UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

COMPARISON OF LIPID PROFILE AND SOME PHYSICO-CHEMICAL PROPERTIES OF BEEF FROM SPAIN AND NETHERLANDS WITH BEEF FROM NATIONAL PRODUCTION WITH DIFFERENT GENETIC BACKGROUND (DAIRY AND CROSSBRED BEEF)

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Abstract

Comparison of Lipid Profile and Some Physic-Chemical Properties of Beef from Spain and Netherlands with Beef from National Production with Different Genetic Background (Dairy And Crossbred Beef)

Some physic-chemical properties and lipid profile of beef from two origins (Holland and Spain) were compared with beef from national production of both dairy and crossbred beef backgrounds.

In comparison, we can find four different groups: two groups of animals from Spain and Holland; n=10 animals per group) and two groups from national production (dairy and beef production systems; n=10 for animals per origin). Animals used in the study were all males of 8-12 months old. In study we can find two different genetics, the Holstein breed (Holland, Spain and Portugal) and crossbred beef genetics (Portugal).

Beef samples were obtained from sirloin (*longissimus lumborum*) in all groups. The fatty acid profile was determinated by GC. Total cholesterol, β-carotene and tocochromanols were quantified by HPLC.

The results of comparison showed that beef from crossbred beef bulls was leaner and displayed a distinct fatty acid profile with lower SFA, MUFA contents and higher PUFA and n-3 PUFA contents than beef from Holstein bulls. Beef from the crossbred beef bulls was the only group in total agreement with P/S and n6/n3 ratios. Relatively to beef color parameters, Portuguese beef presented the highest L* and lowest a* values and intermediary b* values. Beef from Holstein bulls raised in Portugal was on the limit of DFD while beef from the Spanish Holstein bulls was a well-established DFD.

It is however important to highlight that the study was done with a restrict number of samples, and for this reason it is not possible to conclude that beef from national production has a superior quality than beef from abroad.

Key words: beef, fatty acids, vitamin E, cholesterol, pH, color

Resumo

Comparação do perfil lipídico e algumas características físico-químicas de carne de bovino proveniente da Espanha e Holanda com carne de bovino nacional proveniente de animais com genética diferente (leite e cruzados de carne)

Este estudo procurou comparar o perfil lipídico e algumas propriedades físico-químicas da carne de bovino holandesa e espanhola com carne de bovino da produção nacional proveniente de animais com genética diferente (leite e cruzados de carne).

Em comparação podemos encontrar quatro grupos diferentes. Se tivermos em consideração a genética dos animais, podemos encontrar 2 grupos: 1) animais de genética Holstein (acabados em Portugal, Espanha e Holanda) e 2) cruzados de carne de produção nacional. Se tivermos em consideração a origem, podemos também encontrar 2 grupos: 1) do exterior (Espanha e Holanda) e produção nacional (Holstein e cruzados de carne). O estudo contemplou apenas a utilização de touros do sexo masculino e com uma idade ao abate entre os 8 e os 12 meses de idade (vitelão), tendo-se usado 10 animais por grupo.

As amostras de carne usadas no estudo foram obtidas da vazia (*longissimus lumborum*). O perfil de ácidos gordos foi analisado por GC, enquanto a quantificação dos teores de colesterol total, β-caroteno e tococromanóis foi realizado por HPLC.

Os resultados do estudo revelaram que a carne proveniente de bovinos cruzados de carne era mais magra e apresentava um perfil lipídico com menor teor de SFA e MUFA e um teor superior de PUFA e n-3 PUFA comparativamente com os bovinos de raça Holstein. A carne proveniente de bovinos cruzados de carne foi a única a apresentar os rácios P/S e n6/n3 em total concordância com as recomendações internacionais. Relativamente aos parâmetros de cor estudados, podemos afirmar que a carne produzida em Portugal apresentou valores de L* mais elevados, e os valores de a* mais baixos do que a carne espanhola e holandesa, tendo apresentado valores de b*intermédios aos obtidos na carne do exterior. O pH da carne de touros criados em Portugal estava no limite da carne DFD, enquanto o pH da carne proveniente de Espanha se podia considerar uma carne DFD.

Apesar dos resultados aqui apresentados, é importante realçar que o estudo foi realizado com um número restrito de amostras e que por essa razão não é possível concluir que a carne de bovino produzida em Portugal seja superior à carne de bovino produzida no exterior.

Palavras-chaves: carne de bovino, vitelão, ácidos gordos, vitamina E, colesterol, ph, cor

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List of abbreviations

AA: arachidonic acid

AD: Alzheimer’s disease

ADHD: attention-deficit hyperactivity disorder

AHA: American Heart Association

ALA: alpha-linolenic acid

BSE: bovine spongiform encephalopathy

CH_3: methyl

CO_2: carbon dioxide

CVD: cardiovascular diseases

DFD: dark, firm and dry

DGAC: Dietary Guidelines Advisory Committee

DHA: docosahexaenoic acid

EFA: essential fatty acids

EPA: eicosapentaenoic acid

FAO: Food and Agriculture Organization

HDL: high-density lipoproteins

LA: linoleic acid

LCPUFA: long chain polyunsaturated fatty acids

LDL: low-density lipoproteins

MUFA: monounsaturated fatty acids

O_2: oxygen

PUFA: polyunsaturated fatty acids

RA: rheumatoid arthritis

SFA: saturated fatty acids

TFA: trans-fatty acids

VLDL: very low-density lipoprotein

WHC: water-holding capacity

1. Introduction

Beef is a major ingredient in western diets, being the major source of protein to these populations. Meat is the major source of protein and an important source for some micronutrients such as vitamins A, B6, B12, D and E, iron, zinc and selenium, which are not present or have poor bioavailability in vegetal foods. Humans should eat between 0.8 to 1.3 g of protein per kg body weight per day, according with their development or physiological stage. In 85 g of lean beef, which is the recommended serving size, there are about 25 g of protein according with the Dietary Guidelines Advisory Committee (DGAC), which is nearly half of the required daily protein intake (56 g of protein intake daily). Still, humans tend to consume above the needs, contributing to a diet misbalance which is associated with health risks.

Meat also provides fat, particularly saturated fats and cholesterol, which can be harmful to human health. Therefore excessive consumption of meat is associated with increased intake of saturated fat and cholesterol, which are associated with health deleterious effects, particularly with cardiovascular health. In view of its implications in meat quality and human health, the intramuscular lipid composition of beef aroused considerable interest.

Beef production in Portugal is not enough to ensure domestic consumption, representing only 52% of total consumption. For this reason Portugal is forced to import beef. The importation of beef in Portugal features 3 levels: 1) import top quality cuts (chuck round, rump, sirloin and tenderloin) mainly from South America; 2) vacuum packed refrigerated cuts of whole carcasses and 3) refrigerated carcasses exclusively from Europe (Spain 53%, Netherlands 16%).

Such beef importation from different countries, is associated with quality variability, due to different genetics, production systems, feeding management, age at slaughter, slaughter procedures, among other differences, which makes beef quality quite variable. The aim of the study was to evaluate the beef quality obtained from dairy herds of Portugal, Spain and Netherlands, and also compare Portuguese beef quality from dairy and beef breeds.

2. Bibliographic review

2.1 The role of meat during human evolution

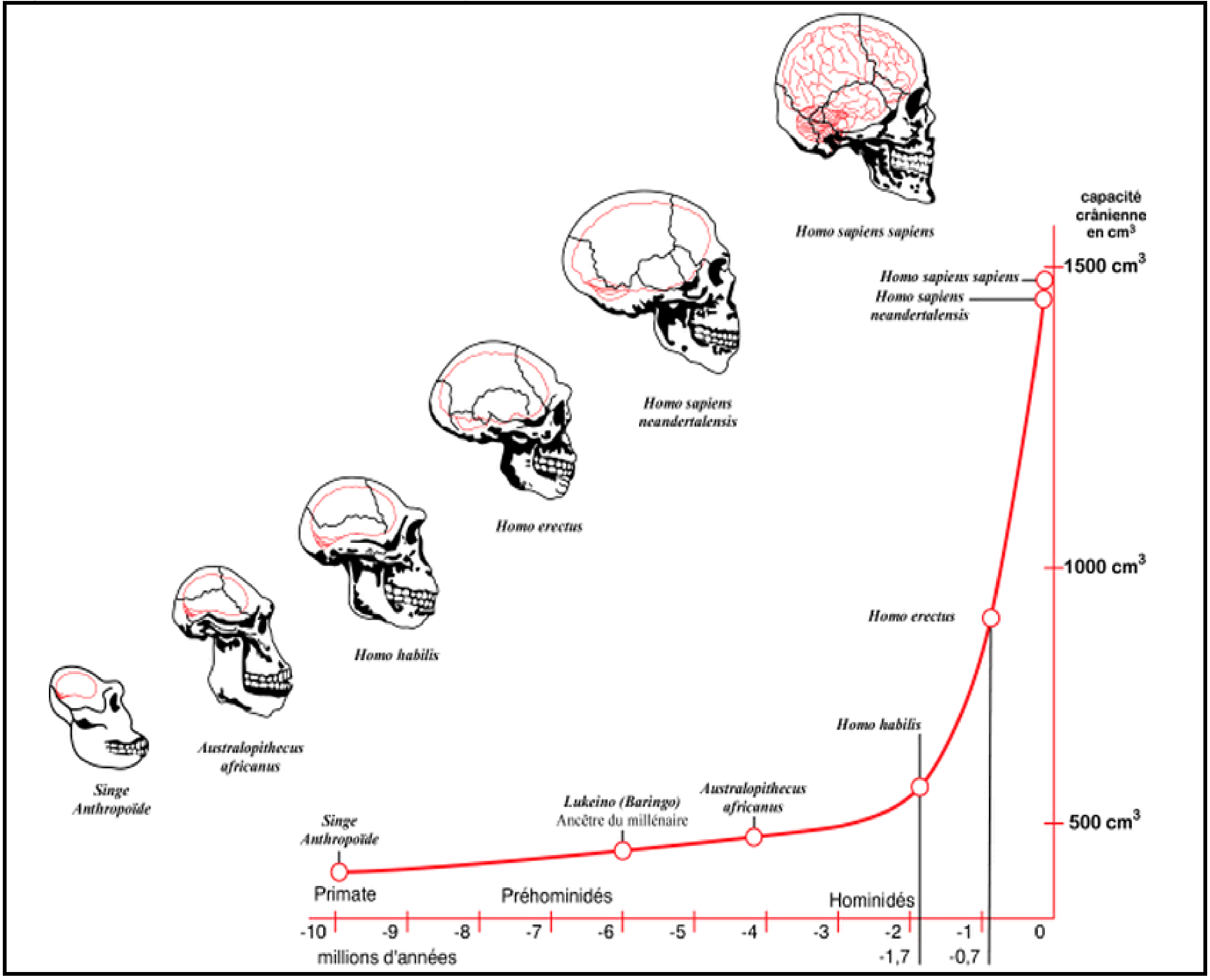
Meat is an important source of high quality protein (in some countries the main source), and some essential micronutrients such as vitamins A, B6, B12, D and E, iron, zinc and selenium, contributing to consumers' health throughout life (Williamson et al., 2005; Scollan et al., 2006). These micronutrients are either not present in plant derived food or have a poor bioavailability in vegetable foods (Biesalski, 2005; Luciano, 2009).

It has been estimated that meat consumption by primitive humans began to rise about 2.5 million years ago, since there is no earlier available archaeological data, but it could have a longer history extending up to 6 to 8 million years ago, as suggests the meat-eating behavior of chimpanzees, which share with us a common ancestor (Larsen, 2003). From an anatomical perspective, modern human beings have not changed compared with their Paleolithic ancestors (Zucoloto, 2011). Before the Agricultural Revolution or Neolithical Revolution (10000 BC), our ancestors lived by hunting and gathering on the African continent and afterwards in other parts of the world, eating animals and plants (Eaton, Eaton, & Cordain, 2002; Larsen, 2003; Richards, 2002; Simopoulos, 2008; Ungar & Teaford, 2002; Zucoloto, 2011).

During the pre-Neolithic period, the consumption of meat, was dependent of hunting or scavenging wild game meat. The consumption of meat seems to have accompanied the increase of stature and brain matter and, in general, the whole transition from *Australopithecus* to *Homo Erectus* via *Homo Abilis* (Richards, 2002; Ungar & Teaford, 2002). Just to figure, the brain size of *Homo Sapiens* is thrice that of *Australopithecus* (Ungar & Teaford, 2002).

There is evidence of human control, or domestication, of wild plants and animals in all continents at the start of the Holocene, about 12000 years ago (Richards, 2002). With the advent of agriculture, novel foods were introduced, varying in accordance with world region and climate, such as rice, or corn for which the human genome had little evolutionary experience (Cordain et al., 2005). Adopting this new food in human nutrition has conducted to a general decline in teeth health and stature, as testified by skeletal evidence, together with the rise of new health disorders (Richards, 2002). In the Western World, the human diet has undergone another major shift the last 100-150 years, due to the so-called Industrial Revolution (Cordain, et al., 2005; Simopoulos, 2006). The industrialization of agriculture practices and the consequent industrialization and mechanization of the oil-seed industry and the large-scale addition of refined vegetable oils to the world's food supply after the Industrial Revolution, significantly altered both quantitative and qualitative aspects of fat intake (Cordain, et al., 2005).

