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# **Modification of Fatty Acid Composition in Meat Through Diet: Effect on Lipid Peroxidation and Relationship to Nutritional Quality – A Review**

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## **1. Introduction**

The use of nutritional strategies to improve quality of food products from livestock is a new approach that emerges at the interface of food science and animal science. These strategies have emphasized in the alteration of nutritional profile, for example increasing the content of polyunsaturated fatty acid (*PUFA*), and in the improvement of the oxidative stability, such as supplementation of animal with natural antioxidants to minimize pigment and lipid oxidation in meat.

The interest in the modification of fatty acid of meat is due to that fatty acid composition plays an important role in the definition of meat quality because it is related to differences in sensory attributes and in the nutritional value for human consumption [1]. Meat is a major source of fat in the diet, especially of saturated fatty acids (*SFA*), which have been implicated in diseases, especially in developed countries, such as cardiovascular diseases and some types of cancer.

One of the key goals of nutritional research focuses on establishing clear relationships between components of diet and chronic diseases, considering that nutrients could provide beneficial health results. The incidence of these diseases in humans is associated with the amount and the type of fat consumed in the diet. Diets high in *SFA* contribute to increase LDL-cholesterol level, which is positively related to the occurrence of heart diseases. However, some monounsaturated fatty acids (*MUFA*) and *PUFA*, in particular long-chain n-3 *PUFA* have favourable effects on human health.

In recent years, consumers' pressure to reduce the composition and quality of fat in meat has led to attempts to modify meat by dietary strategies. Where as in recent years consumers

have been advised to limit their intake of saturated fats and to reach a ratio of *PUFA:SFA* greater than 4 and the type of polyunsaturated fatty acid is now being emphasized and a higher ratio of n-3: n-6 fatty acids is advocated [1]. There is also now concern about the consumption of unsaturated fatty acids that are formed during high-temperature hydrogenation of oils for use in food products: the *trans*-unsaturated fatty acids in which the double bonds are in the *trans*-stereometric position.

Nutritional approaches to improve the oxidative stability of muscle foods are often more effective than direct addition of food ingredients since the antioxidants are preferentially deposited where it is most needed. In addition, diet often represents the only technology available to alter the oxidative stability of intact muscle foods, where utilization of exogenous antioxidants additives is difficult if not impossible. Since product composition is altered biologically, nutritional alteration of muscle composition is more label-friendly since no additive declarations are required.

Among the strategies used, meat and meat products can be modified by adding ingredients considered beneficial for health where the ingredients are able to eliminate or reduce components that are considered harmful. In this sense, several studies have shown that animal diet can strongly influence the fatty acid composition of meat. Scerra et al. [2] showed that feeding ewes with pasture increases the *PUFA* content of intramuscular fat of the lamb infant compared with diets consisting of concentrate. Nieto et al. [3] showed that feeding Segureña ewes with thyme increases the *PUFA* content of intramuscular fat of the lamb meat compared with control diets. Similarly, Elmore et al. [4] showed that feeding lambs with diets rich in fish oil can modify the fatty acid profile of meat (increasing the level of *PUFA*). Moreover Bas et al. [5] used linseed diet and Ponnampalam et al. [6] used fish oil, in order to increase the content of long-chain n-3 fatty acids in lamb meat.

The variation of fatty acid compositions has profound effects on meat quality, because fatty acid composition determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour. It is well known that high *PUFA* levels may produce alterations in meat flavour due to their susceptibility to oxidation and the production of unpleasant volatile components during cooking [7]. Therefore, it's important to study the implications of the modification of fatty acid in the quality of the meat and the lipid stability, for that it would be interesting the use of liposomes to study the lipid oxidation.

Since liposomes mimic cellular structures [8], the feasibility to protect lipid membranes in the presence of natural antioxidants can be investigated in model systems prior to administration trough feeding. Such previous experiments are particularly interesting for meat industry as they furnish preliminary insights with respect to lipid oxidation at relatively short timescales [9].

## 2. Lipid digestion in ruminants and non-ruminants

It is well known that lipid digestion is different in ruminant and non-ruminant and that the nature of lipid digestion by the animal has an important effect on the transfer of fatty acids

from the diet into the animal product. In case of non-ruminant, the principal site of digestion of dietary lipid is the small intestine, where the pancreatic lipase breaks the triacylglycerols down to mainly 2-monoacylglycerols and free fatty acids and the formation of micelles aids absorption, with lipid uptake mediated by the lipoprotein lipase enzyme, which is widely distributed throughout the body. Therefore dietary fatty acids in the non-ruminant are absorbed unchanged before incorporation into the tissue lipids. Dietary lipid sources have a direct and generally predictable effect on the fatty acid composition of pig and poultry products and the supply of unsaturated fatty acids (*UFA*) to tissues may be simply increased by increasing their proportion in the diet [10].

