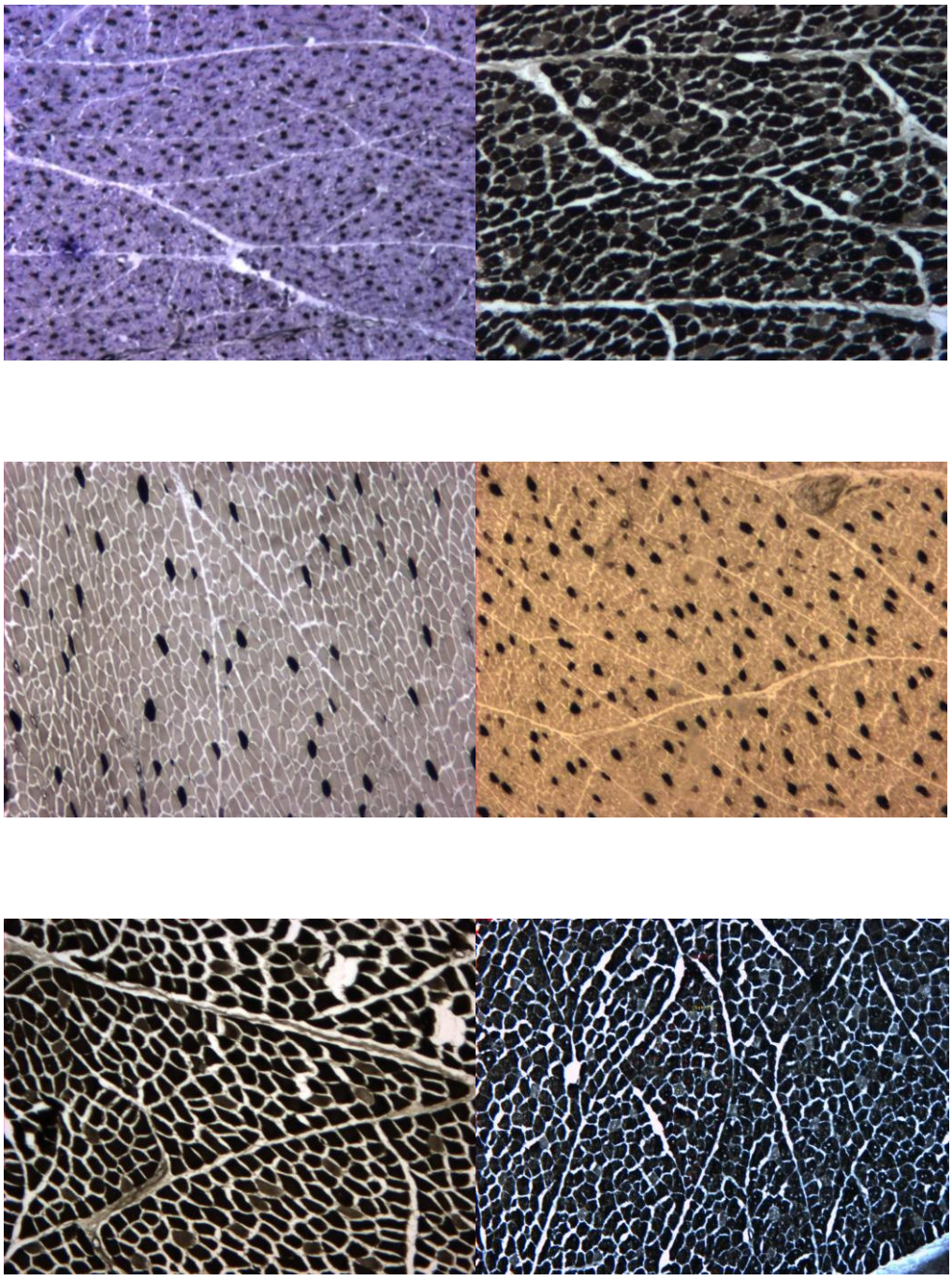

Figure 2- image for type differentiation fibers.

2.6. Effect of gender and prolificacy

The traits, carcass measurements and fiber characteristics for gender and prolificacy are reported in table 10

No differences in birth weight and average daily gain between male and female have been observed. The values are in line with those reported for lambs or other breed (Dwyer and Morgan, 2006, Idris A O, 2010, Sanna et al., 2001) and lower than observed in Merino breed (Miles J. De Blasio, 2006).

As expected, single lambs were heavier than twins at birth and at slaughter. Non effect of gender and prolificacy on dressing percentages has been observed.

