UNIVERSITY OF MOLISE

Department of Agricultural, Environmental and Food Sciences

INTERNATIONAL Ph.D. in
"WELFARE, BIOTECHNOLOGY AND QUALITY OF ANIMAL PRODUCTION"
(XXVI CYCLE)

Related disciplinary scientific section: 07/G1 (Scienze e Tecnologie Animali)

General Coordinator: Prof. Giuseppe Maiorano

Doctorate Thesis Title:

Influence of multiple injections of vitamin E on quality traits and oxidative stability of lamb meat

Ph.D. Candidate:
Dr. Domenico Di Memmo
143440

Supervisors:
Prof. Mario Gambacorta
Prof. Giuseppe Maiorano

ACADEMIC YEAR 2013/2014

To Amy

Acknowledgments

I would like to thank my co-supervisor, professor Giuseppe Maiorano, and my supervisor, professor Mario Gambacorta, for their invaluable inputs towards the completion of this work. Working with You was a big privilege and an unique opportunity to improve my knowledge. I would also like to thank the University of Molise.

Secondly, my heartfelt gratitude goes to all the people that I met during the International PhD study, and to all my collegues in "Welfare, Biotechnology and Quality of Animal Production", in particular to Ms. Abiuso Cinzia, Ms. Cianciullo Daniela, Mr. Di Iorio Michele, Mr. Gentile Andrea, Ms. Mucci Rossella, Ms. Nardoia Maria, Ms. Rocco Martina, Ms. Tavaniello Siria, Ms. Wilkanowska Ania, Mr Vizzarri Francesco. Thank's for their overwhelming support.

I extend my thanks also to my Polish tutor professor Marek Bednarczyk and to all my Polish colleagues.

A big thank you goes to my family, to my friends who have encouraged me through the course of the work. To the farmers that I met during this period I extend my sincere gratitude for your invaluable input without which this work would not have been completed. Thank you so much and keep up the good work.

To all those whose I have not mentioned the name and who, in one way or another, contributed to the success of this work, I want to give my sincere thanks.

Abstract

Transporting animals to the slaughterhouse can increase the susceptibility of meat quality and, therefore, influence the value of pH, TBARS and the oxidation of fatty acids and pigments. Lipid oxidation cause negative changes on structure and nutrition value such as meat flavour, odour and colour: it is well know that colour is the characteristic that more affects the decision of the consumer to purchase. For this reason a supply of antioxidants is recommended to preserve the health of animals and the oxidative stability of their products. One of the most widely used antioxidants in animal diet is vitamin E. It is a lipid soluble and chain breaking antioxidant that protect cellular membranes against oxidative damages. The most active and natural form of vitamin E is α-tocopherol. It is now accepted that dietary supplementation with α-tocopheryl acetate controls lipid oxidation and colour deterioration in red meat like lambs and beef. In literature has been reported that intramuscular injections of tocopherols had a quite similar effect to the supplementation of vitamin E in diet. However, controversial information regarding the productive performances are also showed in some works on lambs. No information exists in literature on performance and meat quality traits of Laticauda lambs injected with Vitamin E. Laticauda is an autochthonous breed of Campania Region in Italy, that is usually reared by small family farms under semi-extensive systems. It derives from crossbreeding local sheep from the Appennines breed with fat-tailed North African sheep, imported in the XVIII century. Laticauda breed has a prevailing attitude toward meat production, associated to a good prolificacy.

In the light of the above mentioned, the aim of the study was to determine the effect of DL-α-tocopheryl acetate injections on carcass and meat quality traits in growing lambs. Twenty-four 15-d-old Laticauda male lambs were randomly allotted to two groups: control (C, n =12) or vitamin E-treated (V, n=12). From the beginning of the study (15 days of age) until day 57 of age, each lamb of the V group received, weekely, i.m. injections of DL-α-tocopheryl acetate (right gluteus) in aqueous solution (Vitalene® E, Fatro, Bologna) for seven weeks for a total dose of 1,500 IU. The first injection consisted of 150 UI (3ml of Vitalene), while the other 6 injections consisted of 225 IU (4.5 ml of Vitalene). C group lambs received injections of physiological saline.

The animals were slaughtered at 64d of age. Hot and cold carcass weights were recorded and dressing percentages were calculated after dressing and chilling (2–4 °C for 24 h). After chilling, pH, colour, area, water holding capacity of *M. longissimus dorsi* (LD) were measured and shrink losses were calculated. After the refrigeration period (24 h at 2-4 °C), from the left side of the carcass the pelvic limb, loin and shoulder were removed and theirs percentage was calculated based on cold carcass weight. *Vastus lateralis* (VL) and *longissimus dorsi* (LD) muscles were removed for laboratory analyses (LD: fatty acids, cholesterol, intramuscular collagen, crosslinks; LD and VL: α-tocopherol content). For the same muscles TBARS (µg MDA/g tissue) at 24, 48, 96 and 192 h *post mortem* were measured during aerobic display at 5°C. Sensory analysis was performed on the pelvic limb from the left side of each lamb carcass, having two panels: one for the students, and the other one for customary consumers of lamb meat. Each panellist was required to evaluate the sample considering the intrinsic characteristics of the meat – tenderness, juiciness, flavour and overall acceptability (general pleasantness) – according to an unstructured line scale ranging from 1 ("very unpleasant") to 9 ("very pleasant").

Vitamin E administration did not influence significantly live weight, carcass traits, except the incidence of pelvic limb, significantly higher in V group lambs; LD quality traits did not differ between the experimental groups, except for the red colour value (a*) that was higher ($P<0.05$) in lambs of vitamin E group.

Treatment with vitamin E did not significantly affect the total SFA content and the proportion of single SFA, except for a slightly higher ($P < 0.08$) content of lauric acid (C 12:0) and lower ($P < 0.01$) content of eptadecanoic acid (C 17:0) in V group. Likewise, vitamin E treatment did not affect the total MUFA amount, except for a lower ($P < 0.01$) content of C 17:1 and higher C 18:1 n-9 *trans* ($P < 0.05$) and C 22:1 ($P < 0.01$) in V group. The oleic acid (C18:1 n-9 *cis*) was the most concentrated fatty acids (40-41%), followed by palmitic (20-21%) and stearic acids (14-15%). Vitamin E treatment positively affected the total PUFA content being higher ($P < 0.01$) in the V group compared with the control group. The vitamin E treated group had a significant higher amount of n-3 long chain PUFA: eicosapentaenoic fatty acid (EPA, C20:5n-3, $P < 0.05$), docosapentaenoic fatty acid (DPA, C22:5n-3, $P < 0.01$) and docosahexaenoic fatty acid (DHA, C22:6n-3, $P < 0.06$). Also vitamin E group had higher ($P < 0.01$) content of C 22:2. In light of this, the total n-3 FA content was higher ($P < 0.01$) in

treated lambs compared with the control group; while, no significant differences were found for the total n-6 FA amount. The n-6/n-3 ratio was lower in treated lambs ($P < 0.01$). The P/S ratio was higher ($P < 0.01$) in treated lambs. Trombogenic index was affected by the treatment being lower ($P < 0.01$) in vitamin E group.

Cholesterol content was found to be not significant ($P > 0.05$) between groups (78.78 *versus* 79.01 mg/100g in C and V, respectively).

Collagen characteristics were not significantly ($P > 0.05$) influenced by the treatment.

The treatment with vitamin E markedly increase ($P < 0.001$) the content of DL-α-tocopherol in both muscles (*longissimus dorsi*: 0.92 *versus* 6.41 µg/g in C and V, respectively; *vastus lateralis*: 0.99 *versus* 7.31 µg/g in C and V, respectively) and increases the meat shelf life. This was evident since muscle tissue lipoperoxidation levels (TBARS) were significantly lower ($P<0.01$) in vitamin E injected lamb meat (*longissimus dorsi* and *vastus lateralis* muscles) at 24, 48, 96 and 192 h *post mortem*, during all the aerobic display at 5°C.

The panel test showed that both the adult and the student judges, enjoyed both types of meat, giving a slightly higher point for the lamb that was injected with vitamin E if compared to the other type of meat (7.24 *versus* 6.97 respectively; $P > 0.05$). Therefore, both expressed a greater pleasure with the meat produced from lamb injected with vitamin E if compared to the meat of control group, as regard the descriptor of tenderness (6.66 *versus* 5.70, respectively; $P< 0.05$) and juiciness (6.39 *versus* 5.00; $P < 0.05$).

At the light of the above, the conclusion of this study is that the intramuscular administration of vitamin E can be recommended to obtain meat with a higher nutritional and commercial value.

Riassunto

Il trasporto degli animali prima della macellazione, ed altri fattori di stress, possono influenzare le caratteristiche chimico-fisiche delle carni, e di conseguenza i valori di pH, TBARS e l'ossidazione di acidi grassi e pigmenti. Questo processo causa peggioramenti qualitativi sia dal punto di vista nutrizionale che commerciale, soprattutto per quanto concerne l'aroma e il colore delle carni, fattori che influenzano enormemente le scelte di acquisto del consumatore. Per questo motivo viene spesso raccomandata una integrazione di antiossidanti tramite la dieta per mantenere gli animali in buona salute e stabilizzare i prodotti dal punto di vista ossidativo. L'antiossidante più utilizzato nelle produzioni animali è senz'altro la Vitamina E. E' un composto liposolubile che ha la funzione principale di proteggere le membrane cellulari dai danni dell'ossidazione. La forma biologicamente più attiva e più presente in natura è l'α-tocoferolo. È ad oggi universalente riconosciuto che una integrazione delle razioni alimentari con α-tocoferil acetato stabilizza e controlla l'ossidazione dei lipidi e il decadimento del colore, nelle carni rosse di specie ovine e bovine. Esiste anche una altra modalità di somministrazione della Vitamina E, ossia attraverso iniezioni intramuscolari di DL-α-tocoferil acetato. Questa modalità ha dimostrato di produrre effetti simili alla somministrazione attraverso la dieta, con risultati però spesso contraddittori, soprattutto per quanto riguarda le performance *in vivo*. Ad oggi non esistono studi di letteratura che descrivono l'effetto di iniezioni intramuscolari di Vitamina E, sulle performance produttive e sulla qualità della carne di agnelli di razza Laticauda. Questa è una razza autoctona della regione Campania, spesso allevata in piccoli gruppi e con sistema di allevamento semi-estensivo. Deriva da incroci di ovini locali degli Appennini con pecore nord africane dalla coda grassa, importate in Italia nel XVIII secolo. E' una razza da carne, che presenta una buona prolificità.

Lo scopo del presente lavoro di tesi è stato quello di determinare l'effetto di iniezioni multiple di Vitamina E (DL-α-tocoferil acetato) sulle performance produttive, sulla qualità e sulla stabilità ossidativa della carne di agnello.

Per la sperimentazione sono stati utilizzati 24 agnelli maschi, di razza Laticauda, di 15 giorni di età. Sono stati suddivisi in due gruppi, somministrando ad ogni animale,

settimanalmente, una soluzione fisiologica (Controllo: n=12) oppure DL-α-tocoferil acetato (Vitamina E: n=12; 1500 UI) fino all'età di 57 giorni.

Gli animali sono stati macellati a 64 giorni. Il peso a caldo e a freddo è stato registrato e sono state calcolate le rispettive rese, dopo 24 ore di refrigerazione (2–4 °C). E' stato misurato il pH, il colore, l'area e la capacità di ritenzione idrica (water holding capacity, WHC) del muscolo *longissimus dorsi*, e le perdite per gocciolamento delle carcasse. Dalla mezzena sinistra, sono stati rimosi i tagli della spalla, della coscia e del lombo e calcolate le relative incidenze sul peso a freddo. Successivamente, sono stati rimossi i muscoli *vastus lateralis* (VL) e *longissimus dorsi* (LD) dalla mezzena destra per le analisi di laboratorio. Sul LD è stata misurato il profilo degli acidi grassi, il contenuto di colesterolo, il contenuto di collagene intramuscolare e legami crociati (Idrossilisilpiridinolina, HLP); su LD e VL è stato quantificato il contenuto di α-tocoferolo (µg/g). Sugli gli stessi muscoli, sono stati determinati i TBARS (specie reattive all'acido tiobarbiturico, µg MDA/g tessuto muscolare) a 24, 48, 96 e 192 ore *post mortem* in conservazione aerobica a 5°C.

L'analisi sensoriale è stata effettuata sul coscio sinistro di ogni animale, utilizzando due panel: uno composto da studenti, l'altro da consumatori abituali di carne di agnello. A ciascun panel è stato chiesto di valutare le caratteristiche intriseche della carne – tenerezza, succosità, sapore e accettabilità generale. I risultati sono stati espressi dai giudici tramite schede di valutazione con scala da 1 (molto sgradevole) a 9 (molto gradevole).

La somministrazione di Vitamina E non ha avuto effetti significativamente rilevanti sulle performance *in vivo* e *post mortem*, ad eccezione dell'incidenza del coscio che è risultata maggiore ($P>0.05$) negli agnelli del gruppo di trattamento. Il pH, la WHC, l'area del muscolo *longissimus dorsi* e il colore non sono risultati significativamente diversi tra i gruppi, ad eccezione dell'indice del rosso (a*) che ha fatto registrare valori più elevati ($P<0.05$) negli agnelli trattamenti.

Il trattamento con vitamina non ha avuto effetti significativamente rilevanti sul contenuto totale degli acidi grassi saturi (SFA), fatta eccezione per una quantità lievemente più elevata di acido laurico (C 12:0) ed inferiore ($P < 0.01$) di acido eptadecanoico (C 17:0) nel gruppo vitamina. Lo stesso risultato è stato riscontrato per gli acidi grassi monoinstraturi (MUFA), fatta eccezione per il contenuto più basso ($P < 0.01$) di C 17:1 e più alto di C 18:1 n-9 *trans* ($P < 0.05$) e C 22:1 ($P < 0.01$) nel gruppo

della vitamina E. L'acido oleico (C18:1 n-9 *cis*) è risultato quello con la concentrazione più elevata (40-41%), seguito dal palmitico (20-21%) e dallo stearico (14-15%). Un effetto significativamente positivo ($P < 0.01$) è stato riscontrato sul contenuto totale di acidi grassi polinstaturi (PUFA), più elevati nel gruppo della vitamina E. Il più abbondante PUFA è risultato essere l'acido linoleico (C 18:2 n-6), con valori simili trai gruppi (7.95 e 8.07%, in C e V rispettivamente). Il secondo più rappresentativo è l'acido linolenico (C 18:3 n-3), che è contenuto principalmente nelle essenze verdi. Significativamente maggiore è risultato essere il contenuto di PUFA a lunga catena n-3 nel gruppo vitamina E: acido eicosapentanoico (EPA, C20:5 n-3, $P < 0.05$), docosapentaenoico (DPA, C22:5 n-3, $P < 0.01$) e docosaesaenoico (DHA, C22:6 n-3, $P < 0.06$). Anche il C 22:2 è risultato significativamente più alto ($P < 0.01$). Alla luce di ciò, il contenuto totale di n-3 è risultato maggiore ($P < 0.01$) negli agnelli trattati rispetto al controllo; mentre non sono state riscontrate differenze significativamente rilevanti per quanto concerne il contenuto totale di n-6. Il rapporto n-6/n-3 è risultato inferiore negli agnelli trattati (2.33 *versus* 3.62 in V e C, rispettivamente; $P < 0.01$). Il rapporto P/S invece è superiore ($P < 0.01$) negli agnelli trattati, anche se il valore ottenuto risulta inferiore a quello raccomandato (0.4-0.7). Anche l'indice trombogenico (TI) è stato influenzato dal trattamento, ed è risultato inferiore ($P < 0.01$) nel gruppo vitamina.

Il contenuto di colesterolo non è risultato diverso ($P > 0.05$) tra i gruppi (78.78 *versus* 79.01 mg/100g in C e V, rspettivamente), ma leggermente più elevato se paragonato con altri studi su altre razze.

La concentrazione di collagene (22.59 e 22.57 μg/mg in C e V, rispettivamente) e di HLP (4.37 e 4.59 μg HLP/mg in C e V, rispettivamente), come anche la stabilità delle fibre di collagene (0.135 e 0.142 mol HLP/mol di collagene in C e V, rispettivamente) non sono state influenzate ($P > 0.05$).

Al contrario il trattamento con la vitamina E ha notevolmente incrementato ($P < 0.001$) il contenuto di DL-α-tocoferolo in entrambi i muscoli (*longissimus dorsi*: 0.92 *versus* 6.41 μg/g in C e V, rispettivamente; *vastus lateralis*: 0.99 *versus* 7.31 μg/g in C e V, rispettivamente) e ha incrementato la conservabilità della carne. Questo è risultato evidente dal momento che i livelli di TBARS sono stati significativamente più bassi ($P < 0.01$) negli agnelli trattati con vitamina E (*longissimus dorsi* and *vastus lateralis* muscles) a 24, 48, 96 e 192 h di conservazione aerobica a 5°C.

Il panel test ha mostrato che sia gli adulti che gli studenti hanno gradito entrambi i tipi di carne, pur preferendo leggermente di più la carne degli agnelli trattati con vitamina E (piacevolezza generale: 7.24 e 6.97 rispettivamente; $P > 0.05$). Sia gli studenti che gli adulti hanno espresso un maggior gradimento per la carne del gruppo sperimentale per quanto riguarda i descrittori della tenerezza (6.66 *versus* 5.70, rispettivamente; $P < 0.05$) e succulenza (6.39 *versus* 5.00; $P < 0.05$).

Alla luce dei risultati ottenuti, si può concludere che l'utilizzo di Vitamina E per via intramuscolare, in agnelli di razza Laticauda, è consigliabile per ottenere carni con un più elevato valore nutrizionale e commerciale.

CONTENTS

List of figures

List of tables

List of abbreviations

AA arachidonic acid
ACAT cholesterol acyltransferase
ADF acid detergent fiber
AI atherogenic index
Ala alanine
AOA antioxidant activity
AP active packaging
Arg arginine
ASI american sheep industries
Asp aspartic acid
ASPA Association for Animal Science and Productions
AV Avellino (City)
BHA butylated hydroxyanisole
BHT butylated hydroxytoluene
BW body weight
C control
CAT catalase
CG control group
CHAOS Cambridge heart antioxidant study
CLA conjugated linoleic acid
CTGF connective tissue growth factor
Cys cysteine
DFD dark firm and dry
DHA docosahexaenoic fatty acid
DM dry matter
DPA docosapentaenoic fatty acid
EBW empty bodyweight
EPA eicosapentaenoic fatty acid
FA fatty acid
FAO Food and Agriculture Organization of the United Nations
GC gas chromatography
GLM general linear model
Glu glutamic acid
Gly glycine
GM gluteus medius
GPX glutathione peroxidase
HDL high density lipoprotein
HFBA n-heptafluorobutyric acid
HHP high hydrostatic pressure
His histidine
HLP hydroxylysylpyridinoline
HPLC high-performance (high-pressure) liquid chromatography
Ile isoleucine
IMCT intramuscular connective tissue
IR infrared
IU international unit

LD *longissimus dorsi*
LDL low density lipoprotein
Leu leucine
LL *longissimus lumborum*
Lys lysine
MAP modified atmosphere packaging
MDA malondialdehyde
Met methionine
MRP Maillard reaction products
MT mile tonns
MUFA monounsaturated fatty acid
NADH nicotinamide adenine dinucleotide
NDF neutral detergent fiber
P:S ratio of PUFA/SFA
Phe phenylalanine
PKC protein kinase
Pro proline
PUFA polyunsaturated fatty acids
RNS reactive nitrogen species
ROS reactive oxygen species
SE standard error
Ser serine
SFA saturated fatty acid
SOD superoxide dismutase
SPSS Statistical Package for Social Science
TBA thiobarbituric acid
TBARS thiobarbituric acid reactive substances
TBHQ tert-butyl hydroquinone
TEP tetraetoxypropane
Thr threonine
TI thrombogenic index
Trp tryptophan
Tyr tyrosine
UI unità internazionale (Italian language)
UK United Kingdom
UNAPOC Unione Nazionale Associazione Produttori ovi-caprini (Italian language)
USDA United States Department of Agriculture
UV ultraviolet
V vitamin
Val valine
VG vitamin group
VL *vastus lateralis*
WHC water holding capacity
WK week
WMD white muscle disease
WOF warmed-over flavour
YG yield grade
µ micro

PART 1. INTRODUCTION (Literature review)

Chapter 1

OVINE PRODUCTION

1.1 Introduction

Sheep farming is an ancient activity in many regions of the world. The reason of the wide diffusion of this species is to attribute to the big capacity to adapt to difficult environmental and climatic conditions (Idda et al., 2010). According to the FAO database (FAOSTAT 2013, the last adjurnment), the number of ovines in the world is 1,172 bilions, rising up if compared with the data of FAO 2002 (FAOSTAT 2002) database (1,034 bilions). Cina, India, Australia and Sudan have the largest flocks (Table 1.1).

Table 1.1. Number of sheeps per country in 2013 (millions)

Cina	185.0
India	75.5
Australia	75.5
Sudan	52.5
Iran	50.2
Nigeria	39.0
United Kingdom	32.8
New Zeland	30.8
World	**1,172.0**
Italy	7.0

Source: FAO (FAOSTAT 2013)

Sheep are an important part of the global agricultural economy. However, their once vital status has been largely replaced by other livestock species, especially the pig, chicken, and cow. China, Australia, India and Iran have the largest modern flocks, and serve both local and exportation needs for wool and mutton. Other countries, such as New Zealand, have smaller flocks but retain a large international economic impact due to their export of sheep products. Sheep also plays a major role in many local

economies, which may be niche markets focused on organic or sustainable agriculture and local food customers. Especially in developing countries, such flocks may be a part of subsistence agriculture rather than a system of trade. Sheep themselves may be a medium of trade in barter economies (Weaver, 2005).

The majority of sheep reared in the world, is used for the production of meat, wool and, in certain regions, the skin (leather). The breeding of dairy sheep is less prevalent, and it is concentrated in few areas in temperate or warm-temperate climate. The volume of the income related to dairy sheep is much lower when compared with those of trade in wool and meat. The production of wool, which represented an important sector in the past, has entered into the crisis resulting from the growth of market for modern and low cost fibers, natural or sinthetics. Over the past fifty years the production has declined by about 10 times, and has become marginal (Idda et al., 2010). In contrast to other commodities, that of sheep meat is steadily increasing in all the world. In 2012 the productions have been 8,480 tons, about a third more compared with the data of 1992, instead the decreasing in the sheep numbers. This trend depends on two factors: the increase in demand and the conversion of wool into meat-wool farms (Mounter et al., 2008).

According to FAO data (FAOSTAT 2012, the last adjurnment), China is the largest producer of sheep meat, followed by Australia, New Zealand, United Kingdom, India, Turkey and Iran. Italy produces about 45,558 tonnes of sheep meat per year (Source: FAOSTAT, 2012) (Table 1.2).

Table 1.2. Sheep meat productions by country (,000 tons)

	2012	**2007**		**1997**		**1987**		**Δ (%)**	
	,000 t	,000 t	%	,000 t	%	,000 t	%	07/97	07/87
Cina	**2,080**	2,000	24.1	1,190	16.6	350	5.5	68.1	471.4
Australia	**607**	683	8.2	566	7.9	584	9.2	20.7	17.0
N. Zealand	**447**	573	6.9	542	7.6	610	9.6	5.7	-6.1
Iran	**130**	390	4.7	301	4.2	208	3.3	29.6	87.5
UK	**275**	325	3.9	342	4.8	296	4.7	-5.0	9.8
Turkey	**226**	272	3.3	324	4.5	310	4.9	-16.0	-12.3
India	**295**	234	2.8	222	3.1	170	2.7	5.4	37.6
Siria	**198**	204	2.5	148	2.1	97	1.5	37.8	110.3
Others	**4,176**	….	….	….	….	….	….	….	….
Italy	**46**	59	0.9	72	1.0	67	1.1	1.4	7.5
TOTAL	**8,480**	**8,308**	**100.0**	**7,177**	**100.0**	**6,352**	**100.0**	**15.8**	**30.8**

Source: FAO (FAOSTAT 2012)

1.2 The importance of sheep sector in Italy

In Italy the sheep population is distributed as shown in Table 1.3.

Table 1.3. Number of sheep in Italy by regions (Istat, 2011).

Zone	**%**	**Region**	**Number**
Nord	6	Lombardy	105,328
		Emilia Romagna	63,758
		Piedmont	91,967
		Aosta Valley	2,258
		Veneto	43,031
		Liguria	10,845
		Trentino Alto Adige	57,300
		Friuli Venezia Giulia	11,290
Center	19	Lazio	588,046
		Tuscany	416,656
		Umbria	107,009
		Marche	150,040
South	18	Apulia	226,829
		Abruzzo	210,573
		Calabria	246,914
		Campania	221,527
		Basilicata	226,829
		Molise	69,164
Islands	57	Sicily	732,376
		Sardinia	3,008,623
ITALY			**6,625,793**

Source: from the ”6° Censimento Dell’Agricoltura” ISTAT

95% of Italian sheep population is localized in the Mediterranean area (center, south Italy and islands). In Italy several breeds exist, classified according to their main productive attitude (Table 1.4).

Table 1.4. Main sheep breeds in Italy

Italian dairy breeds	**Italian meat breeds**	**Italian dairy and meat breeds**	**Italian dairy, meat and wool breeds**	**Foreign breeds**
Altamurana	Alpagota	Bretagana	Brogna	Dorset Down
Comisana	Appenninica	Gentile di Puglia (1)	Garresina	Frisia
Delle Langhe	Bergamasca	Matesina (1)	Rosset Savoiarda	Ile de France
Garfagnina	Biellese	Sopravissana (1)	Tiroler berfgschaf	Merino
Leccese	Brianzola	Varesina(1)	Trimeticcia di segezia	Suffolk
Massese	Cornigliese	Bagnolese (2)	Vissana	
Pinzirita	Ciavenasca	Barbaresca (2)		
Sarda	Fabbrianese	Brigasca (2)		
Sciara o Moscia Calabrese	Finarda	Cornella Bianca (2)		
Valle del belice	Lamon	Fabrosana(2)		
	Marrana	Istriana (2)		
	Merinizzata Italiana	Laticauda (2)		
	Pecora di Coterno	Piezzana (2)		
	Pomarancina	Pusterese (2)		
	Saltasassi			
	Sambucana			
	Villnoesser			
	Zerasca			

Main attitude: (1) Wool and meat (2) Milk and meat. Source: www.federica.unina.it.

As reported in FAOSTAT database (FAOSTAT, 2012), in Italy the most important production is still represented by the dairy herd. The numerical superiority of the dairy breeds, more widespread in the centre-south and in the islands, confirms it. The Sarda breed, in particular, represents more than 70% of the productive sector (Unapoc, 1992). In particular, Sardinia (Italy) is one of the most important EU regions for sheep dairy production. It has more than 3.5 million sheep (3.7% of the EU total in 2009) that totally produce more than 300,000 mile tons (MT) of milk. This quantity corresponds to about 4% of total world production (FAOSTAT, 2012) and the sheep milk is processed into different types of cheese. Sardinia produces approximately 50–60 thousand mile tons of cheese per year, manufactured by more than 50 dairy factories, about half of which are co-operatives (Furesi et al., 2013).

Currently, the italian sheep milk and dairy production is the most modern and technological developed in all the world, also from the commercial point of view (Pulina and Furesi, 2000). Italy has a big sheep biodiversity: most of the local breeds are recorded in the National Studbook ("Libro Genealogico"), the 30% of them has a very low number and are recorded in a national population register ("Registro Anagrafico"). Nowadays the genetic and productive characteristics of many italian breeds are not well known, because is very difficult to study them in their authochtonous environment (Unapoc, 1992). The authochtonous breeds are considered very important for the preservation of the biodiversity, and are nowadays protected by the EC Regulation 1698/2005 (Article 39) through incentives for farmers who chose to raise native breeds in danger of extinction.