However, digestion and metabolism of ingested lipids in the rumen results in the exit of mainly long-chain, saturated fatty acids from the rumen. The rumen microorganisms in the ruminant digestive system have a major impact on the composition of fatty acids leaving the rumen for absorption in the small intestine. Microbial enzymes are responsible for the isomerisation and hydrolysis of dietary lipid and the conversion of *UFA* to various partially and fully saturated derivatives, including stearic acid ( $C_{18:0}$ ). Although linoleic ( $C_{18:2}$  n-6) and linolenic ( $C_{18:3}$  n-3) acids are the main *UFA* in the diet of ruminants, the processes within the rumen ensure that the major fatty acid leaving the rumen is  $C_{18:0}$ . The intestinal absorption coefficient of individual fatty acids is higher in ruminants than nonruminants, ranging from 80% for *SFA* to 92% for *PUFA* in conventional low fat diets. Therefore, the higher absorption efficiency of *SFA* by ruminants has been attributed to the greater capacity of the bile salt and lysophospholipid micellar system to solubilise fatty acids, as well as the acid conditions within the duodenum and jejunum (pH 3.0–6.0).

### 3. Fatty acid in meat

Taking into accounts that fat is currently an unpopular constituent of meat and however contributes to meat quality and is important to the nutritional value of meat. This section considers the fatty acid composition in different species and the roles of the fat in meat quality.

Doing a brief introduction of the importance of fatty acids, firstly we will highlight the essential unsaturated fatty acids, linoleic ( $C_{18:2}$ ), linolenic ( $C_{18:3}$ ) and arachidonic ( $C_{20:4}$ ). They are necessary constituents of mitochondria and cell walls. These fatty acids are special, because contrary to the production from saturated sources, the body can not produce any of the fatty acid mentioned above, unless one of them is available in the diet. Oleic, linoleic and linolenic acids each belong to a different family of compounds in which unsaturation occurs at the n-9, the n-6 and n-3 carbon atoms, respectively, in the hydrocarbon chain numbering from the methyl carbon (n). They are thus referred to as the  $\omega$  -9,  $\omega$  -6 and  $\omega$  -3 series. Linoleic acid is abundant in vegetable oils and at about 20 times the concentration found in meat; and linolenic acid is present in leafy plant tissues [11].

Doing a comparative data between the content of *PUFA* in the muscular tissue of the beef, lamb and pork (Table 1), it is clear that linoleic acid ( $C_{18:2}$ ) is markedly greater in the lean meat of pigs than in that of either the beef or lamb.

	C18:2	C18:3	C20:4	C22:5	C22:6
Beef	2.0	1.3	1.0	Tr.	-
Lamb	2.5	2.5	-	Tr.	-
Pork	7.4	0.9	Tr.	Tr.	1.0

**Table 1.** Polyunsaturated fatty acids and cholesterol in lean meat (as % total fatty acids)

In addition, the Table 2 shows the study of Enser et al. [12], who obtained 50 samples of beef sirloin steaks, pork chops, and lamb chops and determined the fatty acid profile of the muscle portions of these retail meat cuts. In the same way that Table 1, the most notable difference among the ruminant species and pork was the fivefold greater concentration of linoleic in pork and significantly greater proportions of C<sub>20:3</sub>, C<sub>20:4</sub>, and C<sub>22:6</sub>, and C<sub>14:0</sub>. For example, pork have a proportions of linoleic acid (C<sub>18:2</sub> n-6): 302 mg/100g of loin muscle, while beef and lamb contains 89 and 25 mg/100g, respectively. The reason of this is because linoleic acid is derived entirely from the diet. It passes through the pig's stomach unchanged and is then absorbed into the blood stream in the small intestine and incorporated from there into tissues. When linoleic acid is ingested, they are metabolized by animal liver to produce two families of long chain polyunsaturated fatty acids which are specific to animals, respectively, the n-6 and n-3 series.

Fatty acid	Pork	Beef	Lamb
C <sub>12:0</sub> (lauric)	2.6	2.9	13.8
C <sub>14:0</sub> (myristic)	30	103	155
C <sub>16:0</sub> (palmitic)	526	962	1101
C <sub>18:0</sub> (stearic)	278	507	898
C <sub>18:1</sub> (trans)	-	104	231
C <sub>18:1</sub> (oleic)	759	1395	1625
C <sub>18:2</sub> n-6 (linoleic)	302	89	125
C <sub>18:3</sub> n-3 ( $\alpha$ -linolenic)	21	26	66
C <sub>20:3</sub> n-6 (lauric)	7	7	2
C <sub>20:4</sub> n-6 (arachidonic)	46	22	29
C <sub>20:5</sub> n-3 (eicosopentaenoic)	6	10	21
C <sub>22:5</sub> n-3 (docosopentaenoic)	13	16	24
C <sub>22:6</sub> n-3 (docosohexaenoic)	8	2	7
Total	2255	3835	4934
P:S	0.58	0.11	0.15
n-6:n-3	7.22	2.11	1.32

Source: Enser et al. [12]