Table 10. Effects of gender and prolificacy in body weight and carcass measurements

	GENDER (G)		PROLIFICACY (P)		P-VALUE	
	female	male	single	twins	G	P
birth weigh	3.51	3.71	3.9	3.13	ns	***
pre-slaughtered-weight	6.85	7.53	7.68	6.5	†	**
ADG* g	119.16	136.27	135.07	120.49	ns	ns
Cold carcass weight (CCV)	4.32	4.8	4.91	4.08	†	**
Dressing percentage	62.89	63.6	63.68	62.69	ns	ns
pH_	5.5	5.56	5.51	5.6	ns	†

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

ADG*= average daily gain

No differences between male and female have been observed for organs weight (table 11) except for head ($p<0.05$) and brain ($p<0.01$), that resulted significantly higher in males than females. The litter size influenced significantly all organs weight being higher in singles than twin lambs.

Table 11. Effects to gender and prolificacy on organ weight

	Gender (G)		Prolificacy (P)		P-value	
g	females	male	single	twins	G	P
Offal	608.71	661.94	690.75	547.75	ns	***
Spleen	35.47	35.52	37.81	30.88	ns	*
Heart	62.88	69.52	71.94	57.63	ns	***
Liver	168.88	187.58	194.81	153.25	ns	***
Kidney	51.59	52.81	56.06	45.00	ns	***
Perirenal Fat	67.88	62.65	74.06	45.38	ns	**
Head	574.53	626.94	629.88	565.38	*	**
Brain	79.00	84.55	84.56	78.63	**	**

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

The relative weight of kidney and perirenal fat respect the total BW (table 12) was higher in females than in males. A higher perirenal fat relative weight (74.06 g in single respect 45.38 g in twins) was observed also in singles than twins. In addition, twins showed a higher relative weight of head and brain ($p<0.01$) compared to single animals.

Table 12. Effects to gender and prolificacy on relative organ weight

	Gender (G)		Prolificacy (P)		p-value	
Organ /BW	females	male	single	twins	G	P
Offal	8.88	8.74	8.98	8.40	ns	*
Spleen	0.52	0.47	0.49	0.47	ns	ns
Heart	0.92	0.92	0.93	0.90	ns	ns
Liver	2.47	2.49	2.55	2.35	ns	ns
Kidney	0.75	0.70	0.73	0.70	†	ns
Perirenal Fat	0.97	0.79	0.95	0.68	†	**
Head	8.44	8.45	8.27	8.79	ns	*
Brain	1.17	1.16	1.13	1.24	ns	†

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

There were no differences on muscle fiber number between male and female lambs. This is in accordance with previous observation in sheep (Bünger et al., 2009) and chicken

(Scheuermann et al., 2003) where no differences between sexs were observed. However sex related differences in number of fiber have been reported in different species (Klosowska, 2003, Maltin et al., 2003, Maltin et al., 1997, Nougues et al., 1974, Stull and Albert, 1980).

Table n.13 Effects to gender and prolificacy on geometric measures in muscles LD and ST

	GENDER		PROLIFICACY		p-value	
	females	males	single	twins	G	P
Lenght						
LD	33.71	34.9169	35.53	32.88	ns	*
ST	9.45	9.60	9.94	9.11	ns	*
Weight						
LD	109.80	130.07	132.53	107.34	ns	†
ST	23.93	24.75	24.85	23.19	ns	ns

*** P≤0.001 **P≤0.01; *P≤0.05; †P≤0.10; ns = not significant

2.7. CONCLUSIONS

The maternal omega3 supplementation by using linseed during gestation showed a depressing effect on lamb weight and daily gain. On the other hand, the continuous linseed supplementation during lactation restores the growth performances of lambs.

No defined effect of linseed supplementation on the organ weight of lambs has been evidenced. However the lower perirenal fat proportion in lambs fed from LIN mother during gestation support an effects of maternal omega3 supplementation from linseed to contrast the body fat accumulation in lambs.

No effect of the maternal linseed supplementation on morphometric and geometric muscle measurements of suckling lambs have been observed.

Linseed supplementation to ewes did not show a define effect on muscle fiber population of lambs. Therefore, the effect of maternal omega3 supplementation on fiber population of offspring needs further investigation.