Actually, 45,558 tons of ovine meat are produced in Italy (FAOSTAT, 2012). Italian consumption of sheep meat is characterized by regionalization (most in the center-south and islands) and seasonality: greater consumpion occur during the religious festivals (Easter and Christmas) (Credazzi, 1999). About 70% of production is represented by lambs of dairy breeds, sloughtered at 25-40 days of age ("Agnello" and "Agnello da latte"), that are appreciate by italian consumers and have an high demand from the market. The 15% is represented by Merino lambs (mainly from Abruzzo, Molise, Puglia, Calabria and Campania) slaughtered at the age of about 70 days ("Agnellone"). The remaining 15% of production is represented by meat breeds lambs (Appenninica, Bergamasco etc., widespread in north-central Italy) slaughtered at the age of about 100 days (Massi, 1992).

1.2.1 Italian meat breeds

The meat sheep breeds reared in Italy represent a limited percentage. The most common are: Appenninica, Bergamasca, Fabrianese, Laticauda, Merinizzata Italiana, Sopravissana, Gentile di Puglia and Biellese.

Italian breeds, in their ethnographic structure, currently consist of:

- selection nuclei, that consists on groups registered in the herd books;
- production and multiplication flocks used for slaughter, not in the selection and attributable to the breed;
- crossbreeds used for the slaughter (UNAPOC, 1992).

Chapter 2
ATTRIBUTES OF LAMB MEAT QUALITY

2.1 Introduction

Meat is an important edible *post mortem* constituent originating from the live animals that are used as food by the humans. The term meat is generally used in commerce in a more restrictive sense- the flesh of mammalian species (such as cattle, pigs, lambs) raised and prepared for human consumption, except fish, poultry, and certain other animals (Sun and Holley, 2012). Meat and meat products are important component of human diet due to their chemical composition, they are a sources of protein, fat, essential amino acids, minerals and vitamin and other nutrients.

In recent years, the consumer demands meat and meat products with reduced level of fat, cholesterol, and a decreased contents of sodium chloride and nitrite; in addition improved composition of fatty acid profile and incorporated health enhancing ingredients are rapidly increasing worldwide (Zhang et al., 2010). Considering this fact, it is important to highlight that according to the present state of knowledge, lamb is considered to be the daintiest, most easily digestible meat, with high nutritional value and outstanding pro-health values. Increasing requirements can be met by the meat obtained from light lambs (13-30 kg), which is well muscled, low-fat and yields high-quality meat (reviewed by Brzostowski and Tański, 2006).

In Italy, lamb meat is the first meat frequently recommended by many pediatricians. This is related to the presumed lower allergenicity of this meat compared with other types of meat. Moreover, there is some evidence that the introduction of lamb in the diet of children might have positive effects in the treatment of Short Bowel Syndrome and Sandifer Syndrome. The use of lamb meat in the diet of children with atopic dermatitis and multiple food hypersensitivities has also been associated with significant clinical improvement in the severity of the eczematous lesions (reviewed by Nudda et al., 2011).

Meat quality is a term used to describe a range of attributes of meat. It includes attributes such as carcass composition and conformation, the eating quality of the meat,

health issues associated with meat such as *Escherichia coli* 0157 contamination and bovine spongiform encaphalopathy, and production-related issues including animal welfare and environmental impact. These factors combine to give an overall assessment of meat quality by the ultimate arbiter, the consumer (Maltin et al., 2003). For the consumer, quality includes a series of characteristics which make the cooked meat edible, attractive, and nutritious. It is, therefore, more appropriate to speak about the "quality that the consumer perceives" (Beriain et al., 2000).

Quality or "fitness for purpose" is necessary for economic competition, and no production problem can be undertaken without considering this crucial aspect (Alcalde and Negueruela, 2001). Moreover, increased consumer awareness and demand about quality products in the recent past have urged the food manufacturers to produce homogeneous and high quality products. Similarly the meat quality has become an area of great importance and concern in the recent years (Anwer et al., 2013).

2.2 Chemical composition and nutritional aspect of lamb meat

2.2.1 Proteins

There are different ways to define meat quality and various compounds may be employed as indicators of its quality. Progress observed in biological sciences, especially in proteomics, indicates that proteins building meat structures as well as (Pośpiech et al., 2007) play important role in improving meat quality.

Protein is essential for growth and development providing the body with energy and needed for the manufacture of hormones, antibodies, enzymes and tissues. It also helps to maintain the proper acid-alkali balance in the body (Crăciun et al., 2012). Raw red meat contains around 20-25g protein/100g. This protein is highly digestible, around 94% compared to the digestibility of 78% in beans (Wiliams, 2007).

Muscle proteins are categorized as sarcoplasmic, myofibrillar, and stromal proteins based on their solubility. Sarcoplasmic proteins are extracted in aqueous solution with low ionic strength (0.15 or less). Myofibrillar proteins are extracted by salt solutions and require higher ionic strength, called salt-soluble proteins. Stromal proteins include proteins of connective tissues, which are very fibrous and insoluble. Of insoluble proteins, collagen is composed about 0.5 proportions, elastin is about 0.03,

and the remaining 0.47, is a mixture of various proteins such as reticulin (Thu et al., 2006).

The nutritional value of meat is determined not only by the amount of protein contained in it, but also by its biological value, which is most affected by amino acid composition. The nutritional value or quality of structurally different proteins, varies and is governed by amino acid composition, ratios of essential amino acids, susceptibility to hydrolysis during digestion, source and the effects of processing (Crăciun et al., 2012). Amino acids consumed together with meat, are used to synthesize new proteins and take part to the synthesis of many biologically active compounds (Brzostowski and Tański, 2006). According to the importance in human nutrition the amino acids are divided into essential: valine (Val), leucine (Leu), isoleucine (Ile), threonine (Thr), methionine (Met), lysine (Lys), phenylalanine (Phe) and tryptophan (Trp), semi-essential: arginine (Arg) and histidine (His) and non-essential ones: tyrosine (Tyr), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), cysteine (Cys) (Jandasek et al., 2003). Failure to obtain enough of even one of the ten essential amino acids, result in degradation of the body proteins (muscle and other tissues) to counteract the imbalance (Crăciun et al., 2012).

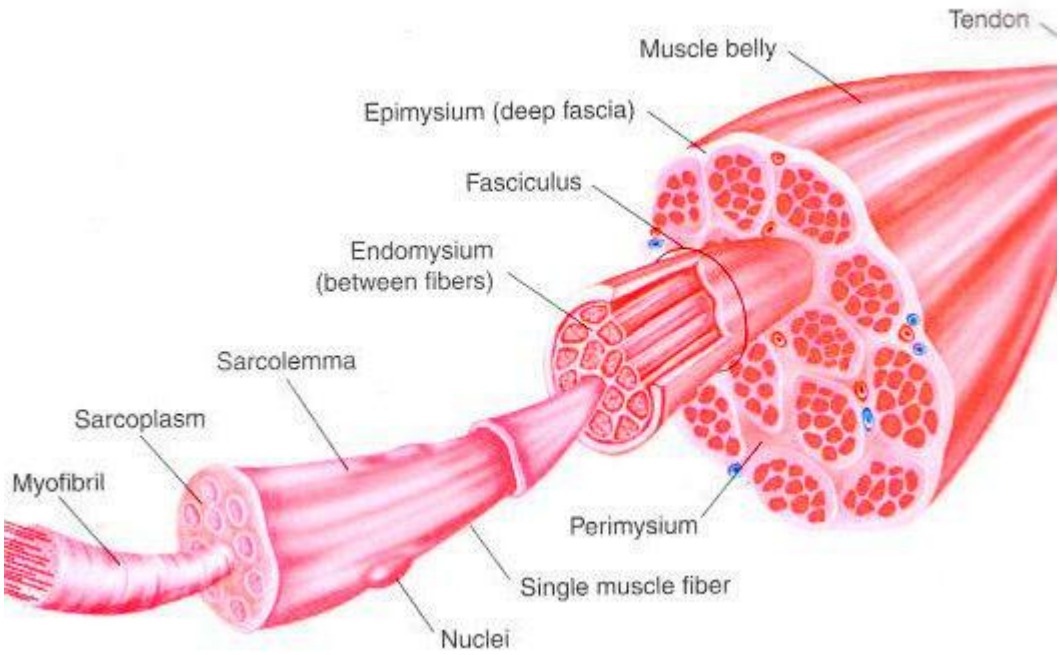
Differentiation of the amino acid composition of meat proteins depending on the lamb genotype, was observed by Jandasek et al. (2003) and Brzostowski and Tański (2006). Except genotype, also age and muscle region affect amino acid composition. In study of Crăciun et al. (2012), the age showed highly significant effects for essential amino acids: isoleucine, tryptophan and histidine and more significant effect for threonine. They observed also that the muscle region had very significant effects for histidine and tryptophan and significant for threonine. Löest et al. (1997) noted that mutton merino lamb carcass essential amino acid composition (g/100g protein) was as follows: 6.94 arginine, 2.61 histidine, 3.19 isoleucine, 7.19 leucine, 7.03 lysine, 2.08 methionine, 4.15 phenuloalanine, 3.79 threonine, 4.28 valine.

2.2.1.1 Collagen

Intramuscular connective tissue is an important constituent when the physical properties of meat are considered, although the amount of collagen and elastin in muscles is low. Both proteins turn over slowly in tissues, and are therefore susceptible

to age-related changes (Bailey, 2001). The connective tissue exists in the muscle as: (1) endomysium- surrounding each muscle fibre, connected with the sarcolemma, which is a continuous structure along the fibre and makes a significant contribution to the mechanical integrity of the muscle; (2) perimysium- surrounding bundles of muscle fibres, is the major part of the intramuscular connective tissue, containing more than 90% of intramuscular collagen and (3) epimysium- surrounding the outside of the muscle (Palka, 1999) (Figure 2.1).

Figure 2.1. Gross composition of muscle indicating epimysium, perimysium and endomysium



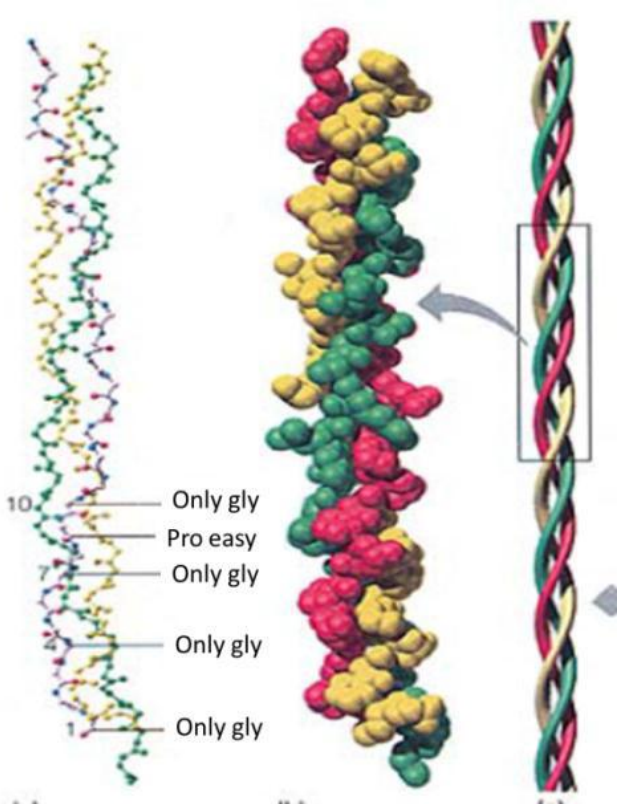
Source: http://panamericanscience10.blogspot.it

The biological diversity of collagenous tissues, can be accounted by the family of collagen molecules, which are, to some extent, tissue-specific (Bailey, 2001). Collagen, the most abundant mammalian and avian protein, is a connective tissue constituent that is present in all tissues. The 19 types of collagen characterized so far are divided into two major structural groups by reference to their ability to associate into macromolecular fibrils, *i.e.* fibrillar and non-fibrillar collagens (Pihlajamaa, 2000). The fibrous collagens are those that self-assemble to form a characteristic band pattern; they include Types I, II, III, V, and XI. Muscle, as well as most tissues, contains a combination of collagen types. Types I and III are the major components of

intramuscular collagen and of the greatest relevance when discussing meat texture. Type IV collagen molecules are the only members of the non-fibrous category found in muscle. Filamentous collagens include the minor types, such as VI and VII. The role that the minor types play in meat texture is unclear (Weston et al., 2002).

The basic structural unit of collagen is tropocollagen, a long, thin molecule with a molecular weight of 300,000 and a length of 280 nm. Each tropocollagen molecule is made up of three polypeptide subunits, called α-chains (Weston et al., 2002), which associate via hydrogen bond to form a superhelix. Both I and III phenotypes possess a large, central triple helical domain consisting of a repeating (GLY-X-Y) triplet and small, nonhelical regions at the carboxyl and amino termini called telopeptides. Interchain hydrogen bonding is enhanced by the large proportions of glycine, proline, alanine, and hydroxyproline amino acids and constitutional water present in collagen α-chains (reviewed by McCormic, 1999) (Figure 2.2).

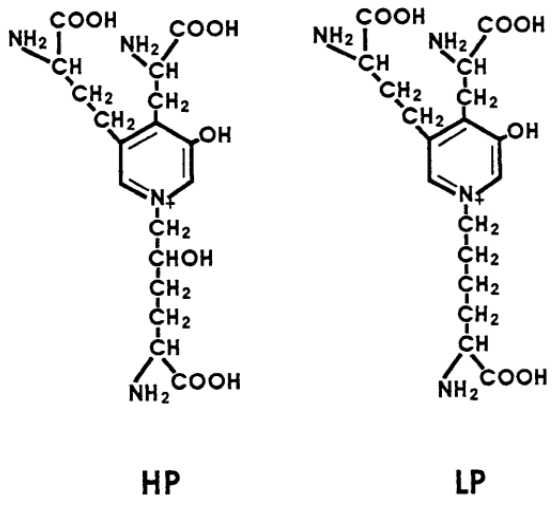
Figure 2.2. Collagen molecule and its organization



Source: https://chempolymerproject.wikispaces.com

Collagen molecules are free to slide past one another and the immature fiber is subject to proteolysis and disruption experimentally by variations in ionic strength or temperature. The high tensile strength of the mature fibril and, thus, its functionality is conferred largely by intermolecular crosslinks (reviewed by McCormic and Thomas, 1998). Two types of crosslinks are known to occur: those intramolecularly within the tropocollagen molecule and those intermolecularly among molecules (Shimokomaki et al., 1972). There are two major pathways by which enzymemediated lysine-aldehyde crosslinks form in Type I and III collagen. The allysine pathway produces aldimine crosslinks formed from lysine aldehydes; the hydroxyallysine path results in ketoamine crosslinks arising from hydroxyaldehydes. The initial condensation products form reducible crosslinks because they contain Schiff base double bonds, which can be reduced. These reducible crosslinks are divalent, capable of linking only two collagen molecules together. Both crosslinks vary in their stability, with ketoamine crosslinks being less heat labile than aldimine. Furthermore, the reducible crosslinks occur transiently and in many tissues can be considered as intermediate products. Divalent crosslinks tend to disappear from many tissues with time and are replaced by mature, nonreducible crosslinks. The crosslinking pathway that apparently predominates in skeletal muscle as well as myocardium is hydroxyallysine based. The known mature crosslinks on the hydroxyallysine pathway are trivalent, hydroxylysylpyridinium (HP), and lysyl pyridinium residues, with the latter present in negligible amounts in most tissues except bone (Figure 2.3).

Figure 2.3. Pyridinium crosslinks. Hydroxylysylpyridinoline (HP) and its dehydroxy analog lysylpyridinoline (LP) (adopted from McCormick, 1999)



Hydroxylysylpyridinium is almost certainly formed in a precursor-product manner from the condensation of two reducible ketoamine crosslinks, a mechanism that is confirmed by the stoichiometric relationship between the disappearance of the reducible crosslink and the accumulation of the trivalent crosslink in tissues. The progression of crosslinks from divalent to trivalent forms is significant because multivalent crosslinks can link adjacent fibrils as well as individual molecules together, thus markedly increasing the strength of the IMCT matrix. Furthermore, HP crosslinks are heat stable; their introduction into muscle collagen would be expected to increase force development (shrinkage) upon denaturation and extend both time and ultimate temperature required for gelatinization of collagen to occur. The progressive nature of crosslink biosynthesis does not mean that there is always a steady, irreversible shift of immature to mature forms. Although there is generally an increase in mature IMCT crosslinks with chronological age, it is also clear that the rate of crosslink formation and directional shifts in the concentration of mature crosslinks, regardless of age, can be altered. Furthermore, wide differences in crosslink type and concentration occur between different tissues and muscle types.

In higher quality muscles of domestic animals of market age, hydroxylysylpyridinium concentrations range from somewhat less than 0.20 to somewhat more than 0.35 mol HP/mol of collagen (reviewed by McCormick, 1999).

2.2.2 Lipids

The lipids are important part of chemical composition. Lipids provide the medium for the absorption of fat-soluble vitamins; are a primary contributor to the palatability of food; and are crucial to proper development and survival during the early stages of life-embryonic development and early growth after birth (FAO, 2008). Lipid content of muscle varies from 1.5% to 13%. Most of lipids are present in fat which can be present as intermuscular fat (between the muscles), intramuscular fat (or marbling, i.e., within the muscles) and subcutaneous fat (under the skin) (Moloney et al., 2002). Significant differences in the composition and different location of the mentioned fat tissues in meat, cause that they have a different meaning for culinary and dietetic quality of the meat. Quantitative proportions of the particular types of fat tissues in the body of animals and lipid profile are subject to big changes with time (age and physiological

state of animals, season of the year, etc.) as well as in relation to genetic and numerous environmental factors (Borys et al., 2012). Most of the lipids are present as glycerol esters, but cholesterol, phospholipids and other fatty acid esters are also present (Moloney et al., 2002).

2.2.2.1 Fatty acids

Fatty acids, especially long-chain polyunsaturated fatty acids, play key role for optimal food quality. Interest in meat fatty acid composition stems mainly from the need to find ways to produce healthier meat. Fatty acids represent 30–35% of total energy intake in many industrial countries and the most important dietary sources of fatty acids are vegetable oils, dairy products, meat products, grain and fatty fish or fish oils (Rustan and Drevon, 2005).

Fatty acids are almost entirely straight chain aliphatic carboxylic acids. The broadest definition includes all chain lengths, but most natural fatty acids are C4 to C22. Naturally occurring fatty acids share a common biosynthesis. The chain is built from two carbon units, and cis double bonds are inserted by desaturase enzymes at specific positions relative to the carboxyl group. This results in even-chain-length fatty acids with a characteristic pattern of methylene interrupted by cis double bonds (Scrimgeour, 2005).

Have been recognized three kind of fatty acids: SFA (saturated fatty acid), MUFA (monounsaturated fatty acid) and PUFA (Polyunsaturated fatty acids). That grouping of fatty acids is based on chemical classifications, but it is clear that individual fatty acids within these groups have distinct biological properties (Rustan and Drevon, 2005).

Saturated fatty acids are ‘filled’ (saturated) with hydrogen. Most saturated fatty acids are straight hydrocarbon chains with an even number of carbon atoms. The most common fatty acids contain 12–22 carbon atoms. Monounsaturated fatty acids have one carbon–carbon double bond, which can occur in different positions. The most common monoenes (alkene with single double bond) have a chain length of 16–22 and a double bond with the cis configuration. This means that the hydrogen atoms on either side of the double bond are oriented in the same direction. Trans isomers may be produced during industrial processing (hydrogenation) of unsaturated oils and in the

gastrointestinal tract of ruminants. The presence of a double bond causes restriction in the mobility of the acyl chain at that point. The cis configuration gives a kink in the molecular shape and cis fatty acids are thermodynamically less stable than the trans forms. The cis fatty acids have lower melting points than the trans fatty acids or their saturated counterparts. In polyunsaturated fatty acids (PUFAs) the first double bond may be found between the third and the fourth carbon atom from the ω carbon; these are called ω-3 fatty acids. If the first double bond is between the sixth and seventh carbon atom, then they are called ω-6 fatty acids. The double bonds in PUFAs are separated from each other by a methylene grouping (Rustan and Drevon, 2005).

Fatty acids are poorly soluble in water in their undissociated (acidic) form, whereas they are relatively hydrophilic as potassium or sodium salts. Thus, the actual water solubility, particularly of longer-chain acids, is often very difficult to determine since it is markedly influenced by pH, and also because fatty acids have a tendency to associate, leading to the formation of monolayers or micelles. The formation of micelles in aqueous solutions of lipids is associated with very rapid changes in physical properties over a limited range of concentration.

Fatty acids are easily extracted with nonpolar solvents from solutions or suspensions by lowering the pH to form the uncharged carboxyl group. In contrast, raising the pH increases water solubility through the formation of alkali metal salts, which are familiar as soaps. Moreover, the influence of a fatty acids structure on its melting point so branched chains and cis double bonds will lower the melting point compared with that of equivalent saturated chains. In addition, the melting point of a fatty acid depends on whether the chain is even- or odd-numbered; the latter have higher melting points (Rustan and Drevon, 2005).

Considering the fatty acids, n-3 PUFA is worthy of for the special attention. The term n-3 indicates that, counting from the methyl (CH_3) end of the molecule, the first double bond is located between the third and fourth carbons. As the degree of unsaturation in fatty acids increases, the melting point decreases conferring more fluidity to n-3 PUFA (Ruxton et al., 2004). Western diets are typically low in n-3 fatty acids, and high in saturated and n-6 fatty acids. This imbalance, developed during the 20^{th} century with the introduction of processed foods, grain fattened livestock, and hydrogenated vegetable fats, all of which dramatically reduced n-3 fatty acid intake. This situation worsened with increased intake of n-6 fatty acids (Azcona et al., 2008).

Omega-6 fatty acids account for the majority of polyunsaturated fatty acids (PUFA) in the food supply. When diets are supplemented with n-3 fatty acids, the latter partially replace the n-6 fatty acids in the membranes of almost all types of cells (i.e., erythrocytes, platelets, endothelial cells, monocytes, lymphocytes, granulocytes, neuronal cells, fibroblasts, retinal cells, hepatic cells and neuroblastoma cells). Competition between the omega-6 and omega-3 fatty acids occurs in prostaglandin formation. Eicosapentaenoic acid (EPA), an omega-3 fatty acid, competes with arachidonic acid (AA), an omega-6 fatty acid, for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level (Simopoulos, 2002).

Omega-3 and omega-6 recognized as beneficial to human health can be found in the composition of sheep meat at levels of up to 72% of the desirable fatty acids. However, high concentrations of lipids and saturated fatty acids classify red meat as one of the main factors responsible for increased plasma cholesterol, which may incur cardiovascular diseases and atherosclerosis (Silva Sobrinho et al., 2014). Polyunsaturated fatty acids concentrations in ruminant meat are low because of biohydrogenation in the rumen (Matsushita et al., 2010). Furthermore, a low ratio of PUFA/SFA (P:S), have been found in lamb meat. The recommended P:S ratio in a normal human diet is 0.4 however, lamb typically has a ratio of 0.1. Another important nutritional index affecting cardiovascular and other diseases is the ratio n-6-3/PUFA. In this case, a ratio below 4.0 is considered optimal for human health, and lamb is well placed with an average ratio of 1–2. For these reasons, researchers have searched for ways to change the fatty acid composition in lamb, specifically to reduce concentrations of SFA and increase n-3 PUFA (Nute et al., 2007).

Like most animal production traits, fatty acid composition is influenced by both genetic and environmental factors. Results of numerous studies confirm that fatty acid composition can be influenced by individual factors such as diet (Silva Sobrinho et al., 2014), breed (Fisher et al., 2000), weight (Matsushita et al., 2010), and level of fatness (Nürnberg et al., 1998).

Among the environmental factors, diet of animals plays an important role in the determination of meat fatty acid profile. Animal nutrition plays an important role due to its regulatory effect on biological processes in muscle that are reflected in the quality of meat (Andersen et al., 2005). It has been reported that to improve the nutritional quality of ruminant meat for the consumer, lipid supplementation of animal diets with omega-3

(n-3) PUFA, is an effective feed strategy to increase the proportion of PUFA in meat (Díaz et al., 2011). Elmore et al. (2000) noted that when linseed and fish oils were fed as supplements to Suffolk and Soay lambs, increased levels of n-3 PUFAs were found in the phospholipids. Bruised whole linseed doubled the amount of C18:3 n-3 and the diet containing fish oil caused a two to fourfold increase of EPA and DHA. These changes in the fatty acid composition of the meat, resulted in changes in the volatile composition of pressure-cooked muscle, particularly in the animals fed on diets containing fish oil, where increases in a number of volatiles formed from the oxidation of n-3 PUFAs were observed. For example 2-(2-pentenyl) furan, 2-ethylfuran and l-penten-3-ol were present in the meat from animals fed on diet containing fish oil at levels 3 times higher than in the meat of the animals fed on a control diet.

However, lamb carcasses and meat quality may vary according to the production system, which is connected with feeding system. The main farming, and consequently feeding, systems practiced for lamb meat production, are the extensive (grazing) and the intensive (indoor), with a great variation between those two. In the extensive grazing systems lambs and dams are continuously stocked on a permanent pasture with no concentrate offered to dams or to lambs. Lambs suckle their mothers and graze until slaughter. In the intensive indoor system, lambs are housed indoors and dams graze without their offspring and receive supplementary feed (roughages and/or concentrate). Lambs are fed on concentrate and hay of higher quality ad libitum, plus milk, until slaughter. It is important to highlight that small ruminants are the most efficient transformers of low quality forage into high quality animal products with distinguished chemical composition and organoleptic characteristics (Zervas and Tsiplakou, 2011).

The most important finding is, favourable fatty acid composition of lamb meat, rearing on pasture with beneficial effect to human health. Feeding systems can play a significant role in improving meat quality, as the changes in fatty acid composition of body fats are primarily linked to the respective fatty acid contents in the diet. Despite hydrogenation process in the rumen, it has been shown, that pasture feeding increases the concentration of n-3 PUFA fatty acids, compared to grain feeding (Enser et al., 1998). Cividini et al. (2008) reported that meat from lambs reared on pasture, contain less saturated fatty acids, less mono unsaturated fatty acids and higher proportion of polyunsaturated fatty acids especial α-linolenic (C18:3) acid and consecutively more EPA. Rowe et al. (1999) compared fatty acid composition in intramuscular fat of LD in

ram lambs slaughtered at 30 kg feeding in stable or grazing on pasture and observed a significant higher content of C20:4 n-6 and lower content of oleic fatty acid in lambs from pasture. Popova (2007) found that lipids of grazing lambs contained relatively more linolenic acid with a lower C18:2n-6/C18:3n-3 ratio, than the concentrate fed animals. Kosulwat et al. (2003) reported that fatty acid composition of lamb meat is affected by increase in fatness. They reported that the levels of MUFA, palmitoleic and oleic tended to rise as fatness score increase and levels of C18:2n-6 and C18:3n-3 tended to decrease as fatness score rose.

