Università degli Studi di Foggia

DIPARTIMENTO DI SCIENZE AGRARIE, DEGLI ALIMENTI E DELL'AMBIENTE

(SAFE)

PhD course on Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region

(XXVIII Cycle)

STRATEGIES TO IMPROVE NUTRITIONAL AND ORGANOLEPTIC QUALITY OF BEEF

Coordinator:
Prof. Giancarlo Colelli
Tutor:
Prof.ssa Rosaria Marino

PhD student:
Dr. Carmela Vassallo

Alla mia Famiglia

"Deus videt, Deus decidit, Deus facet"

A B S T R A C T

The aim of the present thesis was the study of the strategies to improve nutritional and organoleptic quality of beef. Two experiments were performed. The first experiment was undertaken: i) to evaluate the effects of supplementation of sunflower and linseed on growth performance and on nutritional and eating quality of meat from young bulls; ii) to test if a combination of linseed and sunflower seeds have a synergistic effect on fatty acids profile. *Longissimus thoracis* (LT) muscle from 32 Podolian x Limousine young bulls, aged for 8 days was used to evaluate the effect of oilseeds supplementation on nutritional and organoleptic quality of beef. The animals were fed with one four isoenergetic and isoproteic diets: control diet (CO) consisting of oat hay and concentrate and no supplemental fat; Linseed diet (LS): oat hay and concentrate with a supplementation of 10% linseed; Sunflower diet (SS): oats hay and concentrate with supplementation of 10%; Linseeds+Sunflowers seeds diet (LS+SS): oat hay and concentrate with supplementation of (1:1 w/w). The oilseeds supplementation diet increased the content of fatty acids n3 and decreased the ratio of n-6/n-3 in the muscle. The results demonstrate that the oilseeds supplementation is a nutritional strategy to improve the health n3 fatty acid content in beef, also significantly improved the sensory properties of meat.

The second experiment evaluated the effect of linseed supplementation and duration of feeding on fatty acids profile and on bioactive compounds of beef. 48 Friesian steers were randomly allocated during finishing period into six experimental treatments following a 3x2 factorial arrangement. The 6 treatments consisted of 3 different time of administration of diet: 40, 75 or 120 days before slaughter and 2 diet, control diet (CO) with no supplemental oilseeds and linseed diet (LS) containing 10% whole linseed. Linseed supplementation strongly affect the nutritional profile of meat, in particular meat from linseed group showed improvement the content of total monounsaturated ($P<0.05$), CLA ($P<0.05$), n3 polyunsaturated fatty acids ($P<0.01$) and bioactive substances such as creatine

(P<0.05), carnosine and anserine (P<0.001). The duration of feeding and the interaction diet x duration significantly affected the proportions of fatty acids. The cholesterol content in meat was not affected by the dietary treatment while, significant differences (P<0.05) was found in meat after 75 days of linseed somministration. These results confirmed the better nutritional profile of meat from linseed group and demonstrated also that is not necessary to feed bovine for a long time to achieve a high increase of n3 polyunsaturated fatty acids, but 75 days of feeding is enough to obtain meat with beneficial characteristics.

CONTENTS

LIST OF FIGURE

LIST OF TABLES

Chapter 1

Role of red meat in diet

1.1 Introduction

Bovine meat can be considered as a “functional” element since it provides high-quality proteins (amino acids essential) vitamins (mainly those of the B complex, being major sources of B12), folate, minerals (selenium, phosphorus and manganese, iron and zinc of high bioavailability; Mir et al., 2003). Meat consumption is predicted to increase globally over the next twenty years in line with an increasing world population, a greater income potential and the availability of these foods to meet human nutrient requirements as part of an everyday diet (World Health Organization, 2003). Due to its composition and high consumption, meat is important sources of a wide range of nutrients and provide considerable proportions of the dietary intakes of various nutrients that are essential for optimal growth and development (Jiménez et al., 2012). Additionally, meat contributes 10–20% of total calories in the diet in industrialized countries, and meat from ruminants is well documented to be high in saturated fatty acids, with a content of up to 50% (Chizzolini et al., 1999). Relative to pork and poultry, beef contains less cholesterol (Favier et al., 1995), but also contains high levels of SFA, especially, palmitic (C16:0) and stearic (C18:0), which are hyper-cholesterolemic fatty acids. As a consequence, nutritionists recommending a reduction of fat intake, with an intake of 10% for saturated fatty acids. At the same time, it is recommended that the n6/n3 ratio of dietary PUFA should be lower than 4 (Simopoulos, 2004; WHO, 2003) because this ratio is also a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser, 2001). However, ruminant meat also provides n3 long-chain polyunsaturated fatty acids and conjugated linoleic acid (CLA) widely recognized for their beneficial effects on health, i.e. heart attack, depression and cancer and reduction of the inflammation caused by rheumatoid arthritis (McAfee et al., 2010; Enser, 2001). Therefore, fatty acids have different effects on human health, some beneficial and some adverse, and for this reason is important to determine the amount of different fatty acids present in animal foods. Indeed, today's health conscious consumers are

willing to pay higher prices to meat for enhanced levels of fatty acids beneficial to human health.

1.2 Fatty acid classification

Fatty acids can be classified based on carbon chain length and number of double bonds as: saturated (no double bonds), monounsaturated (one double bond) or polyunsaturated (two or more double bonds) fatty acids.

1.2.1 Saturated fatty acids (SFA)

Saturated fatty acids contain only carbon-carbon single bonds in their chain (Figure 1.1). The carbons in these fatty acids are fully loaded with hydrogen atoms, thus forming straight chains. SFA stack tightly, providing rigidity and making food fats, such as butter, solid at room temperature. Since decades lipids from animal foods are a target of dietician's criticism due to the negative effects of excessive consumption of SFA on human health and, for this reason in most developed countries, dietary guidelines produced have proposed reductions in total fat and in SFA intake as a means of reducing the prevalence of coronary heart disease.

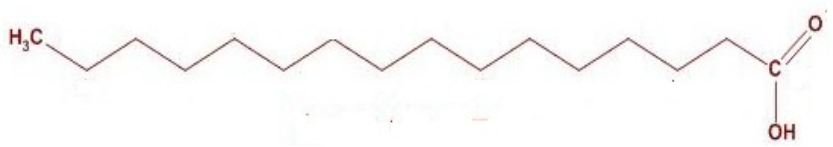


Figure 1.1. Chemical structure of palmitic acid.

Meat is a major source of fat in the diet, especially of saturated fatty acids, the main SFA in red meat are palmitic (C16:0) and stearic acid (C18:0). The content of SFA of the lipid of meat and, especially, in ruminants is due to the regulating mechanisms implemented by rumen microorganisms.

Diets high in SFA contribute to increase Low-density lipoproteins-cholesterol (LDL) level, which is positively related to the occurrence of heart diseases. However, not all SFA have the same effect on blood cholesterol: mainly lauric

(C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids which are responsible for increasing plasma total and LDL-cholesterol concentrations, (Bauman at al., 1999); whilst myristic acid is thought to be the most atherogenic and has four times the cholesterol raising potential of palmitic acid (Ulbricht et al., 1991). The other major SFA, stearic acid has been shown not to increase total cholesterol or LDL-cholesterol concentrations, this is because in human tissues is active the enzyme Δ^9- desaturase which is able to convert about 40% of stearic acid in oleic acid (Chilliard et al., 2001).

1.2.2 Monosaturated fatty acids (MUFA)

The fatty acids containing one double bond are MUFA (Figure 1.2). Common MUFA are palmitoleic acid (16:1 n7), cis-vaccenic acid (18:1 n7) and oleic acid (18:1 n9, OA). In meat, MUFA are composed mainly by oleic acid.

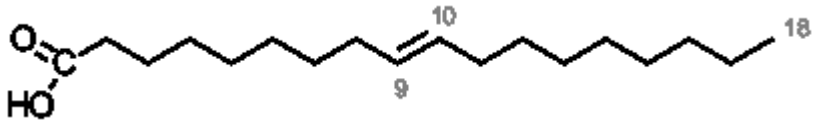


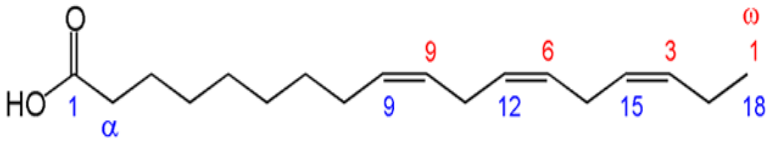
Figure 1.2. Chemical structure of oleic acid (OA, 18: n9).

Several studies reported that MUFA are able to reduce the level of serum LDL-cholesterol, but, contrary to PUFA n6, without effects on HDL-cholesterol (Ulbricht et al., 1991). In light of this beneficial effect on serum cholesterol, foods that contain high amounts of oleic acid can be defined as a functional food in relation to the reduction of cardiovascular disease (Hornstra, 1999).

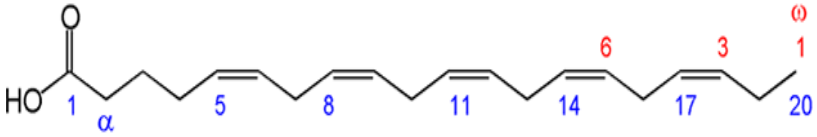
1.2.3 Polynsaturated fatty acids (PUFA)

The fatty acids that contain more than one double bond are called PUFA (Figure 1.3). The position of the first double bond is given by the (nx) notation, counting the number of carbon atoms from the methyl end, according to the

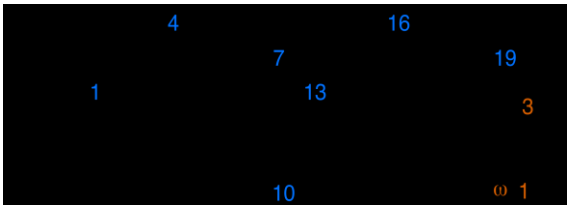
international nomenclature. For example, omega3 and omega6 (also referred as n3 and n6 fatty acids) denote fatty acids, in which the first double bond starts at 3 and 6 carbons from the methyl end respectively (Gurr et al., 2002). Major sources of n6 fatty acids are vegetable oils whereas n3 fatty acid sources are fish and meat (Schmitz et al., 2008). Because these two fatty acids cannot be synthesized in mammals, they are defined as essential fatty acids (EFA).



Alpha-linolenic acid (ALA, 18:3n3)



Eicosapentaenoic acid (EPA, 20:5n3)



Docosahexaenoic acid (DHA, 22:6n3)

Figure 1.3. Chemical structure of alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid.

Moreover these fatty acid play important functions. Linoleic acid (LA, C18:2 n6) is important for the impermeability of the epidermis, while α-linolenic acid (ALA, C18:3 n3) has an important role in the prevention of heart disease, cancer and

disorders of neurological function in both children and adults (Barcelò–Coblijin et al., 2009). Its elongation products, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), are widely recognized for their numerous effects on ~~heart~~ health: improve platelet aggregation, vasodilatation and thrombotic tendency (Siddiqui et al., 2008), are critical for proper brain and visual development in the foetus, and for the maintenance of neural and visual tissues (Calder, 2004). For these reasons is important to maintain a balance between n6 and n3 fatty acids. The optimal ratio between n6/n3 fatty acid may vary with the disease under consideration. In the prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer and suppressed inflammation in patients with rheumatoid arthritis (Simopoulos, 2008).

1.2.4 Conjugated Linoleic Acid (CLA)

Conjugated Linoleic Acid (CLA) is another group of PUFA which has received considerable interest due to a wide range of beneficial effect on human health. Studies demonstrate that CLA has potential anticarcinogenic, immunomodulating and antiatherosclerotic effect (Pariza et al., 2001; Luongo et al., 2003).

CLA consists of a group of geometric and positional isomers of linoleic acid [cis-9, cis-12 (c9,c12)-18:2]. CLA is used as a collective term because all known isomers have double bonds with a single carbon bond in between instead of the usual methylene-separation. These double bonds can either be trans (t) or cis (c) configured and a wide spectrum of isomers with variations in position (7,9; 8,10; 9,11; 10,12 or 11,13) and geometry (c/c; c/t; t/t or t/c) exist (Mulvihill, 2001; Martin et al., 2002). In synthetic CLA preparations the c9,t11 and t10,c12-18:2 isomers are predominant (often in a 1:1 ratio) (Larsen et al., 2003) whereas in CLA rich sources like beef and dairy products about 80% of the CLA is the c9,t11-18:2 isomer (Fritsche et al., 1998). Food sources originating from ruminants are known to have markedly higher CLA concentration than those from monogastric animals. The CLA found in milk and meat of ruminants is obtained by two ways: the manner is an incomplete biohydrogenation of PUFA in the

rumen and the latter is the conversion of VA in animal tissues (Griinari et al., 1999).

1.3 Biosynthesis of fatty acids in ruminants

The lipid composition of forages consists largely of glycolipids and phospholipids, and the major fatty acids are the unsaturated fatty acids linolenic (C18:3) and linoleic (C18:2) acid. In contrast, the lipid composition of seed oils used in concentrate feedstuffs is predominantly triglycerides containing linoleic and oleic acid as the predominant fatty acids. When consumed by ruminant animals, dietary lipids undergo two important transformations in the rumen. The initial transformation is hydrolysis of the ester linkages catalyzed by microbial lipases. This step is a prerequisite for the second transformation: biohydrogenation of the unsaturated fatty acids. Biohydrogenation is the process that converts linoleic acid to numerous isomers of CLA in the gastrointestinal tract through several bacterial species (Crumb, 2011; Figure 1.4). CLA isomers are formed as intermediates during biohydrogenation of C18:2 to C18:0 in the rumen by *Butyrivibrio fibrisolvens* and other rumen bacteria (Khanal et al., 2004). Isomerization of the cis-12 double bond represents the initial step during biohydrogenation of fatty acids containing a cis-9, cis-12 double bond system. The isomerase reaction is unusual because it has no cofactor requirement and occurs in the middle of a long hydrocarbon chain remote from any activating functional groups. Linoleate isomerase is the enzyme responsible for forming conjugated double bonds from the cis-9, cis-12 double bond structure of linoleic as well as α- and γ-linolenic acids. The second reaction is a reduction in which cis-9, trans- 11 CLA is converted to trans-11 C18:1. In vitro studies using labeled linoleic acid cultured with rumen contents demonstrated that isomerization of the cis-12 double bond was followed by rapid conversion of cis-9, trans-11 CLA to trans-11 octadecenoic acid. Hydrogenation of the trans-11 monoene occurred less rapidly, and therefore it increased in concentration. Similar results were obtained in time course studies of linoleic acid biohydrogenation. Therefore, trans-11 C18:1 reduction seems to be rate-limiting in the biohydrogenation sequence of

unsaturated C18 fatty acids. As a consequence, this second to last biohydrogenation intermediate accumulates in the rumen and is, therefore, more available for absorption.

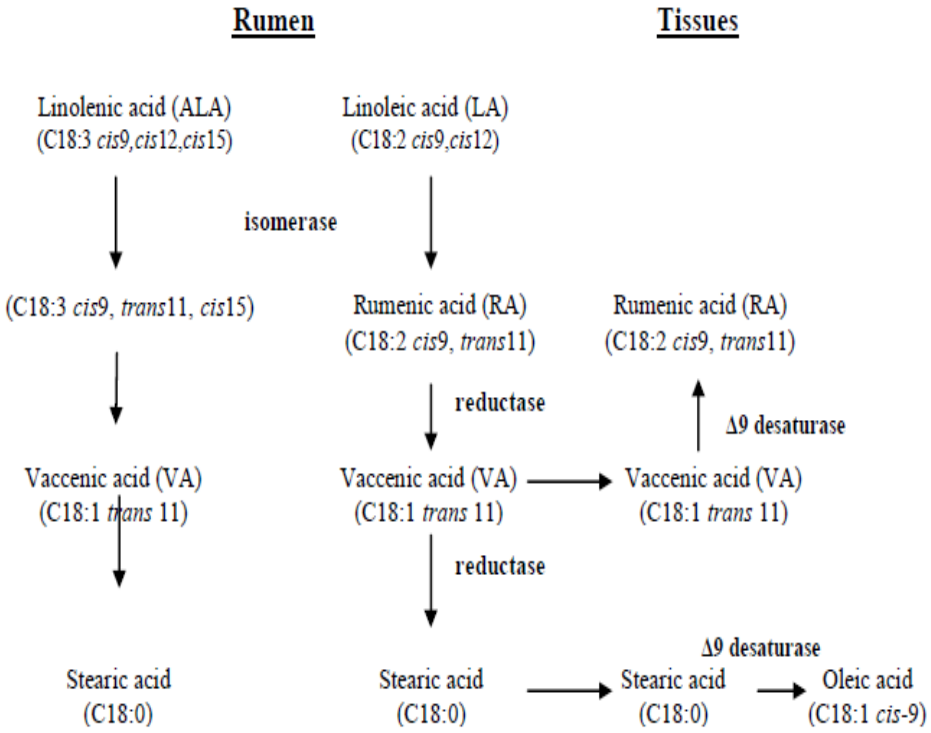


Figure 1.4. Rumen biohydrogenation of linoleic acid.