**Table 2.** Fatty acid Content (mg/100g) of loin muscle in steaks or chops.

However, in ruminants, linoleic acid ( $C_{18:2}$  n-6) and  $\alpha$ -linolenic acid ( $C_{18:3}$  n-3) which are at present in many concentrate feed ingredients, are degraded into monounsaturated (*MUFA*) and saturated fatty acids (*SFA*) in the rumen by microbial biohydrogenation (70–95% and 85–100%, respectively) and only a small proportion, around 10% of dietary consumption, is available for incorporation into tissue lipids. By that reason, beef and lamb contain lower content of linoleic acid, compared with pork meat. Muscle also contains significant proportions of long chain ( $C_{20-22}$ ) *PUFAS* which are formed from  $C_{18:2}$  n-6 and  $C_{18:3}$  n-3 by the action of  $\Delta 5$  and  $\Delta 6$  desaturase and elongase enzymes. Important products are arachidonic acid ( $C_{20:4}$  n-6) and eicosapentaenoic acid (EPA,  $C_{20:5}$  n-3).

Taking into accounts that in ruminants, rumen microorganisms hydrogenate a substantial proportion of *PUFA* diet, resulting in high levels of *SFA* for deposition in muscle tissue, lamb or beef meat contain a low relationship between fatty acids *PUFA* and *SFA* (ratio P/S), which increases the risk of cardiovascular problems and other diseases.

The consequences of a greater incorporation of  $C_{18:2}$  n-6 into pig muscle fatty acids compared with ruminants produces higher levels of  $C_{20:4}$  n-6 by synthesis and the net result is a higher ratio of n-6:n-3 *PUFA* compared with the ruminants. If the nutritional advice is for ratios <4.0, the present value of 7 on pig muscle is unbalanced relative to that of the ruminants (1.32 in lamb and 2.11 in beef). In addition, another ratio is the ratio of all *PUFA* to *SFA* (P:S). The ratio P/S in a normal diet is 0.4 [13] and in lamb meat is 0.15 and in beef 0.11, while in pork is 0.58.

For all these reasons, there is an increase interesting in research intended to modify the fatty acid composition in meat, especially reducing the concentration of *SFA* and increasing *PUFA*.

## 4. Dietary modification of fatty acid in meat

Doing the comparison between ruminants and non-ruminants, the fatty acid composition of stored lipids of the ruminant is relatively unwilling to changes in the fatty acid profile of ingested lipids. This logic has been the basis for expression of the concept that ruminant fats are more saturated than those of non-ruminants. Although effects of ruminal biohydrogenation on ruminant tissue fatty acid profiles are generalized, numerous researchers have demonstrated that ruminant lipids can be manipulated by dietary means to contain a higher proportion of unsaturated fatty acids. The next paragraphs will show the different strategies to modify the fatty acids of ruminants and nonruminants.

### 4.1. Altering quality of muscle from monogastric

Monogastric farm animals are worldwide a main source of high-quality products with a high content of highly available protein, minerals, and vitamins. Pigs and chickens are the main monogastric farm animals [1].

To altering the quality of muscle of monogastric, it's necessary to know the digestion of nutrients in these animals. Anaerobic microorganisms are able to hydrogenate unsaturated fatty acids (*UFA*) preferably polyunsaturated fatty acids (*PUFA*). During these processes they build trans-fatty acid as well as conjugated fatty. While in ruminants these fatty acids are absorbed to a great extent, monogastric animals excrete most of them with the feces as they are produced in the lower parts of the digestive tract.

#### 4.2. Altering quality of muscle from ruminants

Meat from ruminants is a major source of essential nutrients (amino acids, iron, zinc and vitamins from the B group). Meat from ruminants (huge diversity of breeding systems and pieces) is characterised by great variations in fats, quantitatively and qualitatively. Some saturated ( $C_{14:0}$  and  $C_{16:0}$ ) and monounsaturated trans fatty acids are not recommended for human consumption and it is possible to reduce their concentrations in meats by increasing the proportions of polyunsaturated fatty acids absorbed by the animals from their diets. To achieve this goal, fatty acids must be protected against hydrogenation in the rumen. Dietary intake of *PUFA* from the n-3 series and especially from the n-6 series by the animals favour the production of conjugated linoleic acid by the rumen bacteria. Some of these fats, such as CLA (conjugated linoleic acid), could be beneficial to human health. CLA is important in the prevention of specific cancers and in the treatment of obesity that has been demonstrated in animal models and, at least partly, in humans.

Alteration of quality in food products from ruminants requires knowledge of the nutritional and metabolic principles that influence product composition. Ruminants are unique among mammals due to their pregastric fermentation. Microflora and microfauna present in the ruminant forestomach dramatically modify the ingested nutrients and consequently have a large impact on the metabolism and composition of the muscle and milk [1].