Figure 1 - Evolution of cranial capacity in human (copied from Muséum d'Aix-en-Provence 2001)

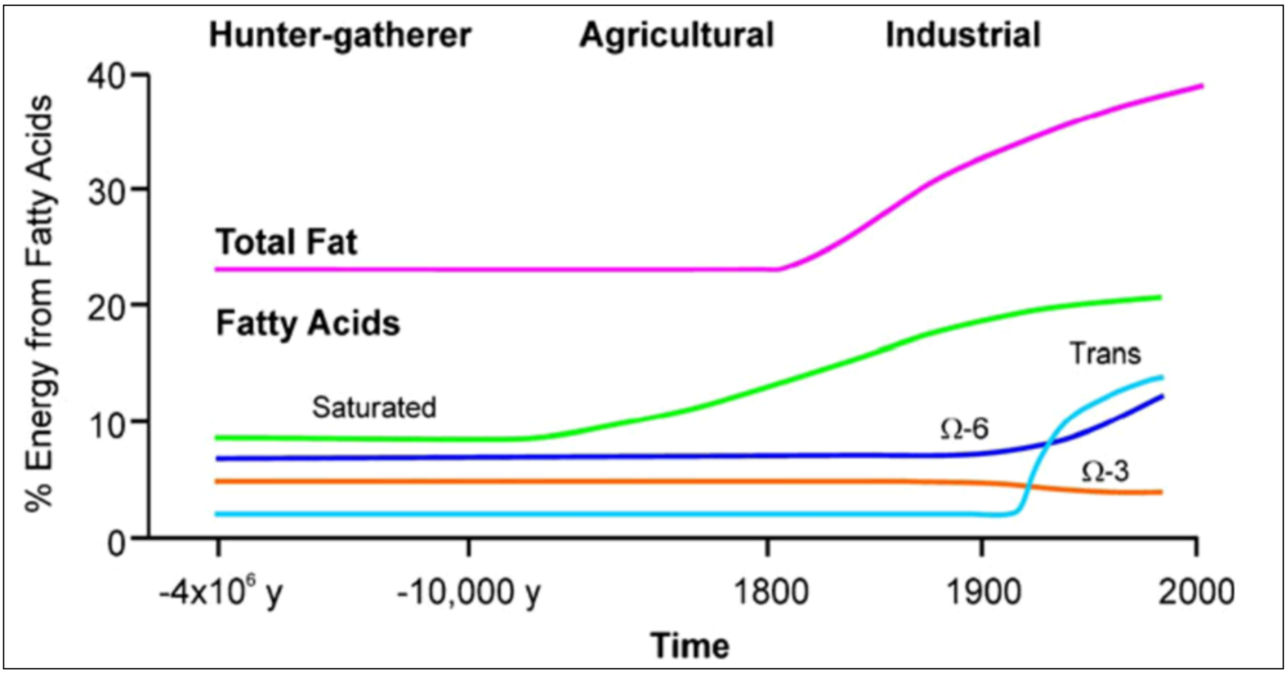


An example of such change was the hydrogenation process in food industry which has produced, and introduced in human diet, novel trans fatty acid isomers (Cordain, et al., 2005). For many centuries, human diet was high in fruits, vegetables, nuts, and berries, industrialization brought in refined grains and sugars (Cordain, et al., 2005; Larsen, 2003; O'Keefe & Cordain, 2004; Simopoulos, 2006). This new diet rich in highly processed and high-glycemic-load foods is at odds with whole, natural, fresh foods early humans used to consumed (Cordain, et al., 2005; O'Keefe & Cordain, 2004). During this period of major social and economic changes, n-3 fatty acids intake saw a reduction, whereas trans-fats boomed along with fried foods, hard margarine, commercial baked goods, and most packaged and processed snack foods (Cordain, et al., 2005; Larsen, 2003; O'Keefe & Cordain, 2004; Simopoulos, 2006).

Early humans consumed diets containing approximately equal amounts of n-3 and n-6, as well as much lower levels of saturated and trans fatty acids compared with current dietary patterns (Kazemian, Kazemi-Bajestani, Alherbish, Steed, & Oudit, 2012; O'Keefe & Cordain, 2004;

Simopoulos, 2006). In the Paleolithic world, n-3 fats were abundant coming from a broad base of the food chain composed of ubiquitous algae in the sea and of grasses and leaves on land (Broadhurst et al., 2002; O'Keefe & Cordain, 2004). The small amount of fat in algae, grasses, and leaves is rich in n-3 fatty acids, which become more concentrated in larger animals through both the terrestrial and marine food chains, especially in fish and larger grazing animals (O'Keefe & Cordain, 2004). Hence, early humans diets contained approximately equal amounts of n-3 and n-6, as well as much lower levels of saturated and trans fatty acids compared with current dietary patterns (Kazemian, et al., 2012; A. P. Simopoulos, 2002). Meat from domesticated animals is low in n-3 fatty acids because these animals are generally grain-fed rather than grass-fed, and grains are rich in n-6 fatty acids (Cordain, et al., 2005; O'Keefe & Cordain, 2004; A. P. Simopoulos, 2002; Simopoulos, 2008). Cereal grains are high in carbohydrates and n-6 fatty acids, but low in n-3 fatty acids and antioxidants (A. P. Simopoulos, 2002). As a result, nowadays, the intake of n-3 fats is much lower compared with our remote ancestors. (O'Keefe & Cordain, 2004; A. P. Simopoulos, 2002).

Figure 2 - Fatty acids trend in human diet throughout history (copied from Kazemian et al., 2012)



2.2 World and Europe bovine meat production

According to FAO database, on the last 48 years, world bovine meat production has more than doubled, passing from 280 million tons to 640 million tons (Figure 3). In 1961, Europe produced approximately 100 million tons of bovine meat, which was about one third of the whole world production. In Europe, bovine meat production kept growing until the 90's of last century and then declined until the turn of millennium, cutting the production to about one half, passing from almost 200 million tons to 110 million tons (Figure 3). Nowadays, Europe produces about one sixth of the world bovine meat.

Figure 3 - Global and European 50-years trend for bovine meat production (FAOSTAT).

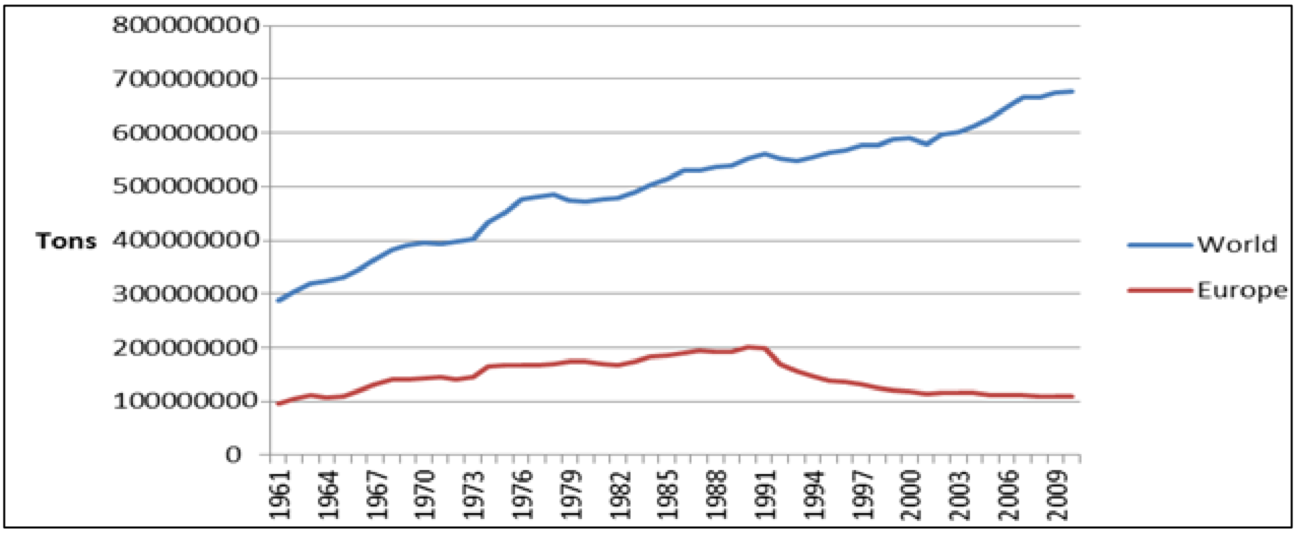
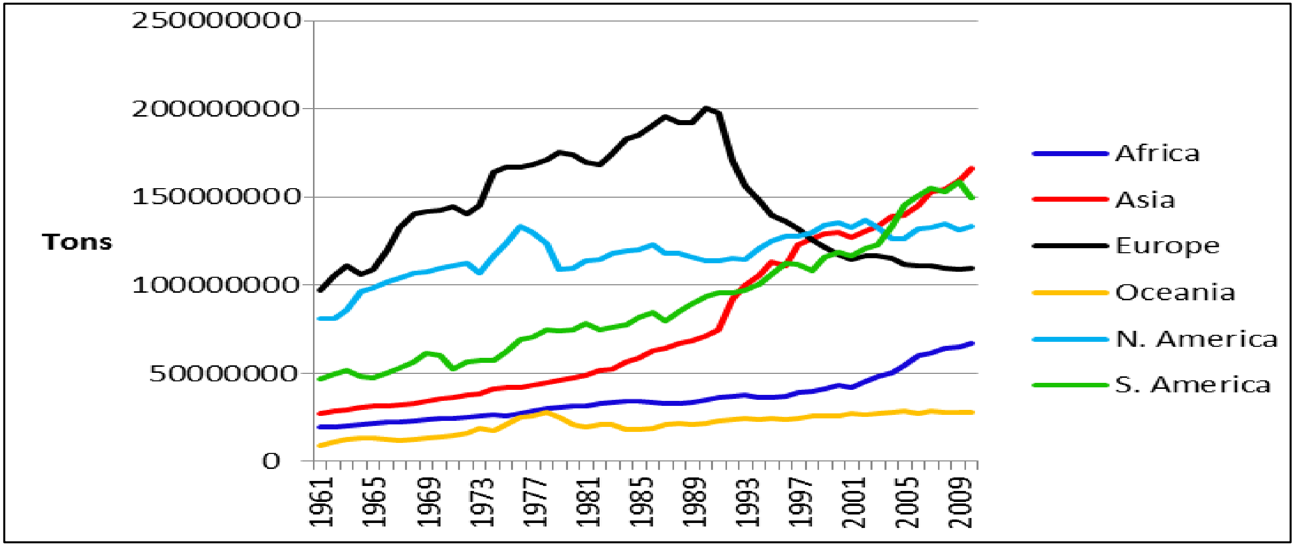
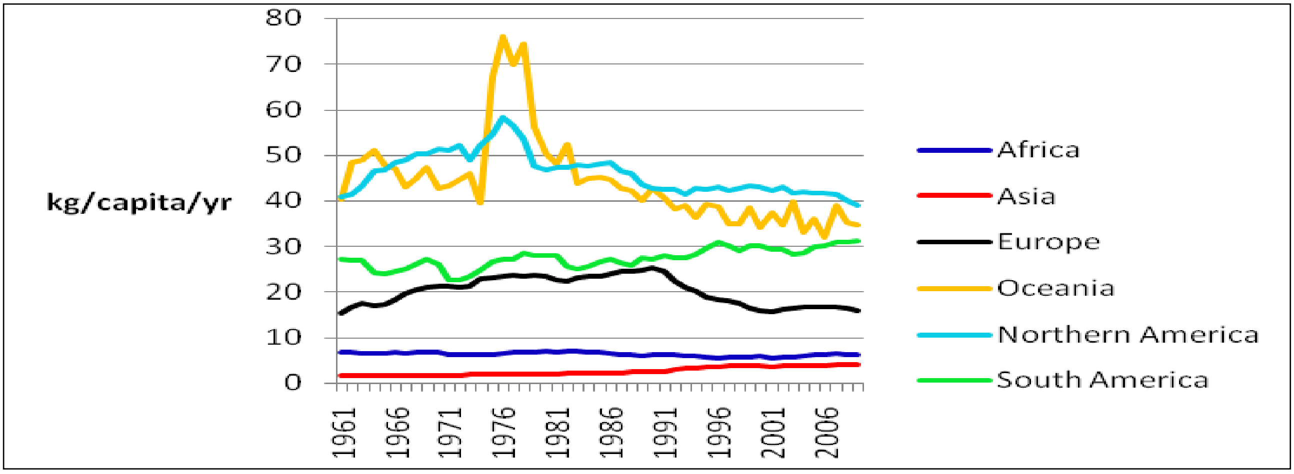


Figure 4 - 50-years trend for bovine meat production by region (FAOSTAT).



During the last forty years, bovine production change extremely. Asia (400%) and Africa (204%) had the steepest production growth during this period, followed by South America (176%) (Figure 4). When considering the consumption, in the last forty years Asia consumption has increased 162,5%, South America increased by 15%, Europe increased by 3,8%, whereas consumption in Oceania decreased by 13,9%, North America decreased by 4,6% and in Africa decreased by 5,9% (Figure 5).

Figure 5 - Global bovine meat supply (kg/capita/yr) 1961-2009 period (FAOSTAT).



In Europe in the last 20 years, bovine meat has been partly substituted by poultry and pork meat. Poultry consumption has increased more than four times while pork meat has increased about 75% (Figure 6).

Figure 6 - Europe bovine, pork, poultry meat supply (kg/capita/yr) 1961-2009 period (FAOSTAT).

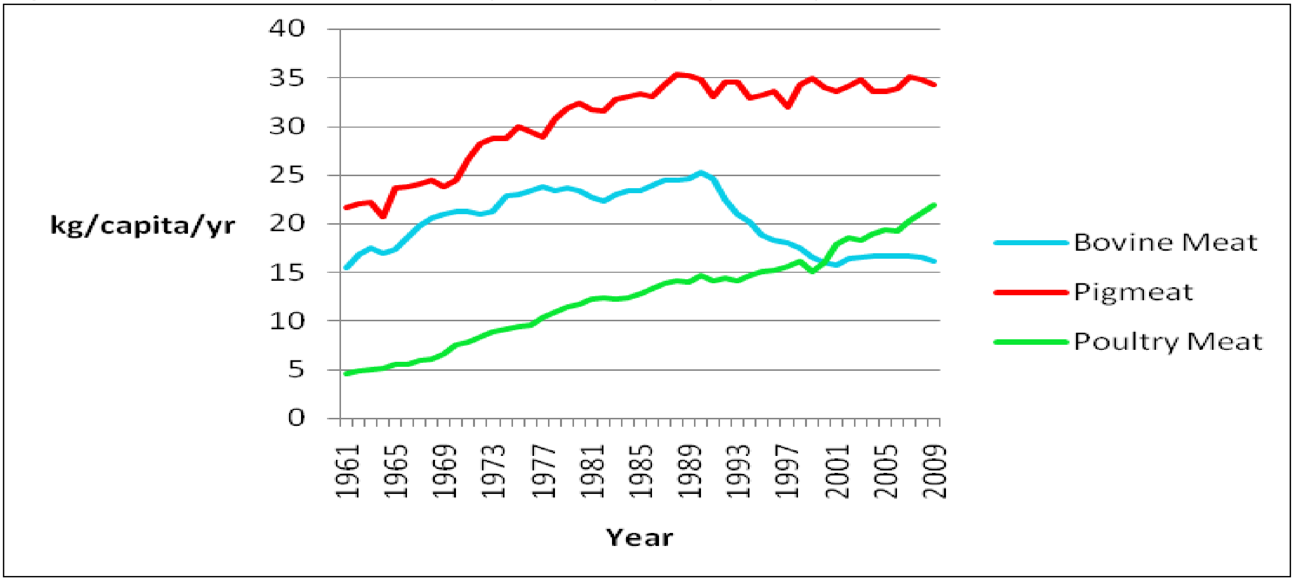
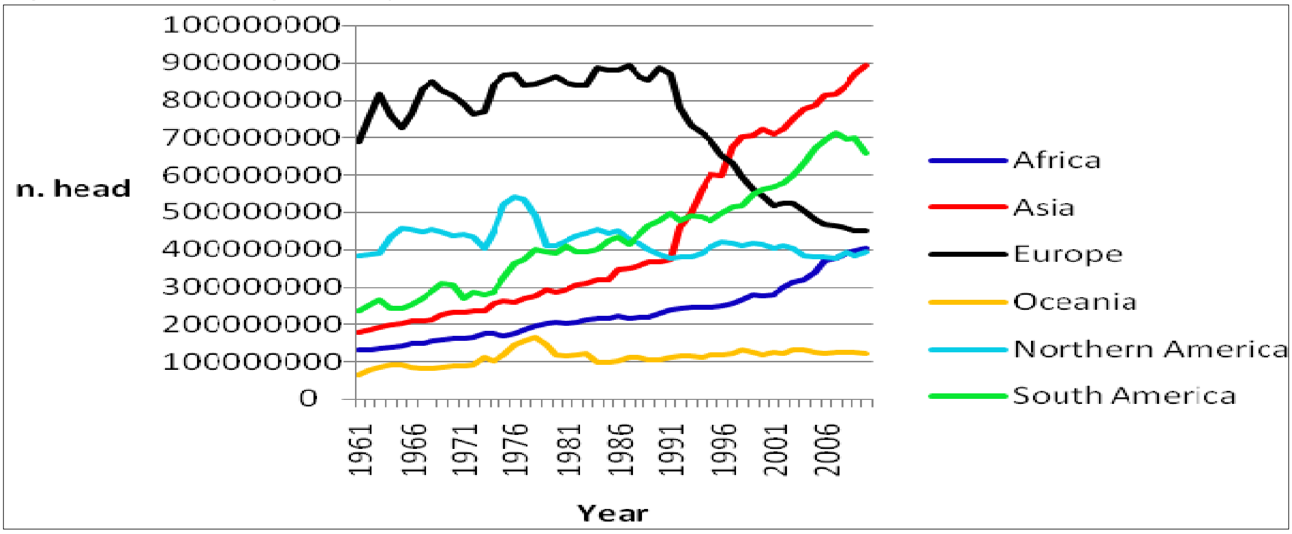