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Experimental 3

Effects of maternal nutrition on fatty acids composition of suckling lambs

Abstract

In the last year was increased the interest of the consumer toward the nutraceutical product. There is apprehension about animal lipid due to its relatively high concentrations of saturated fatty acids (SFA) and low concentration of polyunsaturated fatty acids (PUFA). Maternal nutrition can affect strongly the fatty acid composition of suckling animals. Aims of thois experiment is to evaluate the effect of alpha-linolenic acid (ALA) diet supplementation by extruded linseed to dairy ewes during pregnancy and early lactation on the fatty acid profile in longissimus dorsi and semitendinosus muscles and brain tissue of thirty-eight suckling lambs. The sheep were divided in two groups fed different diets from approximately the last 8 weeks of gestation, to the first 4 weeks of lactation. After parturition half of the ewes were maintained in their initial treatment received during gestation whereas the other half ewes were exchanged from their treatment. The lambs were weighed and then slaughtered at 28 days of age. The *Semitendinosus* and *longissimus dorsi* muscles were analyzed for fatty acid composition. The maternal linseed supplementation during gestation did not modify the lactation increased the content of VA, c9t11 CLA and ALA in milk. In lamb meat, the fatty acid profile of muscle reflect the fatty acid profile of suckled milk. The linseed supplementation during gestation did not increase the ALA and their long chain metabolites in muscles. The continuous linseed supplementation lactation increases the concentration of ALA and EPA, but did not increase the concentration of DHA. The elongase and desaturase indexes evidenced that the efficiency of conversion of ALA into their long metabolites in skeletal muscles of lambs is very low both during fetal life and during the first month of life.

3.1. Introduction

In the last year the interest of the consumer toward the nutraceutical product was increased. There is a certain apprehension about animal lipids due to their relatively high concentrations of saturated fatty acids (SFA) and low concentration of polyunsaturated fatty acids (PUFA). Therefore the reduction of SFA and the increase of PUFA in meat is one of the main goal in meat science. Suckling lamb meat is a traditional food product in Mediterranean countries. Lambs are slaughtered very young, after a suckling period of 20–30 days, because the Italian consumers prefer this kind of product. In period between birth to slaughter, lambs are managed with their mothers and their are fed almost exclusively with maternal milk. Therefore, one of the factors affecting the fatty acid composition of suckling lamb meat is the quality of milk that lamb are receiving. The increasing of the sources of lipids in the mothers diet, can change the composition of the milk, and in particular, the aim is to increase some fatty acids such as omega 3 and CLA. Therefore, maternal nutrition can affect strongly the fatty acid composition of newborn and suckling animals. Some authors have observed that maternal feeding have influenced intramuscular fatty acids composition of suckling lambs (Lanza et al., 2006, Osorio, 2007, Serra et al., 2009) with variation in the content of CLA c9t11 and PUFAn3, and thus a decreased PUFAn6/PUFAn3 ratio. In particular, the maternal linseed supplementation can increase the content of C18:3 n3 in muscles of suckling animals but not of their long chain metabolites as LC-PUFA including eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3) and DHA (22:6n-3) (Nudda et al., 2008). However, during the fetal life, a higher efficiency in the conversion of C18:3 n3 on their long chain metabolites has been observed in the piglet muscles (de Quelen et al., 2010). Therefore a higher conversion of C18:3 into their long chain metabolites in the fetal or mother tissue and a higher transfer of those FA from the mother to the fetus are expected. Therefore maternal linseed supplementation during gestation, as source of C18:3 could be a possible way to increase long chain PUFA n3 content in meat of lambs.

The aim of this experiment was to evaluate the effect of alpha-linolenic acid (ALA) diet supplementation by extruded linseed to dairy ewes during pregnancy and

early lactation on the fatty acid profile in longissimus dorsi and semitendinosus muscles and brain tissue of suckling lamb.

3.2. MATERIAL AND METHODS

3.2.1 Animals and treatments

Thirty-eight Sarda breed suckling lambs were selected from thirty-six Sarda dairy ewes, fed with two different diets, from approximately the last 8 weeks of gestation, to the first 4 weeks of lactation. During gestation sheep were divided in two groups, one of them received the control concentrate (CON group) and the second group received the experimental concentrate containing linseed as source of C18:3 n3 (LIN group). The analytic component of diets are reported in table 1. The ewes received hay ad libitum and 1 kg of the two complete pelleted diet (CPD) formulated specifically for the trial. The CPD was distributed into 3 doses /day for 8 weeks until parturition. In the last 4 weeks of gestation 150g/day of corn flour was added and divided in 3 doses/d. The CON and LIN diets were isoenergetic, isoproteic and isolipidic but differed in the fatty acid composition. The fatty acid composition of the concentrate is reported in table 2. After parturition the sheep were divided in 4 groups.

1) Group CON-CON were fed the control diet during gestation and lactating,

2) Group LIN-CON that received linseed only diet during gestation,and control diet during lactation

3) Group CON-LIN that receive linseed diet only during lactation,

4) Group LIN-LIN that receive linseed diet during both gestation and lactation.

For this experiment, lambs were fed exclusively with maternal milk.