### 5. Fatty acid sources

It's important to take into accounts several factors to choice the ingredient and the form by which it is included in the feed: (a) the cost and availability; (b) the impact of the ingredients and its fatty acid composition on feed digestibility; (3) the influence of consumers and retailers regarding the introduction of ingredients into the food chain and (4) animal feed regulations regarding permitted supplements.

Recognizing that fatty acids are readily absorbed from the diet and incorporated into tissue fat, producers have attempted to improve the nutritional quality of meat by incorporating various sources of n-3-*PUFA* [7, 14]. The main dietary sources of n-3 fatty acids fed to pig are vegetable oils [15-20], fish oil/fish meal [21,22] and forage [23]. Novel oil sources such as chia seed, marine algae, lupin and camelina have been investigated as lipid sources in animal feeds.

## 5.1. Lipid sources for ruminants

### 5.1.1. Forages

Several studies have shown that ruminants consuming fresh pasture have higher content of UFA in their meat than those receiving a cereal-based concentrate diet.

Grass is a good source of n-3 PUFA although there can be variation due to maturity and variety. Grass lipids contain high proportions of the unsaturated linolenic acid (C<sub>18:3</sub> n-3). Other studies have suggested that the (n-6)/(n-3) ratio in phospholipids may be useful to discriminate grass-fed from grain-fed lambs [1, 24].

Therefore, pasture-raised animals have higher proportions of linolenic acid in their fat than stallfed animals [25].

French et al. [15] compared the effect of offering grazed grass, grass silage and concentrates on the fatty acid composition of intramuscular fat in steers. Similar low intramuscular fat contents (<4.5 g/100 g muscle) were determined in meat from all diets offered, hence a possible confounding effect due to differences in the amount of fat deposited was avoided. 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 ( $P < 0.01$ ) and a linear decrease in the concentration of SFA ( $P < 0.001$ ). The highest concentration of PUFA in the intramuscular fat was found in those animals that had consumed grass only (22 kg grazed grass). Grass and grass silage had a much greater proportion of  $\alpha$ -linolenic acid than the concentrates, although levels of linoleic acid were similar. The content of linoleic acid in the intramuscular fat was not significantly different between treatments but concentrations of  $\alpha$ -linolenic acid and total conjugated linoleic acid were significantly higher for grass-fed steers than for steers offered grass silage and/or concentrates.

Moreover, Nuernberg et al. [26] showed that the concentration of lauric acid was higher in subcutaneous fat and muscle of lambs fed on pasture compared to lambs fed concentrate. Similar results were found by Demirel et al. [27], who studied the fatty acids of lamb meat from two breeds fed different forage: concentrate ratio. And Scerra et al. [2], who showed that lamb meat derived from pasture-fed ewes had a lower levels of lauric and palmitic acid (compared with diets with concentrate) that are thought to be a public health risk.

Sañudo et al. [28] studied British lambs compared with lambs fed grass fed grain, the result showed that a higher percentage of linolenic acid in the meat of grass-fed lambs, as result of the introduction of this natural antioxidant.

Realinia et al. [29] studied thirty Hereford steers that were finished either on pasture or concentrate to determine dietary and antioxidant treatment effects on fatty acid composition and quality of beef. These authors reported that the percentages of C<sub>14:0</sub>, C<sub>16:0</sub>, and C<sub>18:1</sub> fatty acids were higher ( $P < 0.01$ ) in the intramuscular fat of concentrate-fed steers, whereas pasture-fed cattle showed greater ( $P < 0.01$ ) proportions of C<sub>18:0</sub>, C<sub>18:2</sub>, C<sub>18:3</sub>, C<sub>20:4</sub>, C<sub>20:5</sub>, and C<sub>22:5</sub>. Total conjugated linoleic acid (CLA) was higher ( $P < 0.01$ ) for pasture- than concentrate-fed cattle. Therefore, these authors reported that finishing cattle on pasture

enhanced the unsaturated fatty acid profile of intramuscular fat in beef including CLA and omega-3 fatty acids. Results from this study suggest that the negative image of beef attributed to its highly saturated nature may be overcome by enhancing the fatty acid profile of intramuscular fat in beef through pasture feeding from a human health perspective.

### 5.1.2. Oilseeds

Many studies to manipulate the fatty acid composition of meat using whole oilseeds have been conducted. For example, the effect of the physical form of linseed offered on the fatty acid composition of meat has been reported by several workers: Raes et al. [30] reported that the replacement of whole soyabean with extruded linseed or crushed linseed in the finishing diet of Belgian Blue young bulls increased  $\alpha$ -linolenic acid. Mach et al. [31] reported that whole canola seed ( $\alpha$ -linolenic acid content 10.6 g/100 g total FA) or whole linseed ( $\alpha$ -linolenic acid content 54.2 g/100 g total FA), at three lipid levels (50, 80 and 110 g/kg DM) to 54 Holstein bulls increased linearly with lipid level the concentration of n-3 PUFA in the longissimus dorsi muscle.