Similar to biohydrogenation of linoleic acid, biohydrogenation of linolenic acid begins with an isomerization followed by a sequence of reductions and terminates with the formation of stearic acid. The predominant C18:3 fatty acid in feedstuffs is α-linolenic acid (cis-9, cis-12, cis-15 octadecatrienoic acid). Rumen biohydrogenation of α-linolenic acid produces cis-9, trans-11, cis-15 conjugated octadecatrienoicacid as the predominant initial isomerization product, and this is followed by reduction of the cis-double bonds. As a consequence, trans-11 octadecenoic acid is a common intermediate in the biohydrogenation of both α-linolenic acid and linoleic acid. In addition, biohydrogenation of γ-linolenic acid,

cis-6, cis-9, cis-12 octadecatrienoic acid, also results in formation of trans-11 C18:1 (Griinari et al., 1999).

The second source of CLA in ruminants is the endogenous synthesis via Δ^9-desaturase of trans vaccenic acid (Khanal et al., 2004). Trans vaccenic acid is a major trans fatty acid, which is a biohydrogenation product of C18:2 (Wood et al., 2008). This fatty acid is converted to CLA in adipose tissue by the action of stearoyl Co-A desaturase, the same enzyme responsible for the production of C18:1c9 from C18:0 (Wood et al., 2008). It is estimated that over 86% of the cis-9, trans-11 CLA isomer in beef fat originates from the desaturation of trans vaccenic acid (Khanal et al., 2004).

1.4 Cholesterol

Cholesterol is an essential element of cell membranes, where it provides structural support and may even serve as a protective antioxidant (Girao et al., 1999). Cholesterol is needed for the synthesis of bile acids, which are essential for the absorption of fats, and of many hormones such as testosterone, estrogen, dihydroepiandrosterone, progesterone, and cortisol. Together with sun exposure, cholesterol is required to produce vitamin D (Colpo, 2005). Cholesterol is the major sterol in the human body and belongs to the class of molecules called steroids, which are derivatives of the perhydrocyclopentanophenanthrene ring system (Figure 1.5). Cholesterol is synthesized in many types of tissue but particularly in the liver and intestinal walls (Christie, 2003). The transport of cholesterol and other triglyceride fats from their sources of origin, to the different parts of the body where they are needed is achieved by very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL) and low-density lipoproteins (LDL). They are also referred to as “bad” cholesterol because if too much LDL cholesterol circulates in the blood, it can slowly build-up in the arteries feeding the heart and brain. This can subsequently lead to formation of plaque, a thick, hard deposit that can clog those arteries, resulting in atherosclerosis. When atherosclerosis affects blood vessels supplying the brain,

this results in cerebral vascular disease, eventually leading to a stroke (Lichtenstein et al., 2006).

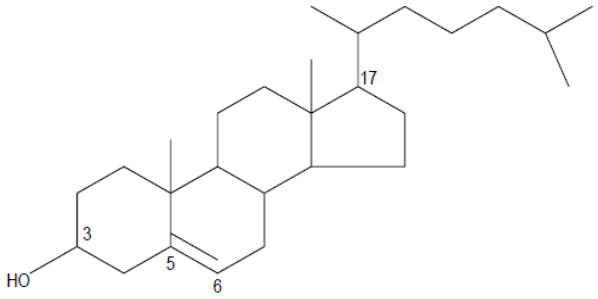


Figure 1.5. Structure of Cholesterol ($C_{27}H_{46}O$).

High-density lipoproteins (HDL), on the other hand, also known as "good" cholesterol, are believed by medical experts to carry cholesterol away from the tissues and back to the liver, where it is then metabolized and passed out of the body. It is also believed that HDL removes excess cholesterol from plaques and thus slows their growth, helping to reduce the risk of heart attack or stroke. So a high HDL level is a good sign of a healthy body; but a low HDL cholesterol level (less than 40 mg/dl for men and less than 50 mg/dl for women) also indicates a greater risk (Lichtenstein et al., 2006).

Several factors can affect the cholesterol levels in the human body. The first factor that we can easily have control over is diet. Meat and meat products are one of the main sources of cholesterol. Chicken meat cholesterol content at 245–627 mg/kg (Al-Najdawi et al., 2002), but lower than for beef (860 mg/kg), pork (850 mg/kg) and lamb (920 mg/kg).

1.5 Bioactive compounds

Meat and meat products also contain additional, physiologically active components that can promote human health, such as bioactive peptides. Indeed, bioactive peptides have been defined as "food derived components (genuine or generated) that, in addition to their nutritional value exert a physiological effect in

the body" (Vermeirssen et al., 2007). Bioactive peptides are inactive within the sequence of their parent protein and can be released by enzymatic hydrolysis either during gastrointestinal digestion or during food processing (e.g., cheese ripening and milk fermentation). They usually contain 2–20 amino acid residues per molecule, but in some cases may consist of more than 20 amino acids. Following digestion, bioactive peptides can either be absorbed through the intestine to enter the blood circulation intact and exert systemic effects, or produce local effects in the gastrointestinal tract. Depending on the sequence of amino acids, these peptides can exhibit diverse activities, including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, and antihypertensive actions (Hartmann et al., 2007). These include L-carnosine, anserine, taurine, creatine, glutathione, lipoic acid, conjugated linoleic acid.

Carnosine is an histidine-containing dipeptide (N-β-alanyl-L-histidine), widely distributed in vertebrate animal tissues, especially skeletal muscle and other mammalian tissues (Decker et al., 1996). Carnosine is synthesised from *β*-alanine and L-histidine by carnosine synthetase. The same enzyme catalyses the formation of homocarnosine from γ-aminobutyric acid and histidine. The methylation of carnosine with *S*-adenosylmethionine is catalysed by carnosine *N*-methyltransferase and yields anserine and *S*-adenosyl-L-homocysteine (Velìšek et al., 2006; Figure 1.6).

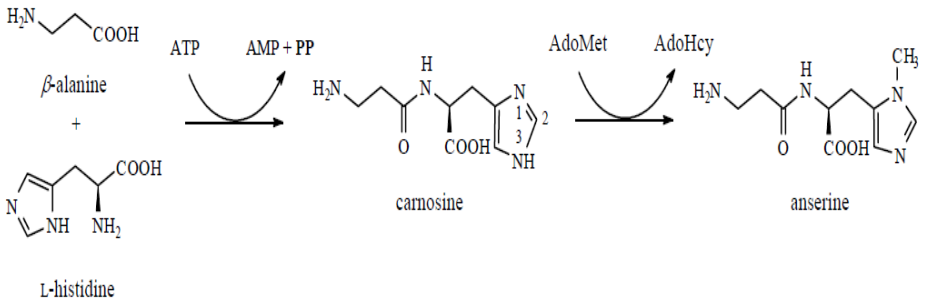


Figure 1.6. Carnosine and anserine are synthesised from *β*-alanine and L-histidine.

Anserine (β-alanyl-N-methy- L-histidine) is widely distributed in vertebrate animal tissues, especially in skeletal muscle, the heart, and the central nervous system (Mora et al., 2007). Carnosine and anserine play a major role in muscle tissue as pH buffer. A high buffer capacity in the muscle can stabilise the intramuscular pH value and thus enlarge capacity for anaerobic performance and tolerance in the case of oxygen deficit (Abe, 2000).

Carnosine and anserine also show antioxidant properties (Guiotto et al., 2005) and can reduce certain proteolytic reactions too. Carnosine and anserine are also able to from complexes with ions such as copper, zinc and cobalt. Depending on the metal ion bonded, the complexes differing biological functions (Baran, 2000). For example, the carnosine zinc complex alleviates injuries to the gastic mucosa, acts against stomach ulcers and inhibits their chief pathogen *Helicobacter plylori*, which is why they said complex is also used as a medicine (Matsukura et al., 2000). Moreover, carnosine reduces certain proteolytic reactions associated with cell ageing (Hipkiss et al., 2002) and, as a result, has been credited with anti-ageing properties. Carnosine and anserine are only found in meat, poultry and same fish, but not in foods of plant. The concentrations of carnosine in meat range from 500 mg per kg of chicken thigh to 2700 mg per kg of pork shoulder. Recent study demonstrated the bioavailability of carnosine by determining its concentration in human plasma after ingestion of beef (Park et al., 2005).

Creatine is an amino acid derivative normally produced in the liver, kidney, and pancreas from glycine, arginine, and methionine (Figure 1.7), and subsequently transported from there into the muscle tissue. Creatine and its phosphorylated derivative phosphocreatine are key components of the energy delivery process in several tissues, particularly those characterized by the transfer of high energy phosphate to ADP in muscle cells (Wyss et al., 2000). These compounds play an important role in the energy metabolism of skeletal muscle, taking part in the post-mortem biochemical processes occurring immediately after slaughter (Toldrá, 2006).

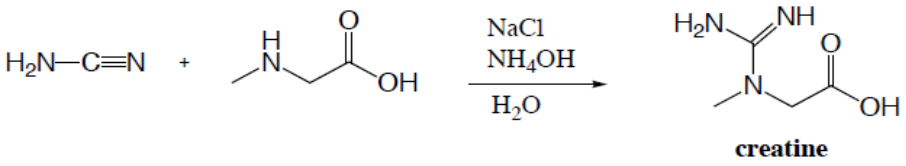


Figure 1.7. Synthesis of creatine.

Muscle creatine is slowly converted non-enzymatically to *creatinine* by the removal of water and the formation of a ring structure (Figure 1.8). The presence of creatine and creatinine in meat has been associated to negative aspects because creatine and creatinine can constitute important precursors of heterocyclic amines, which can be formed on the surface of meat when cooked at high temperatures using dry-heat such as in roasting, frying and grilling (Pais et al., 1999).

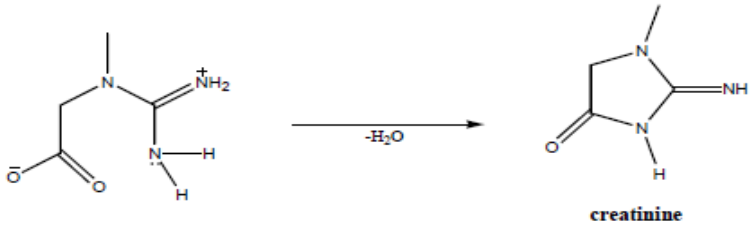


Figure 1.8. Mechanism for transformation of creatine to creatinine**.**

Creatine is absorbed with food, particularly from meat and fish. Purchas et al. (2004) found 401 mg creatine for 100g in fresh beef (*M. semitendinosus*), and levels between 247 and 374 mg/100g are stated for pork (Mora et al., 2008).

Literature Cited

Al-Najdawi, R.; Abdullah, B. (2002). Proximate composition, selected minerals, cholesterol contentand lipid oxidation of mechanically and hand-deboned chickens from the Jordanian market. *Meat Science* 61, 243–247.

Baran, E.J. (2000). Metal complexes of carnosine. Biochemistry (Moscow) *65*, 789–797.

Barceló-Coblijn, G., Murphy, E.J. (2009). Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Progress in Lipid Research*, 48, 355–374.

Bauman, D.E., Baumgard, L.H., Corl, B.A., Griinari, J.M. (1999). Biosynthesis of conjugated linoleic acid in ruminants. *Proceeding of the American Society of Animals Science*,1-15.

Calder, P.C. (2004). n-3 Fatty acids and cardiovascular disease evidence explained and mechanisms explored. *Clinical Science* (London), 107, 1–11.

Chillard, Y., Ferlay, A., Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science*, 70, 31–48.

Chizzolini, R., Zanardi, E., Dorigoni, V., & Ghidini, S. (1999). Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends in Food Science and Technology,* 10, 119–128.

Christie C.C. (2003). *Sterols and sterol esters: Structure, Occurrence, and Biochemistry Analysis.* Lipid Analysis, 3rd Ed., 1-7.

Colpo, A.(2005). LDL Cholesterol:"Bad" Cholesterol, or Bad Science? *Journal of American Physicians and Surgeons*, Volume 10 Number 3.

Crumb, D.J. (2011). Conjugated linoleic acid (CLA) - An overview. *International Journal of Applied Research in Natural Products*, 4, 12-18.

Decker, E. A., & Mei, L. (1996). Antioxidant mechanisms and applications in muscle foods. *Reciprocal Meat Conference Proceedings*, 49, 64–72.

Decker, E. A., & Park, Y. (2010). Healthier meat products as functional foods. *Meat Science*, 86, 49–55.

Enser, M., Scollan, N., Gulati, S., Richardson, I., Nute, G., & Wood, J. (2001). The effects of ruminally-protected dietary lipid on the lipid composition and quality of beef muscle. *Proceedings of the 47th International Congress of Meat Science and Technology,* 1, 12–13.

Favier, J. C., Ireland-Ripert, J., Toque, C. and Feinberg, M. (1995). Répertoire général des aliments 1995. Tables de composition, INRA Editions, 879.

Fritsche, J., & Steinhardt, H. (1998). Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Zeitschrift für Lebensmittel-Untersuchung und -Forschung A – *Food Research and Technology*, 206, 77–82.

Guiotto, A., Calderan A., Ruzza, P., Borin, G. (2005). Carnosine and carnosine-related antioxidants: a review. Current Medicinal Chemistry *12*, 2293–2315.

Girao, H., Mota, C., Pereira, P. (1999). Cholesterol may act as an antioxidant in lens membranes. *Curr Eye Res*;18:448-454.

Griinari, J. M., and D. E. Bauman. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (Ed.) *Advances in Conjugated Linoleic Acid Research*, Vol. 1., 180-200. AOCS Press, Champaign, IL.

Gurr, M.I., Harwood, J.L., & Frayn, K.N. (2002). *Lipid Biochemistry an Introduction 5th Edition*. Oxford, UK: Blackwell Science Ltd.

Hartmann R, Meisel H. (2007). Food-derived peptides with biological activity: from research to food applications. *Curr Opin Biotechnol*;18:163–9.

Hipkiss, A. R., Brownson, C., Bertani, M. F., Ruiz, E., & Ferro, A. (2002). Reaction of carnosine with aged proteins. Another protective process? *Annals of the New York Academy of Science,* 959, 285– 294.

Hornstra, G. (1999). Lipids in functional foods in relation to cardiovascular disease. *Lipids*, 12, S456-S466.

Jiménez-Colmenero, F., Carballo, J., & Cofrades, S. (2001). Healthier meat and meat products: Their role as functional foods. *Meat Science*, 59, 5–13.

Khanal, R.C., & Dhiman, T.R. (2004). Biosynthesis of conjugated linoleic acid (CLA): A review. *Pakistan Journal of Nutrition*, 3, 72-81.

Larsen, T. M., Toubro, S., & Astrup, A. (2003). Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. *Journal of Lipid Research*, 44, 2234–2241.

Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., Franklin, B., Kris-Etherton, P., Harris, W. S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Van Horn L., Winston M. andWylie-Rosett , J. (2006). Diet and Lifestyle Recommendations Revision 2006: A Scientific Statement From the American Heart Association Nutrition Committee. *Journal of the American Heart Association,* 114; 82-96.

Luongo, D., Bergamo, P., Rossi, M. (2003). Effects of conjugated linoleic acid on growth and cytokine expression in Jurkat T cells. *Immunology Letters*, 90, 195– 201.

Martin, J. C., & Valeille, K. (2002). Conjugated linoleic acids: all the same or to everyone its own function?. *Reproduction Nutrition Development* 42, 525–536.

Matsukura, T., Tanaka, H. (2000). Applicability of zinc complex of L-carnosine for medical use. *Biochemistry* (Moscow) *65*, 817–823.

McAfee, A., McSorley, E.M., Cuskelly, G. J., Moss, B.W., Wallace, J.M.W., Bonham, M. P., & Fearon, A.M. (2010). Red meat consumption: An overview of the risk and benefit. *Meat Science*, 84, 1–13.

Mir, P. S., Ivan, M., He, M. L., Pink, B., Okine, E., Goonewardene, L., et al. (2003). Dietary manipulation to increase conjugated linoleic acids and other desirable fatthy acids in beef: A review. *Canadian Journal of Animal Science*, 3, 673–685.

Mora, L., Sentandreu, M.A., Toldra, F. (2008). Contents of creatine, creatinine and carnosine in porcine muscles of different metabolic types. *Meat Science 79*, 709–715.

Mora, L., Sentandreu, M. A., Toldrà, F. (2007). Hydrophilic Chromatographic Determination of Carnosine, Anserine, Balenine, Creatine, and Creatinine. J. Agric. Food Chem., 55, 4664-4669.

Mulvihill, B. (2001). Ruminant meat as a source of conjugated linoleic acid (CLA). *Nutrition Bulletin*, 26, 295–299.