Fatty acids are involved in various ‘‘technological’’ aspects of meat quality including the firmness of fat and the colour, lipid stability and flavour of the meat. Because they have very different melting points, variation in fatty acid composition has an important effect on firmness or softness of the fat in meat, especially the subcutaneous and intermuscular but also the intramuscular fat (marbling). Groups of fat cells containing solidified fat with a high melting point, appear whiter than when liquid fat with a lower melting point is present, so fat colour, is another aspect of quality influenced by fatty acids. The ability of unsaturated fatty acids, especially those with more than two double bonds, to rapidly oxidise, is important in regulating the shelf-life of meat (Wood et al., 2003).

The effect of fatty acid profile on mentioned meat quality traits, has been described by Nute et al. (2007). They investigated the influence of five sources of dietary oil ((1) linseed oil, (2) fish oil, (3) a protected lipid supplement (PLS, 18:2 to 18:3 ratio 3:1), (3) fish oil : marine algae, (4) PLS : marine algae) on the colour and lipid stability of lamb muscle and the flavour of grilled loin chops. In this study it has been observed that lamb fed on dietary containing linseed oil, produced the highest proportion of 18:3n-3 in muscle phospholipid, the highest ratings for lamb flavour intensity and overall liking and the lowest ratings for abnormal flavour intensity. Moreover PLS increased the proportion of 18:2n-6 which reduced lamb flavour intensity and increased abnormal lamb flavour intensity. Diets containing fish oil or marine algae, increased proportions of the longer chain n-3 fatty acids and similar reduced ratings for lamb flavour as the PLS diet. Lambs fed up with marine algae, fish oil and their combination, produced meat that was oxidatively less stable and had a reduced colour and lipid oxidative shelf-life, which was at least partially due to the lower vitamin E content of the muscle.

2.2.2.2 Cholesterol

The high intake of SFA, is associated with a high level of serum cholesterol (Kang et al., 2005) which is a waxy, fat-like substance, found in the organism of all animals. It is an essential part of cells in the body and is used to make certain hormones and digest fats. The two major blood cholesterol carriers are LDL (low density lipoprotein) and HDL (high density lipoprotein) (Bellows and Moore, 2012).

Cholesterol is synthesized from acetyl-CoA via the isoprenoid pathway, and at least four enzymes in the biosynthetic pathway are regulated by cellular cholesterol levels. Essential non-steroidal isoprenoids, such as dolichol, prenylated proteins, heine A and isopentenyl adenosine containing tRNAs are also synthesized by this pathway. In extrahepatic tissues, most cellular cholesterol, is derived from de novo synthesis, whereas hepatocytes obtain most of their cholesterol, via the receptor-mediated uptake of plasma lipoproteins, such as LDL. LDL is bound and internalized by the LDL receptor and delivered to the acidic late endosomes and lysosomes, where hydrolysis of the core cholesteryl esters occurs. The cholesterol that is released, is transported throughout the cell. Normal mammalian cells tightly regulate cholesterol synthesis and LDL uptake to maintain cellular cholesterol levels within narrow limits and supply sufficient isoprenoids to satisfy metabolic requirements of the cell. Regulation of cholesterol biosynthetic enzymes takes place at the level of gene transcription, mRNA stability, translation, enzyme phosphorylation and enzyme degradation. Cellular cholesterol levels are also modulated by a cycle of cholesterol esterification by acyl-CoA : cholesterol acyltransferase (ACAT) and hydrolysis of the cholesteryl esters, and by cholesterol metabolism to bile acids and oxysterols (Liscum, 2002).

Alimentary intake of mono- and polyunsaturated fatty acids, especially of n-6 and n-3 group has positive effects on free cholesterol level in blood (Živković et al., 2002). It has been shown that one type of monounsaturated fatty acid, the *trans* fatty acids (such as t-18: 1), raises cholesterol concentrations relative to *cis* fatty acids (such as 18: 1). *Trans* monounsaturated fatty acids apparently raise LDL-cholesterol concentrations about two-thirds as much as does palmitic acid, and they may have a small HDL lowering action as well (Grundy, 1997).

Some workers have used carbohydrate instead of oleic acid as the neutral nutrient. It is true that carbohydrates and oleic acid have similar effects on total

cholesterol concentrations. Carbohydrates seem to affect lipoprotein metabolism entirely differently from fats. They enhance VLDL concentrations by enriching VLDL particles with triacylglycerol. They reduce the LDL-cholesterol concentration by reducing the cholesterol content of LDL particles, not by reducing the number of circulating LDL particles (30) as dietary oleic acid does. Furthermore, carbohydrates reduce HDL-cholesterol concentrations whereas oleic acid does not (Grundy, 1997).

The cholesterol content of the food products especially from animal source, becomes the prime area of consumers concern, because of the awareness on higher dietary cholesterol and the incidence of coronary disease (Duraisamy et al., 2013). Reducing the amount of cholesterol in meat, which is considered to be a hazardous factor in atherosclerosis, is a challenge for animal husbandry. Compared to the meat of other animal species, the meat of lambs, particularly that obtained from crossbreeds, is considered to be a low cholesterol product. Thus, by the appropriate selection of sheep breeds for crossing, the level of cholesterol in meat can be controlled (Brzostowski and Tański, 2006).

2.2.3 Vitamin and mineral content

Similary to other animal foods, red meat is an excellent source of bioavailable vitamin B12, providing over two thirds of the daily requirement in a 100g serve. Up to 25% RDI of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100g of red meat, but compared to pork it is a relatively poor source of thiamin. Liver is an excellent source of vitamin A and folate, but the levels in lean meat tissue are low. Older animals tend to have higher concentrations of all of these vitamins, so their levels in beef, are generally higher than those in veal, and mutton more than lamb (Dell'Orto, 2000). Levels of vitamin D in meat are low and difficult to measure and have often not been included in food composition data previously. However, recent assays on meat in New Zealand, have reported levels of 0.10μg Vitamin D3/100g and 0.45μg 25-OHD3/100g in beef and levels of 0.04 and 0.93 μg/100g respectively in lamb. Given the higher biological activity of the 25-OH vitamin D, this means that 100g of cooked beef could provide 12% of the estimated adequate intake of 10μg/d for a 51-70 year old, and cooked lamb could provide more than 25%, and hence be an important source of this nutrient, especially for housebound elderly people.(Williams, 2007). Beef and lamb

meat are among the richest sources of the minerals iron and zinc, with 100g, providing at least one quarter of daily adult requirements. The iron in meat is mostly hem-iron which is well absorbed, and meat protein also appears to enhance the absorption of iron from meat. Similarly, absorption of zinc from a diet with high content in animal protein is greater than from plant foods, and the requirements for zinc may be as much as 50% higher for vegetarians. Red meats are also good sources of selenium, providing over 20% RDI per 100g serve, although it is likely that selenium values in meat will be significantly affected by where animals feed and the time of the year of sampling. Lean meat is low in sodium with a potassium/sodium ratio of greater than five. The copper content in raw lean cuts range from 0.055 to 0.190 mg/100g in beef and veal, 0.090 to 0.140 mg/100g in lamb, and 0.190 to 0.240 mg/100g in mutton, all significantly higher than values reported in British meat (reviwed by Williams, 2007).

2.3 Technological properties of meat

Generally consumers decide to purchase meat based on its nutritional value and appearance. However, its technological properties are essential quality parameters for both the industry and the consumer. It can be said that appearance and technological characteristics are connected. Two of the most used measurements, water-holding capacity and pH will be discussed in detail in the following sections

2.3.1 Water Holding Capacity

Water is the main component of meat accounting for approximately 75% of its weight (Borisova and Oreshkin, 1992). The water in muscle has many different forms including (1) bound water, (2) entrapped water, and (3) free water. Bound water is water that exists in the vicinity of non-aqueous constituents and has reduced mobility. This water is very resistant to freezing and to being driven off by conventional heating. Meanwhile, entrapped (also called as immobilized) water is held within the structure of the muscle but is not bound per se to protein. In early *post mortem* tissue, this water does not flow freely from the tissue, yet it can be removed by drying, and can be easily converted to ice during freezing. Entrapped water is most affected by the rigor process

and the conversion of muscle to meat. The last form of water in muscles is free water whose flow from the tissue is unimpeded. Free water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found (reviewed by Huff Lonergan and Lonergan, 2005).

Fluid lost from fresh meat through passive exudation is referred to as muscle exudate or drip. The amount of muscle exudate is an indicator of water-holding capacity (WHC), which refers to the ability of the uncooked meat to retain its inherent or added water through *post mortem* processing and storage (Honikel and Hamm, 1994). The ability of fresh meat to retain water is arguably one of the most important quality characteristics of raw products. For the meat industry, low WHC implies increased economical losses, and consequently there is a strong interest in optimizing this parameter (Maribo et al., 1998; Warris, 1982). Moreover, the WHC of fresh meat is known to influence its technological quality, for example, processing yield. For the consumer, low WHC has a detrimental impact on the appearance of fresh meat cuts during retail (Kauffman et al., 1978; Smith and Lesser, 1982), and may influence the sensory quality of the meat. Poor WHC meat, may be drier or its taste may be negatively affected (Gunenc, 2007).

A number of pre-and *post mortem* factors influence the WHC of meat. During the growth and development of meat animals, genotype and animal diet are important due to their direct influence on muscle characteristics. In the immediate pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC. In the post-slaughter period, chilling, ageing, injecting non-meat ingredients, as well as tumbling, have important influences on WHC. Furthermore, cooking and cooling procedures for the final meat products can also affect the WHC of the product, in particular the cooking and the cooling methods, the heating and the cooling rate, the cooking temperature, and the endpoint temperature (reviewed by Cheng and Sun, 2008). All these factors affect the rate and progress of several biochemical and physical processes, taking place during the conversion of muscle to meat, which are believed to influence the distribution of water within the muscle (Schäfer et al., 2002).

There are many available methods that have been used to measure WHC. Unlike colour, WHC is not definable in absolute units since each method measures slightly different things. A variety of different methods can be found in Table 2.1.

Table 2.1. Different methods of drip loss measurements and water holding capacity of meat (adopted from Borchers et al., 2007).

Methods	**References**
Filterpaper-press method	Grau and Hamm, 1953
Loose bound water	Beutling, 1969
Capillary volumeter	Hofmann, 1975
Tray method	Lundström and Malmfors, 1985
Filterpaper method	Kauffman et al., 1986
Bag method	Honikel, 1987
Centrifugation methods	Honikel and Hamm, 1994
EZ-DripLoss method	Rasmussen and Andersson, 1996
Absorptive material	Walukonis et al., 2002

2.3.2 pH

One of the major factor that influence protein behavior in fresh and processed meat is the pH of the product. Muscle pH is considered to be a measure of *post mortem* muscle metabolism, and as a result can be used as an immediate indicator of the stage of post mortem glycolysis (Newbold and Small, 1985). pH is defined as the negative log of the hydrogen ion concentration. After killing the animal, pH of muscles decreases, until it reaches levels of 5.3–5.6 (depending on species and muscle), usually correlating with muscle glycogen concentrations of about 45–55 mmol/kg wet tissue (Gardner et al., 2005).

Muscle glycogen concentration at the time of slaughter, is one important factor influencing ultimate pH (Rosenvold et al., 2001). After the death of an animal when oxygen is no longer available, the *post mortem* breakdown of muscle glycogen yields lactic acid, the accumulation of which contributes to the change in meat pH (Przybylski et al., 2006). About 45 mmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5 (Immonen et al., 2000). Two enzymes are responsible for degradation of glycogen: glycogen phosphorylase and debranching enzyme (Przybylski et al., 2006). Controlled reduction of muscle glycogen stores might be a potential way to reduce the extent of the pH decline *post mortem*. Reduced glycogen stores, reduce the formation of

lactic acid during the post slaughter conversion of muscle to meat, and thus the extent of the pH fall (Rosenvold et al., 2001).

The concentration of glycogen which greatly varies at the time of slaughter, depends on numerous factors, such as the animal species, the sex of the animal, the type of fiber that is predominant, diet, exercise of animals and the level of stress of the animal in the period prior to slaughter (Lahucky et al., 1998; Scanga et al., 1998; Immonen et al., 2000; Hargreaves et al., 2004, Pösö and Puolanne, 2005). Acute stress in the animal before or during slaughter, results in elevated levels of stress hormones (e.g. cortisol, norepinephrine, and epinephrine) in the muscle. These stress hormones, increase the anaerobic muscle metabolism and lactic acid accumulation, and decrease muscle pH in muscle early *post mortem* (Cannon et al., 1995; Bee, 2006; Luciano et al., 2007). Minimizing factors which cause acute stress before slaughter can reduce the speed of *post mortem* metabolism. There are also post-slaughter factors that influence final pH, such as the packaging and the freezing speed of the meat (Moreno Grande et al., 1999; Hargreaves et al., 2004). It has been observed that, low temperature decreases the glycolytic process by lowering enzymatic activity reducing the pH fall (Joseph, 1996). A low pH can cause inhibition of proteolytic enzymes activity, denaturation of myofibrillar proteins and excessive shortening - and consequently lead to toughness and loss of water-holding capacity (Khan and Cowen, 1977).

The pH of meat is affected not only by the level of lactic acid but also by phosphoric acid and other acid. Phosphoric acid is formed during energy metabolism when ATP is degraded to ADP by the enzyme ATPase and inorganic phosphate, and energy is released. Phosphorylation of ADP results in regeneration of ATP. Creatine phosphate is needed for this regeneration. After depletion of creatine phosphate, ADP is degraded to AMP which is converted irreversibly to IMP by means of the enzyme AMP-deaminase (Mačanga et al., 2009).

The acidification of muscle affects the meat quality, among others, it decreases protein charges and increases their hydrophobicity, thereby reducing water retention. This is confirmed by the very high correlation observed between the increase in extracellular space and muscle pH (Guignot et al., 1993). The pH of meat has also a great impact on three sensory quality characteristics of muscle foods: appearance/colour, texture/tenderness, and flavour, all of which affect the consumer acceptance of meat (Min and Ahn, 2005). It has been noted that the state of the pigment

is largely dependent on the meat pH. At high pH, the iron of heme is predominantly in the ferrous state, and low pH accelerates conventional ferrous iron to the ferric state. It has been also reported, that the rate of autoxidation in oxymyoglobin rapidly increases with increasing hydrogen ion concentration. Moreover, the pH is the very important factor in heme-complex-forming reactions of Mb and hemoglobin, and the colour of the pigments (Ahn and Maurer, 1990). Additionally, the rate of tenderization is also related with pHu; it has been observed that high pHu meat tenderises more rapidly than low pHu meat during ageing (Dutson, 1983; Silva et al., 1999). Considering the effect of pH on meat flavour, it has been observed that at acidic pH, most powerful meat flavourous compounds, such as furans and its derivatives, and most of heterocyclic compound like pyroles, thiazoles pyranones etc., are produced in significant quantity which have antioxidant, anticarcenogenic and antimicrobial efficiency. Moreover, these compounds are responsible for the development of brown colour named as "browning", and have in turn significant antioxidant activity (Tanaka et al., 1988; Yilmaz and Toledo, 2005).

2.4 Sensory aspects of meat

Meat quality is defined by the combination of many factors; however, consumers give a special importance to sensory attributes. Inherent characteristics of animal, long and short-term environmental influences on animal and processing parameters that directly affect carcass or meat, are all factors that influence meat colour, tenderness, flavour and juiciness. The conformance of these attributes to consumers expectations is important and deviating from this, will affect the products marketability. Their importance for meat quality and factors affecting these attributes, are discussed in this section.

2.4.1 Colour of meat

Sensory properties of meat contribute significantly to the perception of quality and value, and this is especially true for the colour of meat (Faustman et al., 2010). Colour is the most important meat quality attribute at the point of sale, since consumers use it as an index of quality and freshness (Carpenter et al., 2001; O'Sullivan et al.,

2002). Data on colour stability of fresh meat, give an idea about possible consumer preferences based on the appearance of the product, but they do not necessarily indicate the safety, flavour, nutritional or functional aspects of the product (Zhu and Brewer, 1998)

Meat colour is measured subjectively or objectively. Subjective measures are generally taken in chillers by people accustomed to it. There are four main problems arising from this kind of measure: (1) the methods utilized sometimes differ from country to country; (2) there is a strong influence of the different lighting in chillers; (3) the subjective assessment is subject to bias; (4) often meat is not allowed to bloom. Objective measurements are generally taken using the CIE colour system. The three fundamental colour parameters are L*, a* and b*. All three values are required to completely describe an object's colour. L* is the lightness and is a measure of the light reflected (100 = all light reflected; 0 = all the light absorbed); a* (red vs. green, where a positive number indicates red and a negative number indicates green.) and b* (yellow vs. blue, where a positive number indicates yellow and a negative number indicates blue) are the other coordinates (Priolo et al., 2001). CIE colour system is commonly used by researchers to measure colour, however it has been also reported that tristimulus coordinates (XYZ) are also useful for measuring meat colour (Alcalde and Negueruela, 2001). Moreover, is worth of mention that currently, many instruments (colorimeters and spectrophotometers) are available for objective colour analysis (Mancini and Hunt, 2005).

The colour of meat is primarily greatly influenced by the chemical stability of meat pigment, myoglobin. Myoglobin is the main protein responsible for meat colour, although other heme proteins such as hemoglobin and cytochrome C may also play a role in beef, lamb, pork, and poultry colour. Myoglobin is a water-soluble protein containing 8 α-helices (A–H) linked by short nonhelical sections. Of the numerous residues in myoglobin, histidine has received the most attention because of its key role in myoglobin structure and function. Myoglobin also contains a prosthetic group located within the protein hydrophobic pocket. The heme ring has a centrally located iron atom that can form six bonds. Four of these bonds are with pyrrole nitrogens, while the 5^{th} one coordinates with the proximal histidine-93. A 6th site is available to reversibly bind ligands. A distal histidine-64 also influences colour dynamics by affecting space relations within the hydrophobic heme pocket. The ligand present and the valence of

iron dictate muscle colour. Therefore, four major chemical forms of myoglobin are primarily responsible for meat colour (Mancini and Hunt, 2005).

Reduced myoglobin is the purple pigment of deep muscle and of meat surface under vacuum. On exposure to air, myoglobin combines with oxygen to form bright red oxymyoglobin (MbO_2) which is thought to indicate freshness (or blooming) and considered attractive to the consumer. With time, discoloration results from conversion of oxymyoglobin to metmyoglobin which is brown and unattractive and the colour of meat, is due to a balance between oxymyoglobin oxidation and metmyoglobin reduction (reviewed by Gatellier et al., 2001).

Reduction of metmyoglobin is crucial to meat colour life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, which is limited in *post mortem* muscle. Unfortunately, both enzyme activity and the NADH pool are continually depleted as time postmortem progresses. Although it is vital for meat colour stability, *post mortem* replenishment of the NADH pool has received little attention (Mancini and Hunt, 2005).

Remarkable effects on meat colour, depend also on several individual factors (such as genotype, diet and age of the animal, the activity undertaken by the animal, pH of meat) and their interactions. Available literature showed that meat colour does vary among different strains of Merinos (Hopkins et al., 2005), this is possibly due to this breed susceptibility to dark cutting, which is caused by a high ultimate pH. Moreover, meat becomes darker and redder as lamb age increases (Warner et al., 2007), but meat colour can also influenced by nutrition. Animals fed on pasture have a yellow fat colour because of the high levels of beta-carotene contained by grass. This yellow fat colour, is measured objectively by b* coordinates. Consumers often perceive meat with yellow fat as meat coming from an old or diseased animal. In addition, forage-based rations, as well as different forage and seasonal changes, are allowed for carcasses with a darker lean appearance or fat that is yellow in appearance (Baublits et al., 2004). Additionally, the effect of ultimate pH on the colour of meat is widely known. It has been reported that poor nutrition and stress can reduce muscle glycogen content at slaughter, resulting in elevated pHu, which, when greater than pH 5.8–5.9, can result in dark, firm and dry meat (DFD) (Gardner et al., 2005).

2.4.2 Meat palatability

Palatability or eating quality of meat, can be defined by three characteristics: tenderness, flavour and juiciness. Their importance for meat quality and factors affecting these characteristics, are presented in the following section.

Tenderness is one of the most discussed features in meat. It is defined as the ease of mastication; however, it is a function of the collagen content, heat stability and the myofibrillar structure of a muscle (Ngambu et al., 2011). Variability in the meat tenderness is the most critical quality problem facing the meat industry. Variable tenderness is a limiting factor for product acceptability, causing a reduction in beef consumption. Tenderness and rate of tenderization depend on many intrinsic (species, type of muscle, and muscle location) and extrinsic factors (preslaughter stress, slaughter conditions, and post slaughter handling) of the animal and on their interaction. (Li et al., 2014). Moreover, It has been also found that tenderness of meat decreases with chronological age. Studies on the quality of raw and cooked lamb, as reported by Batcher et al. (1962), showed decreased tenderness of meat as age of animal increased when cuts from the rib-loin were used. Tenderness is influenced also by meat-related factors, for example content of lipids. There are several possible explanations for a positive effect of lipid on tenderness, including the location of neutral lipid in fat cells within the perimysium which could have a physical effect in separating muscle fibre bundles, beginning the process of tenderisation by ‘‘opening up the muscle structure’’ (Wood et al., 2003). Additionally, have been distinguished the three factors that determine meat tenderness which are: background toughness, the toughening phase and the tenderization phase. While background toughness exists at the time of slaughter and does not change during the storage period, the toughening and tenderization phases take place during the *post mortem* storage period (Koohmaraie and Geesink, 2006).

Meat tenderization process is primarily explained by two different theories. One explains this process as a result of μ-calpain action throughout the muscle cell. The other one characterizes tenderization as a more complex procedure, dependent on many enzymatic processes, among which μ- calpain activity called apoptosis (Luciano et al., 2007). Calpains are calcium-activated proteases consisting of at least three proteases. The two best-characterized isoforms of calpains are μ-calpain and m-calpain, which both degrade the same specific set of myofibrillar and cytoskeletal proteins that are

degraded as muscle is converted into meat (reviewed by Huff-Lonergan and Lonergan, 2005). It was reported that injection of calpain activator (calcium) in muscles accelerates *post mortem* proteolysis and tenderization (Wheeler et al., 1992). Further, it has been shown that activity levels of the calpain proteolytic system, particularly calpastatin, changes with animal age (Ou et al., 1991; Whipple and Koohmaraie, 1992; Northcutt et al., 1998). However, it must be remembered that other novel proteolytic systems, such as caspases, may contribute to *post mortem* proteolysis and meat tenderization. Moreover, tenderness is also related with cytoskeletal degradation processes of titin, nebulin and desmin (Huff-Lonergan et al., 1995, Steen et al., 1997, Koohmaraie and Geesink, 2006). Most research has been focused on determining the contribution of various myofibrillar proteins to meat tenderness. It has been consistently reported that tender meat has faster and more extensive degradation of desmin, troponin-T, nebulin, and titin, compared with tough meat. By monitoring the degradation of these proteins during ageing, researchers have attempted to determine a suitable ageing time to obtain acceptable tenderness (Pulford et al., 2009; Muroya et al., 2010).

A measure of tenderness is the subjective consumer appreciation of meat texture. On the other hand, an objective way to measure tenderness is the force required to shear a standardized piece of meat, with low shear values being desirable (Luciano et al., 2007).

Flavour is a very complex attribute of meat palatability. While it has been known for many years that tenderness plays a large role in acceptability of meat by a consumer, it has become increasingly apparent that flavour also needs to be addressed. Flavour was found to be the most important factor affecting consumers meat buying habits and preferences when tenderness was held constant (Calkins and Hodgen, 2007). Flavour is comprised of aroma (volatile) and taste (non volatile) compounds. Aroma is perceived during eating by olfactory receptors in the nose, and taste is perceived by receptors in the mouth and throat (Watkins et al., 2012).

The characteristic flavour of meat of a particular species is determined by the proportions of different fatty acids in the fat, and, in particular, by the unsaturated fatty acids, which are more susceptible to oxidation to volatile compounds of low molecular weight. Phospholipids, which are rich in polyunsaturated fatty acids, also play a fundamental role in the flavour of meat (Beriain et al., 2000). Sensory analysis showed

that flavour scores are significantly correlated with C14:l (myristoleic acid), C18:0 (stearic acid), C18:l (oleic acid) and C18:3 (α-linolenic acid) of the neutral lipids, with C18:3 of the polar lipid and with water soluble sugar content (Melton et al., 1982).

Additionally, it has been proved that meat flavour is thermally derived. The flavour of raw fresh meat is bland, metallic and slightly salty and only a blood-like taste, whereas desirable meat flavour is apparent only after heating (El-Ghorab et al., 2010). During cooking, a complex series of thermally induced reactions occur between non-volatile components of lean and fatty tissues resulting in a large number of reaction products. However the flavour of cooked meat is affected by compounds contributing to the sense of taste. It is the volatile compounds, formed during cooking, that determine the aroma attributes and mostly contribute to the characteristic flavours of meat. The pivotal reactions during cooking, which result in aroma volatiles, are the thermal degradation of lipid and the Maillard reaction (Mottram, 1998). The latter reaction, helps to explain amine and carbonyl reactions in food. In general, amino compounds condense with the carbonyl group of a reducing sugar in the presence of heat. This produces gylcosylamine which is rearranged and dehydrated to form furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds. All of these compounds contribute to flavour (Calkins and Hodgen, 2007).

A very unique aroma and flavour has a lamb meat, which is the subject of numerous reviews on the subject. Several compounds (branched-chain fatty acids, carbonyl compounds, sulfur-containing compounds, lipid oxidation products, phenols, and basic compounds) are believed to impact lamb flavour. It has been suggested the role of branched-chain fatty acids, 8 to 10 carbons, to lamb characteristic flavour. Lamb and goat fat contains branched-chain fatty acids, which are in much higher concentrations than in other ruminants. Branched-chain fatty acids are in higher concentrations especially when lambs are fed up on barley or diets containing propionate before harvest (reviewed by Duckett and Kuber, 2001).

The flavour of meat is subject to variability especially due to some extrinsic factors (e.g. nutrition) and it affects the overall acceptability of foods. These factors are of utmost importance because they influence the judgement of the consumer, even before the food is consumed (Jayathilakan et al., 2007). Diets probably have their greatest effect on flavour via changes in fatty acid composition which was proved by another research conducted by Sanudo et al. (2000) on British lambs fed up on grass,

and Spanish lambs fed uo pn milk and concentrates. In this study, differences in fatty acid composition were observed; the grass fed animals had higher muscle concentrations of n-3 PUFA and the concentrate-fed animals had higher concentrations of the n-6 PUFA. When the meat was evaluated by British and Spanish taste panels, both found that the British lamb (higher in n-3 PUFA) had a higher odour and flavour intensity, but whereas the British panel preferred the flavour and overall eating quality of the grass-fed lamb, the Spanish panel scored flavour liking and overall liking higher in the Spanish lamb. Also in the study of Fisher et al. (2000) carried out on Suffolk cross lambs, reared on lowland grass or on a standard concentrate diet, effect of nutrition on flavour of meat was observed. In grilled loin chops, taste panellists gave higher scores for lamb flavour and overall liking to the grass group and higher scores for abnormal lamb flavour, metallic, bitter and rancid to the concentrate group.