Elmore et al. [4] reported that the feeding of lamb with diets rich in fat and oils (fish oils, kelp and flax seed) increased the level of polyunsaturated fatty acids. Similarly, Nute et al. (2007) studied the oxidative stability and quality of fresh meat from lambs fed different levels of n-3 PUFA from linseed oil, fish oil, a supplement produced from flax seed (PLS), seed sunflower and soybean meal, seaweed, and combinations of these different oils. They reported that the fatty acid composition of semimembranosus muscle phospholipids was affected by diet.

The rabbit meat was also used in several studies with the objective of fatty acid modification. As the study of Kouba et al. [32], who studied rabbits fed with a diet containing 30 g of extruded linseed/kg. Feeding the linseed diet increased ( $P < 0.005$ ) the content of 18:2n-3 in muscles, perirenal fat, and raw and cooked meat. The long chain n-3 polyunsaturated fatty acid (PUFA) contents were also increased ( $P < 0.01$ ) in the meat. The linseed diet produced a decrease in the n-6/n-3 ratio. These authors highlight that the inclusion of linseed in rabbit diets is a valid method of improving the nutritional value of rabbit meat.

### 5.1.3. Marine algae

Marine algae are an alternative to fish oil as a dietary source of n-3 long chain PUFA (LCPUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

In the study of Cooper [33], the marine algae were included in the sheep diet not only as a source of DHA (fish oil/algae diet supplied 15 g/100 g total FA as DHA) but also because it had been previously shown to undergo a lower level of biohydrogenation than fish oil [33]. In another study of the same author [34] studied the manipulation of the n-3 PUFA

fatty acid content of muscle and adipose tissue lamb was studied. For that fifty lambs, with an initial live weight of  $29 \pm 2.1$  kg, were allocated to one of five concentrate-based diets formulated to have a similar fatty acid content (60 g/kg DM), but containing either linseed oil (high in 18:3n $\omega$ 3); fish oil (high in 20:5n $\omega$ 3 and 22:6n $\omega$ 3); protected linseed and soybean (PLS; high in 18:2n $\omega$ 6 and 18:3n $\omega$ 3); fish oil and marine algae (fish/algae; high in 20:5n $\omega$ 3 and 22:6n $\omega$ 3); or PLS and algae (PLS/algae; high in 18:3n $\omega$ 3 and 22:6n $\omega$ 3). Lambs fed either diet containing marine algae contained the highest ( $P < 0.05$ ) percentage of 22:6n $\omega$ 3 in the phospholipid (mean of 5.2%), 2.8-fold higher than in sheep fed the fish oil diet.

A more limited number of studies have looked into the effects of dietary supplementation with DHA-rich marine algae on the fatty acid composition of muscle tissue of rabbits [35], lambs [4] and pigs [36, 37].

## 5.2. Lipid sources for non-ruminants

The cereal-based diet commonly offered to poultry and pigs supplies mainly n-6 PUFA and a small amount of n-3 PUFA. This is reflected in the fatty acid composition of the animal product. Dietary modification of poultry meat, eggs or pork to increase the n-3 PUFA content requires a supply of n-3 PUFA from the diet.

The actual strategies to non-ruminants are focused on assessing the effect of offering terrestrial versus marine sources of n-3 PUFA and the subsequent implications for product quality.

### 5.2.1. Vegetable oils

Enrichment of poultry diets with plant oils has been shown to have different impacts on abdominal fat and the site of fatty acid deposition depending on the SFA, MUFA and PUFA content of the oil [38].

Crespo & Esteve-García [38] studied broiler chickens fed with a basal diet supplemented for 20 days before slaughter with 10% inclusion of linseed oil, sunflower oil and olive oil. As expected with non-ruminant, the fatty acid profile of the deposited fat in the broiler carcass reflected the dietary fat source. The supplementation with olive oil resulting in the highest proportion of C<sub>18:1</sub>, sunflower oil supplementation resulting in the highest proportion of linoleic acid (51.1 g/100 g total body FA), while linseed oil contributed the highest amount of n-3 PUFA and most favourable n-6:n-3 ratio in the carcass fat.

In addition, Lu et al. [39] investigated the effects of soybean oil and linseed oil on the fatty acid compositions of pork. The three dietary treatments were: (a) no oil supplement; (b) 3% soybean oil supplement; (c) 3% linseed oil supplement. Dietary linseed oil and soybean oil significantly increased the contents of C<sub>18:3</sub> and C<sub>18:2</sub> in the neutral lipids and phospholipids in both longissimus muscle and biceps brachii muscle, respectively.