Table 1 -Analytic component of the experimental concentrates %

	CONTR DIET[1] %	LIN DIET[2] %
DM	89.40	89.41
as % of DM		
Ashes	8.86	8.56
Crude protein	18.42	18.37
NDF	42.94	41.98
ADF	26.02	28.39
ADL	6.00	5.48
EE	7.34	6.99

[1]The ingredient of control diet was: Wheat bran, Dried sugar beet pulp, Calcium carbonate, Soya Meal, Maize, Chloride Sodium, Sodium Bicarbonato, Magnesium Oxide, Alfalfa meal, Distilled from wheat, Wheat bran, Sunflower extraction meal, Corn germ cake.

[2]The ingredient for linseed group was: Wheat bran, dried sugar beet pulp, Calcium carbonate, Soya Meal, Maize, Chloride Sodium, Sodium Bicarbonato, Magnesium Oxide, Soybean hulls, Extruded linseed, Flour of extraction of sunflower, Alfalfa meal, Corn gluten feed

Table 2 – Fatty acid composition of the experimental concentrates

Fatty acids	**CON**	**LIN**
C12:0	1.033	0.147
C14:0	1.130	0.183
C16:0	31.500	10.346
C18:0	27.352	6.071
C18:1 c9	10.731	20.627
C18:2 n6	22.780	26.280
C18:3 n3	2.026	34.217

The predominant FA of the lipid fraction in the CON concentrate were Palmitic acid (C16:0), Stearic and Linoleic acids (C18:2 c9c12, LN). The most abundant FA in LIN concentrate was alfa- linolenic acid (C18:3 c9c12c15, ALA), which accounted for 34% of total FA followed by LN and Oleic acid. At birth the lambs were kept with their mothers and they were fed exclusively with maternal milk. All lambs were slaughtered when they have 28 days of age.

The lambs were weighed before the slaughtered. , The carcass was refrigerated for 24 hours at 4°C and then the *semitendinosus* and *longissimus dorsi* muscles were dissected from each right half-carcass, vacuum packaged and stored at – 80°C until the fatty acid could be analyzed.

3.2.2. Muscle analysis

The dry matter (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) . Fatty acids composition of 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 5.2 and 5.2.2.

The chromatographic conditions and fatty acid identification were carried out as reported in chapter 5 paragraph 5.2.3 The amount of each fatty acid is reported as mg FA/100g of muscle.

3.3. Calculations

The sum of n-3 PUFA, n-6 PUFA and the ratio n-6/n-3 were calculated. 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). The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to (Fernández, 2007). Desaturase index and elongase indix were calculated according to the formulas given in table 3

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

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5\ \Sigma\ MUFA + 0.5\ PUFA(n6) + 3\ \Sigma\ PUFA(n\text{-}3) + (n\text{-}3) / (n\text{-}6)}$$

Table 3. Calculation of desaturase and elongase indices

Desaturase indices		**Elongase indices**	
D5 n-6	C20:4n6/C20:3 n6	**EL1 n-6**	C20:3 n6/C18:3 n6
D5 n-3	C20:5 n3/C20:4 n3	**EL1 n3**	C20:4 n3/C18:4 n3
D6n-6	C18/3n6/C18:2 n6	**EL2 n6**	C22:4 n6/C20:4 n6
D6 n-3	C18:4 n3/C18:3 n3	**EL2 n3**	C22:5 n3/C20:5 n3

3.4.Statistical analysis

Differences in meat composition and fatty acidprofile in of fat of suckling lambs were assessed with ANOVA, using diet, muscle type and diet x muscle type as fixed factor. Statistical analysis was performed using MINITAB® software (Version 16, Minitab, State College, PA, USA), and significant differences were declared for $P \leq 0.05$ and a tendency for $P \leq 0.10$.

3.5. Results and discussion

3.5.1 The milk fatty acid profile

The fatty acid profile of milk fat of ewes is reported in Table 4. The largest proportion of fatty acid in ewe milk was made up by C16:0, C18:0 and C18:1 *cis*-9. The maternal treatments influenced significantly several fatty acids. The content of VA were higher ($P<0.05$) in milk of ewes that received linseed during lactation compared to milk of ewes that received linseed during gestation. The correspondent increased in CLA content in milk of ewes fed linseed during lactation occurred. However, the value of CLA is lower than values normally observed in sheep milk suggesting a low desaturase activity in the mammary gland to convert VA in to CLA.

The content of ALA was positively influenced by linseed supplementation during lactation whereas the LIN supplementation during gestation was not efficient to increase its concentration in milk.

Among the highly unsaturated fatty acids (HUFA), the contents in milk were extremely low and showed differences between ewes that received linseed during lactation compared to gestation for EPA and DPA. The DHA content was not modified by dietary treatment.