Juiciness is an important contributor of eating quality for meat and also plays a key role in meat texture contributing between 10% and 40% to its variability (Winger and Hagyard, 1994). Meat juiciness is considered to arise from moisture released by meat during chewing and moisture from saliva. Juiciness is influenced primarily by meat-related factors (Juarez et al., 2012). Eikelenboom et al. (1996a) and Eikelenboom et al. (1996b) showed that juiciness is correlated both to pHu and IMF. The total fatty acid content of muscle (i.e. neutral lipid plus phospholipid fatty acids), termed marbling fat, has long been recognised as a factor in eating quality, especially juiciness (Wood et al., 2003). The juiciness of the meat after cooking is also affected by the WHC (Gunenc, 2007). Moreover, the concentration of glycogen could influence the juiciness as an increased concentration of glycogen will increase the juiciness in beef with a normal pH (between 5.5 and 5.75) (Immonen et al., 2000). Rearing conditions may also influence the juiciness as meat from indoor reared pigs has been shown to be juicier than meat from pigs reared outdoor (Jonsäll et al., 2001). The impact on juiciness of meat has also been detected considering the diet. Wood et al. (2004) reported that pig receiving low protein diet, produced more tender and juicy meat, compared to individuals fed up with conventional diet. Except meat-related factors, juiciness is influenced also by the cooking procedure. Aaslyng et al. (2003) have reported that, the oven temperature affected the development in juiciness during chewing. In this study, the meat was juicier when cooked at the low oven temperature. In lambs, the pasture and the prence of aromatic plants, can affect positively the meat juiciness, as reported by Smeti et al.

(2014). As regard the cooking methods, Shackelford et al. (1997) reported that the method did not affect juiciness, when cooked at the same temperature, in lamb roasts and chops.

These complex multidimensional sensory characteristics, cannot be readily measured by the use of objective test methods. Therefore, sensory evaluation plays a key role in the quantification of meat quality traits. There are two types of sensory evaluation panel available; the trained panel and the consumer panel. When hedonic information is sought, a consumer panel is used, and when analytical information is sought a trained panel and analytical testing is utilized. Hedonic tests quantify degree of liking, and analytical tests indicate if a difference exists between two samples. The most common method uses a trained panel, to rate variations in sensory characteristics of interest in relation to previously set anchor values. Panellists should be able to discriminate between a series of samples that exhibit a comprehensive range of differences (reviewed by Osman and Aldosari, 2006).

2.5 Classification of lamb carcasses

The meaning of lamb carcass quality depends on the geographic area. The amount of fat appreciated by the consumer, for example, is different in various culinary traditions. In fact, in the less developed countries is appreciated an higher amount of fat if compared to the developed ones. In many countries (e.g. Africa, South America), they prefer to slaughter animals more than one year old and with higher live weights (30-50 kg) (Rubino et al., 1999). In developed countries the quality of ovine carcasses is evaluated according to some enstabilished standard. In USA the American Sheep Industries (ASI) association revisited this standards many times: the last version became effective on July 6, 1992. These standars for grades of lamb, yearling mutton and mutton, are written primarily in term of carcasses. However, they also are applicable to the grading of sides. Ovine carcasses are classified as lamb (until 12 months of age), yearling mutton (12-15 months of age), or mutton (over the 24 months), depending upon their evidences of maturity as indicated by the development of their muscular and skeletal systems (Field et al., 1990).

The official USDA grade standards for lamb carcasses, use a dual grading system of quality grades and yield grades. If a processor wishes to have lamb carcasses

graded, both a quality and yield grade must be applied to each carcass. Quality grades are intended to sort carcasses on eating quality, and as an indicator of intramuscular fat (marbling) is the major determinant of quality grade. The quality grades for lamb are Prime, Choice, Good, Utility and Cull. Prime indicates the highest degree of marbling and eating quality and Cull indicates the lowest degree of marbling and eating quality. Yield grades are intended to sort carcasses on proportion of lean meat in the carcass, and the only measurement used to determine yield grade is subcutaneous fat thickness midway over the loin eye between the 12th and 13th rib. Yield grades for lamb are YG1, YG2, YG3, YG4, and YG5. YG1 indicates the highest and YG5 indicates the lowest proportion of lean meat. Yield grades are calculated by the following formula: Yield grade = 0.4 + (10 x fat thickness, in.). For example, a carcass with a fat thickness of 0.27 has a calculated yield grade of 3.1 (0.4 + (10 x .27)), and is a USDA YG3 carcass. Carcasses with calculated yield grades of 3.0 to 3.9 are USDA YG3 carcasses. Table 2.2 presents the relationships between fat thickness, calculated yield grade, and USDA yield grade. (Thomas, 2014).

Table 2.2. Fat thickness and USDA yield grades for lambs

Fat thickness, in.	**Calculated yield grade[a]**	**USDA yield grade**
0.0 - .15	0.0 - .19	YG1
.16 - .25	2.0 – 2.9	YG2
.26 - .35	3.0 – 3.9	YG3
.36 - .45	4.0 – 4.9	YG4
.46 and greater	5.0 and greater	YG5

[a] .4 + (10 x fat thickness)

Carcass classification of ruminants in the European Union is based on the EUROP carcass classification system (Commission Regulation (EEC) No 461/93, 1993; Council Regulation (EEC) No 2137/92, 1992). The overall aim of the EUROP classification system is to sort carcasses according to their value, for further processing and to ensure fair payment to farmers. The EUROP classification system makes use of four carcass categories or maturity groups of sheep, mutton, yarling mutton, lamb and

suckling lamb. For ruminants EUROP classification is carried out by humans assessment of conformation and fat class in addition to carcass weight. Conformation class describes carcass shape in term of convex or concave profiles and is intented to indicate the amount of meat in relation with bone, where flesh or meati s regarded as the sum off at and lean. Fat class describes the amount of visible fat (subcutaneous) on the outside of the carcass (Fisher & Heal, 2001). Carcasses are given classes from 1 to 15, where grade 1 is P for conformation class and 1 for fat class. Grade 15 is E+ conformation class and 5+ for fat class. High value for conformation class indicates a carcass with well to excellent rounded muscles. High value on fat class indicates a carcass with a high degree of external fat (sub-cutaneous), and utilizes the relationship between external fat and total fat content of carcasses. Classification of ruminant carcasses is traditionally done by trained assessors. The approval limits for certification and validation of assessors for the EUROP classification system are described by the EU Commission regulation (EC) No. 1215/2003. (rewieved by Johansen et al., 2006). Generally, in lamb the yield grading predicts the amount of sellable product. Meat yield is usually classified in 5 categories grade.

In order to obtain uniform offer of any product on the market, it is necessary to develop legislative system for its classification. Due to mentioned, in production and trade of ovine meat in EU countries, as well as in Croatia, there are legislated classification rules that should be conducted as effectively as possible. Classification of lighter carcasses (< 13 kg) is based on three factors: slaughter weight, meat colour and fat cover. In EU for carcasses lighter than 13 kg there is additionally accepted method for assessment of fat cover with photographic standards. Depending on weight class, colour and fat cover, light carcasses are additionally segregated into two classes. Heavy carcasses (> 13 kg) are classified under "S.E.U.R.O.P" classification. Compared to Croatia, in classification of carcasses heavier than 13 kg in EU countries, there is additionally considered degree of fat cover. Within EU, each country itself, i.e. accredited assessors and inspectors are amenable for control of carcass assessments on slaughter lines. In Croatia, the aforementioned classification is accredited by Croatian Agriculture Agency which is under jurisdiction of Ministry of Agriculture. Regarding to current law regulation, classification of lamb and sheep carcasses and pricing in some EU countries as well as in Croatia, is hard to accomplish. Therefore, great efforts are

made into augmentation and in some cases even substitution of current laws that regulate ovine meat production and trade of ovine meat (reviewed by Kaic et al., 2012).

Chapter 3
SHELF LIFE OF LAMB MEAT

3.1 Understanding and estimating the shelf-life of meat

Shelf-life is one of the most important parameters affecting the quality of meat after its distribution to the market (Kozačinski et al., 2012). The Institute of Food Technologists have defined shelf-life as the period between the manufacture and the retail purchase of a food product, during which the product is characterized by satisfactory quality in terms of nutritional value, flavour, texture, and appearance. An alternative definition is that shelf life is that period between the packing of a product and its use, for which the quality of the product, remains acceptable to the product user (Robertson, 1993).

Many factors can influence shelf-life, and can be categorised into intrinsic and extrinsic factors. Intrinsic factors are the properties of the final product, such as: water activity, pH value, redox potential, available oxygen, nutrients, natural microflora and surviving microbiological counts, natural biochemistry of the product formulation (enzymes, chemical reactants), use of preservatives in product formulation (e.g. salt). Meanwhile, extrinsic factors are those factors that the final product encounters, as it moves through the food chain. They include the following: time–temperature profile during processing; temperature control during storage and distribution; relative humidity during processing, storage and distribution; exposure to light (UV and IR) during processing, storage and distribution; environmental microbial counts during processing, storage and distribution; composition of atmosphere within packaging, subsequent heat treatment (e.g. reheating or cooking before consumption); consumer handling (Kilcast and Subrama, 2000).

All factors affecting shelf-life of meat are important, although special attention has to be given to microbiological condition of the freshly processed product (Mead, 2004). All raw meat can have some level of microbial contamination present and cannot be expected to be otherwise without further processing (McDonald and Sun, 1999). However, especially lamb meat, due its higher pH as compared to beef and pork,

comprises an excellent substrate for growth of spoilage bacteria (*Pseudomonads*, *Moraxella*, *Acinetobacter*, *Enterobacteriaceae*, *Br. thermosphacta*, *Aeromonas*) and pathogens (*Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Cl. perfringens* and *E. coli* O157:H7) (reviewed by Karabagias et al., 2011). Moreover, it has been observed that microbial contamination of meat, starts during processing on the slaughter line. First, the microorganisms reach the carcass surface from which they penetrate into deeper layers of the meat (Pipek et al., 2005). The association between microbial development and chemical changes occurring during the storage of meat, is recognized as a potential mean of revealing indicators of meat quality or freshness (Nychas et al., 2008).

Microbial growth and metabolism depends on various factors, mainly related to pre-slaughter and slaughter procedures, to distribution and preservation methods, packaging, handling and storage. The result of microbial contamination is meat spoilage that leads to the development of off-flavours which make the product undesirable for human consumption (Dave and Ghaly, 2011).

Over the last years, have been observed the development of certain preservation techniques, such as high hydrostatic pressure (HHP), modified atmosphere packaging (MAP) and active packaging (AP), natural antimicrobial compounds and biopreservation, which allow to protect products against deteriorative effects and which have replaced traditional methods. The aims of preservation methods are: (1) to inhibit the microbial spoilage and (2) to minimize the oxidation and enzymatic spoilage.

The shelf-life of food, must be determined by both microbiological and sensory analysis (based on smell and taste properties). With regard to microbiological analysis, a number of scientists have proposed different criteria for determining expiry dates and shelf-life (Zurera Cosano et al., 1988; Korkeala and Bjorkroth, 1997).

Meat spoilage, is not only caused by extrinsic factors such as microbial activity, but also by intrinsic factors such as lipid oxidation. *Post mortem* factors can influence lipid oxidation and decrease the shelf-life of meat products due to the initiation of peroxidation which, in fatty acids of animal tissues, starts to occur almost instantly after slaughter (Gray and Pearson, 1994).

Oxidation is a process in which electrons are removed from an atom or group of atoms. It is one of the major causes of quality deterioration in meat. Oxidative stress occurs due to uneven generation of free radicals such as reactive oxygen species (ROS)

and reactive nitrogen species (RNS), which trigger oxidative and/or nitrosative stress and damage of macromolecules, including the lipid and protein fractions (Falowo et al., 2014). ROS is a collective term often used by biologists to include oxygen radicals [superoxide ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$), peroxyl ($RO_2^{\cdot}$) and alkoxyl ($RO^{\cdot}$)] and certain nonradicals that are either oxidizing agents and/or are easily converted into radicals, such as HOCl, ozone (O_3), peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$) and H_2O_2. RNS is a similar collective term that includes nitric oxide radical ($NO^{\cdot}$), $ONOO^-$, nitrogen dioxide radical ($NO_2^{\cdot}$), other oxides of nitrogen and products arising when $NO^{\cdot}$ reacts with $O_2^{\cdot -}$, $RO^{\cdot}$ and $RO_2^{\cdot}$(Wiseman and Halliwell, 1996). When the animals are alive, there are several mechanisms limiting the exposition to ROS and therefore slowing down the lipid oxidation phenomenon. However, during the post slaughter period, which involves the conversion of muscle into meat, the balance between prooxidative and antioxitative factors, favors the prooxidative ones (Estevez et al., 2009). Mutagenesis by ROS/RNS could contribute to the initiation of cancer, in addition to being important in the promotion and progression phases (Wiseman and Halliwell, 1996).

Meat lipids are components that are very susceptible to oxidation processes (Wąsowicz et al., 2004). Oxidation of lipids is affected by a number of factors including: (1) processing and storage conditions (e.g. temperature); (2) content of unsaturated fatty acids and their distribution in triacylglycerol molecule; and (3) the presence of antioxidants (inhibitors) or prooxidants (catalysts) (Wąsowicz et al., 2004). It has been observed that disruption of muscle structures, could promote lipid oxidation and increase the off-flavour problem in meat (Cheng and Ockerman, 2013). Special attention is also deserved for iron. During meat processing, handling, storage and cooking, iron is released from high molecular-weight compounds such as myoglobin and haemoglobin and is available to form chelats through interaction with low molecular-weight compounds such as amino acids, nucleotides, and phosphates. This chelate compounds have a high ability to catalyze lipid oxidation, as do the high molecular-weight iron sources (Estevez et al., 2009).

Oxidation of lipids is a three-step radical chain reaction which consists of (1) initiation, (2) propagation, and (3) termination with the production of free radicals. Initiation reaction produces the fatty acid (alkyl) radical (R•) which in turn reacts with oxygen, to form peroxy radicals (ROO•) in the propagation reaction (Figure 3.1)

A three-step simplified free-radical scheme has been postulated as follows in Figure 3.1.

Figure 3.1. Mechanism of lipid oxidation (adopted from Chaijan, 2008)

1. Initiation

Initiator → free radicals (R°, ROO°)

2. Propagation

$R° + O_2 \rightarrow ROO°$; $ROO° + RH \rightarrow ROOH + R°$

3. Termination

$R° + R° \rightarrow R\text{-}R$

$R° + ROO° \rightarrow ROOR$

$ROO° + ROO° \rightarrow ROOR + O_2$

The peroxy radicals react with unsaturated fatty acids and form hydroperoxides (ROOH) (Falowo et al., 2014). Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odourless and relatively unstable compounds. They decompose to form volatile aromatic compounds, which are perceived as off-flavours and as a warning that food is no longer edible (Wąsowicz et al., 2004). Hydroperoxydes are considered the most important initial reaction product from lipid oxidation. Their breakage results in secondary products such as pentanal, hexanal, 4-hydroxynonenal, and malonaldehyde (MDA).

At the last stage of oxidation, the radical species react with each other and self-destruct to form nonradical product by different mechanisms. At atmospheric pressure, termination occurs first by the combination of peroxyl radicals to an unstable tetroxide intermediate, followed rapidly by its decomposition by the Russell mechanism, which yields nonradical products. Alkoxyl radicals can react with unsaturated lipids to form stable and innocuous alcohols or undergo transformation into unsaturated aldehydes such as MDA and other compounds (Estevez et al., 2009).

It is generally accepted that oxidation of meat lipid, contributes strongly to the development of the warmed-over flavour (WOF). The term warmed-over flavour is used to define the rapid increase in oxidation in cooked meat products, which is characterized by the rancid flavour developed during storage under refrigeration. The cooking temperature, time, and final internal temperature of the meat can influence the

development of WOF. The effects of such cooking parameters are related to differences in the formation of Maillard reaction products (MRP) in the meat, which may include antioxidants suitable for preventing the development of WOF in cooked meat (Evandro Lage et al., 2012). Additionally, lipid oxidation results also in drip losses, discolouration, loss of nutrient value, and the accumulation of toxic compounds, which may be detrimental to the health of consumers (Falowo et al., 2014).

3.2 Antioxidants and their effect on shelf-life of lamb meat

The oxidation of fatty acids reduces the quality of meat and meat products, negatively affecting their shelf-life. Thus, it is important to use active antioxidants to prevent the lipid oxidation development in the meat. Studies have demonstrated that meat shelf-life and quality, can be improved by antioxidant added in the pre-slaughter and post-slaughter stages. Thomas (2000) defines antioxidants as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly retards or inhibits oxidation of the substrate. The antioxidant potential in food, is determined by the antioxidant composition and the antioxidative properties of constituents. The antioxidant activity (AOA) is defined as capability of a compound to inhibit oxidative degradation, e.g. lipid peroxidation. While the antioxidant capacity gives the information about the duration of antioxidative action, the reactivity characterizes the starting dynamics of antioxidation at a certain concentration of an antioxidant or antioxidant mixture. (Roginsky and Lissi, 2005). Antioxidants should not be toxic, display high activity at low concentrations, should concentrate on the surface of the food grease phase, should withstand food processing, and also contribute to the stability of the final product (Mielnik et al., 2003).

As meat is a complex matrix, different models have been developed for studying the balance and the interaction between anti- and pro-oxidant substances. Antioxidant defenses are composed by non-enzymatic water and lipid soluble compounds (vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiols), and enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Together, enzymatic and non-enzymatic systems operate to counteract the action of pro-oxidants in muscle tissues (Descalzo et al., 2008).

Effect of vitamin E on meat will be presented in the next section of introduction part, however describing influence of antioxidants on oxidation of meat lipids, it's necessary to highlight the role of vitamin C, β-carotene and elements constituents of mentioned enzymes (Table 3.1).

Table 3.1. Antioxidant micronutrients (adopted from Machlin and Bendich, 1987).

Nutrient	**Activity**
Vitamin C (ascorbic acid)	Important water-soluble cytosolic chain-breaking antioxidant, reacts directly with superoxide, singlet oxygen, regenerates tocopherol from tocopheroxy radical.
β-carotene	Most potent singlet oxygen quencher, antioxidant properties particularly al low oxygen pressure, lipid soluble.
Zinc	Constituent of cytosolic superoxide dismutase.
Selenium	Constituent of glutathione peroxidise
Copper	Constituent of cytosolic superoxide dismutase and ceruloplasmin.
Iron	Constituent of catalase.

Antioxidants can act by different mechanisms protecting the target lipids from the onset of oxidation or impeding the propagation phase. The antioxidants mechanisms of action, happen when competitively binding to oxygen, slowing the initiation step, interrupting the propagation step by destroying or binding the free radicals, inhibiting the catalyzers or stabilizing the hydroperoxides (Bigolin et al., 2013).

Have been distinguished two kinds of antioxidants: synthetic and natural. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have been commercially used in various food products with the aim of increasing shelf-life and quality (Gharavi et al., 2007). However, the use of these compounds have shown to stimulate the growth of cancer cells in the stomach, liver, and reproductive system of animals. Raising

consumer concern is for their possible deleterious effects on human health. This situation resulted in banning of these compounds in food products in some European countries, Canada, and Japan (reviewed by Avila-Ramos et al., 2013). Meanwhile, using of natural antioxidants offers the potential advantages of a reduction and/or replacement of synthetic antioxidants, lowered assumed toxicity due to their natural origin as components of foods; enhanced masking of off-flavours arising from oxidation and greater consumer acceptability as natural ingredients in foods (Boyd et al., 1993).

The desire for new sources of safe and inexpensive antioxidants of natural origin, has resulted in considerable interest in herbs and spices as sources of natural antioxidants. Essential oils represent a small fraction of the plant composition; the main compounds are terpenes and sesquiterpenes, and several oxygenated derivatives compounds (alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc.) all of them are responsible for the characteristic plant odour and flavour (reviewed by Sampaio et al., 2012).

Many herbs, spices, and their extracts have been added in a variety of foods, firstly to improve their sensory characteristics and secondary to extend shelf-life. Herbs of the *Lambiatae* family, mainly oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), and sage (*Salvia officinalis* L.), have been reported as having significant antioxidant capacity. Some researchers report the effect also of other species, e.g. clove, cinnamon and coriander also exhibit antioxidant properties (Wojdyło et al., 2007).

Natural antioxidants has wide range of activites, as demonstrated in scientific literature. Some reports demonstrate that natural antioxidants can retard meat colour loss by extending the red colour (a*) and delaying metmyoglobin formation. One example of dietary natural antioxidants affecting meat colour is the higher colour parameters (redness a*, yellowness b*) of meat from lambs fed up with oregano essential oil supplementation (1 mL oregano essential oil kg-1) (Simitzis et al., 2008).

Also other members of the Labiatae family, such as rosemary and sage have been extensively studied for antioxidant and antimicrobial activities in a variety of systems. Of special attention is to be worthy rosemary, whose exstract contains antioxidant compounds, the most active being phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methylcarnosate and rosmarinic acid. These compounds help to prevent lipid oxidation and associated colour loss as well as

decrease of microbial growth in red meat packaged in modified atmosphere. The feeding with essential oil and extracts from rosemary, have been successfully used in improving of lamb meat quality. It has been observed that meat of lambs, descendants of mothers whose diet was modified by distillate from rosemary leaves, had higher a* values, better scores for meat and fat colour ($P < 0.05$) and lower TBARS and rancid odour ($P < 0.05$) (Nieto et al., 2010a).

The effect on shelf-life of meat is also influenced by thyme. Nieto et al. (2010b) reported the influence of diet included thyme leaves of pregnant sheep on the quality characteristics of meat obtained from their offsprings. It was noted that the presence of antioxidant compounds in the diet containing thyme leaves delayed colour deterioration, lipid oxidation and bacterial counts, while at the same time it imparted a better appearance to the fresh lamb meat. In general, this effect was more pronounced at the higher level of thyme leaves (7.5%). The positive effect of thyme on meat quality is connected probably with properties of this herb. Thyme essential oil, contains more than 60 ingredients, most of which have beneficial, including antiseptic, carminative, antioxidant and antimicrobial properties (Nieto et al., 2010b).

The influence of herbs essential oils on lipid oxidation of lamb meat have been evaluated also by Karabagias et al. (2011). In this study have been investigated the effect of thyme and oregano essential oils as well as modified atmosphere packaging (MAP1:60% CO_2 / 40% N_2; MAP2: 80% CO_2 / 20% N_2) in extending the shelf life of fresh lamb meat stored at 4 °C. They noted that sensory analysis showed that at the higher concentration both essential oils, gave a strong objectionable odour and taste and were not further used. Of the two essential oils thyme essential oil was more effective as was MAP2 over MAP1 for lamb meat preservation. Microbial populations were reduced up to 2.8 log cfu/g on day 9 of storage with the most pronounced effect being achieved by the combination MAP2 plus thyme essential oil (0.1%).

The influence of dietary verbascoside on meat oxidative status was evaluated in pork meat by Rossi et al. (2014). In this study a verbascoside and vitamin E dietary mixture improved oxidative stability and colour indices in *longissimus dorsi* muscle during refrigerated storage at 4°C. As regard lamb, Casamassima et al. (2014) evaluated the effect of verbascoside dietary supplement on plasma oxidative status, that results slightly positive. The same author, evaluated the dietary verbascoside supplementation effects, at different doses, on Lacaune ewes, that resulted (at an intermedieate dose) in

an increased oxidative stability, highlighted by the lower thiobarbituric acid reactive substances (TBARS) level. Even if there are few studies about the effects of verbascoside on TBARS of lamb meat, we can assume that this effect could be similar to those recorded in pigs and other species.

3.3 The thiobarbituric acid reacting substances (TBARS)

It is suggested that for the determination of oxidative stability, shelf life and consumer acceptance of products, methods for the determination of lipid oxidation can be ranked in the following order: sensory analysis > Headspace analysis of volatiles > Oxygen absorption > Peroxide value > Thiobarbituric acid reactive substances (TBARS) > Carotene bleaching by cooxidation with linoleic acid > Rancimat test (Wąsowicz et al., 2004).

Value of, mentioned in the above pathway, thiobarbituric acid reacting substances (TBARS) is used as an indicator of food quality and is highly correlated with rancidity and warm-over flavour (WOF) in muscle foods (Wilson et al., 1976). TBARS assay is commonly used to measure lipid peroxidation, especially due to its simplicity and cheapness. TBARS is a important tool which let to evaluate the ability of malondialdehyde (MDA), to react with thiobarbituric acid (TBA) (Sochor et al., 2012).

Studies have revealed that above-mentioned MDA is one of the most abundant lipid peroxidation cytotoxins formed in foods, especially in meat. MDA is genotoxic, reaction with DNA to form highly mutagenic adducts in human cells. It has been demonstrated that the level of MDA-DNA adducts in human colorectal tissue correlates with diet and incidence of adenoma. Thus, the presence of MDA in food may have deleterious consequences in human health. From toxicological perspectives, fresh meat is safer than frozen one, because it has been demonstrated that freezing results in increased accumulation of MDA in meat (Okolie and Okugbo, 2013).

The principle of TBARS test, consists in the reaction of MDA with thiobarbituric acid in acidic conditions and at a higher temperature to form a pink MDA-$(TBA)_2$ complex, which can be quantified spectrophotometrically at 532 nm. This method measures the amount of MDA generated during lipid peroxidation, however, other aldehydes generated during lipid peroxidation, which also absorb at 532

nm, may react with TBA. The results of the assay are expressed in μmol of MDA equivalents (Sochor et al., 2012).

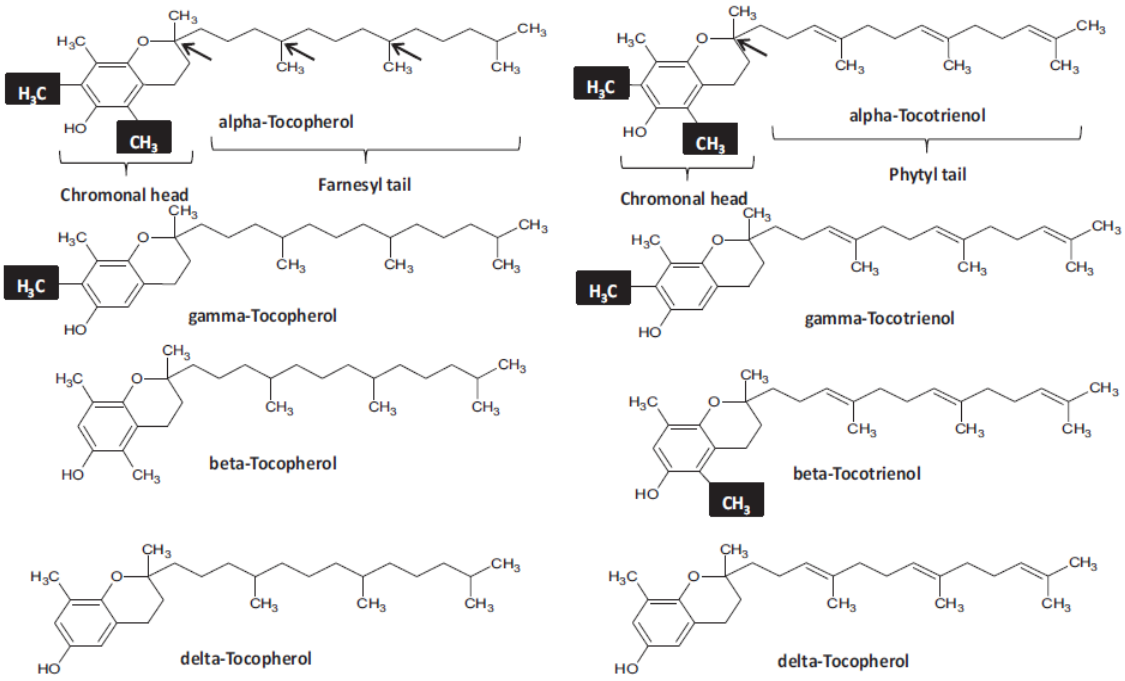
Moreover, TBARS assay has been criticized because it is not specific for lipid hydroperoxides and because several nonlipid molecules in the body produce false positive results (Janero, 1990). It has been reported that the TBARS test cannot be considered reliable for MDA determination. The results of the study conducted by Papastergiadis et al. (2012), suggested that MDA is not the major compound that reacts with TBA and forms a complex that absorbs at 532 nm. Aldehydes, especially MDA, have been found to react with ε-amino and sulfhydryl groups of proteins, resulting in the alteration of their functionality. It has been shown that the TBARS test is reliable when applied for the determination of MDA in vegetable oils and in unprocessed meat and fish products. In processed beef, pork and fish, dry nuts, cheese, and potato crisps, the TBARS test overestimated the content of MDA because of the interference of other compounds with TBA. Furthermore, the results of mentioned studies, also indicated that other than secondary lipid oxidation products interfere with the TBARS test.