### 5.2.2. *Linseed and fish oil*

The n-3 long chain polyunsaturated fatty acids can be incorporated into non-ruminant products from dietary fish oil. The transfer of these fatty acids was found to be influenced by time and duration of feeding and the presence of other oil supplements. Haak et al. [40] offered to pigs a basal diet composed of barley, wheat and soyabean meal ad libitum alone or supplemented with 1.2% linseed or fish oil during: the whole fattening period; the first fattening phase (weeks 1–8) only; or the second fattening phase (6 weeks or 9 weeks, until slaughter at 100 kg). Haak et al. [40] reported that incorporation of  $\alpha$ -linolenic acid into the longissimus thoracis muscle was similar (1.24 g/100 g total FA) when linseed was offered throughout the fattening period or only during the second phase. When fish oil was offered during either of the fattening phases, only the proportion of DHA incorporated was affected, being greater when fish oil was offered during the second fattening phase ( $P < 0.05$ ). Incorporation of EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) following fish oil supplementation for the whole fattening period was 1.37 and 1.02 g/100 g total FA in the longissimus thoracis muscle, representing a six-fold increase compared to the basal diet and a three- and five-fold increase respectively, compared to the linseed diet. In agreement with other animal work [34], Haak et al. [40] concluded that a direct dietary source of DHA was required to increase DHA in animal muscle and that levels in pork could not be substantially influenced by dietary supply of precursors.

### 5.2.3. *Marine algae*

It's well known that microalgae are the original source of DHA in the marine food chain [41], dried marine algae have also been included in animal feeds to improve the DHA level of foods of animal origin. Studies has mainly focused on the quality of eggs [42, 43] and chicken meat [44]. A more limited number of studies have looked into the effects of dietary supplementation with DHA-rich marine algae on the fatty acid composition of muscle tissue of pigs [36, 37].

## 6. Effects of fatty acid modification on the nutritional value of meat

There is a growing consumers resistance to the incorporation of additives into foods, especially where the additives are of synthetic origin, even when they have a nutritional or health advantage. Dietary supplementation of the growing animal provides a unique method of manipulating the content of some micronutrients and other nonnutrient bioactive compounds in meat, with a view to improving the nutrient intake of consumers or improving their overall health.

Research on heart disease in humans has tended to implicate high intakes of saturated fat and cholesterol as contributory factors with a possible protective effect of polyunsaturated fat and a neutral effect of monounsaturated fat [45]. Overall, the advice to consumers has been to control the level of energy consumed as fat to under 35% and in particular, to limit saturated fats to 10% of energy intake [13]. It is also recommended that the proportion of short- and medium-chain saturated fatty acids be reduced and that intake of n-6 fatty acids be reduced relative to n-3 [45].

The nutritional properties of meat are largely related to its fat content and its fatty acid composition. In this sense, long-chain n-3 fatty acids, such as C<sub>20:5</sub> n-3 and C<sub>22:6</sub> n-3 have beneficial health effects, such as reduction in the thrombotic tendency of blood, associated with lower coronary heart disease in humans [46]. In addition, the role of dietary fat in human health is further complicated by the differing biological activity of some fatty acids when present at different stereospecific positions in triacylglycerols [47].

To avoid possible health dangers from the consumption of the meat of ruminants, a greater degree of unsaturation could be introduced into their fats. One example of the modification of fatty acid in meat resulting in an improvement of human health is the study of Diaz et al. [48]. These authors studied the fatty acid content and sensory characteristics of meat from light lambs fed three diets supplemented with different sources of n-3 fatty acids (fish oil, linseed and linseed plus microalgae) and a control diet during refrigerated storage. The meat from lambs fed linseed diets had the highest levels of C<sub>18:3</sub> n-3, while animals fed fish oil, had the highest long-chain n-3 polyunsaturated fatty acids (*PUFA*). Thus, 100 g of meat from lamb fed the fish oil diet provided 183 mg of long-chain n-3 *PUFA*, representing 40% of the daily recommended intake. The levels of n-3, n-6 and long-chain n-3 *PUFA* decreased during a 7-day storage period. These authors reported that consumption of 100 g of lamb muscle from lambs fed control diet would provide about 5% of the daily recommended intake for long chain n-3 fatty acids (500 mg per day, according to EFSA [49]). In case of linseed plus microalgae and linseed diets, would provide nearly 10% of the daily recommended intake for long-chain n-3 fatty acids. The greatest supply of n-3 *PUFA* and long-chain n-3 fatty acids would come from lambs fed fish oil diet, which would provide about 34% of the daily recommended intake for long-chain n-3 fatty acids. Moreover, the highest *PUFA/SFA* ratio was found in lambs fed linseed and fish oil diets, which was close to the recommended value (0.35). The lowest value was observed in lambs fed control diet. During storage, the total content of *PUFA*, including n-3 *PUFA*, n-6 *PUFA* and the long-chain n-3 *PUFA*, decreased. Thus, meat from lambs fed fish oil could supply close to 40% of the daily recommended intake for long-chain n-3 fatty acids on day 0. On day 7 this meat supplies almost 31% and, therefore, this could be considered a reduction in the nutritional value of the meat. This decrease could be a consequence of oxidation changes, since *PUFA* are more prone to oxidation than *MUFA* or *SFA*; the meat from the supplemented groups and especially from animals fed fish diet, are more prone to oxidation than the control diet (with lower content in *PUFA* and long-chain n-3 *PUFA*). Therefore, the importance of the influence of the modification of fatty acid profile on the lipid peroxidation should be studied.