Table 4. Effect of linseed supplementation in fatty acid profile of milk fat of ewes

	Groups (g)				p value
gAG/100gfat	lin-contr	lin-lin	contr-lin	contr-contr	groups
C14:0	3.49	3.63	3.45	3.33	ns
C14:1c9	0.04	0.04	0.04	0.05	ns
C15:0	0.31	0.36	0.34	0.31	ns
C16:0	12.24	11.81	11.83	11.87	ns
C16:1 t9	0.08^{ab}	0.18^{a}	0.17^{a}	0.06^{b}	**
C16:1 c9	0.30	0.29	0.28	0.30	ns
C18:0	7.18	7.35	7.09	6.37	ns
C18:1 t9	0.17	0.27	0.25	0.15	*
C18:1 t11 (VA)	0.94^{ab}	2.44^{ab}	2.32^{a}	0.77^{b}	*
C18:1 c9	10.95	12.76	11.78	10.45	ns
C18:2 n6 (LN)	1.49	2.06	1.89	1.33	†
C18:3 n6	0.02	0.01	0.01	0.02	ns
C18:3 n3(ALA)	0.37^{ab}	0.62^{a}	0.54^{ab}	0.32^{b}	**
CLA c9 t11 (RA)	0.38^{b}	0.98^{a}	0.84^{ab}	0.38^{b}	**
C20:3 n6	0.02	0.01	0.01	0.02	†
C20:4 n6 (ARA)	0.07	0.07	0.09	0.09	ns
C20:3 n3	0.00	0.01	0.01	0.00	†
C20:5 n3(EPA)	0.03^{b}	0.05^{a}	0.04^{ab}	0.03^{b}	*
C22:4 n6	0.01	0.01	0.01	0.01	ns
C22:5 n - 3 (DPA)	0.04	0.07	0.06	0.04	†
C22:6 n-3 (DHA)	0.02	0.03	0.02	0.02	ns

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

3.5.2 Chemical composition of lamb meat

Chemical characteristics of muscle of Sarda suckling lambs (mean±SD) is reported in Table 5. The maternal dietary treatment influenced only the protein content of muscles. The protein content was significantly higher in Lin/Lin lambs. The average protein content was higher than values observed in other suckling lamb meat (Pérez et al., 2002), (18%) but is in accordance with values observed in other Italian lambs (D'Alessandro et al., 2012). The two muscles analyzed were differing in the chemical components except for the fat content. The tight muscle showed a higher content of protein, moisture and ash compared to L.dorsi ($p<0.001$).

Table. 5 Chemical composition of intramuscular fat of Sarda suckling lamb

	Group (G)				Muscle (M)		P-value		
selected nutrient %	lin-con	lin-lin	con-lin	con-con	Thigh	L.D	G	M	G*M
fat	1.57	1.72	1.66	1.63	1.60	1.69	ns	ns	ns
proteins	20.38^{b}	20.85^{a}	20.48^{b}	20.11^{b}	20.75	20.16	**	***	ns
moisture	76.65	76.03	76.46	76.67	76.78	76.10	ns	***	†
ashes	1.19	1.21	1.20	1.22	1.22	1.19	ns	**	ns

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

3.5.3. Fatty acid profile of lamb meat

The sums of fatty acid and values for nutritional indexes of lambs meat are reported in table 6. Lambs fed milk from mother that received linseed in all experimental period have a highest value in MUFA ($p<0.05$), TFA, PUFA n-3 and OBCFA ($p<0.001$) compared to the lambs fro mother that received only control diet. However, the linseed supplementation during lactation affected those fatty acid more than the effect caused by linseed supplementation durin gestation

Table.6. Effects of linseed supplementation in fatty acid partial sums and nutritional ratios.

	Groups (g)				Muscle (M)		p-values		
	lin-con	lin-lin	con-lin	con-con	Thigh	L.D	g	m	g*m
Fatty acids partil sums									
SFA	24.03	22.73	22.35	22.93	21.36	24.67	ns	***	ns
MUFA	23.11^{ab}	24.54^{a}	24.48^{a}	21.44^{b}	22.06	24.73	*	***	ns
TFA	2.26^{b}	3.90^{a}	3.79^{a}	1,49^{c}	2.67	3.05	***	*	ns
PUFA	12.65	13.21	13.54	13.03	13.46	12.76	ns	ns	ns
PUFA-n3	1.83^{b}	2.57^{a}	2.13^{ab}	1.68^{b}	2.21	1.90	***	*	ns
PUFAn-6	9.83	9.10	9.86	10.24	9.97	9.55	ns	ns	ns
HP-PUFA	4.61	4.71	4.91	5.93	5.43	4.64	†	*	ns
OBCFA	1.17^{ab}	1.27^{a}	1.24^{a}	1.03^{b}	1.08	1.28	***	***	ns
nutritionl ratios									
n6/n3	5.65^{b}	3.66^{d}	4.73^{c}	6.46^{a}	4.78	5.47	***	**	ns
PUFA/SFA	0.53	0.59	0.61	0.60	0.64	0.53	ns	**	ns
h/H	2.13^{b}	2.40^{ab}	2.47^{a}	2.28^{ab}	2.47	2.17	*	***	ns
AI ^{c}	0.58^{a}	0.52^{ab}	0.49^{b}	0.58^{a}	0.51	0.58	**	***	ns
TI ^{d}	1.02^{a}	0.86^{b}	0.88^{b}	1.04^{a}	0.89	1.01	**	**	ns