Chapter 4
VITAMIN E

4.1 Introduction

Vitamin E is a term that encompasses a group of potent, lipid-soluble, chain-breaking antioxidants. Structural analyses have revealed that molecules having vitamin E antioxidant activity, include four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ) (Brigelius-Flohe Traber, 1999) (Figure 4.1).

Figure 4.1. Vitamin E isoforms (adopted from Cardenas and Ghosh, 2013).



As is possible to observe in Figure 4.1, methyl groups within the chromonal head, determine alpha, beta, and delta status (highlighted). Arrows point to existing chiral centers located in the farnesyl tail (3) and phytl tail (1) of tocopherols and tocotrienols respectively. An unsaturated tail distinguishes the tocotrienols from the saturated tail of the tocopherol vitamin E isoforms (Cardenas and Ghosh, 2013).

There has been considerable controversy and confusion in the literature concerning the nomenclature to be used for the tocopherols and tocotrienols. Therefore, the IUPAC-IUB Commission on Biochemical Nomenclature recommended that the term vitamin E be used as the generic description for all tocol and tocotrienol derivatives, qualitatively exhibiting the biological activity of α-tocopherol. All tocopherols should be regarded as derivatives of tocol. The natural stereo-isomer of a-tocopherol is (2R, 4'R, 8'R)-a-tocopherol or RRR-a- tocopherol. Vitamin E encompasses a group of eight isomeric molecules, which are characterised by a chromanol ring structure and a side chain (Wang and Quinn, 1999).

The α-tocopherol is the most abundant form in nature, has the highest biological activity based on fetal resorption assays, and reverses vitamin E deficiency symptoms in humans. The molecular functions fulfilled specifically by α-tocopherol have yet to be fully described, but it is unlikely they are limited to general antioxidant functions. (Brigelius-Flohe Traber, 1999).

The term vitamin E was introduced for the first time in 1922 by Evans and Bishop, who described a dietary factor in animal nutrition considered at the time to be especially important for normal reproduction. The multiple nature of vitamin began to appear in 1936, when two compounds with vitamin E activity were isolated and characterized from wheat germ oil: the α- and β-tocopherol. In the following, years two additional tocopherols, γ- and δ- tocopherol as well as the tocotrienols, were isolated from edible plant oils. The American Food and Nutrition Board in 1968 officially recognized the essential nature of vitamin E (Azzi et al., 2000).

The health benefits of consuming vitamin E through diet or supplementation, are believed to be for its antioxidant properties as a peroxyl radical scavenger. Vitamin E protects cells from cell damage caused by free radicals that damage cell membranes through lipid oxidation (lipid peroxidation) leading to DNA damage and cancer development. Vitamin E acts as an efficient antioxidant, by reducing the peroxyl radical and eliminating the chain reaction of fatty acid radical propagation (Cardenas et al., 2013). Recently, α-tocopherol has been found to possess functions that are indipendent of its antioxidant/radical scavenging ability (Azzi et al., 2000), even if some authors (Traber and Atkinson, 2007) propose the hypothesis that all of the observations concerning the *in vivo* mechanism of action of α-tocopherol, result from its role as a lipid soluble antioxidant. Because vitamin E is lipophilic, it partitions preferentially into

hydrophobic environments. The binding affinity to tocopherol binding protein may, in part, be due to this property.

Vitamin E is located in fat deposits, oil storage organs and in cell membranes, and this is certainly due to the hydrophobic character of the vitamin. It is because of this preferential location that the functional role of vitamin E as a lipid antioxidant and membrane stabilizer is thought to be so efficient (Wang and Quinn, 1999).

4.2 Functions

4.2.1 Antioxidant functions

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers. Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defense system normally protects cells from the injurious effects of endogenously produced free radicals as well from species of exosenous origin such as cigarette smoke and polluttants. Should the exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (FAO, 2001).

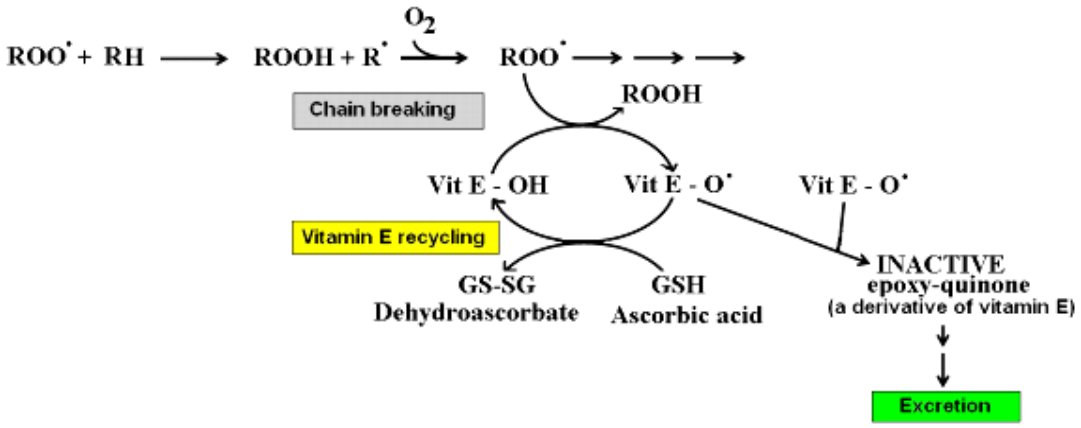
α-Tocopherol and γ-tocopherol constitute essential components of cellular defense mechanisms against endogenous and exogenous oxidants. Unlike many other cellular antioxidants which are constituent enzymes or enzyme-dependent systems, the antioxidant reaction of α-tocopherol is nonenzymatic and fast. The principal role of α-tocopherol as antioxidant, is believed to be in scavenging lipid peroxyl radicals which are the chain-carrying species and propagate lipid peroxidation. The reactions involved in the scavenging function of vitamin E are illustrated in Figure 4.2.

The oxidation of lipids is seen to proceed by a free radical mediated chain process, whereby the lipid peroxyl radical serves as a chain carrier. Chain propagation occurs by abstraction of a hydrogen atom from the target lipid by the peroxyl radical as shown in below (Wang and Quinn, 1999).

Tocopherol reacts with peroxyl radical (ROO•) and neutralize it to ROOH. Tocopherol itself gets converted to tocophryl radical. Tocopheryl radical either interacts with water soluble reactants (e.g., vitamin C, glutathione) to regenerate tocopherol to

take part in scavenging another ROO• (Vitamin E recycle) or reacts with another tocopheryl radical to form an inert product (Figure 4.2). Organs rich in unsaturated fats (e.g., brain) are most vulnerable to damage due to vitamin E deficiency. In spite of many studies, the fact that vitamin E can prevent coronary artery disease, is still controversial (Koner, 2006).

Figure 4.2. Inhibition of lipid peroxidation chain reaction by Vitamin E and mechanisms of recycling (adopted from Koner, 2006)



The efficiency of tocopherols against lipids and membrane phospholipids oxidation and degradation, depends on numerous factors. According to Ohm et al. (2005) is not clear at witch concentration α-tocopherol reaches its maximum activity, even *in vitro*. They observed, in a model consisting of rapeseed oil triglyceride, that the maximum activity was reached at concentration of 50-125 μmol α-tocopherol/kg. Above this range, there is a pro-oxidative effect with a maximum at 250 μmol α-tocopherol/kg. In the animal organism is very difficult to determine the doses of α-tocopherol that is optimal, because it depends on numerous factors: the way of administration, genetic factors (metabolism), the total fat content and some environmental factors (eg. stress) that are difficult to control.

Hence, the biochemical mechanism of chain-braking is observed *in vitro*, but is difficult to demonstrate it *in vivo* because of a lack of sensitive analytical technique. However, many epidemiological studies have demonstrated that vitamin E supplementation can reduce the incidence of stress-related diseases. One example is provided by the Cambridge Heart Antioxidant Study (CHAOS) in 1996, that reported in

over 2000 patients with angiographically proven atherosclerosis, that the vitamin E supplementation (400-800 UI/day) for slightly under 2 years significantly reduced the incidence of cardiovascular death and nonfatal myocardial heart attack by 77%. Decreases in lipid peroxidation of low density lipoproteins (LDL) have been assumed to be responsible of the mechanisms involved in the specific protective activity.

The antiatherogenic results conflict with pro-oxidative vitamin E effects observed, at the same doses, *in vitro.* It has to be considered that vitamin E, like every redox-active compound, may exert anti- and pro-oxidative effects depending on the reaction partners present. Pro-oxidant function of α-tocopherol has been demonstrated in LDL isolated from healthy volunteers and patient with a defect in the α-TTP gene. Knowledge about the importance of a pro-oxidant role of vitamin E *in vivo* has yet to be expanded. Certainly in the presence of other co-antioxidants, including ascorbic acid ubiquinol, vitamin E does not have a pro-oxidant function (Brigelius-Flohe Traber, 1999).

4.2.2 Non-antioxidant functions

According to Azzi and Stocker (2000), α-tocopherol posseses many functions that are indipendent of its antioxidant ability. Vitamin E is an enzymatic activity regulator. For instance, it may inhibit protein kinase (PKC), which plays a role in smooth muscle growth. α-tocopherol has a stimulatory effect on the dephosphorylation enzyme, protein phosphatase 2A, which in turn, cleaves phosphate groups from PKC, leading to its deactivation, calling a halt to the smooth muscle growth. Vitamin E also has an effect on gene expression. Macrophages rich in cholesterol are found in the atherogenetic tissue. Scavenger receptor CD36 is a class B scavenger receptor found to be up-regulated by oxidized low density lipoprotein (LDL) and binds it. Treatment with α-tocopherol was found to downregulate the expression of the CD36 scavenger receptor gene and the class A scavenger receptor (SR-A) and modulates expression of the connective tissue growth factor (CTGF). The CTGF gene, when expressed, is responsible for the repair of wounds and regeneration of the extracellular tissue lost or damaged, during atherosclerosis. Vitamin E also plays a role in neurological functions, and inhibition of platelet aggregation.

Some authors (Traber and Atkinson, 2007) have suggested that all the above mentioned functions can be somehow reconnected to the antioxidant function.

4.3 Metabolism

4.3.1 Digestion and absorption

Even though vegetable oils and derivates are the main dietary source of vitamin E, green leafy vegetables, nuts, and whoole grain also provide a significant amount of this nutrient. In addition vitamin E can be consumed as more chimically stable forms (e.g. α-tocopheryl acetate) incorporated in artificial preparations for nutritional and pharmacological purposes. (Rigotti, 2007). Vitamin E is absorbed in the intestine and enters the circulation via the lymphatic system. It is absorbed together with lipids, packed into chylomicrons, and transported to the liver with the chilomicrone and its remnants. This process is similar for all forms of vitamin E. Only after passage through the liver does α-tocopherol preferentially appear in the plasma. Most of the ingested β-, γ-, and δ- tocopherol is secreted into bile or not taken up and escreted in the feces. The reason for the plasma preference for α-tocopherol is its specific selection by the hepatic α-tocopherol transfer protein (α-TTP) (Brigelius-Flohe, 1999).

4.3.2 Transport

Lipoproteins are the major, rather the only carriers of plasma lipid-soluble antioxidants, including Vitamin E. Indeed, plasma α-tocopherol levels are very well correlated with plasma lipid levels. In humans, relative lipoprotein distribution analysis indicates that tocopherols are mostly transported in LDL and HDL at similar proportions with less than 20% carried in VLDL ad other lipoproteins. However, VLDL accounts for one-third of total α-tocopherol levels after vitamin supplementation (Rigotti, 2007).

4.3.3 Storage

There is no storage site for vitamin E from where vitamin E is released at the time of demand. However, it is interesting to note that 90% of vitamin E is distributed in adipose tissue (Koner, 2006).

4.4 Catabolism and excretion

Due to the great interest in antioxidant function of vitamin E, studies on metabolism, have concentrated on metabolites resulting from oxidation of the chroman moiety. The main hepatic oxidation product was described as α-tocopheryl hydroquinone by NAD(P)H-dependent microsomal and mitochondrial enzymes The quinone and the hydroquinone have both been found in biological membranes treated with azo-bis-amidinopropane, a peroxyl radical generator.

The urinary metabolites of α-tocopherol are the so called „Simon metabolites", α-tocopheronic acid and its lactone: they were found in increased amount in the urine of subjects who consumed 3-5 g all rac α-tocopherol. The chroman ring is opened in the Sinom metabolites. Ring opening starts with the formation of the α-tocopherosyl radical, when α-tocopherol has exerted its antioxidant function. The Simon metabolites were therefore taken as indicators that vitamin E had reacted as an antioxidant. It was paradoxical, however, why a high intake of vitamin E increases its oxidative destruction (Brigelius-Flohe, 1999).

4.5 Use of vitamin E to improve lamb meat quality

One of the basic principles of animal nutrition, is to produce high quality meat. Vitamin E has been shown to improve performance of feedlot ruminant and to increase immune response for ruminant health (Macit et al., 2003a). The diet in animal nutrition is very important, and different diet combinations have been applied to livestock. Vitamin E has frequenty been used in animal nutrition to improve meat quality in recent years. It cannot be synthesized by animal body, and is present in green plants, especially cereal grains (mainly wheat and corn), oats, soybeans, alfalfa meal and green fodders. Vitamin E supplementation to the diet of livestock has a positive effect on the fattening

performance, carcass and meat quality characteristics. Oral supplementation of vitamin E has been also effective in reducing lipid oxidation in meat of livestock (Macit et al., 2003b).

An alternative method of supplementation for vitamin E is the intramuscular injection of massive or physiological doses of vitamin E, generally accomplished using DL-α-tocopheryl acetate (Njeru et al., 1992). This method is more standardized, because natural vitamin E sources (tocopherols) are unstable and are affected by heat, oxygen, moisture, mycotoxins, and oxidizing agents and fats added to the ration. Losses of natural vitamin E may vary from 10% to 60% in blighted, light test weight grain and artificially heat dried grain. Factors leading to inadeguate dietary vitamin E levels, are often related to feeding levels, availability, and stability of these nutrients from natural sources (Vigortone®, 1998).

Vitamin E is part of the body intracellular defense against the adverse effects of reactive oxygen species and free radicals that initiate oxidation of unsaturated phospholipids and critical sulfhydryl groups. It functions as a quenching agent for free radical molecules whith single, highly reactive electrons in their outer shells (Pour et al., 2011). Hence, the most important aspect of vitamin E, is this role on the prevention of lipid oxidation, which is one of the primary mechanisms of the quality deterioration in foods, especially in the meat products. Vitamin E in the muscle is important because prevents the formation of free radical, it is known to improve colour stability and it reduces the drip loss of fresh and frozen meat during display, and extend shelf-life (Wood and Enser, 1997). The changes in quality caused by oxidations, are manifested by adverse changes in flavour, colour, texture and nutritive value, and the possible production of toxic compounds (Gray et al., 1996). A supply of antioxidants is recommended also to preserve the health of animals (McDowell et al., 1996).

Also in ovine, Vitamin E has frequently been known for its role in improving the overall meat quality. The data of literature seem to show that the vitamin E additions in in diet (Strohecker et al., 1997; Okan et al., 2009) or by DL-α-tocopheryl acetate intramuscular injections (Kirby et al., 1996; Maiorano et al.,1999) are inefficient on promoting the ovine growth. However, contradictory data regarding the effect of vitamin E on growth traits in lambs and pigs exist (Maiorano et al., 2007). Some works have demonstrated a beneficial effect of vitamin E treatment on growth traits in lambs (Gentry et al., 1992; Kirby et al., 1997; Macit et al., 2003a). Conversely, other authors

Birch et al. (1994) and Maiorano et al. (2007) observed a lower carcass weight and a negative effect on carcass wholesale cut weights in lambs injected with vitamin E. Maiorano et al. (2001, 2007), recorded negative effects of the vitamin E on the incidence of pelvic limb ($P < 0.05$), that has been attributed to the stimulation of the immune system that, in turn, caused a partitioning of energy away from growth and promoted muscle catabolism (Birch et al., 1994; Hatfield et al., 2000).

Vitamin E seems also to preserve lamb meat from the negative effects of membrane degradation, decreasing the carcass shrink losses (Macit et al., 2003 a,b; Maiorano et al., 2007) and increasing the WHC (Maiorano et al., 2005; Rippol et al., 2013). Mitsumoto et al. (1995, 1998) found that vitamin E treatment, by acting as an antioxidant in cell membranes, stabilized cell integrity and enhanced the ability of beef muscle to retain sarcoplasmatic components; this resulted in less drip loss and greater weight retention *post mortem*.

Vitamin E has also effects on lamb meat colour. The attitude of Vitamin E to preserve the red colour (red index, a*) of meat is described in several studies (Guidera et al., 1997a, b; Maiorano et al., 1999, 2005), but it hasn't effects on the values of luminosity (L*) and the yellow index (b*). Some reports demonstrate that natural antioxidants can retard meat colour loss by extending the red colour (a*) and delaying metmyoglobin formation (Simitzis et al., 2008). This is very important, because the red index is one of the most important commercial characteristic of meat, since it is the colour that consumers associate with freshness (Carpenter et al., 2001; O'Sullivan et al., 2002).

Even if the composition of fatty acids in lambs muscle tissues is strongly influenced by the diet, and then by the genotype, not notable vitamin E effects are reported in literature. Salvatori et al. (2004) observed, in lambs injected with DL-α-tocopheryl acetate, that the vitamin E treatment did not significantly influence the fatty acid profile. A significant ($P < 0:05$) effect was observed only for *Gluteo biceps* muscle with a lower Atherogenic Index for treated lambs, mainly due to a lower proportion of C12:0 and C14:0 in treated groups than in controls. Ponnampalam et al. (2012) described, in lamb slaughtered after 7 weeks of treatment, not significant differences in health-claimable n-3, long-chain n-3, total n-3 fatty acids, SFA, MUFA, or total fatty acid content among the treatment groups (PP=perennial pasture; AP=annual pasture with lucerne hay and oat grain pellet supplement; AP+SF= AP + cracked flaxmeal;

AP+PM=AP with flaxmeal). Kasapidou et al. (2012) also found that vitamin E levels did not affect FA composition.

D'Alessandro et al. (2012) have shown that rearing of suckling lambs by pasture-fed mothers is responsible for a significant decrease in saturated fatty acids and increase in polyunsaturated fatty acids, CLA, and n-3 fatty acids in the lambs meat. Lamb meat from pasture-fed mothers also showed lower n-3/n-6 and SFA/PUFA ratios and thrombogenic index, and a higher tocopherol content. The implications could be important because this fatty acid profile is more favourable for human health, and the higher vitamin E content provides the meat with a greater oxidative stability.

Liu et al. (2013), in Aohan fine-wool sheeps supplemented with different level of Vitamin E for 12 months, found that total muscle (*longissimus lumborum*) MUFA and C18:1n9 contents were significantly higher in the vitamin administred groups (diet containing 200 IU/sheep/d vitamin E) than in the control group. Also the MUFA/SFA ratio was higher in the *longissimus lumborum* m. of vitamin E supplemented groups. They found no significant differences in muscle PUFA content. Probably, this contradiction is due to the fact that in this last study, the age of slaughter of animals is higher compared to the other studies. An hypothesis is that vitamin E supplementation could have effects on fatty acid composition if administered for long periods. The differences in findings suggest that further studies are needed to better understand how vitamin E affects the FA composition in meat.

The data of literature shows that the vitamin E treatment did not modify cholesterol content in different lamb muscles, as reported by Salvatori et al. (2004). They suggest that, besides the genotype, the different type of muscle is a factor that can markedly influence cholesterol levels. An interesting result was obtained by Hidiroglou et al. (2004) in a 90-day feeding study with gerbils, to evaluate the influence of dietary vitamin E levels (25 mg/kg diet, 75 mg/kg, 300 mg/kg, and 900 mg/kg), two levels of dietary methionione and two sources of lipid upon serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol). Low-density lipoprotein cholesterol profile demonstrated a reduction ($P<0.01$) at the higher dietary vitamin E levels (300 and 900 mg/kg) as compared to the 25 mg/kg and 75 mg/kg dietary vitamin E.

Few studies exist in literature, regarding the effects of vitamin E on the muscle HLP concentration (µg/mg) and on collagen maturation (mol of HLP/mol of collagen). Maiorano et al. (2007) observed not significant differences in *longissimus dorsi* m.

collagen charachteristics between lambs injected with 1,500 IU of of DL-α-tocopheryl acetate and control lambs, but ina a previous study was noted an increasing of collagen concentration (P <0.01) in *semitendinosus m.* of lambs injected with 625 IU of DL-α-tocopheryl acetate (Maiorano et al., 1999). Other studies reported an improved collagen synthesis in *semimembranosus m.* (Maiorano et al., 1998a) in lamb injected with 1,000 IU of DL-α-tocopheryl acetate. However, Maiorano et al. (1999) suggested that intramuscular injections of vitamin E, given to growing lambs in adequate form and dose, improve intramuscular collagen properties. An interesting findings was the increase in hepatic hydroxiproline content, as index of collagen, observed by Omer et al. (1989) in rabbits that received 100 IU of vitamin E daily in a cholesterol rich diet. On the opposite, Pietrangelo et al. (1995) and Dennis (1995) noted an inhibitory effect of Vitamin E on the synthesis of collagen, in different tissues of mice and gerbils.

The literature reports, obviously, higher concentrations of vitamin E in muscle tissues of lambs supplemented, or injected, with tocopherols (Ochoa et al., 1992; Wulf et al., 1995; Kerry et al., 2000; Salvatori et al. 2004, Ponnampallam et al., 2012).

Salvatori et al. (2004) reported a concentration of vitamin E of 2.61 versus 6.63 µg/g in control and treated group, respectively, in *longissimus dorsi m.* of lambs (Gentile di Puglia x Sopravissana) injected with 1,000 IU and slaughtered at 64 days. This study also reported significant effects related to the muscles (*semimembranosus, longissimus dorsi, gluteobiceps*), probably due to the different content in fat, and also reported significant effects related to the genotype. Similar results were also reported by Kerry et al. (2000) in Cheviot x Border Leicester lambs, following α-tocopheryl acetate supplementation of 1,000 IU/kg for 56 days, and slaughtered 13 weeks post-parturition. Ochoa et al. (1992) reported a concentration of 15 µg/g in *longissimus dorsi* in 56-day-old lambs fed with 1000 UI/kg/day of vitamin E. Liu et al. (2013) noted that Vitamin E concentrations in *longissimus lumborum (LL)* and *gluteus medius (GM)* increased significantly after 12 months of vitamin E supplementation in Aohan fine-wool lambs (5 months old) fed diets supplemented with 0, 20, 100, 200, 1,000, 2,000, or 2,400 IU/sheep/d vitamin E for 12 months. However, this increase did not occur in a dose-dependent manner, because the muscle vitamin E concentration was highest (7.14 *versus* 5.23 µg/g in *gluteus medius* and *longissimus lumborum*, respectively) in the 200 IU/sheep/d group. The current literature agrees on the fact that vitamin E administration, in diet or by injections, significantly increase the content of α-

tocopherol in different kind of muscle, even though this increase did not occur in a dose-dependent manner. Usually, with the increasing of the administration quantity, there is an increasing in muscle concentration up to reach an optimal dose, to above which the effect of vitamin E in muscle starts to decrease. This effect was firstly observed by Barja et al. (1996) and then confirmed by Maiorano et al. (1999).

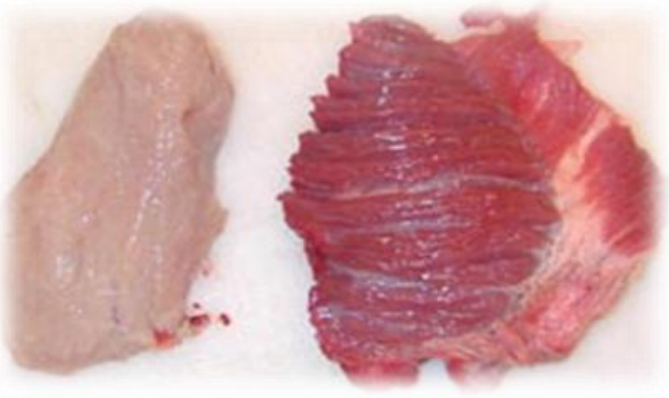
Even if it's clear that treatments, in diet or in muscle, markedly increase the content of Vitamin E in the muscles, it should be noted that the efficiency of tocopherols against the membrane phospholipids oxidation and degradation depends on numerous factors. As described in previously paragraphs, according to Ohm et al. (2005), is not clear at which one concentration α-tocopherol reaches its maximum activity, even in vitro. They observed, in a model consisting of rapeseed oil triglyceride, that the maximum activity was reached at concentration of 50-125 μmol α-tocopherol/kg. Above this range there is a pro-oxidant attitude with a maximum at 250 μmol α-tocopherol/kg. In the animal organism is very difficult to standardize the final concentration of α-tocopherol in the muscle, because it depends on numerous factors: the doses and the way of administration, the metabolism that is different among different breeds, the total fat content and some environmental factors (eg. stress) that are difficult to control. As reported, the importance of the numerous above-mentioned factors are confirmed by Ponnampalam et al. (2012), Salvatori et al. (2004) and Njeru et al. (1994) that recorded a higher concentration of α-tocopherol in *Longissimus dorsi* of treated animals, but the final results in concentration often depends on other agents. Barja et al. (1996) also showed that high doses of vitamin E are not more effective than intermediate ones for optimal protection against lipid peroxidation.

In the light of this considerations, to assess the real antioxidant activity of vitamin E, and in particular its function in preventing lipid peroxidation, is necessary to use other parameters. As described in the previous section of introduction part, the TBARS assay is a reliable test to study this activity in vitro, by subjecting the membrane lipids to oxidant stress and observing the radical-scavenging function of vitamin E. In many studies (Kerry et al., 2000; Salvatori et al., 2004; Rippol et al., 2013) this test confirms the evidence that vitamin E preserve the meat from the lipoperoxidation, allows a better preservation, and prevents warm-over flavour (WOF) in muscle foods. Rippol et al. (2013) found that feeding lambs α-tocopherol enriched concentrate, during the last 10 days of life, or grazing them on alfalfa, drastically

diminished the lipid oxidation (measured as TBARS) of meat. They found that TBARS in muscle, were significantly higher in the control group if compared with the other groups of the experimentation, after 3 and 6 days of display (experimental groups were composed of lambs supplemented with 500 mg of dl-α-tocopheryl acetate/kg concentrate for 0, 10, 20 and 30 d before slaughtering at 22–24 kg BW). Salvatori et al. (2004) reported that the intramuscular lipid peroxidation rate was inversely correlated ($P < 0.05$) to the vitamin E concentration in semimembranosus and gluteobiceps muscles. Oriani et al. (1999) also found a decrease in the level of liver thiobarbituric-acid reactive substances (TBARS), significantly correlated with liver alpha-tocopherol content, in vitamin E treated lambs. Several other studies exist about this topic, but for reason of synthesis, a more detailed comparison of literature data will be provided in the discussion of the present thesis.