Ovesen, L. (2004). Cardiovascular and obesity health concerns. In W. K. Jensen, C. Devine, & M. Dikeman (Eds.), *Encyclopedia of meat sciences*, 623-628. Oxford: Elsevier.

Pais, P., Salmon, C. P., Knize, M. G., & Felton, J. S. (1999). Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *Journal of Agricultural and Food Chemistry*, 47(3), 1098–1108.

Pariza, M.P., Park, Y., Cook, M.E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research*, 40, 283-298.

Park, Y. J., Volpe, S. L., & Decker, E. A. (2005). Quantitation of carnosine in humans plasma after dietary consumption of beef. *Journal of Agricultural and Food Chemistry*, 53, 4736–4739.

Purchas, R. W., Rutherfurd, S. M., Pearce, P. D., Vather, R., & Wilkinson, B. H. P. (2004). Concentrations in beef and lamb of taurine, carnosine, coenzyme Q_{10}, and creatine. *Meat Science, 66*, 629–637.

Schmitz, G., Ecker, J. (2008). The opposing effects of n−3 and n−6 fatty acids. *Progress in Lipid Research*, 47, 147–155.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *The British Journal of Nutrition,* 85, 115–124.

Siddiqui, R. A., Harvey, K. A., Zaloga, G. P. (2008). Modulation of enzymatic activities by n-3 polyunsaturated fatty acids to support cardiovascular health. *Journal of Nutritional Biochemistry*, 19, 417–437.

Simopoulos, A. P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International*, 20, 77–90.

Simopoulos, A.P. (2008). The importance of the omega6/omega3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine* (Maywood), 233, 674-688.

Toldrá, F. (2006). Meat: Chemistry and biochemistry. In *Handbook of food science*. In Y. H. Hui (Ed.). Technology and engineering. Vol. I, 28-1–28-18.

Ulbricht, T.L.V., Southgate, D.A.T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, 985–992.

Valsta, L. M., Tapanainen, H., & Mannisto, S. (2005). Meat fats in nutrition. *Meat Science,* 70, 525‑530.

Velíšek, J., Kubec R., Cejpek K. (2006). Biosynthesis of Food Constituents: Peptides – a Review. *Czech J. Food Sci.* Vol. 24, 4: 149–155.

Vermeirssen, V.; Camp, J.V.; Verstraete, W. (2007). Bioavailability of angiotensin I converting enzyme inhibitory peptides. *Br. J. Nutr.*, *92*, 357–366.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., & Whittington, F.M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.

World Health Organisation (WHO), (2003). Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO Expert Consultation. WHO, Geneva.,160.

Wyss, M., & Kaddurah-Daouk, R. (2000). Creatine and creatinine metabolism. *Physiological Reviews*, 80(3), 1107–1213.

Chapter 2

Effect of diet on meat composition

2.1 Lipid source used in ruminant feed

Several factors influence both the quantity and the quality of lipids in animal products: age of animal, breed and diet (Smith et al., 2009; Wood et al., 1999). Feed supplementation have also become a viable strategy for manipulation of fatty acid profiles. Lipid may be supplied in an animal's diet via various forage sources, oil-rich cereals, oilseeds, or fish oil, and until recently, was offered as a significant energy source (Kennelly, 1996; French et al., 2000; Loor et al., 2003). Nevertheless, the actual choice of fat or oil and the form by which it is included in the feed is decided by a number of factors. These include (1) the cost and availability of the raw material, both locally and on the world market; (2) the impact of fat or oil form (oilseed or extracted oil) and its fatty acid composition on feed digestibility (Garnsworthy, 2002); (3) the influence of consumers and retailers regarding the introduction of genetically modified material into the food chain and (4) animal feed regulations regarding permitted supplements.

2.1.1 Effect of basal diet

The contribution of the basal diet to the fatty acid composition of the animal product can be difficult to quantify. Due to their high carbohydrate content, cereal grains such as barley, maize, oats and wheat, and their by-products, are the main energy sources used in ration formulation for ruminants and non-ruminants. The lipid content of these cereal grains is variable, but their fatty acid profile is similar, with linoleic acid comprising 35–66 g/100 g total fatty acids, and α-linolenic acid making up 4–7 g/100 g total fatty acids. It would appear that the basal diet may impact on the fatty acid composition of the animal product because of its replacement effect in the diet on the targeted fatty acid source. For example, supplementary feeding along with grazing fresh forage has been reported to reduce pasture dry matter (DM) intake, with supplementary silage resulting in a greater reduction than supplementary concentrate (Morrison et al., 2007), an aspect likely to affect the supply of unsaturated fatty acids from the diet.

2.1.2 Forages

Forages are also an important dietary component for ruminant animals reared for meat. Ruminants are generally supplied with unsaturated fatty acids through the forage portion of their diet and it has been documented that animals consuming fresh pasture will have a higher content of unsaturated fatty acids in their milk or meat than those receiving a cereal-based concentrate diet (Dewhurst et al., 2006; Chilliard et al., 2007; Lourenço et al., 2008).

Indeed, fresh grass is an important feed for beef cattle in Northern Europe and there has been much interest in its benefits since, although its total lipid content is low (~50 g/kg DM), α-linolenic acid is the major fatty acid in grass. The majority of α-linolenic acid in forage is present in the form of glycolipids which because of their location within the cell structure are more resistant to rumen hydrolysis, and therefore less susceptible to biohydrogenation than lipid in oilseeds. Once absorbed, α-linolenic acid can have substantial effects on the fatty acid profile of meat lipids. In the study of Warren et al. (2008), a group of steers was fed fresh grazed grass rather than grass silage between 14 and 19 months of age. The results in subcutaneous adipose tissue showed that the proportion of 18:3n-3 was slightly higher in the steers fed grazed grass. This group also had higher proportions of 18:1 trans vaccenic acid and CLA than those fed grass silage, showing that the process of rumen biohydrogenation is different between fresh and conserved grass. A similar result was found by French et al. (2000). In the experiment compared the effect of offering grazed grass, grass silage and concentrates on the fatty acid composition of intramuscular fat in steers. Decreasing the proportion of concentrate in the ration effectively increased the proportion of grass intake and resulted in a linear increase in PUFA:SFA ratio and a linear decrease in the concentration of SFA. The highest concentration of PUFA in the intramuscular fat was found in those animals that had consumed grass only (22 kg grazed grass DM). In effect, grass is a good source of n3 PUFA although there can be variation due to maturity and variety. The ratio of n6/n3 PUFA is particularly beneficial

(low) in ruminant meats, especially from animals that have consumed grass which contains high levels of α-linolenic acid.

2.1.3 Oilseed

Oilseeds, such as soybean, cottonseed, canola seed, linseed and sunflower seed, greatly enhance the quality and nutritional value of animal feeds.

2.1.3.1 Linseed: History and Overview

Linseed is an ancient food that has been used in Europe and Asia since 5000-8000 B.C. to make linen cloth and as early as 650 BC Hippocrates cited linseed for its medicinal purposes (Lagrange, 1995). Linseed (also known as common flax or flaxseed), belongs to family *Linaceae* (*Linum usitatissimum* L.) and is an annual seed crop of the flax plant. Flax is a herbaceous blue flowering plant that is grown in Canada primarily for its oil-rich seeds. Canada is the largest producer and exporter of linseed in the world producing 33% of world production with 74% of the world exports in 2002 (Johnson et al., 2003). North Dakota is the largest producer of linseed in the United States. World production is estimated at 2.1 million tonnes, representing 0.6% of the production of the ten major oilseeds (Agriculture and Agri-Food Canada, 2002). In effect, linseed is best adapted to production in areas with lower growing temperatures and longer day lengths, where oil content and iodine values are optimized. The seeds of flax are smooth, tiny and a light to reddish brown color. This crop is multipurpose and is grown for seed and oil however; it also contains proteins, soluble fiber and polyunsaturated fatty acids (Pradhan et al., 2010). In particularly, linseed is a rich source of lignans. The main plant lignan in linseed is secoisolariciresinol diglycoside (SDG) which is the

precursor of enterdiol, a mammalian lignan (Thompson, 1995). SDG is converted to enterdiol by the bacterial flora in the colon. Linseed is also a good source of minerals and vitamins; especially potassium, magnesium, phosphorus, iron, copper and zinc. Minerals and vitamins are necessary in maintaining the body's normal physiological functions, such as regulating enzyme activity, fluid balance and growth and may act as an antioxidant.

The entire seed may also be ground and referred to as ground linseed. Ground linseed contains both linoleic acid and lignan, and provides protein and energy as a feedstuff. Linseed also can be extruded, a process where heat and pressure are applied as the seeds pass through a die. Extruded linseed contains both linoleic acid and lignan. The heat applied protects the protein from ruminal degradation, providing bypass protein for livestock diets. Overall, linseed in its many forms may be a good source of energy and protein in livestock diets.

2.1.3.2 Sunflower seed: History and Overview

Sunflower (*Helianthus annuus L.*), which originated in North America, is a short season plant that is potentially useful for seed, oil and forage crops. The sunflower belong to the belong to the family of *Compositae Asteracees* and to genus Helianthus (Karleskind, 2003). In India, sunflower is cultivated over an area of about 2.4 million ha with a production of 1.44 million tones. It holds second position in the world in edible oil manufacturing following soyabean oil (Nandha et al., 2014) and grouped among preeminent plant oils for human diet due to its nutritional worth (Skoric et al., 2008). 100 g the seed is made-up to enclose protein 20.78 g, total lipid (fat) 51.46 g, ash 3.02 g, carbohydrate 20 g and fiber 8.6 g with total energy of 2445 kj. Also, its seeds provide minerals like calcium 78 mg, iron 5.25 mg, magnesium 325 mg, phosphorus 660 mg, potassium 645 mg, sodium 9 mg, zinc 5 mg, copper 1.80 mg, manganese 1.95 mg and selenium 53.0 mcg. Regular sunflower oil contains 69% linoleic acid, 20% oleic acid and 11% saturated fatty acids, but a number of strategies have been adopted to present advance range of sunflower oils with elevated oleic acid, stearic acid, linoleic acid, palmitic acid and low saturated acid. In particularly, presence of

Vitamin E content in sunflower oil makes it advantageous for human consumption. Defense system against ROS (reactive oxygen species) is strengthened by the presence of Vitamin E component i.e., α-Tocopherol facilitating oxidation of polyunsaturated fatty acids. Growth conditions have a large impact on the fatty acid organization of the sunflower oil. Warmer climatic conditions generate more monounsaturated fatty acid and oleic acid and less n-6 polyunsaturated fatty acid and linoleic acid (an essential fatty acid, EFA) in comparison to colder climatic conditions (Morrison et al., 1995). In addition, sunflower contains polyphenols such as caffeic, chlorogenic and ferulic acids exert a high antioxidative potential, which might be beneficial both from a technofunctional and biofunctional point of view (Maier et al., 2009).

In recent years, the sunflower crop has interesting characteristic that meet farm requirements; it has higher drought resistance than maize and sorghum high disease and insect resistance and it also improve soil conditions for other crops. Although, sunflower is generally planted for seed production, the green sunflower plant is used as silage and a forage source by livestock producers. While, non-dehulled or partly dehulled sunflower meal has been used as a protein supplement for grazing sheep, lactating ewes and goats (Economides, 1998), lactating cows (Erasmus et al., 1994) and beef steers. Sunflower meal is higher in fiber, has a lower energy value and is lower in lysine, but higher in methionine, than soybean meal. The protein percentage of sunflower meal range from 28%, for non-dehulled seed, to 42% for completely dehulled seeds and it has also been successfully substituted for soybean meal in isonitrogenous diets for swine and poultry feeding (Senkoylu and Dale, 1999).

2.1.3.3 Effect of dietary inclusion of oilseed on meat fatty acid composition

In the last years, different studies have been conducted to manipulate the fatty acid composition of beef using oilseeds. Some studies have investigated the use of canola seed and linseed (Scollan et al., 2001; Raes et al., 2003) to increase the intramuscular n3 content. Linseed inclusion has advantages over the use of linseed oil for technological reasons due to its natural antioxidant content. However, linseed as such cannot be used in animal feeding as the seed coat cannot be penetrated by digestive enzymes. Therefore, to improve accessibility the seeds are broken by treatments, such as crushing, bruising, extrusion or expansion. Another disadvantage of linseed, limiting its inclusion in diets, is the presence of linamarin, an antinutritional compound, which can be destroyed by the technology of thermo-extrusion (Raes et al., 2004a). Also, in ruminant feeding, linseed may be additionally treated with formaldehyde to reduce protein degradation and fatty acid biohydrogenation in the rumen. In a study of Mach et al. (2006), offered whole canola seed (ALA content 10.6 g/100 g total FA) or whole linseed (ALA content 54.2 g/100 g total FA), at three lipid levels (50,80 and 110 g/kg DM) to 54 Holstein bulls and noted that the concentration of n3 PUFA in the *Longissimus dorsi* muscle increased linearly with lipid level. The actual amounts of n-3 fatty acids transferred into the muscle indicated a relatively low level of protection by the seed coat, with a muscle ALA content of 1.9 g/100g total FA from a concentrate level of 38.4 g/100 g total FA. Nevertheless, the n6/n3 PUFA ratio was significantly lower in whole linseed supplemented beef, compared to whole canola seed supplemented beef at increasing levels of supplementation, respectively. These n6/n3 values were substantially higher than when extruded linseed was offered to 48 dairy bulls at 0, 0.4, 0.8 or 1.2 kg/day, with a grass silage/concentrate-based diet (Dawson et al., 2010). Supplementation with extruded linseed significantly reduced the ratio of SFA:UFA (0.99, 0.95, 0.91 and 0.80 respectively) and significantly reduced n6/n3 (5.1, 3.8, 3.3 and 2.8 respectively) in the beef muscle. However, as claimed by Mach et al. (2006), the physical form of linseed offered did not substantially increase ALA levels in the muscle. The effect of the physical form of linseed offered on the fatty acid

composition of meat has been reported by several studies. Raes et al. (2004) reported that the replacement of whole soyabean with extruded linseed or crushed linseed in the finishing diet of Belgian Blue young bulls increased ALA supply equally, irrespective of physical form of the seed. Nevertheless, the inclusion of linoleic acid-rich oilseeds, such as barley based or sunflower, in the diet of ruminants appears to be most effective for increasing CLA concentration. In beef cattle the addition of 3% and 6% sunflower oil to a barley based finishing diet resulted in increased CLA contents in *Longissimus muscle:* 2.0 vs. 2.6 vs. 3.5 mg/g lipid for control, 3%, and 6% sunflower oil, respectively (Mir et al., 2003). A more substantial increase in the CLA concentration can be expected when sunflower oil is added to both the growing and finishing diet of beef cattle. Added to a barley and hay-based diet sunflower oil supplementation increased the CLA content in the lipids of the *Longissimus muscle* to 12.3 compared to 2.8 mg/g FAME in the control group (Mir et al., 2002). Noci et al. (2005) undertook a study to determine the effects of different amounts of sunflower oil in diets fed to crossbred heifers. Biohydrogenation of 18:2n6 to 18:0 in the rumen has been shown to produce CLA isomers as intermediates. While the increased amounts of sunflower oil were shown to significantly increase the amount of CLA isomer *cis*-9, *trans*-11 present in the intramuscular fat of the *Longissimus dorsi* muscle, they also had the effect of increasing the n6/n3 ratio beyond recommended levels.

As previously noted, rumen biohydrogenation is one of the principal obstacles to the alteration of fatty acid composition in beef cattle. A novel approach to overcome this obstacle is protection of the lipid supplement via protein encapsulation or chemical protection. This approach was used by Scollan et al. (2003) in a study that used grass fed cattle supplemented with concentrate treatments containing varying amounts of a ruminally protected lipid supplement (PLS) containing high values of α-linolenic acid (18:3 n3) fatty acids. Feeding the PLS treatments tended to reduce the amount of SFA while increasing overall PUFA and 18:3 n3 in intramuscular fat, showing that the PLS treatments were afforded a high degree of protection from the biohydrogenating action of the rumen.

2.1.4 Fish oil and marine algae

Supplementation of fish oil in the diet has also shown to be an effective method of altering an animal's fatty acid content, as shown by Scollan et al. (2001). The authors conducted a study that used a 60:40 forage:concentrate diet supplemented with either palmitic acid (16:0) as a control, linseed oil, which contains high amounts of 18:3n3, fish oil, or a combination of linseed oil and fish oil. The fish oil contains eicosopentanoic acid (20:5n3) and decosohexanoic acid (22:6n-3), is a more efficient way to provide n-3 fatty acids to humans as these are the eventual products that α-linolenic acid (18:3n3) will provide through chain elongation and desaturation. The linseed oil and linseed oil-fish oil mixtures were able to increase α-linolenic acid (18:3n3) over cattle fed the control diet, while all treatments showed a reduction of linoleic acid (18:2n6) in triacylglycerols (TAG). The long chain PUFA present in fish oil were not detected in the TAG or subcutaneous adipose tissue, suggesting a high susceptibility to biohydrogenation. All three treatments displayed a decrease in n6/n3 ratio. Another problem with oil supplementation (such as fish oil) is that even when small amounts of these fatty acids are assimilated, there appears to be an adverse effect on flavor, color, and shelf life of beef cuts (Sheard et al., 2000).