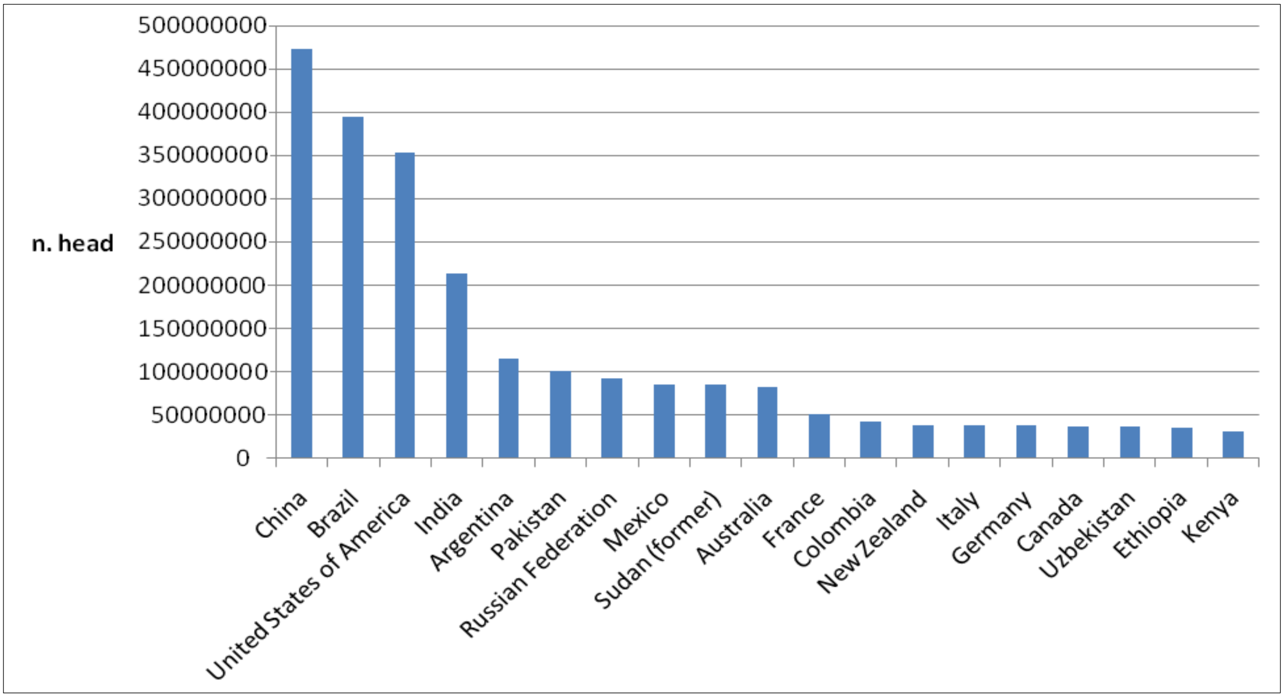
Figure 7 - World's regions 50-years trend for bovine meat production (FAOSTAT).



Western countries of all regions, Europe, North America and Oceania, have seen a major fall in bovine meat consumption, but Europe is the only continent to have an accentuated 25 years negative trend in both production (-49%) and consumption (-36%), since production in North America in the last 25 years saw a stable fluctuation.

In 2010, the five most productive countries were China, Brazil, United States of America, India and Argentina. The most productive European countries were France (11°), Italy (14°) and Germany (15°) (Figure 8).

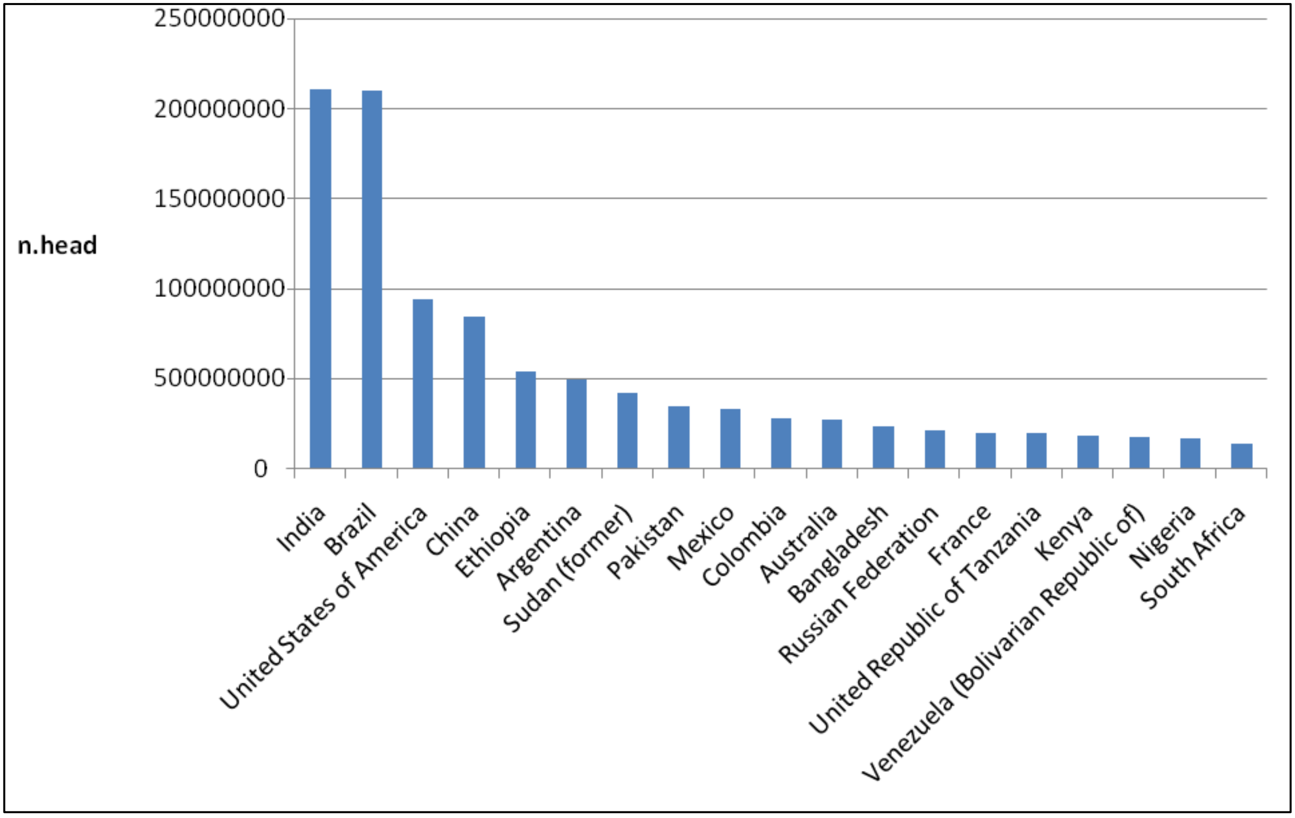
Figure 8 – Beef producing animals/slaughtered in 2010 in most productive countries (FAOSTAT).



In 2010, India, Brazil, United States of America, China and Ethiopia possessed the five biggest world bovine livestock (Figure 9). Together India and Brazil hold more than 400 million heads (Figure 8). The first European country by livestock is France, fourteenth world biggest livestock (Figure 8).

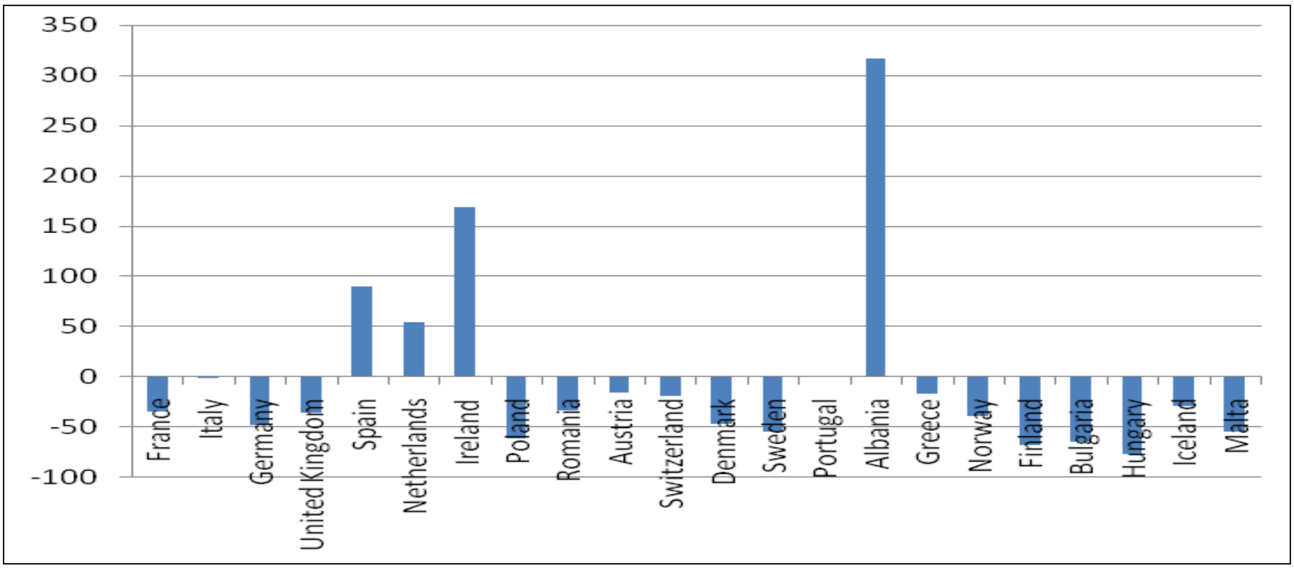
Portugal produces 52% of the national beef consumption, therefore needs to buy from other countries, with Spain representing the first importing market covering 53% of the deficit and Netherlands, which represents the 16% of the imports (*Observatório dos Mercados Agrícolas e das Importações Agro-Alimentares*, 2010). Beef production in Portugal more than doubled since 1961, passing from 46.000 tons to 96.000, while consumption tripled 6,20 kg/capita/year to 18,20 kg/capita/year (FAOSTAT).

Figure 9 - Cattle livestock by country (FAOSTAT).



Globally Europe has suffered an accentuated decline in bovine meat production, but there were some exceptions among European countries, which were associated with some increase in bovine production as Spain (89% increase), Ireland (168% increase), Netherlands (54% increase) and Albania (> 300% increase), (Figure 10).

Figure 10 - Cattle meat production variation (%) among European country in the 1961-2009 period (FAOSTAT).



2.3 The role of meat as the main source of protein in human diet

Red meat is an important dietary source of protein and its contribution to global health has been long established (McAfee et al., 2010). When compared to other sources of protein, such as beans or whole wheat, red meat protein has a higher digestibility (94%), and provides all essential amino acids (Williams, 2007). Meat, as a protein rich product, helps to reduce glycemic index thanks to its low carbohydrate content, which is considered beneficial with respect to overweight, the development of diabetes and cancer (Biesalski, 2005). The recommended protein daily intake is quite variable depending on age and physiologic state, as it is observable in Table 1 (DGAC, 2010b).

Table 1 - Recommended daily intake for protein depending on age and physiologic state (DGAC, 2010b)

Physiological state and age		Recommended daily intake for protein
Children	1 to3 years old	1.05 g/kg/day
	4 to 13 years old	0.95 g/kg/day
	14 to 18 years old	0.85 g/kg/day
Women	during pregnancy	1.1 g/kg/day
	during lactation	1.3 g/kg/day
Adult	average	0.80 g/kg/day

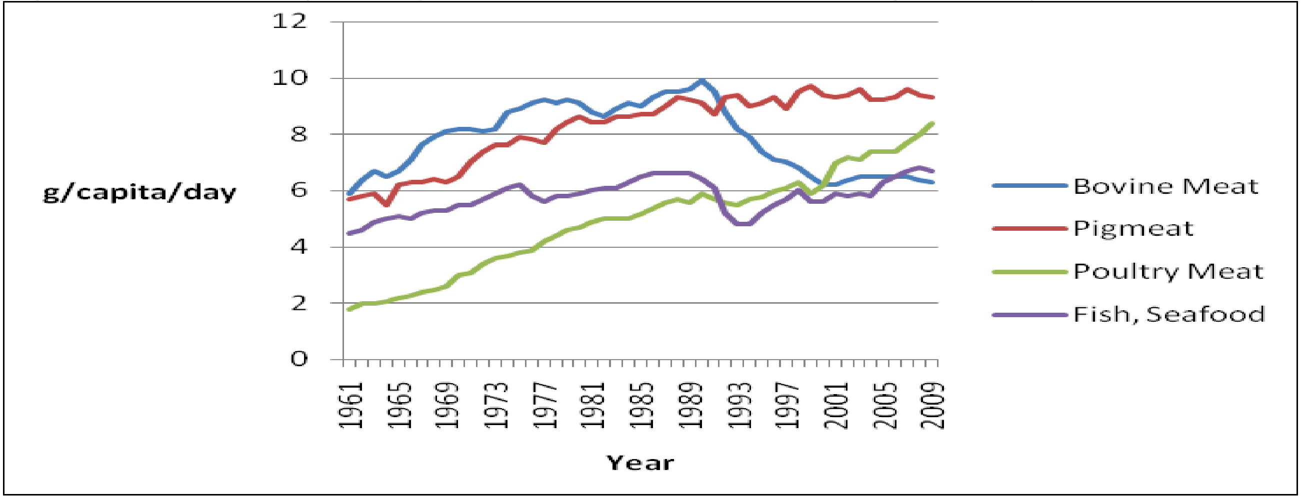
In developing countries, stunting is assumed to be related to improper animal protein intake in the diet (McNeill & Van Elswyk, 2012).