Another important aspect of vitamin E supplementation has to be considered: the health point of view. The most common condition associated with vitamin E (and/or selenium) deficiency in lambs, but also in calves and kids, is white muscle disease. White muscle disease in lambs is sometimes referred to as stiff lamb disease. These conditions result in weakness and/or stiffness associated with a bilateral degeneration of skeletal muscle groups and are recognized at necropsy as pale or white steaks in the affected muscle groups. White muscle disease occurs most often in young nursing animals. Symptoms vary depending upon which muscle groups are affected but usually include stiffness, weakness, lameness, inability to rise, and difficulty in breathing. White muscle disease is more prevalent in calves, lambs, and kids than in pigs or foals (Vigortone®, 1998). White muscle disease (WMD), also known as nutritional muscular is caused by Se deficiency, but is influenced by vitamin E status. White muscle disease occurs with two clinical patterns; the first, is a congenital type of muscular dystrophy in which young ruminants are stillborn or die within a few days of birth after sudden physical exertion, such as nursing or running. The second pattern develops after birth; it is observed most frequently in lambs within 3 to 6 weeks of birth (Figure 4.3) but may occur as late as 4 months after birth (Pour et al., 2011).

Figure 4.3. White muscle disease *vs* normal muscle



Source: http://www.cram.com/flashcards/vet-path

PART 2.

AIM OF DISSERTATION

The aim of study was to determine the effect of multiple DL-α-tocopheryl acetate (Vitamin E) intramuscular injections on carcass and meat quality traits, on meat palatability and on the oxidative stability of meat during aerobic display in growing Laticauda breed lambs.

PART 3.

Chapter 5
MATERIALS AND METHODS

This study was carried out at the University of Molise and conducted at a farm located in southern Italy (Casalbore, 575 m a.s.l., 41°14′06″N, 15°00′27″E), in the spring 2013. For the experiment were used lambs of Laticauda breed. Animal handling followed the recommendations of European Union directive 86/609/EEC and Italian law 116/92 regarding animal care.

5.1 Decription of Laticauda breed

Laticauda sheep (Figure 5.1 and 5.2, called with other local names, such as Barbaresca Campana, Bastarda Arianese, Beneventana, Casalinga, Casereccia, Coda chiatta, Nostrana), is probably an ecotype derived from the adaptation of the Barbaresca sheep, coming from north Africa, crossed with native sheeps, mostly of Appeninica breed.

Currently, its characteristics are well defined and his production performances has been improved by small farmers. This breed is mostly distributed in Campania region, especially in the zone of Benevento and Avellino. It is raised in the hill area in small herds of 10-20 animals. In the past, the most common rearing system was the "transhumance", that is the seasonal movement of people with their livestock between fixed summer and winter pastures. Nowadays the most common rearing system is the semi-intensive, consists of small family farms.

The products of Laticauda sheep typycally consist on:

- meat; due to the high weight gain of the lambs and the high rate of twin births;
- average of milk production per year is approximately 80 kg, with 6-8% of fat content, that results in an high cheese (20%) and "ricotta cheese" (6%) yeld.

The meat of Laticauda is sought by the consumers for its delicate flavour and aroma. Milk is used for the production of a typical "pecorino" cheese charachterized by high organoleptic charachteristics (Balasini, 2001).

Figure 5.1. Young Laticauda Sheeps in Casalbore, Southern Italy *(personal photo)*

Figure 5.2. Laticauda lambs in Casalbore, Southern Italy *(personal photo)*

5.2 Animals

5.2.1 Experimental design, slaughtering procedures, muscle sampling, *in vivo* and *post mortem* measurement

The trial was carried out on twenty-four 15-day-old "Laticauda" suckling male lambs, born as singles in late February from 3-year-old dams of the same weight (58 ± 1.8 kg). The ewes selected for the experiment, were homogeneous in terms of parity and of milk yield and milk protein and fat contents of previous lactations. During the experiment, all dams were reared outdoor when the weather was good and indoors when was reaning. Dams were fed with 1.4–1.7 kg vetch/oat alfalfa and polyphitic hay and 0.5–0.7 kg of concentrate and had freely access to water.

The lambs were randomly allotted to two groups: control (C; n=12; live weight: 11.29±0.66 kg) or vitamin E-treated (V; n=12; live weight: 11.33±0.70). From the beginning of the study (15 days of age) until d 57 of age, each lamb of the V group received, weekely, i.m. injections of DL-α-tocopheryl acetate (right gluteus) in aqueous solution (Vitalene® E, Fatro, Bologna) for seven weeks for a total dose of 1,500 IU. The first injection consisted of 150 UI (3ml of Vitalene), while the other 6 injections consisted of 225 IU (4.5 ml of Vitalene). C group lambs received injections of physiological saline (Figure 5.3). All animals received maternal milk and at 28 days of age they had free access to a starter feed (18% crude protein and 6.89 MJ/kg DM) for an adaptation period of 7 days. Successively and until slaughter, lambs were housed into 6 pens of $4m^2$ with 4 animals per pen (3 pens per treatment) and concentrate (Table 5.1) was offered *ad libitum* (in two daily meals at 0800 h and 1600 h), provided net protein, vitamin, and mineral requirements for the lambs. The pens were cleaned weekly. Lambs had free access to water during the experiment. The lambs stayed with their respective mothers between 1800 h and 0700 h.

Figure 5.3. Schema of the treatments (injections)

INJECTIONS															**SLAUGHTER**
VOL (ml)	**3**	**3**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	**4.5**	
IU		**150 IU***		**225 IU***		**225 IU***		**225 IU***		**225 IU***		**225 IU***		**225 IU***	
ANIMALS (n°)	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**24**
AGE (d)	**15**		**22**		**29**		**36**		**43**		**50**		**57**		**64**

 physiological saline solution (C group)

 *Vitalene E, DL-α-tocopheryl acetate (V group)

Table 5.1. Chemical composition of concentrate fed to lambs.

DM, %	88.42
Crude protein, % DM	18.02
Diethyl ether extract, % DM	4.95
Crude fiber, % DM	12.12
Ash, % DM	7.56
Nitrogen-free extract, % DM	57.15
Hemicellulose, % DM	10.27
Cellulose, % DM	15.17
Lignin, % DM	3.20
ADF, % DM	18.36
NDF, % DM	29.11
Starch and sugar, mg/kg DM	3113.92
DL-α- tocopheryl acetate, mg/kg DM	17.22
Net energy, MJ/kg DM	7.64

5.2.2 Slaughter surveys

Lambs were individually weighted every week, after 12 hours of fasting, and at slaughter (at 64th day of age). At slaughter, after 12 h fasting, lambs were electrically stunned, exsanguinated and processed (ASPA, 1991) at a local slaughterhouse. Hot and cold carcasses, both with offal, were weighed and hot and cold dressing percentage were calculated, after dressing and chilling at 2-4^{o}C for 24h. The empty body weight (EBW: bodyweight excluding contents of the gastro-intestinal tract) was determined. Carcass shrink losses, calculated as the difference between hot and cold carcass weights, were expressed as a percentage of hot carcass weight. After the refrigeration period (24 h at 2-4°C), the main cuts (pelvic limb, shoulder, loin) were removed, weighed and expressed as percentages of cold carcass weight.

5.3. Meat analyses

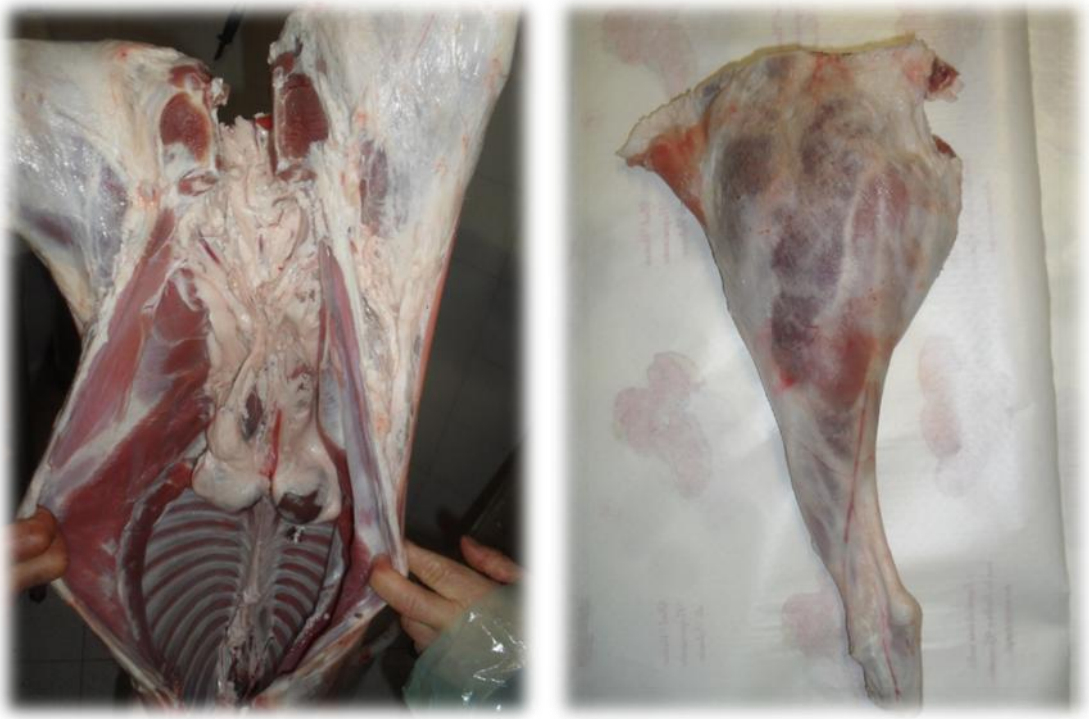
5.3.1 Physico-chemical characteristics

The following analyses were carried out on *m. longissimus dorsi* (LD), between the 12th and 13th ribs:

- pH measured 24h *post mortem* using a portable pHmeter (Crison 507);
- tri-stimulus colour coordinates (*L**, *a** and *b**) were recorded using a Chroma Meter CR-300 (Italia s.r.l., Milano) 24h *post mortem*;
- LD area measured by manually tracing muscles outlines onto acetate sheets and measuring areas by planimeter (Haff-Planimeter No. 317E) (Fernandez et a., 1997);
- water holding capacity (WHC) was measured 24h after slaughter by the method for compression on paper filters. 300 mg of meat was subjected to a pression of 1 kg/cm2 for 5 minutes between two filter papers (Whatman 40, ø 55m, area 23,6 cm2), between two plexiglass sheets (Monetti, 1997). Subsequently was measured the area of the emitted water by a manual planimeter (317E, Haff, Germany). The value of WHC was expressed in percentage as the area of the emitted water divided the area of the filter, multiplied 100.

After the refrigeration period (24 h at 2-4 °C), the left side was weighed and dissected into the main commercial cuts. Pelvic limb, loin and shoulder cuts were weighed and expressed as percentages of the cold carcass weight. From the right side of the carcass the *longissimus dorsi* and *vastus lateralis* muscles were collected, vacuum-packaged and stored at -30°C until laboratory analyses. In addition, left pelvic limb was removed form each carcass for sensory analysis (Figure 5.4)

Figure 5.4. Example of carcass and left-side pelvic limb *(personal photo)*



5.3.2 Preparation and storage of *longissimus dorsi* and *vastus lateralis* muscles for the measurement of oxidative stability

The study of aerobic storage of raw meat was performed using the muscles *longissimus dorsi* and *vastus lateralis*. The muscles were placed in a polystyrene tray, overwrapped with aluminium film and stored at 5°C in condition of aerobiosis. Fatty acid oxidation (TBARS) was measured at 24^{th}, 48^{th}, 96^{th}, 192^{nd} hours (Figure 5.5).

Figure 5.5. *Vastus lateralis* muscles (control and treated group) preparation for storage *(personal photo)*

5.3.3 TBARS (Thiobarbituric acid reactive substances)

Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. TBARS was measured using the colorimetric method of Vyncke (1970). 5 g of meat were homogenized (Ika® Ultraturax T25) with 15 ml of extraction solution (7,5% trichloroacetic acid, 0,1% propylgallate, 0,1% EDTA). The mixture was then filtered in plastic funnels using filters Whatman 1 (ø 70mm). 5 ml of the filtered solution were mixed with 5 ml of 0,02M solution of 2-thiobarbutiric acid (TBA). After incubation at 100°C for 40 min, the absorbance was measured by spectrophotometer (UV 8500, Techomp, Japan) at 532 nm. The calibration curve was prepared by using a dilution of tetraetoxypropane (TEP) instead MDA, because 1M of TEP correspond to 1M of MDA. The value of TBARS was expressed as µg MDA/g of raw meat (or mg/kg) (Figure 5.6).

Figure 5.6. Homogenization, filtration and coloration for TBARS analysis *(personal photo).*

5.3.4 Collagen analysis

Approximately 10 g of *longissimus dorsi* muscle samples were thawed, at room temperature, trimmed of fat and epimysium, lyophilized for 24h (Genesis Pilot Lyophilizer, SP Scientific), and stored frozen (-20°C) until collagen analyses. The lyophilized muscle tissue (100 mg) was hydrolyzed in Duran tubes in 5ml 6N HCl at 110°C for 18 to 20h (Etherington and Sims, 1981) for the determination of hydroxyproline (Woessner, 1961) and crosslinking. The hydrolyzate was filtered (Whatman 1) and diluted with water plus. An aliquot of the hydrolyzate was removed

for hydroxyproline determination and the remaining part was subjected to HLP (Hydroxylysilpyridinoline) crosslink analysis.

5.3.4.1 Intramuscular collagen

The 4-hydroxyproline (intramuscular collagen concentration) was quantified using the colorimetric procedure of Woessner et al. (1961). The hydroxyproline was oxidated with chloramines T (sodium p-toluenesulfonchloramide) that was then destroyed by adding perchloric acid. A solution of p-dimethylaminobenzaldehyde (Ehrlich solution) was added and the tube was placed in water bath at 60°C for 20 minutes. The solution absorbance was then determined by spectrophotometer (UV 8500, Techomp, Japan) at 557nm. The IMC concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as μg hydroxyproline per milligram of lyophilized tissue, comparing the absorbance of the sample with this of the calibration curve.

5.3.4.2 Crosslink analysis

Hydroxylysylpyridinoline (HLP) is the principal non-reducible crosslink of muscle collagen and highly correlated with the thermal stability of collagen (McCormick, 1999). Its concentration was determined using the procedure of Eyre et al. (1984). HLP was measured on *longissimus dorsi* muscle samples from 24 male lambs. Hydrolyzate HLP was concentrated and separated from the bulk of the other amino acids, using the procedure described by Skinner (1982), that consist in elutions from a CF1 cellulose column. The obtained eluate was added of pyridoxamine (internal standard) and concentrated (Speed Vac® Plus SC110A, Savant Instruments, Farmingdale, NY), resuspended in 1% (v/v) n-heptafluorobutyric acid (HFBA) and filtrated (Nylon syringe filter 0.45μm, Whatman).

Quantitation of the HLP was performed using a reversed phase high performance liquid chromatography (RP-HPLC) according to the procedure described by Eyre et al. (1984). For the analysis was used a Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Luna C18 column (250 x 4.6 mm x 5 μm; Phenomenex, Torrance, CA).

5.3.5 Cholesterol analysis

The muscle cholesterol content was determined using the method by Maraschiello et al. (1996). The *longissimus dorsi* muscle sample (100mg) with 2 ml of 0.5 N KOH in methanol was heated in water bath at 80°C for 60min. After cooling, 2 ml of distilled water saturated with NaCl was added. The tubes were vortexed followed by addition of 3 ml ether/hexane (1 : 1, v/v) and centrifuged for 10min at 3000 g. The upper phase was recovered and the hexane/ether extraction step was repeated twice. The extracts were combined and evaporated to dryness and re-dissolved in 1ml of acetonitrile/isopropanol (1:1) for HPLC analysis. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Kinetex 5µ C18 reverse-phase column (150 x 4.6mm x 5 µm; Phenomenex, Torrance, CA), was used. The mobile phase was acetonitrile/2-propanol (55: 45 vol/vol) at a flow rate of 1.2 mL/min. The detection wavelength was 210 nm, retention time was 12.89 min.

5.3.6 Fatty acid analysis

Lipid extraction from muscle samples was performed by modification of Bligh and Dyer (1959) and Folch et al. (1957) methods. Bligh and Dyer extract most classes of lipids and this system requires proportionally smaller volumes of chloroform and methanol than the Folch method.

Lyophilized muscle sample (100mg), added of tridecanoic acid methyl ester (C13:0) as internal standard, was treated with chloroform/methanol/water (1:2:0.8) and stirred for 4 hours. Then to obtain a better separation between the two phases, chloroform, 2N KCl/0.3 N HCl, and H_2O were added consecutively, and centrifuged for 5 min at 3000 g. The upper phase consisting of methanol, water, water soluble compounds such as sucrose or salts and very small amount of chloroform was discarded; while the lower lipid-containing phase was separated from the upper phase, and retained for use. Then, another extraction with chloroform was repeated. The extracted lipids were esterified and then analyzed by gas chromatography (GC). Analysis was performed using a HRGC 5300 Fisons (Rodano, Milan, Italy), equipped with a flame ionization detector and a fused silica capillary Column (CP-Sil RTX 2330), 30 m x 0.25 mm x 0.5 µm film thickness (Restek, Bellefonte, PA, USA). The

carrier gas was helium. The oven temperature program was 120°C for 1 min then increasing at 5°C/min up to 230°C where it was maintained for 20 min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (FAME, Sigma) run under the same operating conditions. Quantification of individual fatty acids, was based on the internal standard method using tridecanoic acid methyl ester. Results were expressed as percentage of the total fatty acids analyzed. To assess the nutritional implications, the n-6 fatty acids/n-3 fatty acids and the PUFA/SFA ratios were calculated. Moreover, in order to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, respectively the atherogenic index (AI) and the thrombogenic index (TI) were calculated, according to the formulas suggested by Ulbricht and Southgate (1991), as follows:

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

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

5.3.7 Alpha tocopherol content

The levels of Vitamin E in the *longissimus dorsi* and *vastus lateralis* muscles were determined and quantified as described by Zapel and Csallany (1983), and then quantified by HPLC (Kontron Instruments, Milan, Italy) model 535 equipped with a C18 reverse-phase column (250 cm x 4.6 mm x 5µm) (Phenomenex, Torrace, CA). 100 mg of muscle was trimmed of fat and epimysium, and then homogenized in 20 ml of acetone for 15 s with Ultraturax (Ika T25 Basic). The homogenized was centrifuged (1300g x 10 min at 20°C) and the supernatant was removed and filtered, with a 0.2 µm nylon filter, in a 10 ml tube. The filtrate was dried with nitrogen stream and then the extract was resuspended in 500 µl of ethanol containing the internal standard (RRR-α tocopheryl acetate). Again the liquid was dried with nitrogen stream and the extract resuspendend in 30 µL of diethyl ether and 80 µL of methanol. 15 ml of this suspension were injected in HPLC. The mobile phase was 100% methanol at a flow rate of 1.5 mL/min. The detection wavelength was 292 nm and retention time was 4.1 min. The α-tocopherol content was expressed as µg/g of muscle tissue.

5.4 Consumer panel

Sensory analysis was performed on the left pelvic limb. The pelvic limbs, removed from the carcasses, were vacuum packaged and aged for 5 days at 4°C. All the pelvic limbs were then cut into slices of 1.5 cm-thickness. From each pelvic limb, the meat was divided into two equal parts, vacuum packaged, coded according to the experimental groups of the animals, and stored frozen (-20°C) until the day before the test.

5.4.1 Quality-quantity test (blind test)

As for the sensorial analysis of the meat, the typology test mainly used regards descriptive methods, that compare two or more samples.

The sensorial analysis conducted on the samples which are the object of this study, has been performed by two panel tests: the first one has been conducted by students who attended the course of "sensorial and rheological analysis of meat and derivates" (academic year 2013/2014), held by prof. Giuseppe Maiorano, and the second one has been conducted by adults, customary consumers of lamb meat.

Nineteen students (10 men and 9 women; age between 22 and 25 years old) participated as initial judges to the first panel that took place at "agriturismo Cassetta" restaurant in San Giuliano Del Sannio town (Figure 5.7).

Figure 5.7. The panel at the "agriturismo Cassetta" *(personal photo)*

The participants were classified as “initial judges” because they had already participated at least to previous sensorial panel, that took place at the “Laboratory of Meat Production and Quality” at the Department of Agricultural, Environmental and Food Sciences.

Twenty adults between 50 and 70 years old participated to the second panel as custumery consumers of lamb meat. This panel session took place at “Di Pietro restaurant” in Melito Irpino town (AV).

During both panel sessions, tables have been set with dishes, pieces of cutlery, glasses and water. Before beginning the test (Quality-Quantity), to every participant was given an information form (Figure 5.8).

Figure 5.8. Information form

Consumer n°……..

A.1 - Where do you live?

.....

B.1 - Gender M □or F□

B.2 - Age

Under 24 years of age	1 □
Between 25 and 44	2 □
Between 45 and 64	3 □
Over 65 years of age	4 □

B.3 – Occupation

…….

B.4 - Education

Elementary school	1 □
Junior high school	2 □
Trade school diploma	3 □
High school diploma	4 □
University degrees	5 □

C.1 - How many times do you eat meat?

0 □ Never 1 □ Rarely 2 □ Many times a month 3 □ Many time at week
4 □ Everyday

C.2 - What type of meats do you eat and how frequently?

	Frequency	**Where predominantly**
Bovine (veal beef etc.)	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Pork	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Ovine/Goatish	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Turkey, poultry, rabbit	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Wild fowl	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Packed meat, ham	□ Never □ Sometimes □ Often	□ At home □ Restaurant
Other ……..	□ Never □ Sometimes □ Often	□ At home □ Restaurant

C.3 - Where do you buy meat?

1 □ Producer 2 □ Butcher 3 □ Supermarket

C.4 – In what way do you buy meat?

1 □ In cuts 2 □ Already prepared

Prof. Maiorano as Panel Leader gave the necessary information regarding how to upfront the analysis, explaining the correct manner to evaluate, in order to fill in the form (Figure 5.9).

Figure 5.9. Professor Maiorano as Panel Leader *(personal photo)*

Samples were cooked in a contact grill, pre-heated to 200 °C until the internal temperature of the muscle reached 72 °C, which was measured using a thermometer with a handheld probe (Koch, Kansas City Missouri), and inserted into the approximate centre of the muscle. Meat samples were served immediately to each consumer, who evaluated tenderness acceptability, flavour acceptability, juiciness acceptability, and overall acceptability. To each panellist was asked to evaluate the sample considering the intrinsic characteristics of the meat – tenderness, flavor, juiciness and overall acceptability (general pleasantness) – according to an unstructured line scale ranging from 1 (“very unpleasant”) to 9 (“very pleasant”) (Figure 5.10a,b). The panel leader was there to help about any doubts and questions about filling in the form. The panel leader did not give precise details of the meat, he only referred to the meat as two samples of lamb meat (blind test). The two samples were given one after the other. The quantity of the samples was big enough to evaluate in a reliable manner. Within the first and the second sample, the judges could use water to rinse their mouth, in order to re-establish the equilibrium of the palate and avoid any interference in the following evaluations.

Figure 5.10a. Panel form, lamb meat

Name…… Surname…… Number…….. Date……

SAMPLE A

You have in front of you a plate with a portion of lamb meat. Taste the sample. Write, on the scale values below, approval parameters.									
	Very unpleasant								Very pleasant
Tenderness	1	2	3	4	5	6	7	8	9
Flavour	1	2	3	4	5	6	7	8	9
Juiciness	1	2	3	4	5	6	7	8	9
Overall acceptability	1	2	3	4	5	6	7	8	9

Judgments and comments

Sample A:

Other:

Figure 5.10b. Panel form, lamb meat

Name…… Surname…… Number…….. Date……

SAMPLE B

You have in front of you a plate with a portion of lamb meat. Taste the sample. Write, on the scale values below, approval parameters.									
	Very unpleasant								Very pleasant
Tenderness	1	2	3	4	5	6	7	8	9
Flavour	1	2	3	4	5	6	7	8	9
Juiciness	1	2	3	4	5	6	7	8	9
Overall acceptability	1	2	3	4	5	6	7	8	9

Judgments and comments

Sample B:

Other:

5.5 Statistical analysis

Data on *in vivo* and *post mortem* performance, and meat quality (pH, colour, WHC, fatty acid and collagen) were analysed by one-way analysis of variance. Body weight at slaughter was included as a covariant. Data on α-tocopherol, and TBARS and pH during the experiment of aerobic preservation were analysed by GLM procedure.

Data on the sensory analysis of meat by panellists, gathered over the course of the panel study, were analysed using nonparametric tests due to the fact that the normality of the distribution of these traits was not confirmed ($P < 0.05$). The differences in terms of panellists' expectations and impressions regarding the quality of meat between samples of meat from control lambs and lambs injected with vitamin E which were kept in two different management systems, during each session were estimated with the use of the Wilcoxon test. The effect of the panellists' (adult versus students) on the assessment of meat was analysed using the U-Mann–Whitney test. All data were performed using SPSS package (SPSS, 2008).

Chapter 6
RESULTS AND DISCUSSION

6.1 *In vivo* and *post mortem* performances

In vivo and slaughter performances are reported in Table 6.1. The vitamin E administration did not influence ($P > 0.05$) the weekly weight gain (data do not shown), final weight gain and consequently the slaughter weight (20.41 and 19.58 kg in C and V group, respectively). The growth shown from the lambs used for this experiment, is in agreement with the breed growth standard (Balasini, 2001).

Vitamin E treatment did not affect ($P > 0.05$) hot and cold carcass weights and cold dressing percentage; conversely, the hot dressing percentage resulted higher ($P < 0.05$) in C group (+8.8%) than in the V group. Also, the weights of the main cuts (pelvic limb, shoulder, loin) and the incidence of shoulder and loin, did not differ ($P > 0.05$) between the evaluated groups. Differently, the incidence of pelvic limb in the vitamin E group was higher ($P < 0.05$) compared to those of control group (11.70 *versus* 11.15% in V and C, respectively).

The data of this research clearly shows that the vitamin E is inefficient on promoting the lamb growth, in agreement with the results of other studies (Maiorano et al., 1999; Schultz et al., 2003), who reported no effect of vitamin E administration on weight gain when given to different lambs breeds. Similarly, Strohecker et al. (1997) and Okan et al. (2009), did not obtain any effect on lamb growth fed dietary vitamin E. The same conclusion was obtained by Kirby et al. (1996) in one experiment of diet supplementation (200 IU of DL-α-tocopherol/head/day) in 84 days old Whiteface lambs. However, several other studies have demonstrated a beneficial effect of vitamin E treatment on growth traits in lambs. Maiorano et al. (2007) reported that vitamin E increased average daily gain during suckling. Gentry et al. (1992) observed increased gains from birth to 30 day of age in Suffolk lambs injected with 900 IU DL-α-tocopheryl acetate, a response observed previously by Horton et al. (1978) in the same breed treated with supplemental vitamin E orally or by intramuscular injection. These results agree with the findings of Macit et al. (2003a) in Morkaraman male lambs fed up

on dietary cointaining vitamin E three fold higher than the basal diet (15 mg vitamin E per lamb per day).