Marine algae are an alternative to fish oil as a dietary source of n3 long chain polyunsaturated fatty acids (LCPUFA), eicosapentaenoic acid and docosahexaenoic acid. Cooper et al. (2004) conducted a study that investigated the effect of protection and the supply of pre-formed n3 LCPUFA on the fatty acid composition of lamb meat. Fifty Suffolk-cross lambs were offered one of five concentrate-based diets having a similar fatty acid content (approximately 60 g/kg DM) from different fat sources, namely (1) linseed oil; (2) fish oil; (3) a 50:50 mixture (oil basis) of marine algae (*Dinophyceae*) and fish oil; (4) a rumen-protected linseed and soyabean supplement (PLS) containing linseed, soyabean and sunflower seed encapsulated in formaldehyde-treated protein and (5) a mixture of equal proportions of PLS and algae (oil basis). The dietary lipid sources offered to the lambs affected the PUFA content of the phospholipid and neutral lipid fractions, with the PUFA concentration being 2.5 times greater when

PLS was offered either alone or with marine algae. Lower contents of EPA and DHA were reported in the phospholipid fraction of the lamb meat when linseed or PLS were supplied compared with diets containing fish oil or marine algae. The combination of marine algae with either fish oil or PLS in the lambs' diet increased DHA transfer by almost 2.5 times that from lambs offered fish oil alone. This high inclusion of DHA was greater than reported by other workers but was attributed to the greater dietary concentration of marine algae in this study and to a lower level of biohydrogenation of the algae as also noted by Sinclair et al. (2005). The low conversion of ALA to n-3 LCPUFA compared to when pre-formed sources of such fatty acids are included in the diet, was in agreement with other workers (Scollan et al., 2001). Cooper et al. (2004) concluded that this supported the dietary requirement for such fatty acids if n3 LCPUFA content is to be increased effectively.

2.2 Oilseed and health

There is now much evidence to show that chronic disease is rapidly increasing worldwide. WHO/FAO (2003) indicate that in 2001, chronic diseases gave rise to approximately 60% of the 56.5 million deaths reported around the world and about 46% of the global burden of disease. It has been projected that by 2020, chronic diseases will account for almost 75% of all deaths worldwide (Given et al., 2006). Almost half of the deaths arising from chronic disease are attributable to cardiovascular disease, although the rapid increase in the obesity/type 2 diabetes-related Metabolic Syndrome (Nugent, 2004) is very concerning, not only because it already affects a large proportion of the population worldwide, but also because it is now starting to affect people earlier in life.

Recently linseed has been considered as a potential source of bioactive food constituents that may supply health boosting properties besides providing basic nutrition (Hasler et al., 2000). The high amounts of protein, soluble fiber, and alpha-linolenic acid, along with other phytochemicals found in linseed, offer many health benefits. The protein and soluble fiber known as linseed mucilage have been shown to have hypolipidemic, and atherogenic effects, as well as to

have positive effects on blood glucose metabolism. In effect, linseed has been shown to lower blood lipid levels, including total cholesterol, LDL cholesterol, apolipoprotein B, and apolipoprotein A-I. The n3 fatty acid component of linseed has also been shown to have a major role in cholesterol metabolism (Gamez et al., 2005; Zhao et al., 2004). Therefore in part, the antiatherogenic property of linseed maybe due to its n3 fatty acid content (Lucas et al., 2002; Lucas et al., 2004). Linseed has also been reported to improve arterial compliance in obese subjects and reduce lipoprotein-a concentrations in postmenopausal women (Arjmandi et al., 1998). In addition to an important phytochemical present in linseed is linoleic acid and alpha linolenic acid are important for normal growth, skin and hair growth, cholesterol metabolism and reproductive performance. Of all of the phytochemicals present in linseed, lignans currently have the most media and consumer interest. As previously noted, linseed is the most abundant source of lignans, ranging from 75 to over 800 times more lignan production than other foods as measured in linseed flour and meal (Thompson et al., 1991). The mammalian lignans found are enterodiol and enterolactone, which are structurally similar to estrogen and exhibit estrogenic and antiestrogenic activities, as well as antitumor, antioxidant, and antimitotic activities. Moreover, in a study done by Allman et al. (1995) the platelet aggregation decreased in hyperlipidemic patients fed linseed. Other phytochemicals present in linseed such as phytates and phytic acid have been shown to prevent cancer, aid in glucose metabolism and act as antioxidants.

Sunflower plays an important role in human nutrition. Indeed, sunflower oil has gained importance due to increased content of oleic and linoleic acid that may help diminishing the cholesterol leading to reduction in heart diseases (Chowdhury et al., 2007). Also, cholesterol content and cancer risk is controlled by the presence of high content of phytosterols in sunflower seeds, i.e., approximately 280 mg per 100 g (Phillips et al., 2005). Sunflower is also a good source of minerals and vitamins; especially vitamin E (α-tocopherol) has a positive effect on coronary system of the body and hence reduces stroke and atherosclerosis (Dutta et al., 2003; Singh et al., 2005). In addition, tocopherols in

sunflower oil protect body from inflammation and tumors by neutralizing free radicals and avoiding oxidative injury to cells, thus helpful in diseases like rheumatoid arthritis and bronchial asthma, cardiovascular and coronary heart diseases (Adams et al., 2002). The antioxidant potential of oil seed crops especially sunflower is of great concern with ever increasing use of this oils seed in different food products; while folic acid in seeds helps in the formation of blood and nucleic acids. Sunflower oil contains magnesium that is supportive for curing migraine, hypertension, and bronchial asthma along with maintaining muscular tone of the body. Mental stress and uneasiness can be resolved by choline and tryptophan in sunflower seeds. Minerals like zinc improve the immune system, while selenium protects from the prostate cancer due to the antioxidant action. Overall, occurrence of several components in sunflower oil make it curative as anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer, cardioprotective and dermoprotective in human beings (Nandha et al., 2014).

Literature Cited

Adams AK, Best TM (2002). The role of antioxidants in exercise and disease prevention. *Phys. Sports Med*., 30:37-44.

Agriculture and Agri-Food Canada, (2002). Bi-weekly Agri-Industries Bulletin. August. Vol. 15:17.

Allman MA, Pena MM, Pang D. (1995). Supplementation with linseed oil versus sunflower seed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. *Eur J Clin Nutr*., 49(3):169-78.

Basarab, J. A.,Mir, P.S., Aalhus, J. L., Shah,M.A., Baron, V. S., Okine, E. K., & Robertson,W.M. (2007). Effect of sunflower seed supplementation on the fatty acid composition of muscle and adipose tissue of pasture-fed and feedlot finished beef. *Canadian Journal of Animal Science*, 87, 71–86.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., Doreau, M., (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *Eur. J. Lipid Sci. Technol*. 109, 828–855.

Chowdhury, K., Banu, L.A., Khan, S., Latif, A. (2007). Studies on the Fatty Acid Composition of Edible Oil. *Bangladesh J. Sci. Ind. Res*., 42:311-316.

Cooper, S.L., Sinclair, L.A., Wilkinson, R.G., Hallett, K.G., Enser, M., Wood, J.D., (2004). Manipulation of the n−3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *Journal of Animal Science,* 82, 1461–1470.

Dawson, L.E.R., Fearon, A., Moss, B., Woods, V.B., (2010). The effect of substitution of a proportion of the concentrate in grass silage/ concentrate-based diets with extruded linseed on performance and meat quality of dairy bulls. *Anim. Feed Sci. Technol.* 156, 10-18.

Dewhurst, R.L., Shinfield, K.J., Lee, M.R.F., Scollan, N.D., (2006). Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.*131, 168–206.

Dutta, A., Dutta, S.K. (2003). Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review. *J. Am. Coll. Nutr*. 22:258-268.

Economides, S. (1998). The nutritive value of sunflower meal and its effect on replacing cereal straw in the diets of lactating ewes and goats. *Livest. Prod. Sci*., Volume 55, Issue 1, 89–97.

Erasmus, LJ, Botha, PM, Meissner, HH. (1994). Effect of protein source on ruminal fermentation and passage of amino acids to the small intestine of lactating cows. *J Dairy Sci,* 77(12):3655-65.

French, P., Stanton, C., Lawless, F., O 'Riordan, E.G., Monahan, F.J., Caffrey, P.J., Moloney, A.P., (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage or concentrate-based diets. *J. Anim. Sci.* 78 (11), 2849–2855.

Gamez R, Maz R, Arruzazabala ML, Mendoza S, Castano G. (2005). Effects of concurrent therapy with policosanol and omega-3 fatty acids on lipid profile and platelet aggregation in rabbits. *Drugs R D.*; 6(1):11-9.

Garnsworthy, P.C., (2002). Fats in dairy cow diets. In: Wisemann, J., Garnsworthy, P.C. (Eds.), Recent developments in Ruminant Nutrition – 4. Nottingham University Press, UK.

Givens, D.I., Kliem, K.E., Gibbs, (2006). The role of meat as a source of n3 polyunsaturated fatty acids in the human diet. *Meat Science*, 74 209‑218

Hasler, C., S. Kundrat and D. Wool, (2000). Functional foods in cardiovascular disease. *Current Atherosclerosis Reports*, 2(6): 467-475.

Johnson, J.B., and Jimmerson, J. (2003). Agricultural Marketing Policy Center. Briefing No. 56. December. Montana State University. Bozeman, MT.

Karleskind A. (2003). Oils & Fats Manuals. Lavoisier. Cap 3, 117-125.

Kennelly, J.J. (1996). The fatty acid composition of milk fat as influenced by feeding oilseeds. *Anim. Feed Sci. Technol.* 60, 137–152.

Lagrange, V. (1995). Linseed in bakery products. *Technical Bulletin-American Institute of Baking Research Department*. 17(4): 1-6.

Loor, J.J., Hoover, W.H., Miller-Webster, T.K., Herbein, J.H., Polan, C.E., (2003). Biohydrogenation of unsaturated fatty acids in continuous culture fermenters during digestion of orchard grass or red clover with three levels of ground corn supplementation. *Journal of Animal Science,* 81, 1611–1627.

Lourenço, M., Van Ranst, G., Vlaeminck, B., De Smet, S., Fievez, V. (2008). Influence of different dietary forages on the fatty acid composition of rumen digesta as well as ruminant meat and milk. *Anim. Feed Sci. Technol.* 145 (1–4), 418–437.

Lucas EA, Lightfoot SA, Hammond LJ, Devareddy L, Khalil DA, Daggy BP, Smith BJ, Westcott N, Mocanu V, Soung do Y, Arjmandi BH. (2004). Linseed reduces plasma cholesterol and atherosclerotic lesion formation in ovariectomized Golden Syrian hamsters. *Atherosclerosis*., 173(2):223-9.

Lucas EA, Wild RD, Hammond LJ, Khalil DA, Juma S, Daggy BP, Stoecker BJ, Arjmandi BH. (2002). Linseed improves lipid profile without altering biomarkers of bone metabolism in postmenopausal women. *J Clin Endocrinol Metab*., 87(4):1527-32.

Mach, N., Devant, M., Díaz, I., Font-Furnols, M., Oliver, M.A., García, J.A., Bach, A. (2006). Increasing the amount of n3 fatty acids in meat from young Holstein bulls through nutrition. *Journal of Animal Science,* 84, 3039–3048.

Maier T, Schieber A, Kammerer DR, Carle R (2009). Residues of grape (*Vitis vinifera* L) seed oil production as a valuable source of phenolic antioxidants. *Food Chem*., 112:551-559.

Mir, P. S., McAllister, T. A., Zaman, S., Jones, S. D. M., He, M. L., Aalhus, J. L. (2003). Effect of dietary sunflower oil and vitamin E on beef cattle performance, carcass characteristics and meat quality. *Canadian Journal of Animal Science*, 83, 53‑66.

Mir, P. S., Mir, Z., Kubert, P. S., Gaskins, C. T., Martin, E. L., Dodson, M. V. (2002). Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, Wagyu times Limousin, and Limousin steers fed sunflower oil-containing diet. *Journal of Animal Science*, 80, 2996‑3004.

Morrison, S.J., Patterson, D.C. (2007). The effects of offering a range of forage and concentrate supplements on milk production and dry matter intake of grazing dairy cows. *Grass Forage Sci.* 62, 332–345.

Morrison, W.H. , Hamilton, R.J. and Kaln, C. (1995). Sunflower seed oil. In: Developments in Oils and Fats, (RJ Hamilton ed.), 132–51. Blackie Academic and Professional, Glasgow.

Nandha, R. , Singh, H., Garg, K. and Rani, S. (2014). A review on "Therapeutic potential of Sunflower seeds". *Int. J. Res. Dev. Pharm. L. Sci*., 3:967-972.

Nassu, R. T., Dugan,M. E. R., He,M. L., McAllister, T. A., Aalhus, J. L., Aldai, N., & Kramer, J. K. G. (2011). The effects of feeding linseed to beef cows given forage based diets on fatty acids of Longissimus thoracis muscle and backfat. *Meat Science*, 89, 469–477.

Noci F, O'Kiely P, Monahan F J, Stanton C, Moloney AP. (2005). Conjugated linoleic acid concentration in m. longissimus dorsi from heifers offered sunflower oil-based concentrates and conserved forages. *Meat Science,* 69:509–18.

Nugent, A. P. (2004). The metabolic syndrome. Nutrition Bulletin, 29, 36‑43.

Phillips, K. M.; Ruggio, D. M.; Ashraf-Khorassani, M. (2005). Analysis of steryl glucosides in foods and dietary supplements by solidphase extraction and gas chromatography. *Journal Food Lipids*,*12*, 124-140.

Pradhan, R.C., V. Meda, P.K. Rout and S. Naik, (2010). Supercritical CO_2 extraction of fatty oil from linseed and comparison with screw press expression and solvent extraction processes. *Journal of Food Engineering*, 98(4):393-397.

Raes, K., Haak, L., Balcaen, A., Claeys, E., Demeyer, D., De Smet, S. (2004). Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. *Meat Sci*ence, 66 (2), 307–315.

Raes, K., De Smet, S., Demeyer, D. (2004a). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef, and pork meat: a review. *Anim. Feed Sci. Technol*. 113 199-221.

Scollan, ND, Choi, NJ, Kurt, E, Fisher, AV, Enser, M, Wood, JD. (2001). Manipulating of fatty acid composition of muscle and adipose tissue in beef cattle. *Bri J Nutr.,* 85:115–24.

Scollan, ND, Enser, M, Gulati, S, Richardson, RI, Wood, JD. (2003). Effect of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle in Charolais steers. *Bri J Nut.,* 90:709–16.

Senkoylu, N., Dale, N., (1999). Sunflower meal in poultry diets: a review. *World Poult. Sci. J*., 55: 153-174.

Sheard, P.R., Enser, M., Wood, J.D., Nute, G.R., Gill, B.P., Richardson, R.I. (2000). Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Sci*ence 55, 213-221.

Sinclair, L.A., Cooper, S.L., Huntington, J.A., Wilkinson, R.G., Hallett, K.G., Enser, M., Wood, J.D. (2005). In vitro biohydrogenation of n-3 polyunsaturated fatty acids protected against ruminal microbial metabolism. *Anim. Feed Sci. Technol.* 7, 579–596.

Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. Poultry Science, 79, 961-970.

Smith, S. B., Gill, C. A., Lunt, D. K., & Brooks, M. A. (2009). Regulation of fat and fatty acid composition in beef cattle. *Asian–Australasian Journal of Animal Science*, 22, 1225–1233.

Singh, U., Devaraj, S., Jialal, I. (2005). Vitamin E, oxidative stress, and inflammation. *Annu. Rev. Nutr.,* 25:151-174.

Skoric, D., Jocic, S., Sakac, Z. and Lecic, N. (2008). Genetic possibilities for altering sunflower oil quality to obtain novel oils. *Can. J. Physiol. Pharmacol*., 86: 215-221.

Thompson, L., Robb, P., Serraino, M., and Cheung, F. (1991). Mammalian lignan production from various foods. *Nutr. Cancer,* 16:43-52.

Thompson, L.U. (1995). Linseed, Lignans, and Cancer. In: Linseed in Human Nutrition (S. Cunanne and L.U. Thompson, eds.). AOCS Press, Champaign, IL. pp. 219-236.

Warren, H.E., Scollan, N.D., Nute, G.R., Hughes, S.I., Wood, J.D., Richardson, R.I. (2008). Effects of breed and a concentrate or grass silage diet on beef quality. II. Meat stability and flavour. *Meat Science*,78 (3), 270-278.