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

The effects of muscle type evidenced differences for SFA, MUFA, OBCFA ($p<0.001$), TFA ($p<0.05$). In particular, L.dorsi had a higher value of SFA, MUFA and trans FA

compared to the tight muscles. On the other hand the tight muscles showed a higher content of PUFA n-3 ($p<0.05$), and HP-PUFA ($p< 0.001$) than L. dorsi.

The n6/n-3 ratio was markedly affected by dietary treatment and muscle type. The lambs from ewes that received only linseed diet had a lower ratio ($p<0.001$) than lambs from the control ewes. In human diet, values of n6/n3 ratio lower than 4 is indicated as the most favorable in order to prevent some cardiovascular diseases and n-6/n-3 ratios are indicators of the nutritional quality of the lipids in a food (Wood, 2003). The hypocholesterolemic/hypercholesterolemic ratio (h/H) was influenced by maternal treatment. It value was the highest in lambs from ewes that received linseed only during lactation whereas the lowest was in lambs that receiving linseed only in gestation ($p<0.05$). Artherogeneticity index (AI) differed significantly between con-conand con-lin groups. Thrombogenicity index (TI) was significantly lower in lambs from lin/lin group. Very low values of AI and TI are recommended for a healthy diet. In table 7 are reported the individual fatty acids of lambs from the 4 treatments. The FA composition of muscles in suckling lambs reflected the FA composition of the maternal milk as observed in previous works with lambs (Lanza et al., 2006) and kids (Lanza et al., 2006, Nudda et al., 2008).

Table 7. Effects of linseed maternal supplementation in fatty acid suckling lamb meat

	Groups (g)				Muscle (m)		p-values		
gAG/100gTQ	**lin-contr**	**lin-lin**	**contr-lin**	**contr-contr**	**Thigh**	**L.D**	g	m	gxm
C14:0	1.91	2.03	1.86	2.00	1.72	2.18	ns	***	ns
C14:1c9	0.06	0.07	0.06	0.08	0.06	0.07	†	ns	ns
C15:0	0.18	0.19	0.19	0.18	0.17	0.20	ns	***	ns
C16:0	11.96^{ab}	10.62^{b}	10.55^{b}	12.47^{a}	10.42	12.38	**	***	ns
C16:1 t9	0.08^{b}	0.15^{a}	0.14^{a}	0.05^{b}	0.10	0.11	***	*	ns
C16:1 c9	0.73^{ab}	0.66^{b}	0.63^{b}	0.87^{a}	0.68	0.77	**	*	ns
C18:0	8.28	8.37	8.51	8.14	7.90	8.75	ns	***	ns
C18:1 t9	0.18^{b}	0.25^{a}	0.24^{a}	0.13^{c}	0.19	0.21	***	*	ns
C18:1 t11 (VA)	0.89^{b}	1.90^{a}	1.80^{a}	0.50^{b}	1.20	1.35	***	**	ns
C18:1 c9	17.99	18.06	18.41	18.63	17.18	19.36	ns	***	ns
C18:2 n6 (LN)	6.65	6.36	6.61	6.19	6.43	6.48	ns	ns	ns
C18:3 n6	0.05^{ab}	0.04^{b}	0.05^{ab}	0.06^{a}	0.05	0.05	**	ns	ns
C18:3 n3(ALA)	0.50^{b}	1.01^{a}	0.88^{a}	0.37^{b}	0.68	0.70	***	ns	ns
CLA c9 t11	0.52^{b}	0.95^{a}	0.89^{a}	0.39^{b}	0.66	0.72	***	ns	ns
C20:3 n6	0.19^{ab}	0.15^{b}	0.17^{b}	0.24^{a}	0.20	0.18	***	*	ns
C20:4 n6 (ARA)	2.22^{b}	1.66^{b}	2.09^{b}	3.03^{a}	2.36	2.14	***	ns	ns
C20:3 n3	0.03^{ab}	0.03^{a}	0.03^{ab}	0.02^{b}	0.03	0.03	*	*	ns
C20:5 n3(EPA)	0.37^{b}	0.52^{a}	0.36^{b}	0.29^{b}	0.40	0.36	**	ns	ns
C22:4 n6	0.13^{b}	0.08^{c}	0.12^{b}	0.24^{a}	0.16	0.14	***	*	ns
C22:5 n 3 (DPA)	0.59	0.62	0.55	0.61	0.66	0.53	ns	**	ns
C22:6 n3 (DHA)	0.32	0.36	0.28	0.34	0.38	0.27	ns	***	ns