In contrast with the previous studies (Maiorano et al., 2007; 2005), vitamin E increased pelvic limb percentage ($P < 0.05$). In fact, the above authors have recorded a negative effect of the vitamin E on the incidence of pelvic limb. Differently, Macit et al. (2003b) did not find any effect by vitamin E supplementation to diet of animals. Others (Birch et al., 1994; Hatfield et al., 2000) have reported that vitamin E-treated lambs had lower leg and shoulder weights; the authors suggested this may have been due to vitamin E stimulation of the immune system that, in turn, caused a partitioning of energy away from growth and promoted muscle catabolism. The incidence of shoulder and loin was found to be similar ($P > 0.05$) between the two experimental groups.

Carcass shrink losses were not influenced ($P > 0.05$) by the DL-α-tocopheryl acetate injections. This finding contrast with the results of Maiorano et al. (2007), who noted a higher value in the control group than in DL-α-tocopheryl acetate-treated lambs; they attributed this result to the treatment with vitamin E and its antioxidant effect on muscle cell membranes. Differently, Macit et al. (2003b) found a higher value of carcass shrink losses of lamb which received a supplement of 45 mg vitamin E/lamb/day for a 75 days fattening period when compared to control animals. Reduced drip losses in lambs, has been attributed to treatment with vitamin E and its antioxidant effect on muscle cell membranes (Macit et al., 2003b). It has been observed that, in muscle, oxidation of membrane phospholipids probably begins immediately after slaughter, leading to decreased membrane fluidity (Dobretsov et al., 1977) and disruption of normal membrane structure and function (Storey, 1996).

The results of *in vivo* and *post mortem* performances obtained in this study confirm partially the findings reported in literature. However, seems that genotype can influence the effect of vitamin E. In fact, Asadian et al. (1996) recorded different performance and different vitamin E requirements in three different lamb genotypes. Another factor could be the mode of administration and the dose of the vitamin: different doses and way of administration, would determine different effects, as suggested by Barja et al. (1996) and Maiorano et al. (1999).

Table 6.1. *In vivo* and slaughter performances (mean ± SE)

Traits	**Control**	**Vitamin E**	***P*-Value**
Number of lambs	12	12	
Initial weight (kg)	11.29 ± 0.66	11.33 ± 0.70	0.966
Slaughter weight (kg)	20.41 ± 0.61	19.58 ± 1.01	0.448
Weight gain (kg)	9.13 ± 0.39	8.25 ± 0.46	0.162
Hot carcass weight (kg)	10.22 ± 0.47	9.32 ± 0.53	0.216
Hot dressing percentage (%)	49.83 ± 0.89	47.53 ± 0.65	0.049
Cold carcass weight (kg)	9.80 ± 0.47	8.92 ± 0.50	0.215
Cold dressing percentage (%)	47.77 ± 0.93	45.56 ± 0.68	0.070
Carcass shrink losses (%)	4.13 ± 0.41	4.11 ± 0.38	0.961
Remarks on left side			
Pelvic limb weight (kg)	1.09 ± 0.05	1.04 ± 0.05	0.516
Pelvic limb percentage (%)	11.15 ± 0.10	11.70 ± 0.08	0.001
Shoulder weight (kg)	0.74 ± 0.03	0.68 ± 0.04	0.202
Shoulder percentage (%)	7.63 ± 0.15	7.68 ± 0.08	0.810
Loin weight (kg)	0.47 ± 0.04	0.42 ± 0.04	0.316
Loin percentage (%)	4.78 ± 0.18	4.76 ± 0.34	0.959

6.2 Physical and chemical properities

6.2.1 Physical properties

The values of pH and colour (L*, a*, b*), measured at 24 h after slaughter, water holding capacity (WHC) and *longissimus dorsi* area, that are considered indicators of meat quality and technical processing characteristics, are reported in Table 6.2.

pH assessment, that is a measure of the acidity reached by the muscle in the *post mortem* period, has not been significantly influenced by the vitamin E treatment ($P > 0.05$). The recorded values (5.66 and 5.68 in C and V groups, respectively) are in the range of acceptability for the ovine meat. The results are in agreement with those recoreded by Velasco et al. (2000) in lambs slaughtered at 10-12 kg, and with those of Vergara et al. (1999) and Maiorano et al. (2007), regarding lamb slaughtered at 20-24 kg.

Vitamin E did not affect ($P > 0.05$) colour parameters, except red index (a*) that was higher ($P < 0.05$) in the lambs of vitamin group compared to those of the control (16.74 *versus* 15.51 in V and C, respectively). The red colour is one of the most important commercial characteristic of meat, because it is the colour that consumers associate with freshness (Carpenter et al., 2001). These data confirm the attitude of the vitamin E to preserve the red colour, that is described in several works (Guidera et al., 1997a, b; Maiorano et al., 1999, 2005).

No vitamin E effect ($P > 0.05$) was found on the water holding capacity (WHC, 23.41 *versus* 23.48% in C and V, respectively). Differently, Maiorano et al. (2005) and Rippol et al. (2013), in injected lambs, and Dell'Orto (2000), in cattle fed in the last 100 days before slaughter with a diet supplementation of 1,500 IU/head/d, reported an increase in WHC. Also, Ramanzin et al. (2002) observed a higher WHC in cattles supplemented with 5,000 and 10,000 IU/head/d in the last 10 d before slaughter compared to the control cattles.

Vitamin E had no effect on *longissimus* muscle area. This finding is consistent with studies involving lambs of Ile-de-France breed (Maiorano et al., 1999) and on the Awassi breed (Macit et al., 2003b), but contrast with the results of Maiorano et al. (2005) who reported a larger ($P > 0.05$) *longissimus dorsi* area in lambs injected with

1,250 IU of α-tocopheryl acetate (C = 0 and V = 250 IU/wk, i.m. injected for 5 wk) in aqueous solution.

Table 6.2. pH, colour, WHC and area of *longissimus dorsi* (mean ± SE)

Traits	**Control**	**Vitamin E**	***P*-value**
Number of lambs	12	12	
pH_{24}	5.68 ± 0.02	5.66 ± 0.02	0.399
Colour 24h post mortem			
L*	50.16 ± 1.04	50.66 ± 0.66	0.692
a*	15.51 ± 0.39	16.74 ± 0.36	0.030
b*	4.68 ± 0.53	4.88 ± 0.33	0.760
WHC (%)	23.41 ± 0.90	23.48 ± 0.90	0.959
Area *longissimus dorsi* (cm^2)	9.39 ± 0.81	9.40 ± 0.60	0.993

6.2.2 Fatty acids (FA) composition and cholesterol content

Muscle lipids are significant in the nutritional quality of meat. Numerous studies have confirmed that there is a strong relationship between the lipids consumed in the human diet and total plasma cholesterol. A low intake of saturated fat and an increased polyunsaturated to saturated fatty acid ratio are associated with a low risk of human coronary heart disease. Not only saturated and unsaturated fats, but also individual fatty acids are important. On the other hand, increasing the unsaturated fatty acids in the muscle cell membranes result in increased oxidative deterioration, and oxidized lipids in food may have adverse effects on health. However, vitamin E exerts a protective effect on the unsaturated fatty acids in tissues in both domestic and laboratory animals and its presence within muscle cell membranes, reduces lipid oxidation, improving the quality characteristics of meat (reviewed in Salvatori et al., 2004).

Fatty acids (FA) composition and cholesterol content of *longissimus dorsi* of lambs are shown in Table 6.3. Treatment with vitamin E did not significantly affect the total SFA content and the proportion of single SFA, except for a slightly higher ($P < 0.08$) content of lauric acid (C 12:0) and lower ($P < 0.01$) content of eptadecanoic acid (C 17:0) in V group. These results are consistent with those reported in literature by Salvatori et al. (2004) who did not observe any significant effect of vitamin E treatment on the SFA composition of different muscles (*longissimus dorsi*, *semimembranosus* and*gluteobiceps*) from two different crossbred lambs. Likewise, vitamin E treatment did not affect the total MUFA amount,except for a lower ($P < 0.01$) content of C 17:1 and higher C 18:1 n-9 *trans* ($P < 0.05$) and C 22:1 ($P < 0.01$) in V group. Quantitatively, the oleic acid (C18:1 n-9 *cis*) was the most concentrated fatty acids (40-41%), followed by palmitic (20-21%) and stearic acids (14-15%). The highest presence of oleic acid in intramuscular lamb fat was in agreement with levels found by other authors (Sanudo et al., 2000; Salvatori et al., 2004). From a nutritional point of view, oleic acid has a relevant importance in the human diet because it acts on lipaemia reducing both LDL cholesterol and the triglycerides and providing other health benefit, such as a reduced risk of stroke and a significant decrease in both systolic and diastolic blood pressure in susceptible populations (reviewed in D'Alessandro et al., 2012). Palmitic acid increases total serum cholesterol, but its effect is lower than lauric (C12:0) and myristic acids (C14:0), which in this study represent approximately 0.2-0.3% and 3%, respectively. Stearic acid is considered a "neutral" fatty acid because it has been shown to have no net impact on the plasmatic level of either LDL or HDL cholesterol in humans. This effect of stearic acid has been attributed to its reduced digestibility and easy desaturation into oleic acid (reviewed in D'Alessandro et al., 2012).

Vitamin E treatment positively affected the total PUFA content being higher ($P < 0.01$) in the V group compared with the control group. On the contrary, Salvatori et al. (2004) did not found any significant vitamin E-effect. It was reported that the increase of PUFA levels observed in the liver of guinea pig treated with vitamin E has been ascribed to a protective effect of vitamin E against PUFA peroxidation (Barja et al., 1996). Clement and Bourre (1993), who observed higher amounts of stearic acid and of total saturated fatty acids and a lower amount of monounsaturated fatty acids and of linoleic acid in liver microsomes of vitamin E-deficient rats, suggested thatvitamin E deficiency, may alter the enzymatic activities of chain elongation–desaturation and the

relation between vitamin E and PUFA. However, Oriani et al. (1999) reported that the effect of vitamin E on fatty acid unsaturation, seems to be lower in muscle than in liver. It is also important to mention that there are several factors in production such as diet, age of weaning, breed, sex, and body weight which can affect the muscle fatty acid composition, besides the action of the rumen (reviewed in Sanudo et al., 2000). Among the PUFA, the most abundant was the linoleic acid (C 18:2n-6), with similar ($P > 0.05$) values in the two experimental lamb groups (7.95 and 8.07%, for C and V group, respectively). The obtained results were similar with those reported by Salvatori et al. (2004), ranging from 7.48 and 8.87% in two different lamb types, and by Sanudo et al. (2000) in two Spanish breeds (approximately 9%). Linoleic acid, is derived entirely from the diet and it is contained at high levels in concentrate feedstuffs (grains and oilseeds). Linoleic acid is degraded into monounsaturated and saturated fatty acids in the rumen by microbial biohydrogenation and only a small proportion, around 10% of dietary C 18:2n-6, is available for incorporation into tissue lipids (Wood et al., 2008). The second most important PUFA is α-linolenic acid (C 18:3 n-3), which is contained at high levels in grass and grass products,and also a high proportion is biohydrogenated to saturated fatty acids in the rumen. It was reported that a variable proportion of dietary linolenic acid is biohydrogenated (85–100%) but this is more than for linoleic acid (70–95%), so less is available for incorporation into tissues(Wood et al., 2008). In the present study, the content of linolenic acid was influenced by the vitamin E treatment, resulting in higher levels ($P < 0.01$) in meat from treated lambs. These results agree with the findings of Salvatori et al. (2004), ranging from 1.03 and 1.42% in two different lamb types, but they did not found any significant effect of vitamin E treatment on the proportion of linolenic acid. In general, concentrated-fed animals, have higher concentration of linoleic acid (C 18:2 n-6) compared to grass-fed animals. Regarding the isomers of CLA detected, C 18:2 *cis*-9, *trans*-11 was the most abundant, even if the weight percentage was less than 1%. Small amount of C 18:2 *trans*-10, *cis*-12 were also detected. Conjugated linoleic acid (CLA) appear mostly in dairy products and are thought to have beneficial effects on health, but are also found at low mg-levels in meats, especially in beef and lamb (Valsta et al., 2005). In general, muscle contains significant proportions of long chain (C20-22) PUFAs which are formed from 18:2n -6 and 18:3n -3 by the action of Δ5 and Δ6 desaturase and elongase enzymes. Important products are arachidonic acid (20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3)

which have various metabolic roles including eicosanoid production (Wood et al., 2003). In the present study, the vitamin E treated group had a significant higher amount of n-3 long chain PUFA: eicosapentaenoic fatty acid (EPA, C20:5n-3, $P < 0.05$), docosapentaenoic fatty acid (DPA, C22:5n-3, $P < 0.01$) and docosahexaenoic fatty acid (DHA, C22:6n-3, $P < 0.06$). Also vitamin E group had higher ($P < 0.01$) content of C 22:2. In light of this, the total n-3 FA content was higher ($P < 0.01$) in treated lambs compared with the control group; while, no significant differences were found for the total n-6 FA amount. The n-6/n-3 and P/S ratios, commonly used criterios to describe the dietetic value of fat, were also affected by the vitamin E treatment. The n-6/n-3 ratio was lower in treated lambs (2.33 *versus* 3.62 for V and C group, respectively; $P < 0.01$). It is reported that an healthy diet should provide the optimal n-6/n-3 ratio of 1:4, even if it has been reported that this balance should range from 1:1 to 1:4 depending on the disease under consideration (reviewed in D'Alessandro et al., 2012). In light of this, the treated lambs provided meat with an "healthier" balance of FA.The P/S ratio has also a great nutritional implications and it is taken as a measure of the propensity of the diet to influence the incidence of coronary disease (Wood et al., 2003). In the present study, the P/S ratio was higher ($P < 0.01$) in treated lambs, even if the obtained values were lower than the recommended range of 0.4-0.7. The atherogenic index (AI) and thrombogenic index (TI) represent criteria for evaluating the level and interrelation though which some fatty acids may have atherogenic or thrombogenic properties, respectively. Only, the TI was affected by the treatment being lower ($P < 0.01$) in vitamin E group. In general, the obtained values of AI were lower than that reported by Salvatori et al. (2004), ranging from 0.71 and 1.12 in two different crossbred lambs and by Sarti et al. (1998) which found higher values of AI for Comisana lambs (0.87) and for Appenninica lambs (0.72) and their crosses.

The muscle fatty acid composition, affects its oxidative stability during processing and retail display and the polyunsaturated fatty acids in phospholipid being liable to oxidative break down at this stage. For this reason, we used a standard test for lipid oxidative stability in foods, that is the thiobarbituric acid reactive substances test (TBARS), to measure the oxidation product malondialdehyde. Values above about 0.5, are considered critical, since they indicate a level of lipid oxidation products which produce a rancid odour and taste which can be detected by consumers (Wood et al., 2008).

Cholesterol content was found not be significant (P > 0.05) between groups (78.78 *versus* 79.01 mg/100g in C and V, respectively). This finding agree with the result reported by Salvatori et al. (2004) in different muscles (*semimebranosus, longissimus dorsi, gluteobyceps*).The values of cholesterol found in the present study are higher if compared with those reported in other works with different breeds (Muci et al., 1992; Rowe et al., 1999; Salvatori et al., 2004).

The discrepancy can be attributed to a number of factors, such as muscle type, age/weight of animal, environmental factors, feeding, and rearing system (Bragagnolo and Rodriguez-Amaya, 2002), as well as the use of different methodologies for cholesterol quantification or for sampling (Bragagnolo and Rodriguez-Amaya, 2002).

In developed countries, a high meat intake contributes to a higher than recommended total and saturated fat and cholesterol intake. There are a number of experimental studies that showed a fat-rich diets, as well as causing obesity, are also directly related to the risk of colon cancer (Roynettee et al., 2004). Fat and cholesterol are also associated with cardiovascular diseases (Jiménez-Colmenero et al., 2001). Therefore, the advice to consumers is to prefer lean meats and low-fat meat products and moderate consumption of meat (Valsta et al., 2005).

Table 6.3. Fatty acids composition (% of total fatty acids) and cholesterol of *longissimus dorsi* (mean ± SE)

Item[1]	**Groups**		
	Control	**Vitamin E**	***P*-value**
C10:0	0.12±0.01	0.13±0.01	0.626
C 12:0	0.20±0.02	0.27±0.03	0.077
C 14:0	3.08±0.14	3.37±0.23	0.300
C 15:0	0.54±0.04	0.53±0.03	0.807
C 16:0	21.01±0.35	20.36±0.52	0.312
C 17:0	1.71±0.18	1.03±0.06	0.002
C 18:0	14.87±0.37	14.37±0.19	0.238
C 14:1	0.21±0.02	0.26±0.03	0.176
C 16:1 n-9	2.16±0.11	2.30±0.05	0.290
C 17:1	0.78±0.08	0.53±0.03	0.009
C 18:1 n-9 *cis*	40.62±0.41	39.93±0.58	0.340
C 18:1 n-9*trans*	0.33±0.03	0.26±0.02	0.054
C 20:1	0.46±0.03	0.50±0.04	0.412
C 22:1	0.44±0.04	0.59±0.05	0.020
C 18:2 n-6	7.77±0.22	7.92±0.16	0.498
C 18:2 *cis*-9 *trans*-11	0.27±0.01	0.30±0.01	0.147
C 18:2 *trans*-10 *cis*-12	0.06±0.01	0.08±0.01	0.057
C 18:3 n-3	0.89±0.04	1.26±0.05	0.000
C 20:4 n-6	2.10±0.10	2.23±0.07	0.294
C 20:5 n-3	0.72±0.12	1.11±0.13	0.037
C 22:2	0.25±0.02	0.41±0.05	0.007
C 22:4 n-6	0.16±0.02	0.14±0.01	0.295
C 22:5 n-3	0.82±0.04	1.50±0.08	0.000
C 22:6 n-3	0.44±0.05	0.63±0.08	0.057

ΣSFA	41.53±0.55	40.06±0.70	0.111
ΣMUFA	44.99±0.36	44.36±0.52	0.355
ΣPUFA	13.48±0.28	15.58±0.35	0.000
n-3	2.87±0.13	4.51±0.13	0.000
n-6	10.02±0.19	10.29±0.22	0.105
n-6/n-3	3.49±0.25	2.28±0.09	0.005
P/S	0.32±0.01	0.39±0.05	0.001
Atherogenic Index (AI)	0.58±0.02	0.58±0.02	1.000
Thrombogenic Index (TI)	1.08±0.03	0.93±0.03	0.002
Cholesterol (mg/100g)	78.79±1.52	79.00±1.82	0.929

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio.

6.2.3 Intramuscular collagen properities

Meat is a complex, composite substance. It consists of myofibers, connective tissue, and lipids. It has been established that collagen, the major component of the intramuscular connective tissue, plays a key role in determining the background toughness of meat from different domestic animals, including birds (reviewed in Maiorano et al., 2012). Furthermore, a marked difference in collagen maturity could affect meat tenderness (McCormick, 2009; Maiorano et al., 2011, 2012) and technological yield (Boutten et al., 2000).

The data regarding the intramuscular collagen are reported in Table 6.4. In current study, collagen concentration (22.59 and 22.57 μg/mg in C and V group, respectively), HLP concentration (4.37 and 4.59 μg HLP/mg in C and V group, respectively) and collagen maturity (0.135 and 0.142 mol HLP/mol of collagen in C and V group, respectively) were not significantly ($P > 0.05$) influenced by the vitamin E treatment. The results of the present study are partially in contrast with the findings provided by Maiorano et al. (2007), who reported that DL-α-tocopheryl acetate did not influence IMC amount but slowed ($P < 0.05$) collagen maturation (HLP/collagen) and reduced ($P < 0.05$) muscle HLP concentration (μg/mg). In addition, in an earlier study involving suckling lambs slaughtered at 40 day of age (Maiorano et al., 1999),

administration of 1,200 IU of DL-α-tocopheryl also reduced mature crosslink levels. According to the results of intramuscular collagen characteristics, it is clear that meat produced from both lambs (C and V groups) would have a similar tenderness.

Table 6.4. Intramuscular collagen (IMC) properties of *longissimus dorsi* (mean ± SE)

Traits	**C Group**	**V Group**	***P*-value**
Number of lambs	12	12	
IMC (μg/mg[a])	22.71 ± 0.85	22.57 ± 0.19	0.931
HLP (μg/mg[a])	4.33 ± 0.19	4.59 ± 0.34	0.510
HLP mol/mol of collagen	0.135 ± 0.0071	0.142 ± 0.0072	0.503

HLP = hydroxylysylpyridinoline;
[a] of lyophilized muscular tissue.

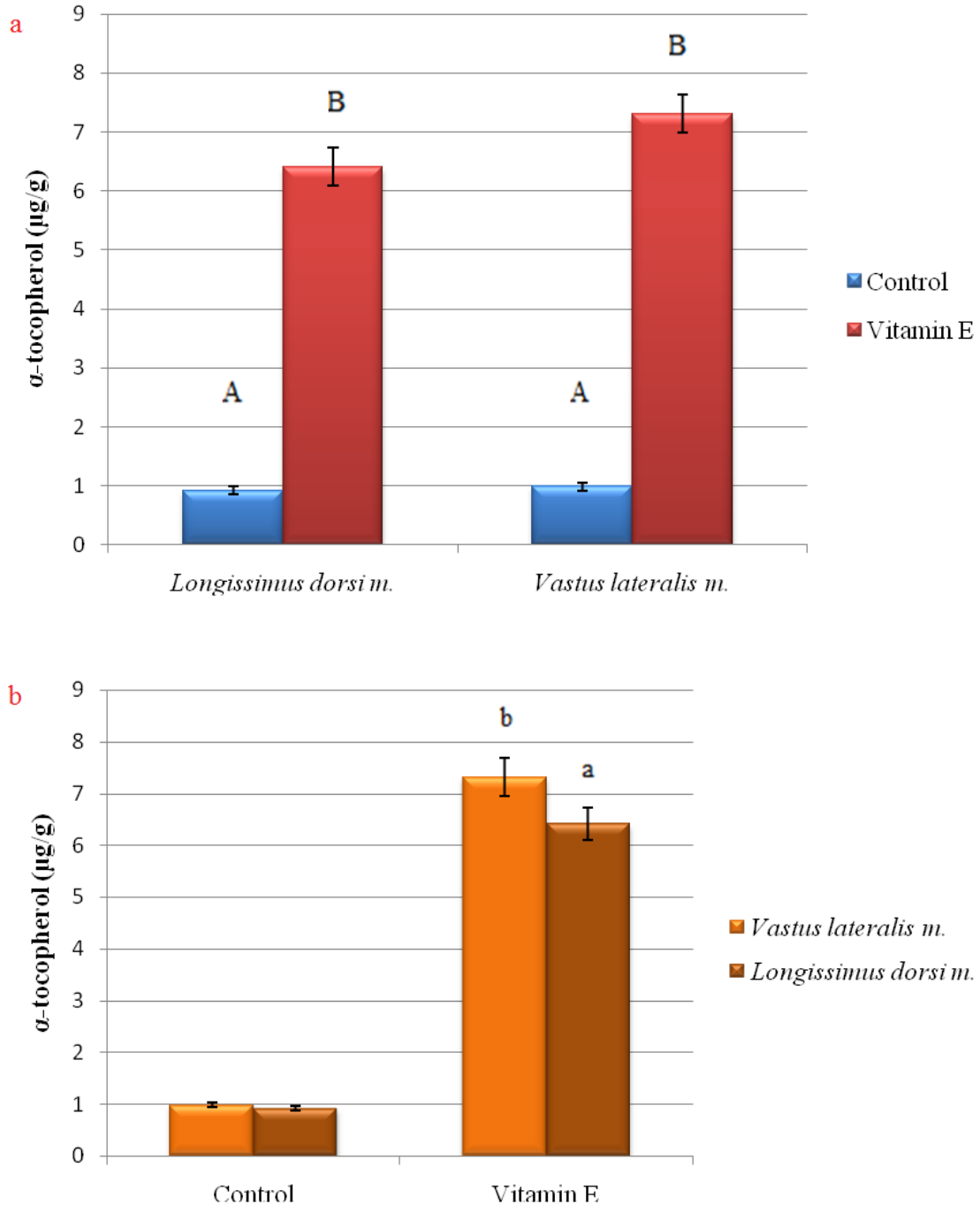
6.2.4 Alpha tocopherol content

The levels of vitamin E in *vastus lateralis* and *longissumus dorsi* muscles is showed in Figure 6.1. In line with the current literature (Ochoa et al., 1992; Wulf et al., 1995; Kerry et al., 2000; Salvatori et al., 2004; Ponnampallam et al., 2012), the treatment with vitamin E markedly increases ($P < 0.001$) the content of DL-α-tocopherol in both muscles (*longissimus dorsi*: 0.92 *versus* 6.41 μg/g in C and V, respectively; *vastus lateralis*: 0.99 *versus* 7.31 μg/g in C and V, respectively) (Figure 6.1 a). Similar results were obtained by Salvatori et al. (2004), who reported a vitamin E concentration of 2.61 *versus* 6.63 μg/g (in control and treated group, respectively), in *longissimus dorsi* of lambs (Gentile di Puglia x Sopravissana) injected with 1,000 IU and slaughtered at 64 days. The above mentioned study also reported different contents of vitamin E in different muscles (*semimembranosus, longissimus dorsi, gluteobiceps*). The authors suggested that the difference is probably due to the different content in fat, and also reported significant effects related to the genotype.

In our study, also, significant differences ($P < 0.05$) were observed in the α-tocopherol concentration between the muscles of treated lambs (6.41 *versus* 7.31 μg/g in *longissimus dorsi* and *vasts lateralis*, respectively), while not significant differences

were observed between the muscles of control lambs (0.92 versus 0.99 µg/g in *longissimus dorsi* and *vasts lateralis*, respectively) (Figure 6.1 b). This diversity is probably due to different fat concentration and to structural, functional and metabolic differences between muscles studied. Similar results were also reported by Kerry et al. (2000) in Cheviot x Border Leicester lambs, following α-tocopheryl acetate supplementation of 1,000 IU/kg for 56 days, and slaughtered 13 weeks post-parturition. Ochoa et al. (1992) reported a concentration of 15 µg/g in *longissimus dorsi* in 56-day-old lambs fed with 1,000 UI/kg/day of vitamin E. Slightly lower concentrations were observed by Liu et al. (2013). They noted that Vitamin E concentrations in *longissimus lumborum (LL)* and *gluteus medius (GM)*, increased significantly after 12 months of vitamin E supplementation ($P < 0.05$) in Aohan fine-wool lambs (5 months old) fed diets supplemented with 0, 20, 100, 200, 1,000, 2,000, or 2,400 IU/sheep/d vitamin E for 12 months. However, this increase did not occur in a dose-dependent manner, because the muscle vitamin E concentration was highest (7.14 *versus* 5.23 µg/g in GM and LL, respectively) in the 200 IU/sheep/d group. The current literature agree with the fact that vitamin E administration, in diet or by injections, significantly increases the content of α-tocopherol in different kinds of muscle, even though this increase did not occur in a dose-dependent manner. Usually, with the increasing of the administration quantity, there is an increasing in muscle concentration up to reach an optimal doses, to above which the vitamin E concentration in muscle starts to decrease. This effect was firstly observed by Barja et al. (1996) and then confirmed by Maiorano et al. (1999).

Figure 6.1. Content of α-tocopherol (µg/g) in *longissimus dorsi* (n=12) and *vastus lateralis* (n=12) muscles between treatments (a) and within treatment (b) (mean±SE).



A, B: P < 0.01; a, b: P < 0.05.