According to dietary recommendation, 0.8 g of protein per kg body weight per day , an 70 kg adult would require 56 grams of high quality protein daily. The recommended serving size (85 g) of lean meat or poultry contains about 25 grams of protein, while 1 cup of milk or yogurt contains 8 grams of protein. Cereals, grains, nuts, and vegetables contain about 2 grams of protein per serving (DGAC, 2010b). It has been argued that the decrease in stature during the agricultural transition in the Neolithic, was due to the reduced protein intake from animal food, which is considerably inferior to the Paleolithic levels (Ungar & Teaford, 2002). Similarly, a change in stature was observed in different stages of the Industrial Revolution, when prices of goods rose, booming urbanization cut off rural areas and a general impoverishment took place in vast number of people (Komlos, 1998). It is also curious to observe how slaves' stature did not decreased, as middle- and upper-class', while low-class' did (Komlos, 1998). The author supports the hypothesis that masters took care of their slaves because they were aware of how a well-nourished worker would contribute to production, whereas low-income families quite exclude the expensive meat from their meal except from special occurrences (Komlos, 1998).

Among animal protein sources, bovine meat has been in the past the major source of protein in human diet, but nowadays, pork, poultry meat and sea food are greater sources of protein to human diet, as it is demonstrated by FAOSTAT (Figure 11).

During growth and development protein needs are high, and consumption of animal products will provide both greater quantity and quality of protein than plant products (DGAC, 2010b), for this reason studying dietary patterns that includes red meat is important in global health (McAfee, et al., 2010).

Figure 11 Protein supply quantity from meat and seafood in Europe (g/capita/day; FAOSTAT)



2.4 The role of meat as animal fat source and health

Fats are a key factor in the maintenance of caloric balance and body weight. Specific fatty acids also serve as precursors for numerous biological pathways that influence inflammation, coagulation, and gene expression among other functions (DGAC, 2010a). Lean meat, after trimming external fat, as revealed by most recent analysis, is relatively low in fat (<7%) and have moderate cholesterol content (Williams, 2007).

2.5 Fatty acids in human nutrition

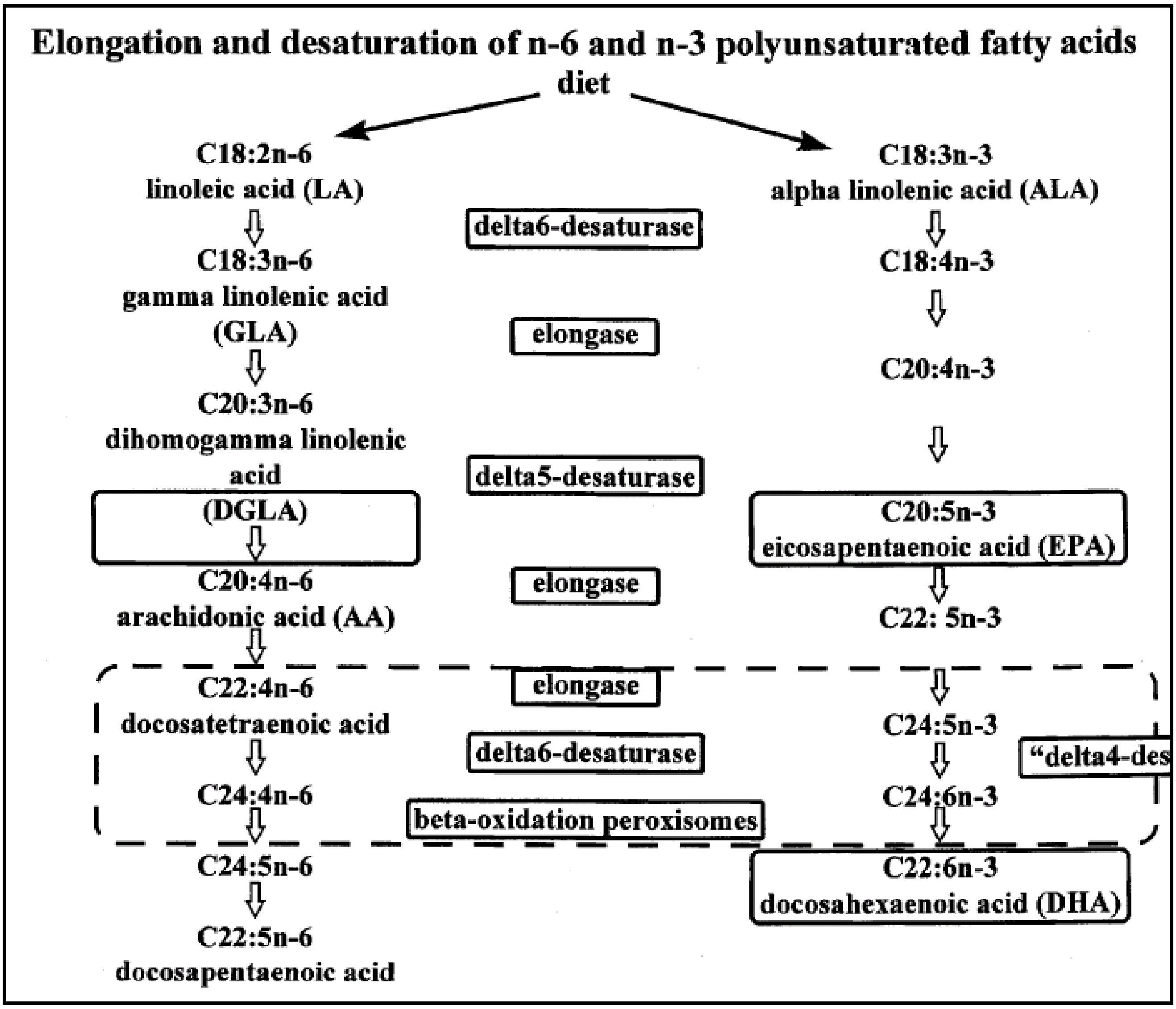
For many years, the intramuscular lipid composition of beef aroused considerable interest because of its implications in meat quality and human health. Dietary fats, or lipids, are a macronutrient class that includes many chemical compounds. Fatty acids, from both phospholipids and triacylglycerides, are of major nutritional importance. Cholesterol is also an important lipid, being present in animal foods only. Lipids are the most energetic nutrient, supplying more energy (9 kcal/g, approximately 37.66 kJ/g) than carbohydrates and protein. Lipids supply the essential fatty acids, as linoleic (LA) and alpha-linolenic (ALA) acids, lipid-soluble vitamins (vitamins A, D, E, K) and carotenoids (DGAC, 2010a). However, red meat was recognized as one of the main sources of saturated fat in the human diet (with a low PUFA/SFA ratio), which are associated to a number of health disorders (Luciano, 2009).

Fatty acids are heterogeneous lipids mostly found in cell membranes and fat depots. Their classification is based on their chain length, the number of double bonds, the position of the first double bond from the methyl end, and a *cis* versus *trans* configuration across a double bond. These structural heterogeneities among fatty acids are important determinants of the significant variation in biological effects of the different fatty acids. Fatty acid quantity and quality also vary by their source, with important differences between meat, fish, and plant sources, as well as natural versus synthetic sources (DGAC, 2010a).

Fatty acids typically have an even number of carbon atoms, in the range of 8–26, and can be separated in two classes, according with the presence of double bonds in the carbon chain. Those with only single bonds are referred to as saturated, whereas those with at least one C=C double bond are called unsaturated (Abayasekara & Wathes, 1999; Baghurst, 2001; Ruxton, Reed, Simpson, & Millington, 2004). The monounsaturated fatty acids (MUFA) have just a single double bound, while polyunsaturated fatty acids (PUFA) have two or more double bonds and are named according to the position of these bonds and the total chain length. For example, eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:6n-3) are both omega-3 or n-3PUFAs with five six double bonds, respectively.

There are two classes of essential fatty acids (EFA), n-6 and n-3 (Figure 12). The distinction between n-6 and n-3 PUFA relies on the location of the first double bond, counting from the methyl end of the fatty acid molecule (Ruxton, et al., 2004; Simopoulos, 2008). The n-6 and n-3 fatty acids are essential because humans like all mammals, cannot synthesize them and must obtain it from the diet (Simopoulos, 2008). A balanced intake of both n-6 and n-3 fatty acids is essential for health (Simopoulos, 1999).

Figure 12 - Elongation and desaturation of n-6 and n-3 polyunsaturated fatty acids (copied from Simopoulos, 2008)



There is competition between n-6 and n-3 fatty acids for the desaturation enzymes (Simopoulos, 2008). Desaturase and elongase are vital enzymes for the conversion of ALA to DHA and EPA. The Desaturase enzymes have a preference for ALA but the presence of high levels of plasma LA (caused by high n-6 PUFA intakes) can shift its actions towards the n-6 pathway (Ruxton, et al., 2004). There is increased evidence of beneficial effects from EFA supplementation during pregnancy, lactation, infancy and throughout life (Brenner, 2003). However, increased amounts of n-6 fatty acids are common in the Western diet (Givens, Kliem, & Gibbs, 2006; Simopoulos, 2008), consequently the eicosanoid metabolic products from arachidonic acid (AA), specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, are formed in larger quantities than those formed from n-3 PUFA, specifically EPA (Simopoulos, 1999). A diet rich in n-6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time (Simopoulos, 1999).

The n-3 PUFAs, especially long-chain EPA and DHA fatty acids, exert a strong positive influence on human health. At present, fish oil is the major source of n-3 PUFA (Arab-Tehrany et al., 2012). The n-3 PUFAs are considered a key element for an adequate development and functioning of the nervous system. Numerous studies have pointed out the relationship between n-3 PUFA and mental illnesses. (Fenton, Hibbeln, & Knable, 2000; Lin & Su, 2007; Mamalakis, Kiriakakis, Tsibinos, & Kafatos, 2004; Stoll, Locke, Marangell, & Severus, 1999; Su, Huang, Chiu, & Shen, 2003). In animal studies, n-3 PUFA have been shown to influence body composition and to reduce the accumulation of body fat, thereby affecting body weight homeostasis (Martinez-Victoria & Yago, 2012). In reproduction, alteration of the concentration and ratio of n-6 and n-3 PUFAs has been shown to affect number and size of ovarian follicles, the ovulation rate, progesterone production by the *corpus luteum*, the timing of luteolysis and gestational length (Abayasekara & Wathes, 1999).

2.5.1 Saturated fat and health

Saturated fatty acids (SFA) are associated with a poor lipid/lipoprotein profile and increased risk of cardiovascular disease (CVD) (Hu et al., 1999; Hunter, Zhang, & Kris-Etherton, 2010; Khosla & Hayes, 1994; Willett, 2012). However, different saturated fatty acids can have different effects on plasma lipid and lipoprotein concentrations. The myristic acid is considered as the saturated fatty acid which increases CVD risk (Loison, Mendy, Sérougne, & Lutton, 2007), while has been shown the neutrality of stearic acid, which has been found the most suitable candidate to replace *trans*-fatty acids (TFA) in food (Hunter, Zhang, & Kris-Etherton, 2010). Lin et al. (2004) in their prospective cohort study provide little support for an association between dietary fat and colorectal cancer risk, but intake of fried foods, which means high levels of SFAs, and/or other factors related to their intake may be associated with colorectal cancer development.

2.5.2 Mono- and polyunsaturated fat and health

Monounsaturated fats (MUFA) have one double-bonded in the carbon chain. Eating monounsaturated are believed to lower cholesterol levels and reduce the risk of heart disease and stroke (Masala et al., 2007). Polyunsaturated fats are not synthesized by human body and for that are also referred to as essential fatty acids. They can be divided into two categories: n-6 fatty acids and n-3 fatty acids (Ruxton, et al., 2004). N-3 fatty acids play a role in normal growth and development and proper brain function. They also reduce widespread inflammation and decrease the risk of chronic diseases, such as heart disease, arthritis and cancer (Kazemian, et al., 2012). Like n-3 fatty acids, n-6 fatty acids play a role in growth, development and brain function, but they also regulate metabolism, stimulate hair growth and keep the reproductive system healthy. Unlike n-3, some n-6 fatty acids can promote inflammation. In order to follow healthy is to consume a balance diet of both types of fatty acids (Calder, 2012).

2.5.2.1 Cardiovascular health and disease

n-3 PUFAs competitively displace n-6 PUFAs for incorporation into cell membranes (Ander, Dupasquier, Prociuk, & Pierce, 2003; Shaikh & Edidin, 2006). AA is potentially one of the most important of the n-6 PUFAs and upon cell activation, is released from the cell membrane and is transformed into powerful cellular inflammatory/prothrombotic mediators such as thromboxanes, prostaglandins, and leukotrienes leading to a plethora of cellular effects. n-3 PUFAs have specific regulatory pathways such as insulin sensitization, anti-inflammatory, electrical remodeling of cardiomyocytes, hemodynamic effects and reverse myocardial remodeling (anti-fibrotic effect) (Kazemian, et al., 2012).

Over the past few decades, there was collected a considerable amount of data supporting the benefits of n-3 PUFAs on overall cardiovascular health. Reasons for the potential cardiovascular benefits of n-3 PUFAs are multifactorial and not completely understood (Jarreau, Lee, Lavie, & Ventura, 2012).

Epidemiological and clinical studies suggest that consumption of n-3 PUFA contributes to the reduction of cardiovascular mortality through different mechanisms including modulation of cellular metabolic functions, gene expression and beneficial effects on lipid profile or blood pressure (Cabo, Alonso, & Mata, 2012).

Clinical studies suggest that EPA and DHA from fish oil not only prevent the incidence of detrimental cardiovascular events, but also lower the cardiovascular mortality rate (Abayasekara & Wathes, 1999; Balakumar & Taneja, 2012). It has also been shown that the use of very long chain n-3 polyunsaturated fatty acids (n-3LCPUFA) could potentially benefit the Metabolic Syndrome

(MS), a disease characterized by accumulation of CVD risk factors, by exerting an hypotriglyceridemic effect in metabolic syndrome (MS) patients (Lopez-Huertas, 2012). The triglyceride lowering may produce further benefits by reducing pro-atherogenic LDL levels and also perhaps by ameliorating the inflammatory process associated with MS (Lopez-Huertas, 2012).(Ann Chao, 2005)

2.5.2.2 Inflammatory diseases

Inflammation plays a key role in many common conditions and diseases. Fatty acids can influence inflammation through a variety of mechanisms acting from the membrane to the nucleus. They act through cell surface and intracellular receptors that control inflammatory cell signalling and gene expression patterns (Calder, 2012). Modifications of membrane fatty acid composition can modify membrane fluidity, lipid raft formation and cell signaling leading to altered gene expression and can alter the pattern of lipid and peptide mediator production (Calder, 2012). Cells involved in the inflammatory response usually contain a relatively high proportion of AA in their membrane phospholipids (Calder, 2012). Eicosanoids produced from AA have well-recognised roles in inflammation. Oral administration of the marine n-3 fatty acids EPA and DHA increases the contents of EPA and DHA in the membranes of cells involved in inflammation by decreasing the amount of AA present (Calder, 2012). This is accompanied by a decrease in the amount of AA present. EPA is a substrate for eicosanoid synthesis and these are often less potent than those produced from AA (Calder, 2012). EPA gives rise to E-series resolvins and DHA gives rise to D-series resolvins and protectins. Resolvins and protectins are anti-inflammatory and show remarkable potency in resolving inflammation-related diseases (Xu et al., 2010). Thus, the exposure of inflammatory cells to different types of fatty acids can influence their function and so has the potential to modify inflammatory processes (Calder, 2012).