WHO/FAO (2003). Diet, nutrition and the prevention of chronic diseases (p. 148). Report of a Joint WHO/FAO Expert Consultation. Geneva: WHO.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I., & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society*, 58, 363-370.

Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. (2004). Dietary alphalinolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr.,* 134(11):2991-7.

Chapter 3

Experiment 1

3.1 Introduction

Beef is often perceived as detrimental to health because is associated with an increase in the risk of cardiovascular diseases due to its high content of saturated fatty acids (SFA) (Givens, 2006). However, meat from ruminants is a good dietary source of some nutrients with health benefits including omega 3 polyunsaturated fatty acids (n3 PUFA) and conjugated linoleic acid isomers (CLA). The decrease of SFA and the increase of health-beneficial PUFA has been a main topic of ruminant meat research (Dilzeret al., 2012; Molendi-Coste et al., 2011). Strategic feeding provide an opportunity to improve the nutritional value of the fat by altering its composition. Linseed contains ~40% oil, and of this 50–60% is linolenic acid (LNA), making it one of the richest plant sources of n3 fatty acids. The increased levels of n3 fatty acids through linseed feeding have been demonstrated in different animal species (Scollan et al., 2006; Mapiye et al. 2013) and have helped to maintain red blood cell and plasma n3 fatty acid levels in humans. Sunflower seed is rich in linoleic acid (LA, C18:2 n6) and seems to contribute to the increase of CLA levels 18:2 cis-9,trans-11 in muscle (Bessa et al. 2007; Noci et al. 2007). However, rumen biohydrogenation of dietary PUFA is modulated by several factors, such as the amount and type of the lipid supplement (Harfoot et al., 1997) and the basal diet (Bessa et al., 2005), resulting in differences in the amount of PUFA that escapes from rumen biohydrogenation and in the type and distribution of biohydrogenation intermediates.

Few studies have been undertaken to study the synergistic effect of different oilseeds supplementation of diet on meat. Therefore, the objective of this study was: i) to evaluate the effects of supplementation of sunflower and linseed on growth performance and on nutritional and eating quality of meat from young bulls; ii) to test if a combination of linseeds and sunflower seeds have a synergistic effect on fatty acids profile.

3.2 Materials and Methods

3.2.1 Animals and dietary treatments

The experiment was conducted on an animal farm located in the Torre Guaceto Reserve (Brindisi), Southern Italy. 32 Podolian x Limousine young bulls that averaged 413±8.98 (SE) days of age with a mean initial body weight of 335 ±15.88 (SE) kg were randomly assigned to four groups: CO – control diet: consisting of oat hay and concentrate and no supplemental fat; LS – diet: oat hay and concentrate with a supplementation of 10% linseed; SS-diet: oats hay and concentrate with supplementation of 10% sunflower; LS+SS diet: oat hay and concentrate with supplementation of linseeds and sunflowers seeds (1:1 w/w). All diets were isonitrogenous and isocaloric but differed in FA profile, mainly due to differences in 16:0, 18:0, *cis*-9-18:1, *cis*-9, *cis*-12-18:2 (LA), and ALA. To ensure that the concentrates were isocaloric, the increase in lipid level was counterbalanced by a decrease in nonfibrous carbohydrates (NFC), mainly by reducing concentrate. Chemical composition of the diets is shown in Table 3.1.

Table 3.1. Chemical composition and fatty acid content (%) of the dietary ingredients.

	Diet			
	CO	**LS**	**SS**	**LS+SS**
Chemical composition (%DM)				
CP	13.90	14.31	14.10	14.20
Ether extract	4.50	5.50	5.90	5.70
NDF	46.61	44.55	44.31	44.51
ADF	23.36	22.65	22.55	22.58
Fatty acids composition (% of total fatty acids)				
C14:0	0.35	0.26	0.25	0.25
C16:0	25.48	19.55	18.63	19.02
C18:0	4.68	3.84	3.95	3.81
C18:1	21.06	18.11	20.87	20.55
C18:2c9c12	19.35	15.71	34.67	25.19
C18:3n3	19.59	48.55	19.84	34.21

CO = control, LS = linseed, SS = sunflower, LS+SS = linseed + sunflower.

3.2.2 Measurements and sample collection

At the end of finishing period, all animals were weighed on the morning of slaughter to record final body weight (BW) and transported to a commercial abattoir. Slaughter occurred according to industrial routines used in Italy and to the EU rule n.119/1993. After slaughter the hot carcass weight was recorded and the dressing percentage calculated. pH was determined 1 and 24 h after slaughter using a portable pH meter (Hanna Instruments, Woonsocket, RI) and combined glass electrode, inserted approximately 5 cm into the *Longissimus thoracis* muscle. After 8 days of ageing *Longissimus thoracis* muscle was removed from each half carcass and cut into steaks for chemical analysis, fatty acids profile and sensory analysis evaluation.

3.2.3 Chemical analysis

Feed samples of each experimental diet were collected and air-dried at 105°C to a constant weight prior to analysis. Chemical analysis of oat hay, concentrate, linseed and sunflower seed were carried out according to the AOAC methods (1995). Meat sample was ground to homogeneous consistency using a food processor. Moisture, protein, lipid and ash contents were determined according to AOAC methods (1995). All the chemical determinations were performed in duplicate.

3.2.4 Fatty acid methyl esters (FAME) analysis

The fatty acid composition of feeds and meat was carried out according to O'Fallon et al., (2007). Briefly, 1 g of sample was added into a screw cap (16x25 mm) reaction tube in which 0.7 mL of 10*N* KOH in water, 5.3 mL of MeOH and 0.5 mg of C13:0/ml of internal standard were added. The tubes were incubated in a 55°C water bath for 1.5 h with vigorous hand shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After the incubation, the tubes were cooled in a cold tap water bath for 15 min and 0.58 mL of 24*N* H_2SO_4 was added. The tubes were mixed by inversion and incubated again in a 55°C water bath for 1.5 h. After cooling 3 mL of hexane was added into each tube and

vortex for 5 min. The tubes were centrifuged at 500 × *g* (Eppendorf 5810 R, Eppendorf AG, Hamburg, Germany) for 5 min at 21°C, and lipid extracts were transferred into a GLC vial. Fatty acid profile was quantified using an Agilent 6890N instrument equipped with a HP-88 fused-silica capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25μm). Operating conditions were: a helium constant pressure of 175 kPa; a FID detector at 250°C; a split-splitless injector at 250°C with an injection volume of 1μl. The temperature program of the column was: 4 min at 70° C, with a subsequent increase to 175ºC (13ºC/min), 27 min at 175 ºC, a subsequent increase at 215°C (4°C/min) and held for 45 min. Retention time and area of each peak were computed using the 6890N NETWORK GC system software. Individual FAME peaks were identified by comparing their retention times with those of standards (GLC Reference standard 674 and GLC Reference standard UC-59M; Nu-Check Prep, Inc. Elysian MN 56028, USA).

Activities of the Δ^9-desaturase and elongase enzymes were determined as described by Oliveira et al. (2011) :

Δ^9-desaturase C16 = 100[(C16:1 *cis*-9)/ (C16:1 *cis*-9 + C16:0)],

Δ^9-desaturase C18 = 100[(C18:1 *cis*-9)/ (C18:1 *cis*-9 + C18:0)],

elongase = 100[(C18:0 + C18:1 *cis*-9)/ (C16:0 + C16:1 *cis*-9 + C18:0 + C18:1 *cis*-9)].

Atherogenic and thrombogenic indices were calculated according to Ulbricht and Southgate (1991) as follow:

Atherogenic index = (C12:0 + 4 x C14:0 + C16:0) / [(ΣMUFA + ΣPUFA (ω-6) and (ω-3)];

Thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 x ΣMUFA + 0.5 x ΣPUFA (ω-6) + 3 x ΣPUFA (ω -3) + (ω -3)/(ω -6)].

3.2.5 Sensory analysis

The sensory properties were evaluated on steak loin of 1.5 cm-thick, grilled at 250°C to an internal temperature of 72°C as assessed by a thermocouple probe inserted into the meat. Samples were kept warm until serving within 10 min after

cooking to a trained 10 member sensory panel previously selected for their flavour and texture sensitivity. Six preliminary sessions were used to develop attributes and to train assessors for attribute intensity evaluation. The sensory traits and their definitions are summarized in Table 3.2. After a further training in scale use (Stone and Sidel, 1985), the assessors rated the attributes on the basis of 10 cm unstructured lines with anchor points at each end (0 = absent, and 10 = very strong; therefore, greater scores corresponded to more tender products). Scores were the distances (cm) from the left anchor point. The sensory analysis was performed in a controlled sensory analysis laboratory with individual booths which were provided with red light to mask any differences in meat color. Panelists received a set of 4 samples per session, representing the 4 different groups. Meat was served using a randomized design for order and carry over effects, and panelists were asked to drink a bit of natural water at the beginning of the sensory evaluation and between samples to try to make the palate conditions similar for each sample.

Table 3.2. Sensory traits and their definitions (Marino et al., 2011).

Tenderness	The opposite of the force required to bite through the sample with the molars;
Juiciness	The amount of liquid expressed from the sample during the first and second chews;
Fibrousness	Perception of particles of different consistency from the rest of the mass;
Sensory chewiness	The opposite of the number of chews necessary to reduce the sample to a consistency ready for swallowing;
Intensity of odour	Intensity of the sum of all odors;
Intensity of flavour	Intensity of the sum of all flavors;
Off-flavour intensity	Intensity of the sum of all off- flavors.

3.2.6 Statistical analysis

All data were subjected to an analysis of variance using the GLM procedure of the SAS statistical software (SAS Institute, 2011), with treatment as a fixed effect. When significant differences were found (at $P<0,05$ unless otherwise noted), the Student *t-test* was used to locate significant differences between means. Least Squares means were evaluated using the PDIFF and STDERR options of SAS.

Sensory values were normalized standardizing each assessor by his standard deviation in order to reduce the effect of the different use of the scale, (Naes, 1991).

3.3 Results and Discussion

3.3.1 Beef production

The effect of oilseed supplementation on beef production are shown in Table 3.3. The source of oilseeds supplementation in the diet did not affect dressing percentage in agreement with other studies conducted with finishing cattle fed linseed, canola seed or sunflowers seeds (Corazzin et al., 2012; Hussein et al., 1995; Raes et al., 2004). The pH changes during 24 h postmortem (data not shown) were found to be regular and there was no differences due to diet effect. pH_{24} values in all experimental groups fell within the normal range (5.5–5.8) to avoid dark-cutting.

In addition, dietary oilseeds source did not influence chemical composition. The lack of effect of linseed or sunflowers inclusion in the concentrates on chemical composition and especially on intramuscular fat content could be due to the similar energetic and proteic level of the diets.

Table 3.3. Effect of different oilseed supplements on beef production (means±SEM).

	Diet					
	CO	**LS**	**SS**	**LS+SS**	**SEM**	**Effect, P**
Slaughter weight (kg)	500	490	505	512	15.33	NS
Carcass weight (kg)	297.75	295.44	298.56	301.47	2.45	NS
Dressing percentage (%)	59.55	58.05	59.12	58.88	0.65	NS
Intramuscular fat of LT muscle (%)	2.55	2.68	2.66	2.72	0.28	NS

NS = not significant.

CO = control, LS = linseed, SS = sunflower, LS+SS = linseed + sunflower.

3.3.2 *Fatty acids composition of Longissimus thoracis muscle*

As shown in Table 3.4 diet significantly affected the fatty acid profile of meat. Including linseed or/and sunflower seeds significantly decreased the percentage of saturated fatty acids ($P<0.001$), in particular meat from all oilseed supplemented groups showed lower percentage of myristic ($P<0.05$) and palmitic ($P<0.01$) acids compared to control group. It is known that the highest content of saturated fatty acids, particularly myristic and palmitic acids, led to a greater cholesterol levels in human plasma increasing the risk of atherosclerosis and cardiovascular diseases (Oostindjer et al., 2014). Differences in SFA percentage might be related to a reduction in endogenous fatty acids synthesis in young bulls fed with oilseeds supplementation, this hypothesis is supported by the lowest palmitic percentage, the major endogenous FA synthesis product, found in meat from linseed and/ or sunflower seeds groups.

The levels of MUFA were affected by diet ($P<0.05$) showing higher percentage in beef from oilseeds supplemented groups compared to control. In particular oleic acid, the most abundant MUFA in muscle, is considered protective factors being able to reduce low density lipoprotein concentration in blood.

An increasing of vaccenic acid (C18:1 t11) and of total CLA and CLA 9c, t11 were found in LS, SS and LS+SS groups. Vaccenic acid is an intermediary product of bio-hydrogenation of linoleic and linolenic acids. The increased ruminal concentration of C18:1t11 acid may account for the increase in CLA in meat from oilseed supplemented groups *via* stearoyl Co-A desaturase. This enzyme converts vaccenic acid to its corresponding CLA c9,t11 isomer, the main CLA isomer, during bio-hydrogenation process (Griinari et al., 2000).

The CLA and vaccenic acid are considered potential bioactive compounds for human health, and supplementation of diet with linseed and/or sunflower seeds may be a feasible strategy to enrich meat with these fatty acids.

Total PUFA were affected by oilseeds supplementation. Beef from LS, SS and LS+SS groups had higher PUFA ($P<0.05$) percentage than control group. Total n6 PUFA and linoleic acid (LA, C18:2 n6) were influenced by oilseed type with young bulls fed diets containing SS having the greatest ($P<0.01$) percentage and

animal fed diets with LS and LS+SS showing the lowest value. Inclusion of linseeds alone or in association with sunflower seeds in the diet significantly increased the proportion of total n3 PUFA ($P<0.01$) and of the majority of individual n3 PUFA as linolenic (ALA, C18:3 n3; $P<0.01$), eicosapentaenoic (EPA, C20:5 n3; $P<0.001$), docosapentaenoic (DPA, C22:5 n3, $P<0.01$) and docosaeasoenoic (DHA, C22:5 n3; $P<0.05$) fatty acids that showed the highest value in LS and LS+SS groups.

The result of the present study highlights that not all oilseed supplements exert the same effect. Feeding sunflower seeds was more effective in raising the total PUFA in beef through an increase in n6 PUFA. On the other hand, linseed supplementation and combination with linseeds and sunflower seeds resulted in an increase of n3 fatty acid deposition in meat. Increasing linolenic acids and its long-chain derivatives, especially DHA and EPA in beef is a remarkable result from nutritional point of view. In particular long chain fatty acids have a wide range of biological effects, which are beneficial for human health (Givens et al., 2006) and are essentials element for the development and function of the brain and retina for neonatal growth (Koletzko et al., 2008).

Previous studies found that diet with greater content of linoleic acid had lower rates of biohydrogenation in the rumen compared to diet enriched in linolenic acid resulting in beef with great PUFA content (Mapiye et al., 2013, Shingfield et al., 2013). In the present study, the somministration of linseed as whole seed might have protected PUFA from rumen biohydrogenation and increase passage of PUFA to the duodenum. Therefore, we retain that differences in proportions of n6 and n3 PUFA among oilseed supplemented groups were attributed to different enzymatic activities and/or to the competition between LA and LNA for the same elongation and desaturation enzymes.

Table 3.4. Effect of different oilseed supplementation on fatty acids profile (%) of *Longissimus thoracis* muscle (means±SEM).

	Diet					
	CO	**LS**	**SS**	**LS+SS**	**SEM**	**Effect, P**
SFA	44.78a	41.05b	42.84b	41.62b	0.71	***
C12:0	0.12	0.06	0.08	0.08	0.02	NS
C14:0	2.75a	1.95b	2.15b	2.11b	0.12	*
C15:0	1.04	0.86	0.98	0.89	0.08	NS
C16:0	24.85a	23.05b	23.35b	23.16b	0.41	**
C17:0	0.75	0.61	0.71	0.65	0.05	NS
C18:0	14.42	13.73	13.85	13.88	0.48	NS
C20:0	0.45	0.25	0.29	0.35	0.08	NS
MUFA	38.14b	40.27a	40.22a	40.07a	0.55	*
C14:1	0.28	0.61	0.55	0.51	0.09	NS
C15:1	0.41	0.45	0.38	0.42	0.09	NS
C16:1 *cis 9*	2.15	2.65	2.57	2.62	0.24	NS
C17:1	1.11	1.25	1.19	1.08	0.09	NS
C18:1 *cis 9*	32.25	32.76	32.58	32.51	0.31	NS
C18:1t11	1.68b	2.45a	2.85a	2.49a	0.18	**
PUFA	15.21c	16.35b	17.30a	16.79ab	0.31	*
n6	13.84b	12.82b	15.16a	13.75b	0.35	**
C18:2 n6	11.85ab	10.78c	12.46a	11.35bc	0.31	*
CLA 9c,11t	0.19b	0.52a	0.57a	0.54a	0.05	*
CLA, tot	0.28b	0.64a	0.69a	0.66a	0.06	*
C20:4n6	1.43	1.15	1.47	1.42	0.13	NS
C20:3n6	0.28	0.29	0.55	0.32	0.11	NS
n3	1.37c	3.64a	2.13b	3.26a	0.18	**
C18:3n3	0.95c	2.45a	1.58b	2.22a	0.15	**
C20:5n3 **EPA**	0.15b	0.46a	0.22b	0.41a	0.05	***
C22:5n3 **DPA**	0.25b	0.58a	0.29b	0.52a	0.05	**
C22:6n3 **DHA**	0.02b	0.15a	0.04b	0.11a	0.02	*

NS = not significant; * = P<0.05; ** =P<0.01; *** P<0.001.
CO=control, LS=linseed, SS=sunflower, LS+SS=linseed +sunflower.