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

The most abundant FA of the muscles, i.e. C18:0, C18:1 c9 and C18:2 n6 were not influenced by maternal diet, whereas the content of C16:0 was reduced by linseed supplementation during lactation. The reduction of palmitic acid is interesting from a

nutritional point of view because it is considered hyperlipidemic and responsible for increased plasma cholesterol contents (Ulbricht and Southgate, 1991).The content of the most abundant FA of the muscles, i.e. C16:0, C18:0 and C18:1 c9, was higher in L.dorsi than tight muscle as previously observed by other authors in the Longissimus muscle in beef (Orellana et al., 2009, Realini et al., 2004). Otherwise, no differences in the content of C18:0 among muscles were observed by Velasco et al.,(2001). Because stearic acid, is derives from elongation of C16, the higher content of C18:0 could be partly related to the higher content of C16 in this muscle. Oleic acid (C18:1cis-9) differed between muscles being higher in L.dorsi than in tigh muscle. Usually, oleic acid increases with fatness. In this case the content of fat was higher in L. dorsi muscle compared to tight muscle, even if the differences did not reach the level of significance. The increase of oleic acid is also related to an increase in the activity of the enzyme D-9 desaturase, which synthesizes oleic acid from stearic acid (C18:0) (Bauman, 2000). This could be an explanation by the fact of the higher content of oleic acid in L. dorsi compared to tight muscle. The monounsaturated oleic acid has been reported to be hypolipidemic, reducing both plasma cholesterol and triglycerides, and it can be considered a desirable component of the diet (Mattson and Grundy, 1985). The lambs from ewes that received linseed continuously or only during lactation showed a higher concentration of vaccenic acid (C18:1 t11; VA) than group that received milk from mother feed with linseed only in gestation. The higher content of VA in lamb was expected because an effect of maternal linseed supplementation on muscles fatty acid profile of the suckling kids was previously observed (Nudda et al., 2008). This FA is the main trans fatty acid isomer present in milk and meat fat (Destaillats et al., 2005) because 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 (Nuernberg et al., 2008, Santos-Silva, 2002) rich in C18:3 n3, the most important precursor of VA in the rumen. The lambs from ewes that received linseed continuously or only during lactation showed a higher concentration of CLA and ALA than lambs from mother feed with linseed only in gestation. This result can be explained by the fact that the lambs at this stage are "functional monogastric" and so there is no the ruminal biohydrogenation of dietary LA and ALA fatty acid before being absorbed by the intestine. Therefore, the contents of RA accumulated in the meat of suckling lambs derived in part from RA present in milk and in part the activity of Δ9-desaturase enzyme on VA (Bauman, 2000). However, our data on

lambs contrast with recent observations of Berthelot et al. (2012) that reported that feeding ewes with linseed did not increase the proportion of CLA and C18:3 in lamb muscle. Among the long chain fatty acid of the omega6 family, C20:3 n-6, C20:4 n6 (ARA) and C22:4 in muscles were lowered by maternal linseed supplementation. Linoleic acid provides the arachidonic acid needed for membrane synthesis, if linoleic acid is not presence in adequate quantities the non-essential n-9 oleic acid is desaturated and elongated to form eicosatrienoic acid (20:3n-9) which is incorporated into membranes (Palmquist, 2009). Deficiency symptoms appeared in rat when linoleic acid was <2% of energy intake (Holman, 1960).In the omega3 family only the EPA was significantly increased in lambs by maternal linseed supplementation both during gestation and lactation. The DPA and DHA were not influenced by the ALA supplementation to the mothers in accordance with other work with lambs (Bas et al., 2007), beef (Raes et al., 2003a, Raes et al., 2003b) and pig (Ahn et al., 1996, Riley, 2000, Romans et al., 1995a, Romans et al., 1995b). Lamb supplemented directly with low amount of omega 3 (9%) showed that low quantity was sufficient to increase the production of the active metabolites EPA and DPA, but not to increase the DHA production (Bas et al., 2007). DHA has been found in fetal tissue already at 145 days of gestation (0.83%; Nudda et al., 2007) suggesting that it is mainly accumulated in lamb tissue during intrauterine life through the mother blood, independently of the diet.