N-3 LCPUFA, such as EPA and DHA can also reduce the development of allergic disease by affecting both the innate and adaptive immune system involved in the initiation and persistence of allergic disease (Van den Elsen, Garssen, & Willemsen, 2012).

Although the inflammation may afflict different body compartments, one common characteristic of these conditions and diseases is excessive or inappropriate production of inflammatory mediators, including eicosanoids and cytokines (Calder, 2012). The roles of n−6 and n−3 PUFAs in shaping and regulating inflammatory processes and responses suggest that the balance of these fatty acids might be important in determining the development and severity of inflammatory diseases (Simopoulos, 2008). For example, a high intake of n−6 PUFAs, especially AA, could contribute to inflammatory processes and so could predispose to or exacerbate inflammatory diseases.

Conversely, the recognition that the long-chain n−3 PUFAs have anti-inflammatory actions suggests that increasing their intake by patients with inflammatory diseases, for example, through dietary supplementation, may be a clinical benefit (Calder, 2006).

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of the joints and bones. The n-6 PUFA and AA are the precursor of inflammatory eicosanoids which are involved in RA (Miles & Calder, 2012). Some therapies used in RA target AA metabolism. Marine n-3 PUFAs (EPA and DHA) found in oily fish and fish oils decrease the AA content of cells involved in immune responses and decrease the production of inflammatory eicosanoids from AA (Miles & Calder, 2012). EPA and DHA give rise to eicosanoid mediators that are less inflammatory than those produced from AA and both give rise to resolvins that are anti-inflammatory and inflammation resolving, although little is known about these latter mediators in RA (Miles & Calder, 2012). Despite their well-known anti-inflammatory actions, the clinical usefulness of n-3 PUFA in inflammatory bowel disease is still controversial (Cabre, Manosa, & Gassull, 2012).

2.5.2.3 Neuronal function and diseases

n-3 PUFA may be key for an adequate development and functioning of the nervous system. This notion is further supported by the high concentrations of n-3 LCPUFA such as docosahexaenoic acid (DHA) in the cerebral cortex and the retina, a brain-derived neuronal network specialized for photo-signal transduction and processing (Mohajeri & Winwood, 2012; Uauy & Dangour, 2006).

Docosahexaenoic acid (DHA) is the major polyunsaturated fatty acid (PUFA) in the brain and a structural component of neuronal membranes (Innis, 2007; Mohajeri & Winwood, 2012; Sparks, Martin, Gross, & Hunsaker, 2000; Tanaka, Farooqui, Siddiqi, Alhomida, & Ong, 2012; Uauy & Dangour, 2006).

n-3 PUFA deprivation may affect brain development at multiple levels, from membrane biogenesis, through gene expression, protection against oxidative stress, and altered neurotransmission (Innis, 2007).

Evidence suggests that dietary DHA supplementation may have beneficial effects on neurotransmission (Tanaka, et al., 2012). Several studies, but not all, reported improvements of the offspring in some neurodevelopmental tests as a result of DHA supplementation during gestation, or, at least, positive relationships between maternal or cord serum DHA percentages and cognitive skills in young children (Larque, Gil-Sanchez, Prieto-Sanchez, & Koletzko, 2012), while severe DHA deprivation leads to mental retardation (Hibbeln et al., 2007). Maternal seafood consumption in pregnancy provide an healthy fetal neurodevelopment rather than harm (Hibbeln, et al., 2007).

Numerous studies have pointed out the relationship between n-3 PUFA and mental illnesses. (Fenton, et al., 2000; P. Y. Lin & Su, 2007; Mamalakis, et al., 2004; Stoll, et al., 1999; Su, et al., 2003). Neurocognitive disorders such as attention-deficit hyperactivity disorder (ADHD), dyslexia, dyspraxia and autism spectrum disorders are often associated with a relative lack of n-3 fatty acids (Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010). n-3 PUFA indeed are considered therapeutically against schizophrenia (Fenton, et al., 2000), bipolar disorder (P. Y. Lin & Su, 2007; Stoll, et al., 1999) and depression (Mamalakis, et al., 2004), given that patients suffering those conditions were lacking these fatty acids within the body.

Children and adolescents with ADHD have been shown to have significantly lower plasma and blood concentrations of PUFA and, in particular, lower levels of n-3PUFA (Gillies, Sinn, Lad, Leach, & Ross, 2012; Schuchardt, et al., 2010).

2.5.2.4 Cancer risk and protection

Consumption of meat, rather than fatty acids intake, is considered to increased cancer risk, particularly those affecting large intestine, prostate and breast (Chao, 2005; Biesalski, 2005; Broitman, 1986; Gerber, 2012; Giovannucci et al., 1993; Kuriki & Tajima, 2006; Kushi & Giovannucci, 2002; Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004; Lin, et al., 2004; McAfee, et al., 2010; Simopoulos, 2002; Stacewicz-Sapuntzakis, Borthakur, Burns, & Bowen, 2008; Sun-Il et al., 2008; Wang & Jiang, 2012). Recommendations to decrease red meat intake, particularly processed meat or beef intake, may decrease the risk of colorectal cancer and prostate cancer (Kushi & Giovannucci, 2002). Evidence showed that prolonged high consumption of red and processed meat may increase the risk of cancer in the distal portion of the large intestine (Chao, 2005).

Several studies reveal that low-fat diets high in n-3 fatty acids reduce cancer risk, while high-fat diets rich in n-6 fatty acids may promote cancer cells growth (Larsson, et al., 2004; Stacewicz-Sapuntzakis, et al., 2008). In the past, the strong correlation between consumption of fat and mortality rate from prostate cancer has raised the hypothesis that dietary fat increases the risk of this malignancy (Giovannucci, et al., 1993), but epidemiologic studies have been inconsistent (Gann et al., 1994). Studies on long-term consumption of large amounts of linoleic acid associated with increased cancer risk revealed to be inconsistent (Zock & Katan, 1998).

Lin et al. (2004) in their prospective cohort study provide little support for an association between dietary fat and colorectal cancer risk, but intake of fried foods, which means high levels of SFAs, and/or other factors related to their intake may be associated with colorectal cancer development.

Experimental models showed consistently a modulation of carcinogenesis by n-3 PUFA and that fish intake is often described as part of a beneficial dietary pattern (Gerber, 2012).

2.5.3 Cholesterol and health

Cholesterol is a steroid-based alcohol with a hydrocarbon side-chain. Cholesterol has both hydrophilic and hydrophobic properties; hydrophilic property is due to its hydroxyl end, while its hydrophobic properties are due to its hydrocarbon side-chain (DGAC, 2010a). It is an essential structural component of mammalian cell membranes and is required to establish the proper membrane permeability, fluidity and a precursor of bioactive lipids such as bile acids and steroid hormones (Ohlsson, 2010).

Cholesterol is carried throughout the body by specific lipoproteins, the very low-density lipoprotein (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). VLDL and LDL are responsible for carrying dietary and biosynthesized cholesterol to the tissues, while HDL is responsible for carrying it back to the liver. (Amarenco, Labreuche, & Touboul, 2008; Burillo et al., 2009; Chirovsky, Fedirko, Cui, Sazonov, & Barter, 2009; Elsøe et al., 2012; Loison, et al., 2007; Ohlsson, 2010; Olson, 1998; Savel et al., 2012; Willett, 2012; Zoratti, 1998).

Cholesterol is an essential molecule to humans, due to structural and functional reasons. However, abnormally high levels of cholesterol (from the diet or atypical liver production) and abnormal proportions of low-density lipoproteins (LDL, also known as the bad cholesterol) and high-density lipoproteins (HDL, also known as the good cholesterol) are associated with cardiovascular diseases (Muchenje, et al., 2009).

Plasma cholesterol levels are influenced by the cholesterol content of diet and by the fatty acid composition of the diet. Some fatty acids of the SFA group, namely lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0) have been shown to promote endogenous (liver) cholesterol production and thereby increase serum cholesterol level (Muchenje, et al., 2009). Among them, myristic acid was considered as the SFA responsible for the highest increase in plasma total cholesterol and in LDL levels, in both human subjects and animals (Loison, et al., 2007). Trans fatty acids (TFA) present in the diet can also affect cholesterolemia. LDL and cholesterol synthesis appears to increase as a result of high dietary TFA intake, while high-density lipoprotein-cholesterol has been shown to decrease (French, Sundram, & Clandinin, 2002; Hunter, 2006).

LDL and LDL oxidation is thought to play a key role in the development of atherosclerosis (Elsøe, et al., 2012; Hajjar & Haberland, 1997; Leonarduzzi, Sottero, & Poli, 2002; Pietras, Pieszka, Bederska-Łojewska, & Orczewska-Dudek, 2012). Cholesterol pathological levels have also been associated with neuropathies such as Parkinson’s and Alzheimer’s disease (Hu, 2010; Sparks, et al., 2000). Heart disease may be the forerunner to the Alzheimer's disease (AD), and cholesterol may be involved in the production and accumulation of b-amyloid protein in the brains of AD patients (Sparks, et al., 2000). Hu (2010) reviewed associations of serum cholesterol with Parkinson's disease, but to date the risk associations between the two remain inconsistent.

Clear cholesterol intake recommendations are not easy to establish, as there is no precise basis for selecting a target level, however, the American Heart Association (AHA) suggest 300 mg/d as maximum daily intake (Krauss et al., 2000).

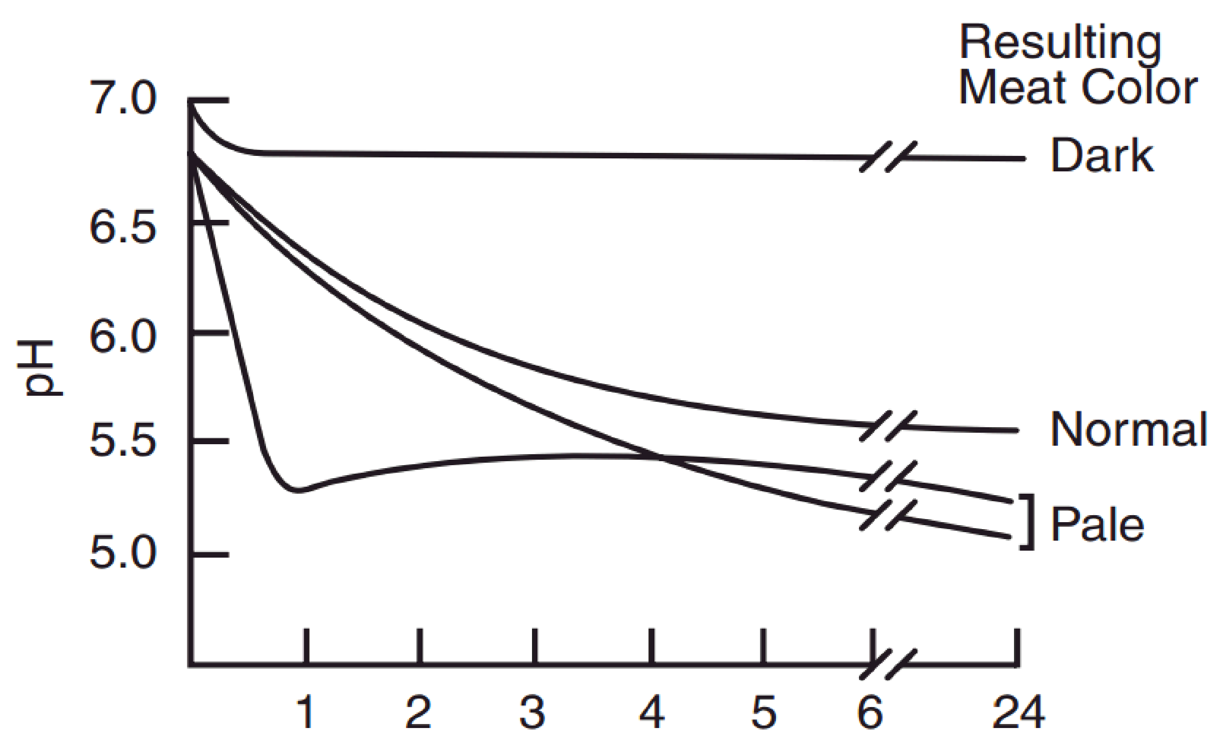
Chizzolini et al. (1999) measured the average content of cholesterol in beef that is 60 mg in100 g, while from Dinh et al. (2011) estimates it ranges between 36 and 58 mg in100 g, averaging 48,2 mg in 100 g. Is to be pointed out that cholesterol content differs according to muscle with oxidative and glycolytic fiber (Dinh et al., 2011). Oxidative muscles are richer in phospholipids this means high cholesterol content (Chizzolini et al. 1999).

2.6 Importance of pH in meat quality evaluation

The pH level of meat can affect the shelf-life of the meat, its color, its tenderness, and the eating quality. The pH is useful to predict meat tenderness. The normal or ultimate pH level of meat should be between 5.3 and 5.7. For example, an ultimate pH within this values promotes a bright red attractive color in beef, whereas, lower pH (below 5.3) causes pale, soft, weepy meat, which is rare in beef but very common in pork meat. After death, glycogen consumption leads to lactic acid accumulation in meat, which is responsible for the pH decline from 7.0 to about 5.7 (Figure 13) during normal *rigor mortis* development (Miller, 2007). The normal pH decline of meat during rigor mortis can be altered by low levels of glycogen at slaughter, causing meat to retain a high pH (Miller, 2007). At high pH (above 5.9) the meat will become darker and quality begins to decline. During the handling, transport and pre-slaughter period, muscle glycogen is consumed rapidly, after slaughter there is little lactic acid production, the main responsible of pH decline, which results in DFD (dark, firm and dry) meat (Muchenje et al., 2009). 'Dark cutting" meat spoils quickly, is sticky, unacceptable to consumers and unsuitable for ageing. This DFD meat is of inferior quality as the less pronounced taste and the dark color is less acceptable to the consumer and it has a shorter shelf-life, due to the abnormally high pH value, which promote bacterial growth (Muchenje, et al., 2009). Stress, prior to slaughter, is said to be one of the most important influences to ultimate meat tenderness by affecting water-holding capacity of meat. Water-holding capacity (WHC) is defined as the ability of meat to retain its water during application of external forces, such as cutting, heating, grinding or pressing (Muchenje, et al., 2009). Low pH and high temperature during the onset of rigor promote exudative meat as myofilaments at this condition are more loose (Muchenje, et al., 2009). To understand better the importance of the pH and temperature profile at this stage, it affects also myoglobin and enzymes stability, which are meat color crucial factors in early postmortem (Hwang & Thompson, 2003).

Production system and feeding can influence the ultimate pH as well. Grass-fed animals have darker meat than have grain-fed ones. This is caused by the higher pHu values found in beef from grass-fed compared to grain-fed cattle. It has been argued that grass-fed steers may be more susceptible to pre-slaughter stress and associated pre-slaughter glycogen depletion than are grain-fed steers, as the latter would be better accustomed to penning and handling (Muchenje, et al., 2009).