The differences in the proportions of SFA, MUFA, n3 and n6 PUFA have affected nutritional indices as shown in Table 3.5. Meat from all oilseed supplemented groups showed higher P/S ratio (P<0.05) and lower atherogenic (P<0.01) and thrombogenic (P<0.01) indices than control groups giving rise to a beef with better nutritional profile. n6/n3 ratio were influenced by oilseed type with young

bulls fed linseed diets alone or combined with sunflowers containing the lowest values. This result shows that the main differences concerning n6/n3 ratio are linked to the addition of whole linseed in diet highlighting that combining a supplementation with linseed and sunflower seeds can contribute strongly to a reduction of this ratio that is considered an important parameters for nutritional assessment of fat (World Health Organization, 2003).

Table 3.5. Effect of different oilseed supplementation on nutritional indices of *Longissimus thoracis* muscle (means±SEM).

	Diet					
	CO	**LS**	**SS**	**LS+SS**	**SEM**	**Effect, P**
P/S	0.34b	0.40a	0.41a	0.41a	0.02	*
n6/n3	10.06a	3.49c	7.12b	4.21c	0.27	***
A.I.[1]	0.70a	0.54b	0.57b	0.56b	0.04	**
T.I.[2]	1.43a	1.03b	1.18b	1.08b	0.07	**

NS = not significant; * = P<0.05; ** =P<0.01; *** P<0.001.
[1]AI= atherogenic index, [2]TI= thrombogenic index
CO = control, LS = linseed, SS = sunflower, LS+SS = linseed + sunflower.

3.3.3 Sensory characteristics of meat

Palatability attributes of beef from young bulls fed with different oilseed supplements are shown in Table 3.6. Steaks from oilseed supplemented diet was more tender and juicy with higher flavour intensity than meat from control group (P<0.05). These results may be linked to the differences in proportions of saturated and unsaturated fatty acids found among diets. Fatty acids have different melting points, in particular saturated fatty acids have the highest melting points while as unsaturation increases, melting point decreases (Campo et al., 2008). Therefore the greater tenderness of beef from animals fed oilseed supplements could be attribute to a lower SFA content and higher MUFA and PUFA found in LS, LS+SS and SS groups.

Table 3.6. Effect of different oilseed supplementation on sensory properties of *Longissimus thoracis* muscle (means±SEM).

	Diet					
	CO	**LS**	**SS**	**LS+SS**	**SEM**	**Effect, P**
Tenderness	6.33b	7.58a	7.25a	7.45a	0.25	*
Juiciness	5.42b	6.55a	6.42a	6.48a	0.29	*
Fibrousness	4.75	4.25	4.42	4.17	0.25	NS
Chewiness	14.75	12.92	13.50	13.05	0.65	NS
Odour	5.08	4.92	5.5	5.55	0.31	NS
Flavour	4.83b	5.87a	6.05a	59.16a	0.36	*
Off-flavour intensity	3.83	4.73	4.97	4.66	0.36	NS

NS = not significant; * = P<0.05
CO = Control, LS = linseed, SS = sunflower, LS+SS = linseed + sunflower.

The different flavour intensity could be due to oxidation of PUFA that produces volatile compounds which may contribute to desirable or undesirable meat flavour depending on type, amounts and proportions in meat (Elmore et al., 1999). It is worth to note that supplementation with linseed and/or sunflower seeds did not provoke the increase of off flavour intensity as, on the contrary, reported in a previous study (Mapiye et al., 2013).

3. 4. Conclusions

Linseed and/or sunflower seeds supplementation of oat hay and concentrate basal diet decreased saturated fatty acids, particularly myristic and palmitic acids, and increased MUFA and total CLA content. Dietary supplementation with whole linseed alone or in combination with sunflower seeds increased linolenic and long chain n3 percentage and reduced n6/n3 ratio confirming as an effective strategies to improvement fatty acids profile of meat.

Literature Cited

Association of Official Analytical Chemists (1995). Official Methods of Analysis, 16th ed. AOAC International, Arlington, VA.

Bessa, J.B., Portugal, P.V., Mendes, I. A., Santos-Silva, J. (2005). Effect of lipid supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs fed dehydrated lucerne or concentrate. *Livestock Production Science*, Volume 96, Issues 2–3, 30 185–194.

Bessa, R. J. B., Alves, S. P., Jerónimo, E., Alfaia, C. M., Prates, J. A. M., & Santos-Silva, J. (2007). Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acid in lambs. *European Journal of Lipid Science and Technology*, 109(8), 868–878.

Campo, G., Brito, J.M., Soares de Lima, J.M., Vaz Martin, D., Sañudo, C., San Julián, R., Hernández P., Montossi, F. (2008). Effects of feeding strategies including different proportion of pasture and concentrate, on carcass and meat quality traits in Uruguayan steers. *Meat Science* 80, 753–760.

Corazzin, M., Bovolenta, S., Sepulcri, A., Piasentier, E. (2012). Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. *Meat Sci*ence, 90, 99–105.

Dilzer, A., Park, Y. (2012). Implication of conjugated linoleic acid (CLA) in human health. Critical Reviews in *Food Science and Nutrition*, 52, 488–513.

Elmore, J. S., Mottram, D. S., Enser, M., Wood, J. D. (1999). Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry*, 47(4), 1619–1625.

Direttiva 93/119/CE del Consiglio, del 22 dicembre 1993, relativa alla protezione degli animali durante la macellazione o l'abbattimento Gazzetta ufficiale n. L 340 del 31/12/1993.

Givens, D.I., Kliem, E.K. & Gibbs, A.R. (2006). The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Science*, 74, 209-218.

Griinari, J. M, Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela K. V. V., & Bauman, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. *Journal of Nutrition*, 130, 2285–2291.

Hussein, H. S., N. R. Merchen, and G. C. Fahey, Jr. (1995). Effects of forage level and canola seed supplementation on site and extent of digestion of organic matter, carbohydrates and energy by steers. *Journal of Animal Science,* 73, 2458–2468.

Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I et al. (2008). The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* 36, 5–14.

Mapiye, C., Aalhus, J.L., Turner, T.D., Rolland, D.C., Basarab, J.A., Baron, V.S., McAllister, T.A., Block, H.C., Uttaro, B., Lopez-Campos, O., Proctor, S.D. , Dugan, M.E.R. (2013). Effects of feeding linseed or sunflower-seed in high-forage diets on beef production, quality and fatty acid composition. *Meat Science,* 95, 98–109.

Marino, R., Albenzio, M., Caroprese, M., Napolitano, F., Santillo, A., Braghieri, A. (2011). Effect of grazing and dietary protein on eating quality of Podolian beef. *Journal of Animal Science*, 89, 3752-3758.

Molendi-Coste, O., Legry, V., & Leclercq, I. A. (2011). Why and how meet n−3 PUFA dietary recommendations? Gastroenterology Research and Practice.

Naes, T. (1991). Handling individual differences between assessors in sensory profiling. *Food Quality and Preference*, 6, 217–220.

Nakamura, M.T. and Nara T. Y. **(**2004). Structure, function, and dietary regulation Δ^6, Δ^5, and Δ^9 desaturases. *Annual Review of Nutrition.* Vol. 24: 345-376.

Noci, F., French, P., Monahan, F. J., & Moloney, A. P. (2007). The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. *Journal of Animal Science*, 85, 1062–1073.

O'Fallon, J.V, Busboom J.R., Nelson M.L., Gaskins C.T. (2007). A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science,* 85:1511–1521.

Oliveira, D. M., Ladeira, M. M., Chizzotti, M. L., Machato Neto, O. R., Ramos, E. M, Gonçalves, T. M., Bassi, M.S., Lanna, D. P. D., & Ribeiro, J. S. (2011). Fatty acid profile and qualitative characteristics of meat from Zebu steers fed with different oilseeds. *Journal of Animal Science*, 89, 2546–2555.

Oostindjer, M., Alexander, J., Amdam, G. V., Andersen, G., Bryan, N. S., Chen, D., Corpet, D. E., De Smet, S., Dragsted, L. O., Haug, A., Karlsson, A .H., Kleter, G., de Kok, T. M., Kulseng, B., Milkowski, A. L., Martin, R. J., Pajari, A. M., Paulsen, J. E., Pickova, J., Rudi, K., Sødring, M., Weed, D. L., & Egelandsdal, B. (2014). The role of red and processed meat in colorectal cancer development: a perspective. *Meat Science*, 97, 583–596.

Raes, K., De Smet S., & Demeyer D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Animal Feed Science and Technology,* 113(1–4), 199–221.

SAS (SAS Institute, 2011). SAS/STAT user's guide (version 9.2) Cary, NC: Statistical Analysis System Inst.

Scollan N., Hocquette J. F., Nuernberg K., Dannenberger D., Richardson I., and Moloney A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science,* 74:17–33.

Shingfield, K. J., Bonnet, M. and Scollan N. D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal* 7:s1, 132–162.

Stone, H., and Sidel. V. L. (1985). Sensory Evaluation Practices. Academic Press Inc., New York.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, 985-992.

WHO (World Health Organization) (2003). Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultationWHO technical report series 916, Geneva.

Chapter 4

Experiment 2

4.1 Introduction

In the last years, much attention has been paid at the role of food components in preventing diseases by modulating physiological system (Dilzer et al., 2012; Molendi-Coste, 2011). Meat and meat products are generally recognized as good sources of high biological-value proteins, group B vitamins, minerals and trace elements as well as some other bioactive compounds (Kauffman, 2001) that can promote the human health. Particularly, carnosine and anserine also show antioxidant properties and anti-ageing effects (Hipkiss, 2009) whereas, creatine is important to muscle energy metabolism and has been shown to be useful as an ergogenic aid in some studies (Juhn, 2003) and a protective effects in neurodegenerative diseases such as Parkinson's and Huntington's disease (Adhihetty et al., 2008). On the hand, meat contributes to the intake of fat, saturated fatty acids, cholesterol, and other substances that, in inappropriate amounts, may result in negative physiologically effects (Toldrà et al., 2011). Studies on beef production have shown that the composition of meat could be improved through manipulation of animal feed and, consequently, on the type of fat added to the diet. In particular, several research has been conducted on how diet supplementation with oilseeds (sunflower seed, soya, linseed, canola seed) and vegetable oils, cold present an opportunities for producing beef with enhanced levels of components with potential health benefits such as polyunsaturated n3 fatty acids series and CLA (Choi et al., 2000; Scollan et al., 2001; Raes et al., 2003; Kudrna et al., 2008). To substantially increase PUFA and their ruminal biohydrogenation intermediates in beef, linseed is the most important oilseed, being source of α-linolenic acid of about 53% of ALA (Oomah, 2001). However, the time required for the incorporation of significant amount on n-3 PUFA into tissues is poorly documented. Only few studies on pork (Kouba et al., 2003; Haak et al., 2008) have been undertaken to valuation the effect of duration and time of feeding of n-3 PUFA source on the fatty acid composition of meat. Therefore, the aim of this study was to examine how the duration of linseed feeding may affect the incorporation of n3 PUFA in beef in order to determine the optimal supplementation strategy. In addition, in this experiment were also studied the

effects of supplementation of linseed on fatty acids profile, cholesterol and bioactive compounds.

4.2 Materials and methods

4.2.1 Animals and dietary treatments

The experiment was conducted on a commercial farm located approximately 20 km north-east of Foggia, Apulia, Southern Italy (latitude 41°27′6″, longitude 15°33′5″). Forty-eight Friesian steers (290 ± 12.88 kg means ± SE of initial body weight) were randomly allocated during finishing period into six experimental treatments following a 3 × 2 factorial arrangement. The 6 treatments consisted of 2 diets, control diet (CO) with no supplemental fat and linseed diet (LS) containing 10% whole linseed (Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy) and 3 different time of administration of the diet: 40, 75, or 120 days before slaughter. The ingredients and chemical composition of the diets are reported in Table 4.1. The CO and LS diets were isonitrogenous and isocaloric but differed in FA profile, mainly due to differences in C16:0, C18:0, C18:1*cis*-9, C18:2 n6 (LA), and C18:3 n3 (ALA). To ensure that the concentrates were isocaloric, the increase in lipid level was counterbalanced by an equivalent decrease in non fibrous carbohydrates (NFC), mainly by reducing concentrate. All concentrate ingredients were ground with the exception of the linseeds that were included as whole seeds.

4.2.2 Feed analysis

Analysis on feed were performed in the pooled feed samples collected throughout the trial period. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest, Roberts and Lewis (1991) while the AOAC (1995) methods were used for crude fat (Ether extract; method 935.38) and crude protein (CP; method 984.13) measurements. Fatty acids composition were determined using the procedure described by O'Fallon et al. (2007).

Table 4.1.Ingredients, chemical and fatty acid composition of the diets.

	Control diet	Linseed diet
Ingredients (%)		
Barley silage	51.68	49.31
Concentrate	37.17	30.83
Soy bean	4.53	3.63
Linseed	0.00	10
Straw	4.53	4.53
Sodium bicarbonate	0.73	0.73
Vitamin mineral premix[1]	0.91	0.91
Chemical composition (% DM basis)		
CP	13.90	14.31
Ether extract	4.50	5.50
NDF	46.61	44.55
ADF	23.36	22.65
Fatty acids composition (% of total fatty acids)		
C14:0	0.35	0.26
C16:0	15.94	13.44
C18:0	4.45	3.24
C18:1	19.55	20.11
C18:2c9c12	48.55	35.66
C18:3n3	9.85	27.55

[1] Ingredients: vit. A, 1150,000 IU; vit. D, 390,000 IU; vit. E, 50 mg; vit. PP, 8500 mg; vit. B1, 112 mg; vit. B2, 112 mg; vit. B6, 80 mg; vit. B12, 1 mg; d-Pantotenic acid, 2400 mg; ChoIine, 15,000 mg; Iron, 150 mg; Manganese, 800 mg; Zinc, 2200 mg; Cobalt, 8 mg; Iodine, 30 mg; Selenium, 5 mg; Molibden, 10 mg.

4.2.3 Measurements and sample collection

All animals were individually weighed every 15 days. At the end of each feeding period, steers fed with control and linseed diet were weighed on the morning of slaughter to record the final body weight (BW) and transported to a commercial abattoir. Animals were handled by specialized personnel following

the European Union Guidelines (2010/63/ EU Directive) on the protection of animals used for experimental and other scientific purposes. Slaughter occurred according to industrial routines used in Italy and to the EU rule n.1099/2009. After slaughter the hot carcass weight was recorded and the dressing percentage calculated. pH was determined 1 and 24 h after slaughter using a portable pH meter (Hanna Instruments, Woonsocket, RI) and combined glass electrode, inserted approximately 5 cm into the *Longissimus thoracis* (LT) muscle. After 8 days of aging *Longissimus thoracis* muscle was removed from each half carcass and cut into steaks for chemical analysis, fatty acids profile, cholesterol content and bioactive compounds determination.

4.2.4 Meat chemical composition and Fatty acid methyl esters (FAME)analyses

Meat sample was ground to homogeneous consistency using a food processor. Moisture, protein, lipid and ash contents were determined according to AOAC (1995) methods. All the chemical determinations were performed in duplicate. The fatty acid composition of meat was carried out according to O'Fallon et al. (2007) with some modifications. Briefly, 1 g of sample was added into a screw cap reaction tube in which 0.7 mL of 10*N* KOH in water, 5.3 mL of MeOH and 0.5 mg of C13:0/ml of internal standard were added. The tubes were incubated in a 55°C water bath for 1.5 h with vigorous hand shaking for 5 sec every 20 min to properly permeate, dissolve, and hydrolyze the sample. After incubation, the tubes were cooled in a cold tap water bath for 15 min and 0.58 mL of 24*N* H_2SO_4 was added. The tubes were mixed by inversion and incubated again in a 55°C water bath for 1.5 h. After cooling 3 mL of hexane was added into each tube and vortex for 5 min. The tubes were centrifuged at 500 × *g* (Eppendorf 5810 R, Eppendorf

AG, Hamburg, Germany) for 5 min at 21°C, and lipid extracts were transferred into a GLC vial. Fatty acid profile was quantified using an Agilent 6890N instrument equipped with a HP-88 fused-silica capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25μm). Operating conditions were: a helium constant pressure of 175 kPa; a FID detector at 250°C; a split-splitless injector at 250°C with an injection volume of 1μl. The temperature program of the column was: 4 min at 70° C, with a subsequent increase to 175ºC (13ºC/min), 27 min at 175 ºC, a subsequent increase at 215°C (4°C/min) and held for 45 min. Retention time and area of each peak were computed using the 6890N NETWORK GC system software. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards (FIM-FAME-7-Mix, Matreya LLC, Pleasant Gap PA, USA), added of C18:1-11t, C18:2-9c,11t, C18:2-9c, 11c, C18:2-9t, 11t, and C18:2-10t, 12c (Matreya LLC, Pleasant Gap PA, USA).