## 7. Quality of *PUFA* enriched animal products and relation with lipid peroxidation

One of the main factors limiting the quality of meat and meat products is lipid oxidation. Lipid oxidation results in rancid odour and flavour, sometimes referred to as warmed-over flavour. Fatty acids are oxidised into aldehydes, alkanes, alcohols and ketones by chemical (auto-oxidation) or enzymatic ( $\beta$ -oxidation) reactions. In this sense, rancid aroma is

apparently due to the dominance of alkanal (hexanal, nonanal) or certain alcohols (1-penten-3-ol, 1-octen-3-ol). The reason is due to the first step of lipid oxidation, which involves the removal of hydrogen from a methylene carbon in the fatty acid. This becomes easier as the number of double bonds in the fatty acid increases, which is why polyunsaturated fatty acids are particularly susceptible to oxidation. Therefore, increasing the degree of unsaturation of muscle membranes reduces the oxidative stability of the muscle. In addition, the relative oxidation rates of fatty acids containing 1, 2, 3, 4, 5 or 6 double bonds are 0.025, 1, 2, 4, 6 and 8, respectively [50].

It is very interesting to correlate the fatty acid profile of the meat with the development of off-odour and off-flavour in order to understand the susceptibility of oxidative damage in the meat. For example, If the ratio of *PUFA* to *SFA* is higher in the meat, this softer fat is more susceptible to oxidative damage, and this may cause difficulties for the retailers who are increasingly turning toward centralized butchery and modified atmosphere packaging, both of which lead to meats being exposed to higher levels of oxygen for a longer period of time prior to retail.

There are few studies that examine the effect of an enrichment of the diet in n-3 *PUFA* and the oxidation potential of muscle. Some of these studies are made in rabbit meat [51-52, 32] and lamb meat [53]. While Kouba et al. [32], reported that the enriched *Longissimus dorsi* did not exhibit a lower oxidative stability, Castellini et al. [51] and Dal Bosco et al. [52] found that feeding a n-3 *PUFA* enriched diet lowered significantly TBARS level in, raw meat and *Longissimus dorsi*. One plausible explanation could be that these authors only supplemented the experimental diet with a high amount of vitamin E, which led to an increase of vitamin E level in tissues of rabbits fed this diet, as already described by Oriani et al. [54], and it is well known that the susceptibility of lipids to oxidation can be reduced by vitamin E, as described by Lin et al. [55] in poultry and Monahan et al. [56] in pigs.

The oxidative processes in living animals are dependent on the endocrine and enzymatic activities in the tissues. There is some evidence that differences between species and breeds of animals exists. However, a high individual variation has also to be assumed. Oxidative processes can occur at many different stages in animal nutrition. During digestion the nutrients are soluble and therefore can be easily oxidized. Extrinsic influences on the oxidative processes mainly derive from the composition of the feedstuffs and feed additives. Therefore, feed should be protected against oxidative damage already during storage.

With increased content of polyunsaturated fatty acids (*PUFA*) a higher oxidation rate in the feed, in the digest as well as in the intermediate metabolism, occurs. But feedstuffs can also contain antioxidants like vitamins, carotenoids, or phenols, or prooxidative compounds like some trace elements. To improve the oxidative stability of the feed, antioxidative additives are often used as supplements to the diets.

Notwithstanding the beneficial attributes of polyunsaturated fatty acids, it should be noted that lipid oxidation products are believed to adversely affect the health of cells. Fortunately muscular tissue contains several enzymes that protect cells against such change, the most important of which is glutathione peroxidase [57].

To avoid the lipid oxidation tendency shown in meat rich-PUFA, Díaz et al. [48], recommended the inclusion of antioxidants in the diet of lambs, in order to avoid the negative impact on the flavour and to prevent fatty acids from oxidation of these on lamb meat enriched in n-3 fatty acids. Therefore, the inclusion of antioxidants with the incorporation of the ingredients responsible of the fatty acid modification through the feed could be an interesting strategy to prevent oxidation of the meat. Similarly, it has been shown that some fatty acids (such as conjugated linoleic acid) can exert antioxidant activity in meat by reducing lipid oxidation [58]. In a previous study made by our group, the effectiveness of thyme leaves diet (during pregnancy and lactation of ewes) to improving the lamb meat lipid stability was attributed to the antioxidant effect of the phenolic compounds present in the thyme leaf. These bioactive compounds in the leaves may interfere with the propagation reaction of lipid oxidation, besides inhibiting the enzymatic systems involved in initiation reactions [59]. It has been shown that diet with natural antioxidants interferes with the metabolism of fatty acids in ruminants [60]. Taking into accounts another studies using plants of the family Labiatae in the diet, Youdim and Deans [61] showed that a dietary supply of thyme oil or thymol to ageing rats showed a beneficial effect on the antioxidative enzymes superoxide dismutase and glutathione peroxidase, as well as on the polyunsaturated fatty acid composition in various tissues. Animals receiving these supplements had higher concentrations of polyunsaturated fatty acids in phospholipids of the brain compared to the untreated controls. Similarly, Lee et al. [62] showed that the pattern of fatty acids of the abdominal fat of chicken was also altered by oregano oil and dietary carvacrol lowered plasma triglycerides. In animals for food productions, such effects are of importance for product quality: these supplement may improve the dietary value and lead to a better oxidative stability and longer shelf-life of fat, and meat [63].