Figure 13 - Postmortem pH decline and the resulting meat color, copied from Miller (2007).



2.7 Meat characteristics in consumer choice

In the last decades, meat production has been transformed, going through a process of increasing productivity and efficiency of production that raise several issues, such as animal welfare, food safety and environment safety. All these transformations have gained strength, especially, due to the outbreaks of some important diseases, like the foot and mouth disease and bovine spongiform encephalopathy (BSE) (Angulo & Gil, 2007; Euclides Filho, 2004). Along with meat health and environmental concerns, several factors influence the changes in consumer demand including: demographic characteristics of population (sex, age, ethnicity, etc.), convenience, type of distribution from little butcher’s shop to big supermarket and price. Consumer preferences for meat, from a sensory stand point, are influenced by appearance, tenderness, flavor, and juiciness (Resurreccion, 2004).

2.7.1 Color at display

Meat color is a very important quality parameter for both industry and consumer (Delmore, 2009; Gray, et al., 1996; Smith, et al., 1996). Meat cuts that have lost their fresh appearance are discriminated by consumers (Gray, et al., 1996). Color is a very important shelf-life spoiler, as consumer notices easily the dark appearance of meat and would not purchase it (Smith, Morgan, Sofos, & Tatum, 1996). Shelf-life is the time meat takes to become unpalatable or unfit for human consumption because of surface oxidation or the growth of spoilage organisms (Delmore, 2009; Gray, et al., 1996; Smith, et al., 1996). Meat shelf-life is evaluated by color, odor and flavor (Delmore, 2009). Although a change in color doesn’t mean that meat is unpalatable or unfit to be consume or, worse, unsafe, yet color and odor have the tendency to spoil together over time (Wood et al., 2004). Grass-fed animals have darker meat than have grain-fed ones.

It is often referred to as case life rather than shelf-life, as color play major role, that is the length of time meat can be displayed under refrigeration before a color change occurs (Delmore, 2009; Smith et al., 1996). Therefore, meat color stability is a great concern in meat industry as affects strongly consumer’s purchasing will (Renerre, 2000). Vitamin E supplement in bovine feeding has been shown to increase shelf-life in retail display (Arnold et al., 1992; Yang, Lanari, Brewster, & Tume, 2002). To meet consumer expectations, meat should have an attractive bright-red color compatible with long shelf-life and good eating quality (Kerry, Buckley, & Galvin, 2000).

2.7.2 Vitamin E

Vitamin E represents the major lipid-soluble antioxidant found in animal cells (Gray, Gomaa, & Buckley, 1996; Lucarini & Pedulli, 2007). Vitamin E is the generic name for various tocopherols and tocotrienols homologous with α-tocopherol being the most active (Lucarini & Pedulli, 2007). The main function of vitamin E is to prevent the peroxidation of membrane phospholipids, and to avoid cell membrane damage through its antioxidant action (Cameron Faustman & Wang, 2000; Gray, et al., 1996; Lucarini & Pedulli, 2007; Schaefer, 2007; Smith, et al., 1996; Yang, Lanari, Brewster, & Tume, 2002). Due to its lipophilic nature, vitamin E is found mostly in cell membranes (Cameron Faustman & Wang, 2000; Kerry, Buckley, & Galvin, 2000; Lucarini & Pedulli, 2007; Michelle Renerre, 2000; Smith, et al., 1996) Oxidation of red oxymyoglobin to brown metmyoglobin is responsible for the color change, while the propensity of unsaturated fatty acids to oxidize leads to the development of rancidity over time (Lucarini & Pedulli, 2007; Schaefer, 2007; Smith et al., 1996; Wood et al., 2004). These biochemical reactions generally proceed in parallel, indeed, it has been shown that lipid oxidation products can promote pigment oxidation and *vice versa* (Wood et al., 2004). The ability of unsaturated fatty acids, especially those with more than two double bonds, to rapidly oxidize, is important in regulating the shelf-life of meat (rancidity and color deterioration) (Wood et al., 2004). Radical species, such as peroxides produced during lipid oxidation, act directly to promote pigment oxidation (Gray, et al., 1996). Others factors such as stress, postmortem pH, carcass temperature and slaughter techniques (i.e. electrical stimulation) can induce oxidation (Gray, et al., 1996). Vitamin E prevents both lipid and myoglobin from being oxidized (Lucarini & Pedulli, 2007; Smith et al., 1996; Wood et al., 2004), being for that reason of prime importance to the preservation of meat red cherry color and meat nutritional quality.

Two classes of antioxidants can be found in animals: enzymatic and non-enzymatic. Enzymatic antioxidants continue to exert their function post-mortem though their effectiveness diminishes with increasing time post-mortem (Monahan, 2000). Non-enzymatic antioxidants, such as Vitamin C, Vitamin E, β-Carotene and other reducing peptides exert their functions at their own site individually, remaining effective longer in postmortem conditions (Niki, Noguchi, Tsuchihashi, & Gotoh, 1995). A single molecule of Vitamin E is enough to protect thousands of phospholips (Pryor, 1982). Vitamin C protects against both lipid oxidation and oxyhemoglobin oxidation (Cameron Faustman & Wang, 2000).

2.7.3 Tenderness, flavor and juiciness at consume

The importance of flavor, tenderness, and juiciness traits to the consumer in the purchasing-decision process is widely recognized (Huff Lonergan, Zhang, & Lonergan, 2010, Koohmaraie, Wheeler, & Shackelford, 1995, Resurreccion, 2004). Immediately after slaughter meat is tender (low shear force) (Koohmaraie et al., 1995). However, because of the muscle shortening which accompanies *rigor mortis* development, meat toughens during the first 12 to 24 hours after death (Koohmaraie et al., 1995). Simultaneously, an opposite phenomenon (i.e., tenderization) also begins either at slaughter or shortly after slaughter (Koohmaraie et al., 1995). Postmortem proteolysis degradation of a few key structural proteins by endogenous enzymes is responsible for postmortem tenderization (Koohmaraie et al., 1995).

Among the proteolytic systems endogenous to skeletal muscle, the only enzyme system involved in meat tenderization is the calpain (calcium-dependent) proteolytic system (Koohmaraie et al., 1995). To improve tenderness beef should be aged at least 14 days, lamb should be aged for 10 days and pork for 5 days (Koohmaraie et al., 1995). Stress, prior to slaughter, is said to be one of the most important influences on pHu and ultimate meat tenderness. As it was explain above, it may result from transportation, rough handling, extreme temperatures, or anything that causes the animal to draw on its glycogen reserves before slaughter (Muchenje, et al., 2009). Protein degradation and protein oxidation have been identified as processes that modify proteins as well as the tenderness of meat (Huff Lonergan, Zhang, & Lonergan, 2010).

Flavor is a cooking-derived meat quality (Calkins and Hodgen, 2007, Williamson, 2012). Sulfurous and carbonyl compounds liberated from the Maillard reaction, or non-enzymatic browning, are responsible for meat flavor (Calkins and Hodgen, 2007). In general, amino compounds condense with the carbonyl group of a reducing sugar in the presence of heat (Calkins and Hodgen, 2007, Williamson, 2012). This produces gylcosylamine which is rearranged and dehydrated to form furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds. All of these compounds contribute to flavor (Calkins and Hodgen, 2007, Williamson, 2012). As the reaction progresses, the intermediates can react with other amines, amino acids, aldehydes, hydrogen sulfide, and ammonia through the Amadori rearrangement, Strecker degradation, and Schiff bases pathways, and all these molecules contribute to meat flavor (Calkins and Hodgen, 2007).

Juiciness refers to the liquid or moisture liberated during eating. As flavor, depends also on consumer preference and on cooking (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003).

It has however been shown that the correlation between cooking loss and the initial juiciness depends on the raw meat quality, especially on the pH, as the class with the low pH acts differently from the other classes (Aaslyng, et al., 2003; Toscas, Shaw, & Beilken, 1999). It is also known from previous studies that the pH, due to its effect on electrostatic repulsion, affects the distance between the myofilaments and, moreover, it has recently been shown that the distance between the myofilaments influences the distribution of water and how tightly the water is bound in the meat (Toscas, et al., 1999). If the liquid lost from the meat during cooking came from different populations of water, and the water assessed as juiciness early in the chewing circle only came from the loosely bound water, this could explain why the pH influences the correlation between cooking loss and juiciness (Toscas, et al., 1999).

3. Materials and methods

3.1 Animals and samples

Meat samples were obtained from carcasses of pure bred Holstein young bulls (aged between 8 and 12 months old)) from Dutch (n=10), Spanish (n=10) and Portuguese (n=10) production. Young bulls from crossbred beef background with similar age and raised in Portugal were also included in the study (n=10).

Beef used in this study represented the regular bovine meat commercialized in Portugal by Pingo Doce and was collected in June of 2012. Carcasses arrived at the butchery with different periods between slaughter and sampling, 4 days for Dutch carcasses, 3 days for Spanish carcasses and 2 days for Portuguese carcasses. Meat samples were collected from carcasses in the butchery at the first day of their arrival, two meat samples from each carcass were collected from the *longissimus lumborum* muscle at the L1-L3 location. The two meat samples were collected with different thickness (1 sample with 3 cm, and the other with 1 cm). The 3 cm thickness sample was used for physic-chemical analysis (color assessment, pH and Warner Bratzler shear force (WBSF), while the 1 cm thickness sample was used to the biochemical evaluation.

Samples were treated in a differentiated manner according to the analysis to be performed. The 3 cm thickness sample was just trimmed of major connective and adipose tissues in the muscle margins. On the other hand, the 1 cm thickness sample was trimmed of major connective and adipose tissues present in the muscle tissue, and then blended in a food processor (Moulinex, France).

The blended meat was then divided in two portions: one of the portions was weighted and stored frozen -20°C in a 50 ml Container (Greiner, Germany). The second portion was *vacuum* packaged, frozen and stored at -18 ºC until the laboratory analysis.

3.2 Beef color measurements, pH determination and Warner Bratzler shear force quantification

Beef samples with 3 cm thickness were used for and in the following sequence:

1) Beef color measurements were carried out with a Minolta CR 300 colorimeter (Konica Minolta Holdings Inc., Tokio, Japan) with a C iluminant and a 2º standard observer in the CIELAB space, after 1 hour of blooming to allow oxygenation. The color coordinates derived directly from the colorimeter were lightness (L*), redness (a*), yellowness (b*).

Chroma (C*) and hue (h*) were calculated from a*and b* values according to the following the equations (Hue = $\tan^{-1}$ (b*/a*); Chroma= $(a^{*2} + b^{*2})^{1/2}$);

2) The beef pH was measured with a HI 99163 portable pH-meter (Hanna Instruments, USA). The pH was measured three times in each sample, and the value expressed was the mean of the three determinations;

3) The samples were grilled until it reached 70 ºC of internal temperature. Grill cooking was conducted with a Modular 65/70 FTES electric griddle (Modular System Ltd., Italy) preheated at a temperature of 250 ºC. The temperature was controlled with a needle thermocouple probe, which was inserted horizontally at the midpoint of the steak's width. The steaks for WBSF were chilled until reached room temperature. Each sample provided a minimum of eight strips with a 1 cm^2 cross section. The cores were removed parallel to the muscle fibre orientation and were sheared perpendicular to the longitudinal orientation of the muscle fibres, using a TA-TX Plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a Warner-Bratzler shear blade. The beef sample resistance to shearing was recorded in a force-deformation plot. The maximum shear force in kg corresponded to the highest peak of the curve.

3.3 Analytical methods

3.3.1 Lyophilization

Meat stored frozen in the 50 ml container was submitted to lyophilization (−60 °C and 2.0 h Pa) until constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, West Sussex, UK). Lyophilized muscle samples were then ground and homogenized using a home-style coffee grinder and maintained desiccated at room temperature, and analyzed within two months.

3.3.2 Fatty acid analysis

Fatty acid methyl esters were prepared from directly from lyophilized muscle samples using a solution of 14% boron-trifluoride in methanol (Sigma, USA), as preiously described by (Rule, 1997). Briefly, 100 mg of lyophilized muscle were weighted and mixed with 2 ml of boron-trifluoride in methanol, 2 ml of methanol and 1 ml of the internal standard solution (2 mg of nonadecanoate per ml of *n*-Hexane). The instrumental tubes were then closed, vortex-mixed and placed in water bath at 80 °C for two hours with maximum agitation. Afterwards, tubes were allowed to cool, and 3 ml of water and 3 ml of hexane were added, tubes were capped and vortex-mixed for 15 s, and submitted to centrifugation at 2500 rpm for 5 min to accelerate phase separation, 2.0 ml of the upper phase were transferred to GLC auto-sampler vials and sealed.

The identification of fatty acid methyl esters was detected by gas-liquid chromatography using an Shimadzu GC2010-plus (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and a fused-silica capillary column (BPX70, 60 m × 0.25 mm × 0.25 μm, SGE Europe Ltd, UK), helium was used as the carrier gas, in a flow of 1 ml/min. One ml of the sample was injected in split mode 1:30. The oven temperature was programed to begin a 50ºC (kept within a 1 min), raise from the 50° to 150° C at 50° C/min (kept within a 1 min), and then raise 1° C/min until the 200ºC (maintained for 2 min) and finally raise 3°C/min to the 220°C. The injector and detector were maintained at 250° C.

3.3.3 Cholesterol β-carotene and tocopherols determination

The simultaneous determination of total cholesterol, β-carotene and tocopherols was performed as previously described (Prates, Quaresma, Bessa, Fontes, & Alfaia, 2006). Briefly, the compounds in analysis were extracted from fresh meat samples with a saponification solution. Afterwards, samples were submitted to chromatography analysis for compound separation, using HPLC methodology and a normal-phase silica column. A couple of detectors in tandem (UV-visible photodiode array detector, and fluorescence detector) were used for compound detection.

Tocopherol detection was performed using fluorescence detection (excitation wavelength of 295 nm and emission wavelength of 325 nm), while the UV-visible photodiode array detector was used for simultaneously detection of both cholesterol (202 nm) and β-carotene (450 nm).

The injection volumes used varied between 10 and 100 μl in order to get values inside the linearity range of the standard curves. The contents of total cholesterol, tocopherols and β-carotene in meat were calculated, in duplicate for each muscle sample (values accepted for CV <6%), based on the external standard technique, from a standard curve of peak area vs. concentration.

3.3.4 Statistical analysis

All the parameters in study were subjected to analysis of variance (ANOVA), considering their origin as a single effect, using the GLM procedure of SAS. Least squares means were presented and compared, using the LSD test, when the effect of feeding system was significant ($p < 0.05$).