Atherogenic and thrombogenic indices were calculated according to Ulbricht and Southgate (1991) as follow:

Atherogenic index = (C12:0 + 4 x C14:0 + C16:0) / [(ΣMUFA + ΣPUFA (ω-6) and (ω-3)];

Thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 x ΣMUFA + 0.5 x ΣPUFA (ω-6) + 3 x ΣPUFA (ω -3) + (ω -3)/(ω -6)].

4.2.5 Meat cholesterol determination

Cholesterol content was determined using a quantitative colorimetric kit (BioVision, California, USA) with a UV–Vis spectrophotometer (Biotek PowerWaveXS2, Biotek Instruments, Inc. Highland Park, Winooski, Vermont, USA). Absorbance was measured at 570 nm against a standard curve obtained using scalar dilution of cholesterol standard. The cholesterol content was expressed as mg/100g of muscle.

4.2.6 Meat bioactive compounds determination

Creatine, creatinine, carnosine and anserine were extracted from raw meat and analyzed by HPLC according to Mateescu et al. (2012) with small modifications. Briefly, 6 ml of 0,01 N HCl were added to 2 g of ground sample and vortexed for 15 s. The mixture was placed on an orbital shaker (with ice) for 15 min and centrifuged for 20 min at 12,000 × g and 4 °C. Supernatant was filtered through PTFE Syringe Filters (13 mm, 0,45 um) and 250µL of this solution were deproteinized by adding 750 µL of acetonitrile. Samples were held at 4°C for 20 min then centrifuged for 10 min at 10,000 × g and 4°C. Finally, 20 µL of each sample were injected into the HPLC system.

The chromatographic separation was performed using an Infinity 1260 HPLC (Agilent Technologies) equipped with Kinetex HILIC Silica column (4,6 × 75 mm, 2,6 µm) and a DAD detector. Mobile phases consisted of solvent A, containing 0,65 mM ammonium acetate, pH 5.5, in water/acetonitrile (25:75, vol/vol), and solvent B, containing 4,55 mM ammonium acetate, pH 5.5, in water/acetonitrile (70:30, vol/vol). The separation conditions were a linear gradient from 0% to 100% of solvent B in 13 min at a flow rate of 1.4 mL/min. Before each injection the column was equilibrated for 10 min under the initial conditions. Creatine, carnosine and anserine were identified at a wavelength of 214 nm, whereas creatinine at 236 nm. For the quantification, sample peak areas were correlated to standard curves, previously obtained under the same chromatographic conditions.

4.2.7 Statistical analysis

All data were subjected to analysis of variance, using the GLM procedure of the SAS statistical software (SAS Institute, 2011). The mathematical model for growth performance, dressing percentage, chemical composition, fatty acid analysis, cholesterol content and bioactive components included fixed effects due to diet, duration of feeding and diet × duration, and random residual error. All effects were tested for statistical significance ($P<0.05$) and significant effects were reported in tables. When significant differences were found ($P<0.05$ unless

otherwise noted), the Student *t-test* was used to locate significant differences between means.

4.3 Results and Discussion

4.3.1 Growth performance, dressing percentage and intramuscular fat content

In table 4.2 are reported growth performance, dressing percentage and intramuscular fat content as affected by diet and feeding duration. Diet did not affect growth performance and dressing percentage in agreement with other studies conducted with finishing steers fed linseed or other fat sources (Corazzin et al., 2012). As expected higher average daily gain was found at 75 and 120 days of feeding than 40 days, regardless of the diet. Linseed supplementation and duration of feeding did not influence chemical composition of meat. The lack of effect of linseed inclusion on chemical composition and especially on intramuscular fat content could be due to the comparable energetic and proteic level of the diets. Particularly, Alberti et al. (2008) reported that supplementation of an isoenergetic and isoproteic concentrate diet with 10% whole linseed and/or 2% rumen-protected CLA did not result in significant differences in adipocyte size and number or lipogenic enzyme activity in Holstein young bulls. 24 hours pH values (data not shown) in all experimental groups fell within the normal range (5.5–5.8) to avoid dark-cutting.

Table 4.2. Growth performance, dressing percentage and intramuscular fat percentage as influenced by different diet and feeding duration (40, 75 and 120 days).

	Diets							Effects, P		
	CO			LS						
	40	75	120	40	75	120	SEM	Diet	Duration	Diet x Duration
Initial body weight (kg)	283	301	288	276	299	290	8.21	NS	NS	NS
Final body weight (kg)	328b	384ab	412a	322b	381ab	425a	12.8	NS	*	NS
Average daily gain (kg/d)	1.13	1.11	1.03	1.15	1.09	1.13	0.11	NS	NS	NS
Carcass weight (kg)	182	195	186	179	194	184	5.40	NS	NS	NS
Dressing percentage	55.49	55.56	56.19	55.43	55.75	55.42	0.31	NS	NS	NS
Intramuscular fat percentage	2.35	2.44	2.58	2.48	2.57	2.65	0.21	NS	NS	NS

NS = not significant; * = P< 0.05.
CO = control, LS = linseed
a,b,c = means with different superscripts in the same column are statistically different among feeding time (P< 0.05).

4.3.2 Fatty acids composition of Longissimus thoracis muscle

The effect of linseed supplementation and duration of feeding on fatty acid composition of *Longissimus thoracis* muscle are shown in Table 4.3. Diet significantly affected the proportion of saturated fatty acid (SFA, $P<0.01$), in particular meat from linseed group showed lower percentage of myristic (C14:0, $P<0.05$) and palmitic (C16:0, $P<0.01$) acids compared to control group. The reduction of SFA content in meat from LS group may be related to a reduction of endogenous fatty acids (FA) synthesis from rumen due to the inhibitory effect of linolenic acid yielded by LS diet; this hypothesis is supported by the lowest palmitic percentage found in LS group, that represent the major endogenous FA synthesis product. Comparable to our results, previous studies (Corazzin et al., 2012; Nassu et al., 2011) highlighted a reduction in the proportion of saturated fatty acids ≤ C16:0 in muscle tissue of beef cattle fed linseeds. From a nutritional point of view, myristic and palmitic fatty acids are significantly associated to a higher cholesterol in human plasma increasing the risk of atherosclerosis and cardiovascular diseases (Kim et al., 2013; Oostindjer et al., 2014).

Including linseed in the diet significantly increased total monounsaturated fatty acid (MUFA, $P<0.05$), polyunsaturated fatty acids (PUFA, $P<0.05$), total CLA ($P<0.05$) and n3 polyunsaturated fatty acids ($P<0.01$) and decreased n6 polyunsaturated fatty acids ($P<0.05$). In particular, the highest percentage of vaccenic (C18:1t11, $P<0.01$), CLA 9c,11t ($P<0.05$), linolenic (C18:3n3; $P<0.001$), eicosapentaenoic (EPA C20:5n3, $P<0.05$), docosapentaenoic (DPA C22:5n3, $P<0.05$) and docosehaxanoic (DHA- C22:6 n3, $P<0.05$) fatty acids were found in meat from steers fed linseed.

These results highlighted that dietary long chain PUFA increased the levels of trans-11 18:1 acid as product of rumen bio-hydrogenation of dietary linoleic and linolenic acids. The increased ruminal concentration of C18:1t11 acid may account for the increase in CLA in meat from linseed group *via* stearoyl Co-A desaturase. This enzyme converts vaccenic acid to its corresponding CLA c9,t11 isomer, the main CLA isomer, during bio-hydrogenation process (Griinari et al., 2000).

Linseed supplementation resulted in an enrichment of n3 fatty acid in meat, which was accompanied by a decrease of n6. Differences between the content of n3 and n6 fatty acids are related with the amount of linseed in the diet and the ratio of 18:3n3 to 18:2n6. In particular these fatty acids are in competition for inclusion into tissue lipids and for the enzymes responsible for desaturation and elongation (Diaz et al., 2011). The results of the present study are in agreement with other studies showing that 18:3n3 and 20:5n3 increased with high level of linseed in the diet (Enser et al., 2000; Mapiye et al., 2013).

Diet supplemented with linseed increased the availability of linolenic acid into the rumen resulting in enhanced synthesis of its elongation and desaturation products such as EPA, DPA and DHA. The proportions of long chain n3 PUFA in the current study are comparable to those reported by Nassu et al. (2011), and Mapiye et al. (2013). Increasing linolenic acids and its long-chain derivatives, especially DHA and EPA in beef is a remarkable result from a nutritional point of view. In particular long chain fatty acids have a wide range of biological effects, which are beneficial for human health (Givens et al., 2006), and they are essentials element for the development and function of the brain and retina for neonatal growth (Koletzko et al., 2008).

The duration of feeding and the interaction diet x duration significantly affected the proportions of several fatty acids. The percentage of myrisitc fatty acid increased with duration of feeding regardless of diet. On the contrary, it was observed a decline of PUFA and n6 through the experiment in control diet and in linseed diet, respectively. Furthermore, meat from steers fed with linseed supplementation showed an increased percentage of n3, linolenic and EPA fatty acids passing from 40 to 75 days of administration remaining constant thereafter, while, vaccenic acid, CLA 9c,11t, total CLA increased ($P<0.05$) between 40 and 75, but declined at 120 days.

The present result suggested that supplying 10% linseed for 75 days before slaughter is a sufficient feed duration to reach the saturation plateau for the synthesis of the PUFA n3 metabolites. A comparable trend was observed on swine by Kouba et al. (2003) that found an increased content of n3

polyunsaturated fatty acids and decreased the n6/n3 ratio in pork muscle and adipose tissue within 20 to 60 days on feed.

Table 4.3. Fatty acid composition (%) of meat from Longissimus thoracis muscle as influenced by diet (CO= Control; LS= linseed) and feeding duration (40, 75 and 120 days).

	Diets									
	CO			LS				Effects, P		
	40	75	120	40	75	120	SEM	Diet	Duration	Diet x Duration
C12:0	0.20	0.15	0.09	0.17	0.21	0.22	0.08	NS	NS	NS
C14:0	4.22b	4.46 ab	4.85a	3.13b	3.45ab	3.77a	0.21	*	*	*
C15:0	1.12	1.65	1.55	1.33	1.22	1.28	0.37	NS	NS	NS
C16:0	28.38	27.44	27.97	26.25	25.92	25.78	0.51	**	NS	NS
C17:0	0.91	0.84	0.73	0.64	0.75	0.84	0.27	NS	NS	NS
C18:0	12.45	12.65	12.85	12.25	12.12	11.85	0.46	NS	NS	NS
C14:1	1.05	1.03	0.87	1.21	1.15	1.35	0.14	NS	NS	NS
C15:1	0.35	0.37	0.38	0.62	0.65	0.75	0.11	NS	NS	NS
C16:1	4.42	3.97	4.15	3.99	3.88	3.95	0.28	NS	NS	NS
C17:1	1.75	1.48	1.09	1.77	1.66	1.78	0.21	NS	NS	NS
C18:1t11	1.08	1.13	1.08	3.18b	3.93a	3.21b	0.16	**	**	NS
C18:1	32.55	32.15	32.48	31.77	31.47	31.55	0.52	NS	NS	NS
C18:2n6	7.48	7.28	7.25	7.02	6.88	6.68	0.32	NS	NS	NS
CLA 9c,11t	0.26	0.33	0.24	0.37b	0.53a	0.42b	0.02	*	**	*
C20:3n6	0.95	0.85	0.79	0.72	0.55	0.52	0.10	*	NS	NS
C20:4n6	2.49	2.83	2.41	2.58	2.37	2.25	0.15	NS	NS	NS
C18:3n3	0.76	0.75	0.52	1.35b	1.78a	1.64ab	0.08	***	*	NS
C20:5n3	0.14	0.11	0.10	0.32b	0.45a	0.41a	0.03	*	*	NS
C22:5n3	0.31	0.25	0.21	0.61	0.56	0.59	0.05	*	NS	NS
C22:6n3	0.02	0.02	0.02	0.08	0.14	0.12	0.02	*	NS	NS
SFA [1]	47.28	47.19	48.04	43.77	43.67	43.74	0.68	**	NS	NS
MUFA [2]	41.21	40.13	40.05	42.54	42.74	42.59	0.61	*	NS	NS
PUFA [3]	12.47a	12.52a	11.64b	13.24	13.49	12.78	0.27	*	**	NS
n6	11.24	11.40	10.79	10.88a	10.56ab	10.06b	0.21	*	*	NS
CLA tot [4]	0.32	0.44	0.34	0.56b	0.76a	0.61b	0.05	*	*	*
n3	1.23	1.13	0.85	2.36b	2.93a	2.76a	0.13	**	*	NS

NS = not significant; * = P< 0.05; **= P<0.01; ***= P<0.001.

a, b, c = means with different superscripts in the same column are statistically different among feeding time (P< 0.05).

[1] Saturated fatty acids = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0.

[2] Monounsaturated fatty acids = C14:1 + C15:1 + C16:1 + C17:1 + C18:1t11 + C18:1.

[3] Polyunsaturated fatty acids = C18:2n6 + CLA 9c,11t + C20:3n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3.

[4] Total Conjugated linoleic acids = CLA 9c,11t + CLA 10t,12c+ CLA 9c,11c+ CLA 9t,11t.

The increase at 75 days and the subsequent drop at 120 days of vaccenic acid and CLA 9c,11t in meat from linseed group could be attributed to the down-regulation of Δ9-desaturase activity and/or its bio-hydrogenation products (Juarez et al., 2011b; Kronberg et al., 2006). Previous studies (Waters et al., 2009) found that dietary n3 PUFA reduce expression of the gene that codes the enzyme required to desaturate VA to CLA in bovine muscle tissue. The same authors highlighted that was not established the minimum time required to evoke a significant reduction in Δ9-desaturase mRNA expression, although they found that 50 days was sufficient and extending the supplementation period beyond this did not further depress Δ9-desaturase expression.

The differences in the proportions of SFA, MUFA, n3 and n6 PUFA due to the effect of linseed supplementation and duration of dietary treatment have significantly affected nutritional indices as shown in Table 4.4. Meat from linseed group showed lower n6/n3 ratio ($P<0.001$), atherogenic ($P<0.05$) and thrombogenic ($P<0.05$) indices than control group. The n6/n3 ratio, that it has been suggested should not exceed 4, is considered one of the most important parameters for nutritional assessment of fat (British Department of Health, 1994). Feeding linseed strongly reduced the values of n6/n3 ratio, reaching values below 4. Furthermore, meat from linseed group was also characterized by low AI and TI indices. The nutritional indices (AI and TI) represent criteria for evaluating the level and interrelation through which some fatty acids may affect the risk of the potential aggregation of blood platelets. Regarding to the effect of duration of feeding, n6/n3 ratio showed an opposite trend in meat from CO and LS through all experimental period. It was observed a gradual increase of n6/n3 ratio in control, whereas, in linseed meat a decrease was found passing from 40 to 75 days of administration remaining constant thereafter. This result confirms that the main differences concerning n6/n3 ratio are linked to the addition of whole linseed in diet.

Table 4.4. Nutritional indices of meat as influenced by different diet and feeding duration (40, 75 and 120 days).