The efficiency of tocopherols against the membrane phospholipids oxidation and degradation depends on numerous factors. According to Ohm et al. (2005), is not clear at witch concentration α-tocopherol reaches its maximum activity, even *in vitro*. They noted, in a model consisting of rapeseed oil triglyceride, that the maximum activity was reached at concentration of 50-125 μmol α-tocopherol/kg. Above this range there is a pro-oxidant attitude with a maximum at 250 μmol α-tocopherol/kg. In the animal organism is very difficult to standardize the final concentration of α-tocopherol in the muscle, because it depends on numerous factors: the doses and the way of administration, the metabolism that is different among several breeds, the total fat content and some environmental factors (eg. stress) that are difficult to control. The importance of the numerous above-mentioned factors, are confirmed by Ponnampalam et al. (2012), Salvatori et al. (2004) and Njeru et al. (1994). They recorded a higher concentration of α-tocopherol in *longissimus dorsi* of treated animals, but the final results in concentration often depends on other agents. Barja et al. (1996) also showed that high doses of vitamin E are not more effective than intermediate ones for optimal protection against lipid peroxidation.

In the light of this considerations, additional analyses are been necessary to assess the antioxidant activity of vitamin E, and in particular its function in preventing lipid peroxidation. The TBARS assay, during the aerobic storage at 5°C of longissimus dorsi and vastus lateralis, a reliable test to study this activity in vitro, was realizable by subjecting the membrane lipids to oxidant stress and observing the radical-scavenging function of vitamin E.

6.3 TBARS: oxidative stability

TBARS values during storage are provided in Table 6.5 and 6.6 and Figure 6.2 and 6.3. During all the storage period in *longissimus dorsi* (Table 6.5) and in *vastus lateralis* (Table 6.6), the values of TBARS were significantly lowest ($P < 0.000$) in treated group respect to the control.

It is important to underline that in both muscles, from treated lambs, the levels of TBARS were not significantly ($P > 0.05$) different for 96 hours (after four days of display) of aerobic preservation, and then started to increase ($P < 0.01$) (Figures 6.2, 6.3). On the contrary, in the LD and VL of not treated animals, the oxidation start

immediately (24 hours of display; Figures 6.1 and 6.2). The values obtained in the treated animals at 96h (0.13 in LD and 0.11 MDA/kg in VL) are very interesting because are lowest than 0.5 that is considered critical, since indicate a level of lipid oxidation products, which produce a rancid odour and taste, which can be detected by consumers (Wood et al., 2008). This value, while is reached in the meat of treated animals at 192 hours, in that of control group values above 0.5 are already achieved at 48 hours, in both muscles. The above described trend confirms the evidence that vitamin E preserves the meat from the lipoperoxidation, and allows a better preservation and a prevention against bad odours. Results similar to our work were obtained by Rippol et al (2013), Okan et al (2009) and Kerry et al. (2000). Analysis of TBARS at different times during the aerobic storage at 5°C, is described in several works regarding the addition of vitamin E in the diet, but few works exist in literature regarding TBARS in animals injected with vitamin E (Salvatori et al., 2004). The range of TBARS in our study is between 0.05 (LD, 24h) and 1.61 (VL, 192h) mg/kg, in line with other studies on storage of lambs meat (Luciano et al., 2013; Rippol et al., 2013; Salvatori et al., 2004). The values of TBARS measured at different storage times in the meat from vitamin E group, are quite low if compared with the results of other works on lamb meat preservation, and if compared with the use of other antioxidants; differently, the values of TBARS in the control groups are in line with those of literature (Luciano et al., 2013; Rippol et al., 2013).

Rippol et al. (2013) found that feeding lambs α-tocopherol enriched concentrate, during the last 10 days of life, or grazing them on alfalfa, drastically diminished the lipid oxidation (measured as TBARS) of meat. They found that TBARS in muscle, were significantly higher ($P < 0.05$) in the control group if compared with the other groups of the experimentation, after 3 and 6 days of display (experimental groups were composed of lambs supplemented with 500 mg of DL-α-tocopheryl acetate/kg concentrate for 0, 10, 20 and 30 d before slaughtering at 22–24 kg BW). Salvatori et al. (2004) reported that the intramuscular lipid peroxidation rate was inversely correlated ($P < 0.05$) to the vitamin E concentration in *semimembranosus* and *gluteobiceps* muscles. Oriani et al. (1999) also found a decrease in the level of liver thiobarbituric-acid reactive substances (TBARS), significantly correlated with liver alpha-tocopherol content, in vitamin E treated lambs.

Ponnampalam et al. (2012) detected, 24 hours after slaughter, a TBARS content ranging between 0.24 and 0.36 mg MDA/kg in *longissumus luborum* m. of lambs reared on the pasture. This result is quite interesting, because the content of vitamin E, in the same animals, range between 3.63 and 5.88, that are values similar to those of the present study. From this study we might assume that the effects of vitamin E injections and of the pasture rearing system, on the evolution of lipid peroxidation, could be similar.

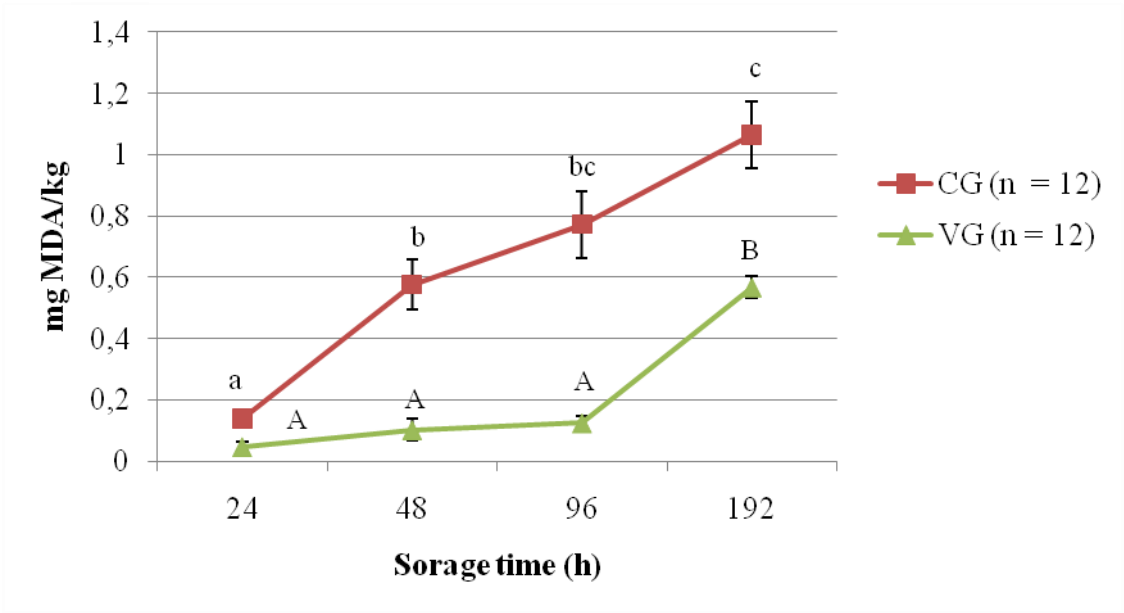
Table 6.5. Effect of the treatment (controll *versus* vitamin E) on the evolution of TBARS (mg MDA/kg, means±SE) in *longissimus dorsi* muscles during the aerobic storage period at 5°C

Storage time (hours)	**Control**	**Vitamin E**	***P*-value**
24	0.1392±0.0186	0.0483±0.0147	0.000
48	0.5758±0.0809	0.1033±0.0362	0.000
96	0.7725±0.1096	0.1258±0.0219	0.000
192	1.0633±0.1098	0.5667±0.0355	0.000

Table 6.6. Effect of the treatment (controll *versus* vitamin E) on the evolution of TBARS (mg MDA/kg, means±SE) in *vastus lateralis* muscles during the aerobic storage period at 5°C

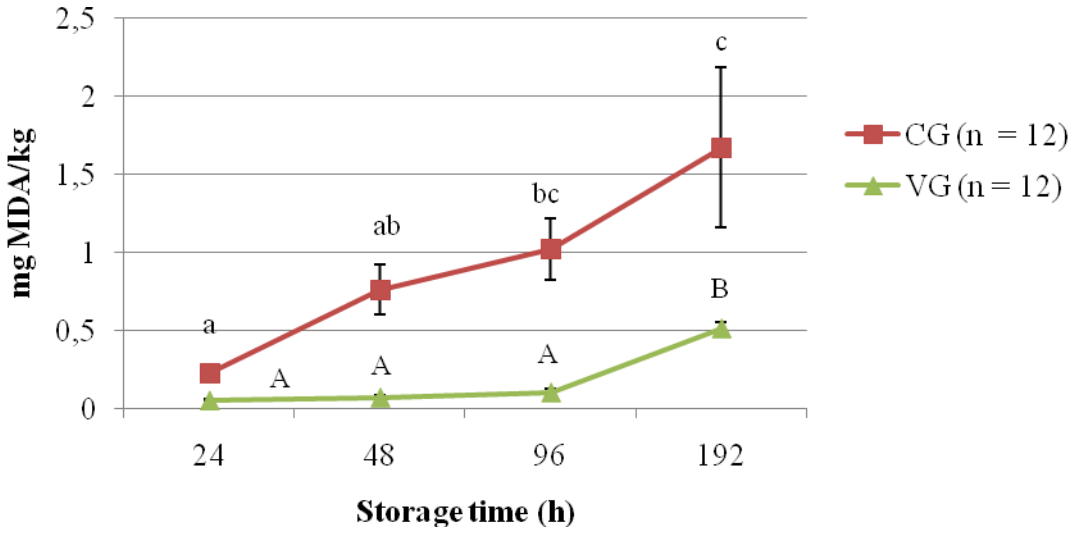
Storage time (hours)	**Control**	**Vitamin E**	***P*-value**
24	0.2283±0.0580	0.0550±0.0044	0.000
48	0.7633±0.1612	0.0742±0.0107	0.000
96	1.0217±0.1991	0.1067±0.0176	0.000
192	1.6717±0.5142	0.5142±0.0393	0.000

Figure 6.2. TBARS (mg MDA/kg, means±SE) in *longissimus dorsi* muscle, within lambs treated (VG) and no treated (CG), during the aerobic storage at 5°C



[a,b,c] values within the same group differ significantly (P < 0.05)
[A,B] values within the same group differ significantly (P < 0.01)

Figure 6.3. TBARS (mg MDA/kg, means±SE) in *vastus lateralis* muscle, within lambs treated (VG) and no treated (CG), during the aerobic storage at 5°C



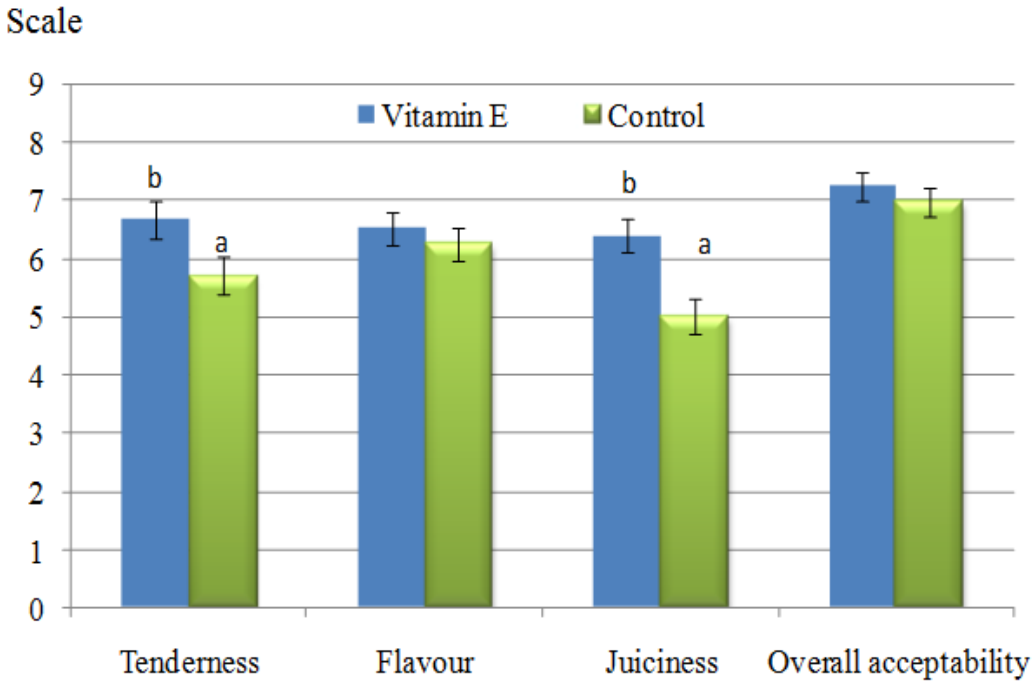
[a,b,c] values within the same group differ significantly (P < 0.05)
[A,B] values within the same group differ significantly (P < 0.01)

Figure 6.4. Cuvette of Vitamin E group (left side) and Control group (right side) during the spectrophotometer reading (96h). It is possible to observe the different coloration *(personal photo)*

6.4 Panel test

From the statistical analysis, the results evidenced that all the judges (students and adults) enjoyed both types of meat (Figure 6.5), giving an extra point higher for the lamb that was injected with vitamin E if compared to the other type of meat (7.24 and 6.97 respectively; $P > 0.05$). Therefore, both expressed a greater pleasure with the meat produced in the vitamin E group if compared to the meat of control group, as regard the descriptor of tenderness (6.66 *versus* 5.70, respectively; $P < 0.05$) and juiciness (6.39 *versus* 5.00, respectively; $P < 0.05$). Also the flavour received a slightly higher score for V group meat if compared to that of control group ($P > 0.05$). There are few work in literature regarding the effect of vitamin E on sensorial characteristic of meat. Bielanski et al. (2008) demonstrated a significative effect of vitamin E, supplemented in diet, on the tenderness of meat, but not significative effects on other sensory traits (aroma, juiciness, taste, overall acceptability). D'Alessandro et al. (2012) demonstrated that maternal feeding system in the stall or in pasture affects the nutritional value of suckling lamb meat and consumer acceptability (after they are given information on the production system). The authors suggested that this effect could be due to an higher α-tocopherol content in the meat of pasture feeding lambs. On the contrary, Maiorano et al. (2010) found that meat from lamb fed with maternal milk, from mother reared on stall, received higher hedonic scores than lamb fed with maternal milk from mother reared on the grass, on the blind test; while, with label information on animal feeding system, the panellists gave a opposite result than that of blind test, except for the tenderness.

Figure 6.5. Sensory analysis (rated on scale 1-9) of meat lambs (Vitamin E *versus* Control) perceived by judges (adults and students, mean±SE)

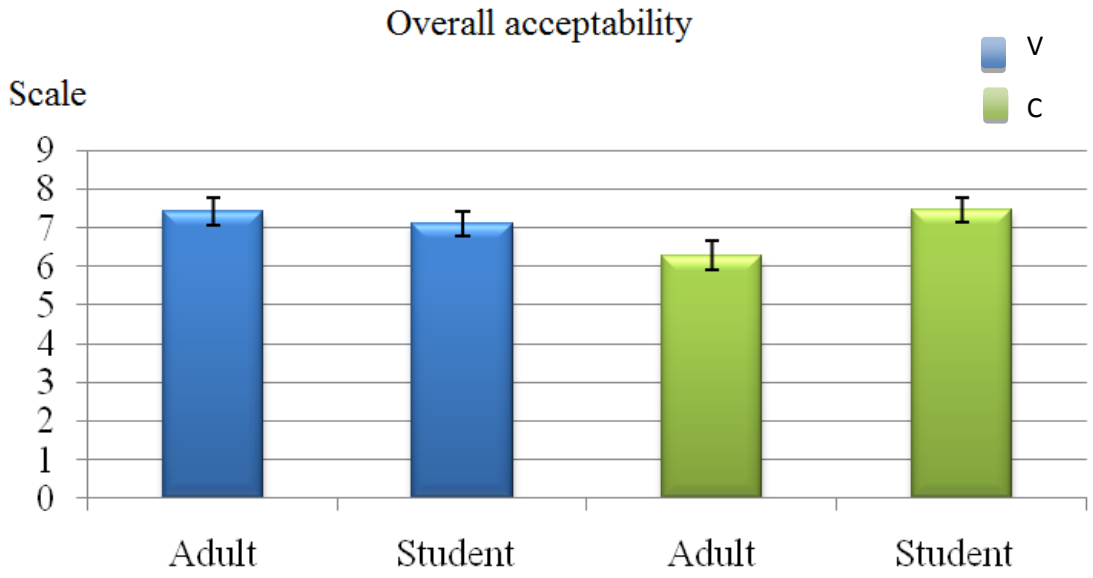


a, b: $P < 0.05$

A further analysis of data regards the different level of perception and approval expressed by the students, considered as initial judges, compared with the adults, considered as custumary consumer of lamb meat.

The results shown in Figure 6.6 demonstrated that the age of the panellists did not significantly influence ($P = 0.211$) the values of overall acceptability of the two lamb meat samples, obtained from the treated and control group, respectively.

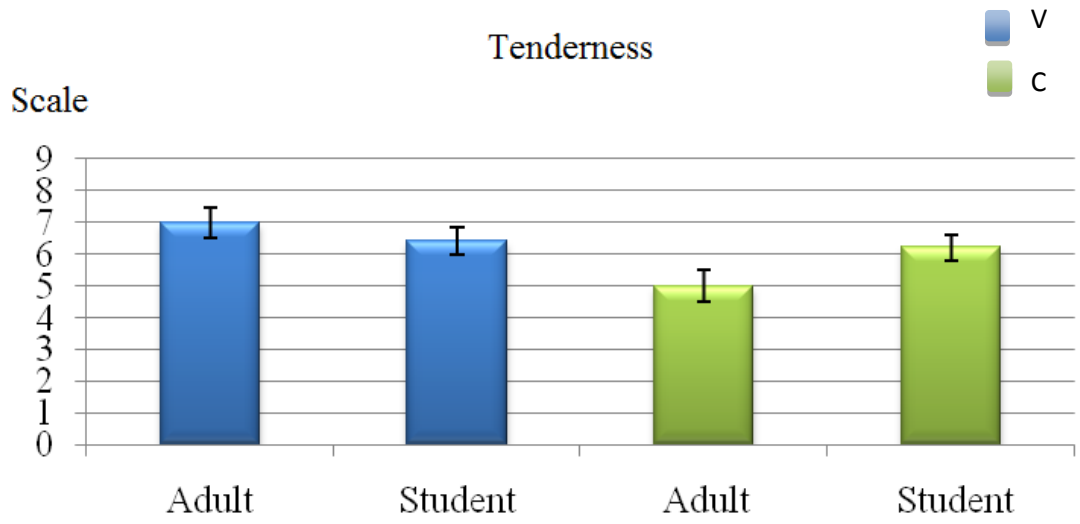
Figure 6.6. Level of general approval of lamb meat, obtained by vitamin E treated (V) and control (C) lambs, perceived by the judges (adults *versus* students, mean ± SE).



P = 0.211

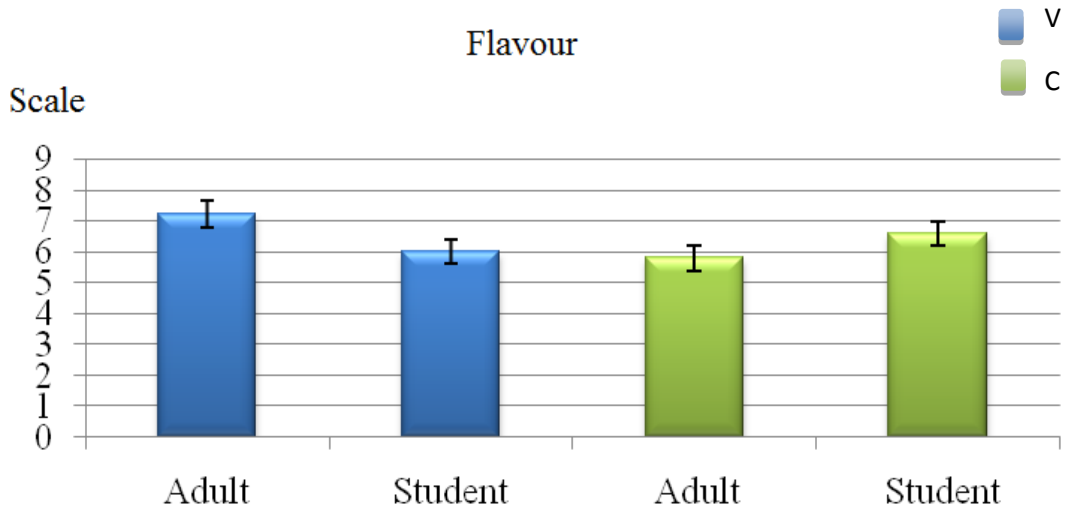
Similarly, tenderness (Figure 6.7), flavour (Figure 6.8) and juiciness (Figure 6.9) were not affected by the age of the panellist. These results point out a particular relevant aspect: two different generations (adults: between the ages from 50 to 70 years old, students between the ages from 21 to 25) having different taste and eating habits, expressed a very similar evaluation. This is notable, considering that this type of meat is normally consumed by adult consumers.

Figure 6.7. Level of tenderness of lamb meat, obtained by vitamin E treated (V) and control (C) animals, perceived by the judges (adults *versus* students, mean ± SE)



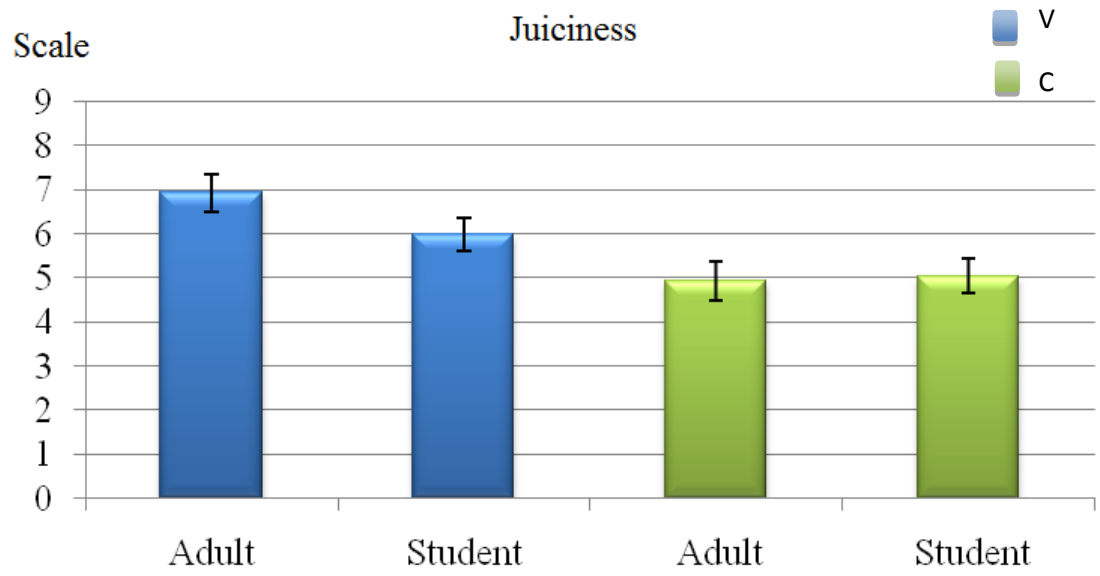
P = 0.4899

Figure 6.8. Level of flavour of lamb meat, obtained by vitamin E treated (V) and control (C) animals, perceived by the judges (adults *versus* students, mean ± SE)



P = 0.604

Figure 6.9. Level of juiciness of lamb meat, obtained by vitamin E treated (V) and control (C) animals, perceived by the judges (adults *versus* students, mean ±SE)



P = 0.331

Chapter 7

CONCLUSION

This study confirmed that a supply of antioxidants is important to improve the commercial charachteristic and preserve the oxidative stability of animal products. In literature many studies exist regarding the effects of vitamin E addition on the diet, but few works exist about in muscle injection, especially as regard the effects on TBARS during the storage period.

The current study clearly demonstrated that vitamin E administration did not influence significantly live weight, carcass traits, except the incidence of pelvic limb, significantly higher in V group lambs; *longissimus dorsi* quality traits did not differ between the experimental groups, except for the red colour value (a*), that was higher ($P < 0.05$) in lambs of vitamin E group.

Treatment with vitamin E did not significantly affect the total SFA content and the proportion of single SFA, except for a slightly higher ($P < 0.08$) content of lauric acid (C 12:0) and lower ($P < 0.01$) content of eptadecanoic acid (C 17:0) in V group. Likewise, vitamin E treatment did not affected the total MUFA amount, except for a lower ($P < 0.01$) content of C 17:1 and higher C 18:1 n-9 *trans* ($P < 0.05$) and C 22:1 ($P < 0.01$) in V group. The oleic acid (C18:1 n-9 *cis*) was the most concentrated fatty acid (40-41%), followed by palmitic (20-21%) and stearic acids (14-15%). Vitamin E treatment positively affected the total PUFA content being higher ($P < 0.01$) in the V group compared to the control group. Among the PUFA, the most abundant was the linoleic acid (C 18:2n-6). The second most important PUFA was α-linolenic acid (C 18:3n-3). In the present study, the vitamin E treated group had a significant higher amount of n-3 long chain PUFA: eicosapentaenoic fatty acid (EPA, C20:5n-3, $P < 0.05$), docosapentaenoic fatty acid (DPA, C22:5n-3, $P < 0.01$) and docosahexaenoic fatty acid (DHA, C22:6n-3, $P < 0.06$). Also vitamin E group had higher ($P < 0.01$) content of C 22:2. In light of this, the total n-3 FA content was higher ($P < 0.01$) in treated lambs compared to the control group; while, no significant differences were found for the total n-6 FA amount. The n-6/n-3 and P/S ratios were also affected by the vitamin E treatment. The n-6/n-3 ratio was lower in treated lambs (2.33 *versus* 3.62 for V and C group, respectively; $P < 0.01$). The P/S ratio was higher ($P < 0.01$) in treated

lambs, even if the obtained values, were lower than the recommended range of 0.4-0.7. Also the TI was affected by the treatment being lower ($P < 0.01$) in vitamin E group. In light of this, the treated lambs provided meat with an "healthier" balance of FA.

Cholesterol content was found to be not different ($P > 0.05$) between groups (78.78 and 79.01 mg/100g in C and V, respectively) and slightly higher when compared with other works, and with other breeds.

Collagen concentration (22.59 and 22.57 μg/mg in C and V group, respectively), HLP concentration (4.37 and 4.59 μg HLP/mg in C and V group, respectively) and collagen stability (0.135 and 0.142 mol HLP/mol of collagen in C and V group, respectively) were not significantly ($P > 0.05$) influenced by the treatment.

The treatment with vitamin E markedly increases ($P < 0.001$) the content of DL-α-tocopherol in both muscles (*longissimus dorsi*: 0.92 *versus* 6.41 μg/g in C and V, respectively; *vastus lateralis*: 0.99 *versus* 7.31 μg/g in C and V, respectively) and increases the meat shelf life. This was evident since muscle tissue lipoperoxidation levels (TBARS), were significantly lower ($P<0.01$) in vitamin E injected lamb meat at 24, 48, 96 and 192 h *post mortem*, during all the aerobic display at 5°C.

The panel test showed that the both adult and the student judges, enjoyed both types of meat giving a slightly higher point for the lamb that was injected with vitamin E if compared to the other type of meat (7.24 and 6.97 respectively; $P > 0.05$). Therefore, both expressed a greater pleasure with the meat produced in the experimental group if compared to the meat of control group, as regard the descriptors of tenderness (6.66 *versus* 5.70, respectively; $P < 0.05$) and juiciness (6.39 *versus* 5.00; $P < 0.05$).

At the light of the above mentioned, the conclusion of this study is that the intramuscular administration of vitamin E can be recommended to obtain meat with an higher nutritional and commercial value.

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