3.5.4 Effect of linseed supplementation in desaturation and elongation activity index

The desaturation and elongation activity indexes in ST and LD muscles of suckling lamb of the 4 experimental treatments are reported in table 8. Both essential fatty acids are metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA) and ALA was metaboliuzed to EPA and DPA, increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl group (Simopoulos, 2002). Almost all desaturase index were influenced by maternal dietary treatment, except for the D5 n-3. The D5n-6 (C20:4 n6/ C20:3 n6) was significantly higher ($p<0.001$) in lambs suckling milk from mothers fed with control diet during gestation and lactation (CON/CON group) compared to lambs from LIN/LIN group. Also the desaturase index D6 n-3 (C18:4 n3/C18:3 n3) result lower in linseed group compared to lambs from ewes fed control diet for all experimental period despite the higher ALA

concentration in lin-lin group. Regarding the index of elongation the EL1n-6 was not influenced by maternal dietary treatment. However the EL1n3 was the highest in lambs from ewes with continuous linseed supplementation and the lowest in lambs from the control group. On the other hand, the EL2 n-3 was higher in contr-contr group compared to groups that received linseed in gestation, lactation or both. The elongases and desaturases appear to have a greater affinity for ALA than for LA because 10 fold more LA is required to have an equal effect on n-3 metabolism as ALA does on LA elongation (Holman, 1998). In fact, evidences from numerous in vivo and in vitro studies, showed that these two families of fatty acid not only share these enzymes, but they also compete for the same enzymes (Brenner and Peluffo, 1966, Holman, 1968) and an excess of one of them can cause a important decrease in the conversion of the other (Hague and Christoffersen, 1986). Even though the affinity of desaturases is higher for ALA than for LA, the lower concentrations of ALA in control group can probably have resulted in a greater conversion of the latter to longer chain PUFA. Some authors (Palmquist, 2009, Ward et al., 2010) reported that both Δ5- and Δ6- desaturase are related with variantion in the intramuscular fat content, but in our trial no differences in the intramuscular fat content among lambs groups have been observed.

Table 8- Estimated desaturation and elongation activity indexes in thigh and longissmus dorsi muscle of Sarda suckling lambs

	Groups				Muscle		P -values		
gAG/100gTQ	**lin-con**	**lin-lin**	**con-lin**	**con-con**	**Thigh**	**L.D**	**g**	**m**	**G*m**
D5 n-6	11.299^{b}	10.612^{b}	12.072^{ab}	13.343^{a}	11.871	11.792	***	ns	ns
D5 n-3	23.405	25.587	23.989	22.451	24.383	23.332	ns	ns	ns
D6n-6	0.008^{ab}	0.007^{b}	0.007^{b}	0.010^{a}	0.008	0.008	**	ns	ns
D6 n-3	0.039^{a}	0.020^{b}	0.022^{b}	0.049^{a}	0.033	0.034	***	ns	ns
EL1 n-6	3.688	3.601	3.994	4.471	4.294	3.583	ns	**	ns
EL1 n3	0.853^{ab}	1.042^{a}	0.879^{ab}	0.685^{b}	0.937	0.792	**	**	†
EL2 n6	0.061^{bc}	0.052^{c}	0.071^{b}	0.081^{a}	0.068	0.064	***	ns	ns
EL2 n3	1.595^{b}	1.229^{c}	1.559^{b}	2.180^{a}	1.700	1.581	***	†	ns

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

3.6.Conclusion

The maternal linseed supplementation during lactation increased the content of VA, c9t11 CLA and ALA in milk. Similar results have been observed in lamb meat, whereas the fatty acid profile of muscle reflected the fatty acid profile of suckled milk. In particular, the maternal linseed supplementation during gestation did not change the content of ALA and their long chain metabolites EPA, DPA and DHA compared to control group. The continuous linseed supplementation increases the concentration of ALA and EPA, but did not increase the concentration of DHA. The maternal linseed supplementation only during lactation increased the concentration of ALA in intramuscular fat of suckling lambs but did not affect their long chain metabolites. The elongase and desaturase indexes evidenced that the efficiency of conversion of ALA into their long metabolites in skeletal muscles of lambs is very low both during fetal life and during the first month of life. Finally, the higher value of D5 and D6 desaturase activity in control group suggest an inhibitory effect of the high dose of ALA in the diet on itself desaturation pathway. The conversion of ALA into longer metabolites was efficient in two specific steps of elongation pathway and particularly in C20:4 n3 and in C22:5 n3. The overall results showed that the use of linseed in the maternal diet during gestation and lactation is not efficient increasing the concentration of long chain fatty acid of the omega3 family in meat of suckling lambs.

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