## 7.1. Liposomes

Oxidative stress leads to oxidation of low-density lipoproteins (LDL), which plays a key role in the pathogenesis of atherosclerosis, which is the primary cause of coronary heart disease [64]. The nutritional manipulations of the fatty acid composition of meats increase the susceptibility of their lipids to peroxidation; because as have been explained in the previous sections, PUFA are known to act as substrates initiating the oxidative process in meat. In this sense, much attention has been paid to the use of the natural antioxidants, since potentially these components may reduce the level of oxidative stress in the feed.

Several methods have been described in the literature for assessing antioxidant activity. These include radical scavenging assays, ferric reducing assay, or inhibition of the oxidation of oils, emulsions, low-density lipoproteins (LDL), or liposomes. The use of LDL is an interesting method of assessing antioxidant properties relevant to human nutrition, since these systems allow investigation of the protection of a substrate by an antioxidant in a model biological membrane or a lipoprotein. Assessment of the activity of mixtures of lipid-soluble and water-soluble antioxidants in liposomes has clear advantages over other commonly used methods. The liposome system allows the lipid-soluble components to be present in the lipid phase without the presence of a cosolvent, while the water soluble antioxidants can be added to the aqueous phase of the liposome [8].

The liposome system also allows study the synergy between different antioxidants ingredients used in the manufacture of feed, as tocopherols or other water-soluble antioxidants to be demonstrated [65- 66], whereas synergy is not normally observed if these components are present in homogeneous solution.

Therefore, the use of a liposome system is an interesting strategy for a preliminary assessment of the antioxidant activity of ingredients used in the manufacture of feedstuffs.

This was the objective of a previous study [9] where the use of liposomes as biological membrane models to evaluate the potential of natural antioxidants as inhibitors of lipid peroxidation was described. For that, the antioxidative effects of by-products from manufacturing of essential oils, i.e., distilled rosemary leaf residues (*DRL*), distilled thyme leaf residues (*DTL*), and the combined antioxidative effects of *DRL* or *DTL* with  $\alpha$ -tocopherol (*TOH*), ascorbic acid (*AA*), and quercetin (*QC*) on peroxidation of L- $\alpha$ -phosphatidylcholine liposomes as initiated by hydrophilic azo-initiators, were investigated. The results showed that the extracts from *DRL* and *DTL* all had an obvious antioxidative effect as evidenced by a lag phase for the formation of phosphatidylcholine-derived conjugated dienes. Combination of *TOH* or *QC* with *DRL* and *DTL*, respectively, showed synergism in prolonging of the lag phase. Distilled leaves of rosemary and thyme were found to be a rich source of antioxidants as shown by the inhibition of the formation of conjugated dienes in a liposome system. Based on this study, it can be concluded that rosemary and thyme residues, as by-products from distillation of essential oils, are a readily accessible source of natural antioxidants, which possibly provides a good alternative to using synthetic antioxidants in the protection of foods and meat products in particular.

After this study, it was reported that both distilled leaves (rosemary and thyme) were readily accessible source of natural antioxidants in animal feedstuffs, these by-products were added to the feed of pregnant ewes [67- 71]. As shown previously with the liposomes model system study, the meat of lambs from ewes fed with distilled rosemary and thyme leaf had lower levels of lipid oxidation and these additives were considered a good alternative to using synthetic antioxidant in animal diets.

## 8. Conclusions

This review suggest that the negative image of meat attributed to its highly saturated nature may be overcome by enhancing the fatty acid profile of intramuscular fat through feeding from a human health perspective. Increasing the n-3 *PUFA* content of animal feedstuffs can be a promising and sustainable way to improve the nutritional value of meat, without forcing consumers to change their eating habits.

It's well known that although dietary *PUFA* improves meat nutritional qualities, such meats are more susceptible to lipid oxidation during processing. Therefore, there is a need to study the differences in oxidative stability of the muscles in order to understand the effect of dietary on lipid peroxidation. For that the use of liposomes is an interesting strategy to study the lipid peroxidation in model system as preliminary studies (prior the administration of fatty acid sources through feeding).

When all of these considerations are taken into account, the possibility of preserving the nutritional qualities of processed meat rich in *PUFA* by an original dietary antioxidant strategy is recommended, in order to prevent the lipid peroxidation and the decrease of overall liking of meat.

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