4. Results and Discussion

This research was planned to assess the composition of intramuscular lipid fraction and some physic-chemical parameters from beef commercialized in Portugal. Considering that national production of beef is quite below the country needs (52% of beef requirements), it is essential to import beef. In beef trade there are three major ways: 1) refrigerated and vacuum-packed premium pieces (as rump, sirloin and tenderloin), 2) refrigerated and vacuum-packed individual pieces from whole carcasses or 3) refrigerated whole carcasses. Considering price as a major factor in beef trade (Monteiro, 2012), the importation of whole carcasses represents an important way to assure a stable deliver of beef at reasonable cost to Portuguese market. In this trade, proximity is a prime issue, and for this reason purchasing of refrigerated bovine carcasses is made exclusively from Europe. Considering the European Union bovine production numbers (FAOSTAT, 2012), Spain and Netherlands were respectively the fifth and sixth major beef producers and these two countries are among major suppliers of beef to Portugal. Beef value is determinate by different aspects and carcass conformation is among the most important ones, for this reason, carcasses from Holstein animals are less valorized than those of beef breeds, and because of their low market value, carcasses from Holstein bulls are the predominant kind of beef sold to Portugal.

The need of constant supply of refrigerated beef carcasses from different European countries makes the beef marketed in Portugal quite variable. Beside this, the beef quality of these carcasses is unknown, not allowing suppliers to make decisions based on beef quality. Therefore, the objective of this study was to compare the nutritional quality of beef lipid fraction and some physic-chemical parameters from different origins. In comparison we have 4 different groups, 2 groups of beef both with Holstein genetics, from Spain and Netherlands, and 2 groups of beef from national regular production (one with the Holstein genetics, and another with crossbred beef background.

4.1 Total fatty acid content and fatty acid profile

The intramuscular total fatty acid content presented here includes all fatty acids in meat, from both polar and neutral lipid fractions *i.e.* phospholipids and triacylglycerols. Differences observed among groups in the total fatty acid content strongly suggests that animals were slaughtered in different body conditions. Such suggestion was made considering that phospholipid content in muscle cell membranes remains quite constant (Smet, Raes, & Demeyer, 2004), while the fatty acid component associated with triacylglycerols is dependent on the degree of lipid reserves deposited at the intramuscular level. Differences in the intramuscular proportion of neutral and polar lipids are associated with differences in the fatty acid composition, since triacylglycerols and phospholipids possess a different fatty acid composition, and the increase in triacylglycerols dilutes the phospholipid fraction changing the global fatty acid profile (Noci, Monahan, French, & Moloney, 2005; Smet, et al., 2004).

Table 2- Total fatty acid content (mg/g muscle), fatty acid profile (g/100 g of beef) from young bulls from Spain and Netherlands and national production (dairy and beef)

Fatty acids	Young bulls				Statistics	
	Dairy farm		Portuguese production		SEM	P
	Spain	Netherlands	Dairy farm	Beef farm		
C12:0	0.042^{b}	0.043^{b}	0.042^{b}	0.067^{a}	0.005	0.0026
C14:0	1.87^{a}	1.83^{a}	$1.41^{a,b}$	1.06^{b}	0.134	0.0003
i-C15:0	0.03^{a}	0.05^{b}	0.04^{b}	0.09^{a}	0.004	<0.0001
C14:1c9	$0.31^{a,b}$	0.34^{a}	$0.20^{b,c}$	0.16^{c}	0.031	0.0004
a-C15:0	0.05^{b}	0.10^{a}	$0.09^{a,b}$	0.11^{a}	0.008	0.0003
C15:0	0.33^{a}	0.34^{a}	0.26^{b}	0.32^{a}	0.017	0.0073
i-C16:0	0.04^{c}	$0.06^{b,c}$	0.08^{b}	0.15^{a}	0.007	<0.0001
C16:0	23.04^{a}	22.06^{a}	19.71^{b}	17.93^{c}	0.443	<0.0001
C16:1c7	0.14^{c}	0.22^{a}	$0.16^{a,c}$	$0.19^{a,b}$	0.009	<0.0001
C16:1c9	2.24^{a}	2.48^{a}	1.61^{b}	1.19^{b}	0.130	<0.0001
a-C17:0	0.13^{c}	0.23^{b}	0.23^{b}	0.32^{a}	0.014	<0.0001
C17:1c9	0.49^{b}	0.81^{a}	0.47^{b}	0.43^{b}	0.050	<0.0001
C18:0	14.90^{a}	15.28^{a}	15.94^{a}	13.45^{b}	0.396	0.0008
C18:1t10	3.39^{a}	$1.37^{b,c}$	$2.26^{a,b}$	0.97^{c}	0.336	<0.0001
C18:1t11	0.35^{b}	0.52^{b}	0.55^{b}	1.08^{a}	0.102	0.0001
C18:1t12	0.30^{a}	0.21^{b}	0.17^{b}	0.17^{b}	0.013	<0.0001
C18:1c9	23.05^{b}	31.05^{a}	$22.09^{b,c}$	18.20^{c}	0.020	<0.0001
C18:1c11	2.24	2.26	2.21	2.38	0.067	0.339
C18:1c12	0.21	0.21	0.24	0.22	0.027	0.746
C18:1c13	$0.19^{a,b}$	0.25^{a}	$0.15^{b,c}$	0.12^{c}	0.016	<0.0001
C18:1c15	0.15^{b}	0.27^{a}	0.15^{b}	0.22^{a}	0.015	<0.0001
C18:2n-6	13.54^{a}	8.16^{b}	15.40^{a}	14.71^{a}	0.909	<0.0001
C18:3n-6	0.09^{b}	0.09^{b}	0.10^{b}	0.15^{a}	0.006	<0.0001
C18:3n-3	0.25^{b}	0.42^{b}	0.34^{b}	2.70^{a}	0.127	<0.0001
C20:0	0.13^{a}	0.11^{b}	$0.12^{a,b}$	0.08^{c}	0.006	<0.0001
CLA(c9t11)	0.03^{b}	0.08^{b}	0.06^{b}	0.19^{a}	0.015	<0.0001
C20:1c11	0.13^{a}	0.12^{a}	0.13^{a}	0.08^{b}	0.007	0.0001
C20:3n-9	0.15^{a}	0.09^{b}	0.18^{a}	0.09^{b}	0.012	<0.0001
C20:3n-6	0.77^{c}	0.65^{c}	1.09^{b}	1.36^{a}	0.069	<0.0001
C20:4n-6	3.35^{b}	2.91^{b}	4.84^{a}	5.43^{a}	0.295	<0.0001
C22:0	0.04^{b}	0.05^{b}	0.04^{b}	0.11^{a}	0.006	<0.0001
C20:5n-3	0.14^{b}	0.20^{b}	0.19^{b}	2.18^{a}	0.051	<0.0001
C22:4n-6	0.49^{b}	0.47^{b}	0.77^{a}	0.28^{c}	0.042	<0.0001
C22:5n-6	$0.10^{b,c}$	0.11^{b}	0.16^{a}	0.06^{c}	0.010	<0.0001
C22:5n-3	0.36^{b}	0.61^{b}	0.59^{b}	2.85^{a}	0.085	<0.0001
C22:6n-3	0.05^{b}	0.08^{b}	0.07^{b}	0.55^{a}	0.017	<0.0001
Others*	0.98^{b}	1.05^{a}	1.06^{a}	1.15^{a}	0.037	0.036
DMAs						
DMA-C16:0	3.19^{b}	2.45^{c}	3.32^{b}	4.96^{a}	0.186	<0.0001
DMA-C18:0	1.89^{b}	1.80^{b}	2.59^{a}	3.07^{a}	0.187	<0.0001
DMA-C18:1	0.80^{b}	0.54^{c}	0.87^{b}	1.06^{a}	0.083	<0.0001

*Representing the sum to co-eluted fatty acids (namely i-C17:0+C16:1, C18:1t6 to t9, C18:1c14+t16 and C20:2n-6+C21:0)

The fatty acid profile of beef from young bulls of different origins, presented in Table 2, embraces 35 fatty acids in all the four groups in comparison, being composed of 11 saturated fatty acids (SFA; 7 of which were of linear chain and 4 were of branched chain), 12 monounsaturated fatty acids (MUFA; 9 with *cis* configuration and 3 with *trans* configuration) and 12 polyunsaturated fatty acids (PUFA; including one from the *n*-9 family, 4 from the n-3 family, 6 from the n-6 family and the 18:2 *c*9,*t*11 CLA isomer).

The predominant fatty acids in beef were the 16:0 and 18:0 among SFA, representing 17.9-23% and 13.5-15.9% of total fatty acids, respectively. The 18:1 *cis*-9 was the prime representative of MUFA, being responsible for 18.2-31.1% of total fatty acids while the 18:2n-6 and 20:4n-6 were the major representatives of PUFA with 8.2-15.4% and 2.9-5.4% of total fatty acids, respectively. Similar results have been previously reported in beef (Alfaia et al., 2009; Nuernberg et al., 2005; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004). Beyond these 5 prime fatty acids, only a few fatty acids were associated with a relative percentage above 1% (14:0, 16:1*cis*-9, 18:1 *cis*-11 and the 18:1 *trans*-10), in all the groups in comparison.

Differences between groups in the fatty acid composition where expected considering differences in edafo-climatic conditions between different origins, different traditions and practices in cattle husbandry and the use of different production and feeding methods. Moreover, the observed differences in the total fatty acid content between groups suggest differences in their total lipid content.

Considering the absence of information regarding the nutrition finishing plan used by different groups, it is quite difficult to discuss in detail all the results, therefore discussion will solely focus on major results.

Among the most remarkable results was the relative parity associated to beef from bulls with Holstein background of Portugal, Spain and Netherlands, which showed no significant differences in the relative percentage of 11 fatty acids (C12:0, C18:0, C22:0, C18:1*trans*-11, C18:2*cis*-9,*trans*-11 (CLA), C18:3n-3, C18:3n-6, C20:1*cis*-11, C20:5n-3, C22:5n-3, C22:6n-3). In all these fatty acids, beef from Holstein bulls displayed an inferior relative percentage relatively to beef from crossbred beef bulls. Differences in feeding management between dairy and beef producers could be at least partially responsible for differences observed. The superior relative percentages of C18:0, C18:1*trans*-11, C18:2*cis*-9,*trans*-11, C18:3n-3, 18:3n-6, C20:5n-3, C22:5n-3, C22:6n-3 have been found in beef from pasture grazing systems relatively to beef fed on concentrate feeding (Alfaia, et al., 2009; Leheska et al., 2008; Noci, et al., 2005; Nuernberg, et al., 2005; Realini, et al., 2004), suggesting that the group of Portuguese crossbred beef bulls was finished on a feeding system that included pasture grazing or green vegetal feeding, while Holstein bulls were finished on a more concentrate based diet.

Moreover, results revealed that beef from Dutch and Spanish Holstein animals had equal percentage of 8 fatty acids (C14:0, C15:0, C16:0, C16:1c9, C20:3n-6, C20:4n-6, C22:4n-6, C22:5n-6), which were significantly different from beef of Holstein bulls raised in Portugal. In these differences, it was observed that non-Portuguese Holstein beef had a superior percentage of SFA (C14:0, C15:0, C16:0) and MUFA (C16:1c9) and an inferior percentage of n-6PUFA (C20:3n-6, C20:4n-6, C22:4n-6) than Holstein bulls raised in Portugal. Such differences in the fatty acid profile are probably consequence of two conditions: 1) the superior content of SFA found in non-Portuguese beef could be a consequence of increased intramuscular lipid deposition, as it is well known that triacylglycerols contribute to meat fatty acid with a more saturated composition than muscle phospholipids (Marmer, Maxwell, & Williams, 1984 ; Noci, et al., 2005; Wood et al., 2008); 2) previous proposal, suggests differences in the total fatty acid content which is not the case, since Holstein bulls raised in Portugal had a total fatty acid content in between those from Dutch and Spanish Holstein bulls, therefore differences in the fatty acid profile of finishing diet and particularly in their n-6 PUFA should be responsible for such differences in the n-6 PUFA (Alfaia et al., 2009; Nuernberg, et al., 2005).

4.2 Fatty acid partial sums and nutritional ratios

The fatty acid partial sums and nutritional ratios of the four groups in comparison are presented in Table 3. As previously observed in the fatty acid profile, the most relevant result of the comparison was associated with some similarities among groups with Holstein background and their divergence with the Portuguese group of crossbred beef background. Beef from bulls of the crossbred beef group was associated with an inferior relative percentage of total SFA (but superior relative percentage of branched SFA), inferior relative percentage of total MUFA, but superior relative percentage of total PUFA, n-3PUFA and the long chain n-3PUFA (n-3LCPUFA), and a superior relative percentage of total DMA than beef from bulls with Holstein background, independently of their origin. Beef from bulls with Holstein genetic background were characterized by superior relative percentages of total SFA and MUFA and inferior relative percentage of PUFA. Such result could be a consequence of different factors: 1) the genetic pattern of the Holstein breed, makes the Holstein an early maturing breed (Schreurs et al., 2008), which contributes to an earlier lipid deposition in fat depots; 2) the Holstein bulls were submitted to an intense finishing feeding plan with increased concentrate proportion; 3) the combination of genetics and feeding plan.

The suggestion previous made on differences in the finishing feeding plan between bulls of the crossbred beef group and Holstein bulls is sustained by differences observed in branched fatty acids. Pasture is poorer in energy and richer in fiber, leading to a more intensive cellulolytic rumen-bacteria activity inducing an increase in the proportion of branched fatty acids in meat (Kraft, Kramer, Schoene, Chambers, & Jahreis, 2008). Contrastingly, corn silage and concentrate rations are rich in dietary starch and poor in neutral detergent fiber, leading to a reduction in the rumen pH and the subsequent shift in the microbial population from cellulolytic to amylolytic bacteria, which are not linked to the synthesis of branched fatty acids (Kraft, et al., 2008; Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006).

Dimetylacetals (DMA) were also identified by Gas Chromatography, corresponding to plasmalogenic lipids, which were converted into DMA after methylation (Kraft, et al., 2008). Plasmalogens are a special group of glycerophospholipids in which the sn-1 position of the glycerol backbone is linked with a long chain fatty aldehyde via a vinyl-ether bond (Dannenberger et al., 2006). Plasmalogens occur primarily as phospholipids (Horrocks, 1972), for this reason lean beef should contain an higher content of DMA. Such data is in agreement with the study results, since beef from bulls of the crossbred beef group was the one with the with the lowest total fatty acid content and the highest DMA percentage, and beef from Dutch Holstein bulls were those with the highest total fatty acid content and the lowest DMA percentage. However, there was an apparent divergence between DMA percentage and total fatty acid content in beef from Holstein bulls raised in Portugal and Spain, such discrepancy may be a consequence of: 1) diet and 2) differences in the period between slaughtered and sampling. Beef from pasture-based feeding systems have been associated with increased contents of plasmalogens (Dannenberger, et al., 2006). Differences in the period between the slaughter time and sampling could also be responsible for differences observed in the percentage of plasmologens, since plasmologens are located in the cells membranes and show a high sensitivity to acids and to reactive oxygen substances (ROS), being for these reasons the first shield against oxidative damage (Brites, Waterham, & Wanders, 2004; Lee, 1998). Beef from the Spanish Holstein bulls was sampled one day later than Holstein bulls slaughtered in Portugal, such difference may explain the unexpected lower DMA percentage in Spanish bulls, which showed an inferior total fatty acid content.