	Diets									
	CO			LS				Effects, P		
	40	75	120	40	75	120	SEM	Diet	Duration	Diet x Duration
P/S	0.26	0.27	0.24	0.30	0.31	0.29	0.04	NS	NS	NS
n6/n3	9.14c	10.09b	12.69a	4.61a	3.60b	3.65b	0.15	***	*	*
AI [1]	0.85	0.86	0.92	0.70	0.71	0.74	0.03	*	NS	NS
TI [2]	1.50	1.52	1.63	1.22	1.16	1.19	0.04	*	NS	NS

NS = not significant; * = P< 0.05; ***= P<0.001.
a,b = means with different superscripts in the same column are statistically different among feeding time (P< 0.05).
CO= control; LS= linseed
[1]Atherogenic index = (C12:0 + 4 x C14:0 + C16:0)/[MUFA + PUFA (n-6) and (n-3)];
[2]Thrombogenic index = (C14:0 + C16:0 + C18:0)/[(0.5 x MUFA + 0.5 x PUFA (n-6) + 3 x PUFA (n-3) + (n-3)/(n-6)];

4.3.3 Cholesterol content

The effect of diet and duration of feeding on cholesterol content of meat from *Longissimus thoracis* muscle is shown in Figure 4.1. Cholesterol received more attention because of its suspected role in atherosclerosis (Valenzuela et al., 2003). The cholesterol content in meat was not affected by the dietary treatment while, significant differences (P<0.05) were found due to duration of feeding in linseed group, showing the lowest cholesterol content in meat after 75 days of feeding. This result could be related to the highest polyunsaturated fatty acids content of meat found after 75 days of linseed supplementation that might have affected the meat cholesterol content. An increase in the concentration of polyunsaturated fatty acids maybe decrease the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, which is the rate-limiting enzyme in cholesterol synthesis. It is known that n3 polyunsaturated fatty acids, prevent the synthesis and accumulation of lipoprotein-cholesterol and cholesterol esters in tissues and body fluids (Litwińczuk et al., 2015). These results highlight that the level of cholesterol synthesis is regulated by dietary intake of polyunsaturated fatty acids and suggest that cholesterol content is not simply related to the amount of intramuscular fat (Smith et al., 2004).

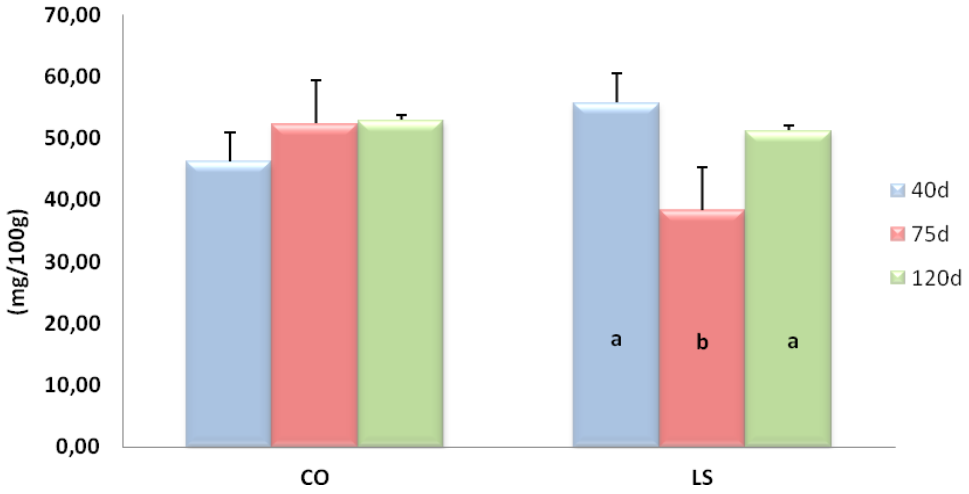


Figure 4.1. Cholesterol content of meat from *Longissimus thoracis* muscle as influenced by different diet and feeding duration (40, 75 and 120 days).

4.3.4 Bioactive compounds content

Creatine, creatinine, carnosine and anserine contents in meat from *Longissimus thoracis* muscle as influenced by diet and duration of dietary treatment are reported in Table 4.5. Diet significantly affected the profile of bioactive substances, in particular, meat from linseed group showed the highest concentration of creatine ($P<0.05$), carnosine ($P<0.001$) and anserine ($P<0.001$) than control meat.

Many studies have focused on the benefit of linseed supplementation related to its high content of ALA, while the other linseeds compounds have received less attention and their activity is not well described. Linseed is rich in essential amino acids such as histidine, methionine, isoleucine, valine, leucine, threonine and lysine (Bernacchia et al, 2014). Histidine is one of two main components of carnosine (*beta*-alanyl-L-histidine) and anserine (*beta*-alanyl-*N*-methylhistidine) whereas methionine is essential for creatine formation which is limited when methionine in not sufficiently taken in the diet. Therefore the highest contents of creatine, creatinine, carnosine and anserine found in meat from linseed group

could be linked to the amino acids composition of linseed. Although we retain that also the interaction with other linseed components such as n3 polyunsaturated fatty acids could have contributed to the great content of these bioactive compounds. Fatty acid effects on muscle protein synthesis have not been thoroughly investigated but there are some evidence that FA may account for muscle protein synthesis. Gingras et al. (2007) found that supplementation with n3 fatty acids to the diet of growing steers increased sensitivity to insulin improving protein metabolism through the stimulation of a greater provision of amino acids.

In addition n3 FA supplementation affects in a complex manner the expression of various muscle biology and meat quality-related genes in bovine longissimus muscle. Particularly, Hiller et al. (2011) found that genes encoding cell function and signalling-associated proteins are also modulated by dietary n3 FA intervention.

Table 4.5. Levels of bioactive compounds (mg/g meat) in meat from *Longissimus thoracis* muscle as influenced by different diet and feeding duration (40, 75 and 120 days).

	Diets							Effects, P		
	CO			LS						
	40	75	120	40	75	120	SEM	Diet	Duration	Diet x Duration
Creatine	2.61	2.72	2.83	2.88b	3.15b	3.28a	0.08	*	*	*
Carnosine	2.12	2.38	2.30	3.45	3.68	3.77	0.12	***	NS	NS
Creatinine	0.43	0.36	0.38	0.39	0.44	0.33	0.05	NS	NS	NS
Anserine	0.65	0.80	0.74	1.11	1.15	1.03	0.05	***	NS	NS

NS = not significant; * = P< 0.05; ***= P<0.001.
CO = control, LS = linseed.
a, b, c = means with different superscripts in the same column are statistically different among feeding time (P< 0.05).

Duration of feeding significantly affected creatine (P<0.05) concentrations, while no significative differences were found in carnosine, creatinine and anserine concentration. In particular, in linseed group creatine concentration showed an increase passing from 40 to 75 days of feeding whereas after 120 days no differences were found. The reason of the highest content of creatine in meat from

linseed group after 75 days is not easy to explain but could be related to the highest content of n3 polyunsaturated fatty acids found at this feeding time confirming what has been previously postulated.

4.4 Conclusions

Results highlight that linseed supplementation not only contributed to ameliorate fatty acids profile of meat in terms of lower content of SFA, higher content of PUFA, CLA tot and consequently favorable n6/n3 ratio, AI and TI indices but also enriched meat of bioactive compounds including carnitine, carnosine and anserine. Furthermore this study demonstrated that is not necessary to feed steers for a long time to achieve a high increase of n3 polyunsaturated fatty acids, but 75 days of linseed supplementation is enough to obtain n3-enriched meat with a reduced cholesterol content. Short-term diet manipulation can be of interest for meat industry with an economic sustainability of linseed feeding.

Literature Cited

Adhihetty, P. J., & Beal, M. F. (2008). Creatine and its potential therapeutic value for targeting cellular energy impairment in neurodegenerative diseases. *Neuromolecular Medicine,* 10, 275-290.

Alberti, P., Panea, B., Sanudo, C., Olleta, J. L., Rpoll, G., Ertbjerg, P., Christensen, M., Gigli, S., Failla, S., Concetti, S., Hocquette, J.F., Jailler, R., Rudel, S., Renand, G., Nute, G.R., Richardson, R.I., & Williams, J. L. (2008). Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. *Livestock Science*, 114, 19-30.

AOAC (1995). Official Methods of Analysis (16th ed.). Association of Official Analytical Chemists, Arlington, VA.

Arihara, K. (2006). Functional properties of bioactive peptides derived from meat proteins. In N.M.L. Nollet, F. Toldrá (Eds.), *Advanced technologies for meat processing* (pp. 245–274), CRC Press, Boca Raton.

Bernacchia, R., Preti, R., & Vinci, G. (2014). Chemical Composition and Health Benefits of Linseed. *Austin Journal of Nutritional Food Sciences*, 2(8), 1045.

British Department of Health (1994). Nutritional aspects of cardiovascular disease Report of Health and Social Subjects No 46. London, HMSO.

Choi, N. J., Enser, M., Wood, J. D., & Scollan, N. D. (2000). Effect of breed on the deposition in beef muscle and adipose tissue of dietary n-3 polyunsaturated fatty acids. *Animal Science*, 71, 509–519.

Corazzin, M., Bovolenta, S., Sepulcri, A., & Piasentier, E. (2012). Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. *Meat Science*, 90, 99–105.

Diaz, M. T., Caneque, V., Sanchez, C. I., Lauzurica, S., Perez, C., Fernandez, C., Alvarez, I., & De la Fuente J. (2011). Nutritional and sensory aspects of light lamb meat enriched in n-3 fatty acids during refrigerated storage. *Food Chemistry*, 124, 147-155.

Dilzer, A., & Park, Y. (2012). Implication of conjugated linoleic acid (CLA) in human health. *Critical Reviews in Food Science and Nutrition*, 52, 488–513.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P., & Sheard, P.R. (2000). Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science*, 55, 201–212.

Gingras, A.A., White, P.W., Chouinard, P.Y., Julien, P., Davis, T.A., Dombrowski, L., Couture, Y., Dubreuil, P., Myre, A., Bergeron, K., Marette, A., & Thivierge, M.C. (2007). Long-chain omega-3 fatty acids regulatebovine whole-body protein metabolism by promoting muscle insulin signalling to the Akt-mTOR-S6K1 pathway and insulin sensitivity. *Journal of Physiology,* 579, 269-284.

Givens, D.I., Kliem, K.E., & Gibbs, R.A. (2006). The role of meat as a source of $n-3$ polyunsaturated fatty acids in the human diet. *Meat Science*, 74, 209–218.

Griinari, J. M, Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela K. V. V., & Bauman, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. *Journal of Nutrition*, 130, 2285–2291.

Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K., & Raes, K. (2008). Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *Journal of Animal Science*, 86 (6), 1418–1425.

Hiller, B., Herdmann, A. & Nuernberg, K. (2011). Dietary n-3 fatty acids significantly suppress lipogenesis in bovine muscle and adipose tissue: a functional genomics approach. *Lipids*, 46, 557–567.

Hipkiss, A. R. (2009). Carnosine and its possible roles in nutrition and health. *Advanced in Food and Nutrition Research,* 57, 87–154.

Juárez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S., & McAllister, T. A. (2011b). Effects of vitamin E and linseed on rumen-derived fatty acid intermediates in beef intramuscular fat. *Meat Science*, 88(3), 434–440.

Juhn, M. S. (2003). Popular sports supplements and ergogenic aids. *Sports Medicine, 33*, 921–939.

Kauffman, R. G. (2001). Meat composition. In Y. H. Hui, W. K. Nip, R. W. Rogers, & O. A. Young (Eds.), *Meat Science and Applications*,1–19. New York: Marcel Dekker, Inc.

Kim, K., Jung, N., Lee, K., Choi, J., Kim, S., Jun,J., Kim, E.,Kim, D. (2013). Dietary omega -3 polyunsaturated fatty acids attenuate hepatic

ischemia/reperfusion injury in rats by modulating tall-like receptor recruitment into lipid rafts. *ScienceDirect*, 855-862.

Koletzko, B., Lien, E., Agostoni, C., Bohles, H., Campoy, C., Cetin, I., Decsi, T., Dudenhausen, J. W., Dupont, C., Forsyth, S., Hoesli, I., Holzgreve, W., Lapillonne, A., Putet, G., Secher, N. J., Symonds, M., Szajewska, H., Willatts, P., & Uauy, R. (2008). The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *Journal of Perinatal Medicine*, 36, 5–14.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., & Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science*, 81(8), 1967–1979.

Kronberg, S. L., Barcelo-Coblijn, G., Shin, J., Lee, K., & Murphy, E. J. (2006). Bovine muscle n-3 fatty acid content is increased with linseed feeding. *Lipids*, 41(11), 1059–1068.

Kudrna V., Marounek M. (2008). Influence of feeding whole rapeseed and extruded linseed on production of dairy cows, rumen and plasma constituents, and fatty acids composition of milk. *Archives of Animal Nutrition*, 62 (1), s. 60-69.

Litwińczuk, Z., Domaradzki, P., Grodzicki, T., Litwińczuk, A., & Florek, M. (2015).The relationship of fatty acid composition and cholesterol content with intramuscular fat content and marbling in the meat of Polish Holstein-Friesian cattle from semi-intensive farming. *Animal Science Papers and Reports*, 33 (2), 119-128.

Mapiye, C., Turner, T. D., Rolland, D. C., Basarab, J. A., Baron, V. S., McAllister, T. A., Block, H. C., Uttaro, B., Aalhus, J. L., & Dugan, M. E. R. (2013). Adipose tissue and muscle fatty acid profiles of steers fed red clover silage with and without linseed. *Livestock Science*, 151, 11- 20.

Mateescu, R. G., Garmyn, A. J., O'Neil, M. A., Tait, R. G., Abuzaid, A., Mayes, M. S., Garrick, D. J., Van Eenennaam, A. L., VanOverbeke, D. L., Hilton, G. G., Beitz, D. C., & Reecy, J. M. (2012). Genetic parameters for carnitine, creatine, creatinine, carnosine, and anserine concentration in longissimus muscle and their association with palatability traits in Angus cattle. *Journal of Animal Science*, 90, 4248-4255.

Molendi-Coste, O., Legry, V., & Leclercq, I. A. (2011). Why and how meet n−3 PUFA dietary recommendations? Gastroenterology Research and Practice.

Nassu, R. T., Dugan, M. E. R., He, M. L., McAllister, T. A., Aalhus, J. L., Aldai, N., & Kramer, J. K. G. (2011). The effects of feeding linseed to beef cows given forage based diets on fatty acids of longissimus thoracis muscle and backfat. *Meat Science*, 89, 469–477.

O'Fallon, J. V, Busboom J. R., Nelson M. L., & Gaskins C. T. (2007). A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science*, 85,1511–1521.

Oomah, B. (2001). Linseed as a functional food source. *Journal of Science of Food and Agriculture*, 81, 889-894.

Oostindjer, M., Alexander, J., Amdam, G. V., Andersen, G., Bryan, N. S., Chen, D., Corpet, D. E., De Smet, S., Dragsted, L. O., Haug, A., Karlsson, A .H., Kleter, G., de Kok, T. M., Kulseng, B., Milkowski, A. L., Martin, R. J., Pajari, A. M., Paulsen, J. E., Pickova, J., Rudi, K., Sødring, M., Weed, D. L., & Egelandsdal, B. (2014). The role of red and processed meat in colorectal cancer development: a perspective. *Meat Science*, 97, 583–596.

Raes, K., De Smet, S., Balcaen, A., Claeys, E., & Demeyer, D. (2003). Effect of diets rich in N-3 polyunsaturated fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. *Reproduction Nutrition Development,* 43(4), 331–345.

SAS (SAS Institute, 2011). SAS/STAT user's guide (version 9.2) Cary, NC: Statistical Analysis System Inst.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition*, 85(1), 115–124.

Smith, S. B., Smith, D. R., & Lunt, D. K. (2004). Adipose tissue. In W. K. Jensen, C. Devine, & M. Dikeman (Eds.), Encyclopedia of meat sciences (pp. 225–238). London, UK: Elsevier Academic Press.

Toldrá, F., & Reig, M. (2011). Innovations for healthier processed meats. *Trends in Food Science and Technology*, 22, 517–522.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, 985-992.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre, and no starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597.

Waters, S. M., Kelly, J. P., O'Boyle, P., Moloney, A. P., & Kenny, D. A. (2009). Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of delta 9-desaturase in muscle of beef cattle. *Journal of Animal Science,* 87, 244-252.

Ringraziamenti

Il lavoro di questa tesi giunge a conclusione di un percorso che è stato accompagnato dalla presenza di numerose persone che hanno contribuito a rendere il corso di dottorato una bellissima esperienza professionale.

Per questo motivo desidero esprimere la mia gratitudine verso

la prof.ssa Rosaria Marino, per avermi consentito la realizzazione di questo lavoro ed essere stata sempre una preziosa guida durante il mio percorso di dottorato;

il prof. Giancarlo Colelli, in qualità di coordinatore di questo corso di dottorato di ricerca, per la cortesia durante questi tre anni;

il prof. Agostino Sevi, la prof.ssa Marzia Albenzio, la prof.ssa Mariangela Caroprese, la prof.ssa Antonella Santillo per la loro disponibilità e le piacevoli conversazioni;

la dott.ssa Concetta Perilli e la dott.ssa Francesca D'Angelo per il loro prezioso aiuto in laboratorio;

i miei colleghi Antonella, Roberta, Donatella, Lucia, Rosanna, MariaGiovanna, AnnaLisa, Cristina e Francesco, grazie per il supporto, l'amicizia e anche per le bellissime serate passate insieme che hanno reso questa esperienza meravigliosa.

Un grandissimo ringraziamento va inoltre alla mia famiglia per avermi sempre sostenuta e incoraggiata ogni giorno per raggiungere questo importante traguardo professionale.

Grazie a tutti.