Concerning nutritional ratios, it was observed that P/S ratio ranged from 0.22 to 0.54. The lower P/S ratios were observed among beef from non-Portuguese carcasses (0.22 and 0.35 in Spanish and Dutch bulls, respectively), while the P/S ratio in beef from Portuguese production ranged between 0.44-0.54. In the n6/n3 ratio it was observed an even greater divergence of values, the lowest value was observed in beef from bulls of the crossbred beef group (2.7). Among beef obtained from animals with Holstein background, the highest n6/n3 ratio was observed among Spanish bulls and the lowest among the Dutch group.

Differences in the fatty acids partial sums and nutritional ratios, previously presented were probably the result of the combined effect of three major features: 1) differences in the genetic background of crossbred beef bulls and Holstein bulls; 2) differences in the total fatty acid content; 3) differences in the feeding management, particularly between those of Holstein genetics and the crossbred beef ones.

The genetic influence on fatty acid profile has been considered of minor importance (Smet, et al., 2004), however, in this study we have a quite divergent genetic background, the dairy and beef backgrounds, which could have increased the importance of this effect. Differences in the total fatty acid content are a consequence of increased lipid deposition at the intramuscular level and it is well known that triacylglycerols and phospholipids possess a quite distinct fatty acid profile (Marmer, et al., 1984 ; Smet, et al., 2004). Differences in the feeding management is a prime factor influencing beef fatty acid composition (Alfaia, et al., 2009; Leheska, et al., 2008; Smet, et al., 2004; Wood, et al., 2008).

Considering the importance of fatty acids nutritional ratios in the evaluation of beef quality, it was advisable the comparison of the study results with international recommendations. In this respect, beef from national production possess a P/S ratio in accordance with nutritional recommendations, which stated that it should be above 0.4 (Health, 1994; A.P. Simopoulos, 2002; WHO, 2003; Wood et al., 2003), which was not the case for non-Portuguese beef. Considering the n6/n3 ratio, it was observed that beef from national crossbred beef bulls was the only beef in agreement with international recommendations, which stated that it should not exceed 4.0 (Wood, et al., 2003). Therefore, beef from national crossbred beef bulls was the only beef variety in total agreement with international recommendations on P/S and n6/n3 ratios.

According to Givens and Gibbs (2008), the nutritional quality of beef lipid fraction is not only dependent of nutritional ratios, but is also dependent on the amount of long chain fatty acids with health beneficial effects as the n−3 PUFA (20:5 n−3, 22:5 n−3 and 22:6 n−3). Therefore, a 150 g portion of beef from the Portuguese crossbred beef group contributes with 79.8% of the recommended adult daily intake (450 mg) (I. D. Givens & Gibbs, 2008).

4.3 Total cholesterol

The total cholesterol content in beef of all the four groups in comparison is displayed in Table 3. The total cholesterol content presented here includes the free and esterified cholesterol present in muscle as well as in the intramuscular fat depots. Cholesterol is an essential component of cell membranes and is also stored as cholesterol esters in lipid droplets (Chizzolini, Zanardi, Dorigoni, & Ghidini, 1999; Dinh, et al., 2011).

Table 3 - Total cholesterol content (mg/100 g of muscle), total fatty acid content (g/100 g of muscle), partial sums of fatty acids (% w/w) and nutritional ratios of intramuscular fat in the *longissimus lumborum* muscle of young bull meat from Spain and Netherlands and national production from dairy and beef farms

	Young bulls				Statistics	
	Dairy farm		Portuguese production		SEM	P
	Spain	Netherlands	Dairy farm	Beef farm		
Total Cholesterol	44.55	60.27	46.85	42.78	7.694	0.379
Total fatty acids	1,40	1,88	1,57	0,94	0.517	<0.0001
Partial sums						
∑ SFA	40.60^{a}	40.14^{a}	37.95^{a}	33.68^{b}	0.693	<0.0001
∑ SFA linear	40.35^{a}	$39.70^{a,b}$	37.52^{b}	33.02^{c}	0.694	<0.0001
∑ SFA branched	0.25^{c}	0.44^{b}	0.43^{b}	0.67^{a}	0.024	<0.0001
∑ DMA	$5.88^{b,c}$	4.79^{c}	6.79^{b}	9.09^{a}	0.396	<0.0001
∑ MUFA	33.83^{b}	40.67^{a}	30.87^{b}	25.72^{c}	1.275	<0.0001
∑ MUFA *trans*	4.69^{a}	2.64^{b}	$3.46^{a,b}$	2.52^{b}	0.337	0.0003
∑ MUFA *cis*	29.14^{b}	38.02^{a}	$27.41^{b,c}$	23.19^{c}	1.171	<0.0001
∑ PUFA	$19.30^{b,c}$	13.82^{c}	23.75^{b}	30.47^{a}	1.441	<0.0001
∑ n-3PUFA	0.81^{b}	1.32^{b}	1.20^{b}	8.36^{a}	0.272	<0.0001
∑ n-6PUFA	18.34^{a}	12.40^{b}	22.37^{a}	22.01^{a}	1.289	<0.0001
∑ n-3LCPUFA	0.55^{b}	0.90^{b}	0.86^{b}	5.66^{a}	0.152	<0.0001
∑ n-6LCPUFA	4.71^{b}	4.14^{b}	6.87^{a}	7.15^{a}	0.406	<0.0001
∑ Others	0.38^{c}	0.57^{b}	0.64^{b}	1.03^{a}	0.037	<0.0001
Nutritional Ratios						
P/S	$0.35^{b,c}$	0.22^{c}	$0.44^{a,b}$	0.54^{a}	0.038	<0.0001
n6/n3	22.77^{a}	9.60^{c}	18.77^{b}	2.70^{d}	0.501	<0.0001

No significant differences were observed among groups in comparison in total cholesterol content, which ranged between 42.8-60.3 mg/100 g of muscle. The absence of significant differences in the total cholesterol content was expected, since beef samples collected to the study were all obtained from the similar portion and muscle (the cranial portion of sirloin), and for that reason it was expected no significant differences in muscle fibre composition, which is the major cause of differences in total cholesterol content (Chizzolini, et al., 1999). Moreover, it has been shown that muscle total cholesterol content is not significantly influenced by the finishing regimen (Leheska, et al., 2008).

The total cholesterol content quantified in this study is in agreement with cholesterol contents previously quantified in beef and veal of several Portuguese autochthonous breeds slaughtered as veal or beef (Alfaia, et al., 2009; Alfaia, Quaresma, et al., 2006; Alfaia, Ribeiro, et al., 2006; Costa et al., 2011; Costa et al., 2006; Monteiro, 2012; Pestana, Costa, Alfaia, et al., 2012; Pestana, Costa, Alves, et al., 2012; Pestana, Costa, Martins, et al., 2012; Prates, Quaresma, Bessa, Fontes, & Alfaia, 2006) and in the range of international standard values for beef and veal (Chizzolini, et al., 1999; Dinh, et al., 2011).

4.4 Lipid soluble antioxidants

The *longissimus lumborum* β-carotene and vitamin E (α- and γ-tocopherols) contents are depicted in Table 4. α-Tocopherol was the major vitamin E homologue in beef, being responsible for 84.3-96.6% of total vitamin E present in beef, while γ-tocopherol was responsible for the remaining 3.4-15.7% of total vitamin E. α-Tocopherol content ranged between 1.0 and 2.8 μg/g of meat, while the γ-tocopherol content ranged between 0.1-0.37 μg/g of meat.

Beef from Portuguese production displayed superior contents of α-tocopherol and β-carotene contents than Spanish and Dutch beef, significant differences in the α-tocopherol contents were also observed among the Portuguese production, but that was not the case for β-carotene content, which showed similar values. On the other hand, Spanish and Dutch beef displayed the lowest contents of both α-tocopherol and β-carotene contents of all groups and showed no significant differences in between. Regarding γ-tocopherol it was observed significant differences between the dairy groups. Beef from Portuguese crossbred beef bulls revealed the lowest γ-tocopherol of all groups in comparison, despite not being significantly different from Dutch bulls beef.

Despite differences previously pointed out, beef from all groups in comparison displayed a vitamin E content below the content required to retard lipid oxidation (3.5 μg/g of meat) (Faustman et al., 1989; Mitsumoto, Cassens, Schaefer, Arnold, & Scheller, 1991). The β-carotene content found on beef from bulls raised in Portugal confirms that the finishing feeding of these animals included fresh green forage considering previous results on controlled feeding study (Quaresma, Trigo-Rodrigues, Lemos, & Bessa, 2013). The suggestion that the finishing diet included green forage is supported not only by the β-carotene content but also by the α-tocopherol content. Several studies have shown that cattle finished on pasture produce higher levels of α-tocopherol in the final meat product than cattle fed high concentrate diets (Arnold et al., 1992; De la Fuente et al., 2009; Descalzo et al., 2005; Descalzo & Sancho, 2008; Insani et al., 2008; Realini, et al., 2004).

Table 4 – Major lipid soluble (α-tocopherol, γ-tocopherol and β-carotene) in beef from in the *longissimus lumborum* muscle of young bull meat from Spain and Netherlands and national production from dairy and beef farms

	Young bulls				Statistics	
Lipid soluble antioxidants	Dairy farm		Portuguese production		SEM	P
	Spain	Netherlands	Dairy farm	Beef farm		
α-tocopherol*	1.23^{c}	1.01^{c}	1.98^{b}	2.81^{a}	0.143	<0.0001
γ-tocopherol*	0.21^{b}	0.13^{c}	0.37^{a}	0.10^{c}	0.013	<0.0001
β-carotene*	0.05^{b}	0.03^{b}	0.11^{a}	0.11^{a}	0.006	<0.0001

* µg/g of meat

4.5 Beef color, pH and Warner Bratzler shear force

Meat color is the first criterion consumers use to judge meat quality and acceptability (Conforth, 1994). Meat purchasing decisions are influenced by color more than any other quality factor, once it is the only available attribute at the point of sale without opening the packages (Suman & Joseph, 2013).Thus, a bright cherry-red color is commonly utilized by consumers as an indicator of freshness and wholesomeness (Mancini & Hunt, 2005).

Table 5 - Beef color parameters (L*, a*, b*, h* and C*), Warner Bratzler shear force (WBSF) and pH in beef from in the *longissimus lumborum* muscle of young bull meat from Spain and Netherlands and national production from dairy and beef farms

	Young bulls				Statistics	
	Dairy farm		Portuguese production		SEM	P
	Spain	Netherlands	Dairy farm	Beef farm		
WBSF (kg)	4.87	6.78	5.54	7.75	0.837	0.0825
L*	30.87^{b}	35.20^{a}	36.11^{a}	37.59^{a}	1.0025	0.0003
a*	15.52^{b}	18.03^{a}	14.34^{b}	14.20^{b}	0.5742	<0.0001
b*	1.42^{b}	3.66^{a}	3.00^{a,b}	3.08^{a,b}	0.5629	0.0481
Hue angle	3.51	5.73	14.87	4.90	7.0543	0.3486
Chroma	15.79^{b}	18.44^{a}	14.75^{b}	14.55^{b}	0.5741	<0.0001
pH	6.18^{a}	5.51^{b}	5.87^{a,b}	5.42^{b}	0.1124	<0.0001

Hue = $\tan^{-1}(b^*/a^*)$ and Chroma= $(a^{*2} + b^{*2})^{1/2}$

The meat color is mainly determined by the amount and chemical state of heme pigments, mainly myoglobin, responsible for 95% of total meat iron after slaughter (Fenemma, 2007). However, the greatest influence on meat color is the ultimate pH of meat (Shorthose & Harris, 1991), which is mainly determined by glycogen content at slaughter. The low muscle glycogen at slaughter results in high ultimate pH post-mortem and dark meat. On the other hand, high muscle glycogen at slaughter promotes extended anaerobic glycolysis and lactate accumulation that generates a lower ultimate pH and favors a lighter meat color (Hunt, 2009). Color in meat is therefore related to ultimate pH, which in turn, is related to tenderness (Monteiro, 2012). That is the reason we have integrated color, pH and shear force in the same chapter.

Color measurements are done using the Commission International de I' Eclairage color system (CIE, 1986). The CIE L* a* b* system, the L* component is called lightness (value), and a* and b* are called chromaticity coordinates. L* represents the difference between dark (0) and light (100). The coordinate a*(redness) measures the ratio between green (-60) and red (+60) and the coordinate b* measures the ratio between blue (-60) and yellow (+60). The chromaticity coordinates were used to calculate the hue angle (h*) and chroma (C*). The hue angle is function of the wavelength of reflected light and corresponds to what is commonly called color, and angle depends mainly on pigment content and their chemistry (Renerre, 2000). On the other hand, Chroma indicates how pure the color is (Sahin & Sumnu, 2006) and depends mostly on the myofibril structure and on the ultimate meat pH (Renerre, 2000).

Data on beef color parameters (L*, a*, b*, Hue, Chroma), pH and Warner Bratzler shear force (WBSF) is presented in Table 5. Meat pH showed some significant differences between groups. The lowest pH value (5.4) was observed in beef from the Portuguese crossbred beef group, this pH value was numerically but not statistically inferior to the pH value of beef from Dutch Holstein bulls. The Spanish beef displayed the highest pH value (6.2), this value was significantly superior to Dutch beef, while beef from Portuguese Holstein bulls had an intermediate pH value, being no statistically different from the other beefs from Holstein bulls. No statistical significant differences were observed in WBSF between groups in comparison ($p>0.05$), despite the observed variability.

Beef color measurements showed significant differences between groups in comparison in L*, a*, b* and C*, but not in h*. Spanish beef displayed the lowest value on L* or lightness value, which was significantly inferior to all other beefs in comparison.

Significant differences between groups were observed in *L a* and *b* coordinates. L`* or lightness reached its lowest value in beef from Spanish bulls, which was significantly lower than all other beefs in comparison, no significant differences were observed between Dutch and Portuguese beef on *L* value. Beef redness or a* coordinate reached maximum value in Dutch beef, which was significantly superior to all other beefs in comparison, no significant differences were observed between the Spanish and Portuguese beef on a* value. Beef yellowness or b* coordinate reached maximum value in Dutch beef and lowest value in Spanish beef, which were significantly different in between, while the Portuguese beef of both background showed a halfway b* value, being no significantly different from Spanish and Dutch beef.

Portuguese beef from crossbred beef bulls showed the lowest pH value of all groups in comparison and the highest *L* value. Such result is consequence of intense postmortem glycolysis, which decreases muscle pH, making it brighter and superficially more wet allowing it to scatter more light (Swatland, 1989).

5. Conclusion

The comparison done in this study showed that beef from national crossbred beef bulls was leaner and possessed a more favorable fatty acid profile than beef from Holstein bulls, independently of their origin. Beef from crossbred beef bulls was the only one in accordance to international recommendations regarding P/S and n6/n3 ratios. The beef α-tocopherol content of all groups is below the recommendations. The pH value of beef from Portuguese Holstein bulls was on the limit of DFD while beef from the Spanish Holstein bulls was a well-established DFD. However, Spanish Holstein beef presented contradictious results, considering slaughter date occurred one day before sampling, ageing could have already started at this time and this might explain lower shear force and high pH value. It is however important to highlight that the study was done with a restrict number of samples, and for this reason it is impossible to extrapolate.

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