



UNIVERSITÀ DEGLI STUDI DI PERUGIA

DIPARTIMENTO DI SCIENZE AGRARIE, ALIMENTARI E  
AMBIENTALI

CORSO DI LAUREA MAGISTRALE IN  
TECNOLOGIE E BIOTECNOLOGIE DEGLI ALIMENTI

EFFETTO DELLA RAZZA E DEL TIPO DI  
ALIMENTAZIONE SU ALCUNI PARAMETRI  
QUALITATIVI DELLA CARNE SUINA

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*Anno Accademico 2012/2013*

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## Riassunto

In questa tesi si sono valutati gli effetti di due strategie di alimentazione, su alcuni parametri qualitativi della carne suina. Nella prima strategia, Normal (NORM) si prevedeva la somministrazione del 100% di concentrati necessari, nella seconda, Restrictive (Rest) invece, solo il 60% di concentrati veniva reso disponibile. Due tipologie di ibridi commerciali DLY e TLY sono stati allevati nella piattaforma biologica di ricerca dell'università di Aarhus, in Danimarca, da Agosto a Ottobre 2012. La ricerca è stata svolta sulla generazione F1 di 32 scrofe di razza Landrace x Yorkshire (LY) inseminate con seme di verro Duroc (D) e sulla generazione F1 di 32 scrofe di razza Landrace x Yorkshire (LY) fecondate con seme di verro Tamworth (T). I suini sono stati allevati all'aperto in paddocks di 1625 m<sup>2</sup> e macellati a circa 90 giorni di età. Tutti i soggetti, al momento della macellazione, sono stati pesati e per ogni carcassa sono stati prelevati due fasci muscolari, *biceps femoris* e *longissimus dorsi* da cui a loro volta sono stati prelevati i campioni da sottoporre ad analisi. I parametri qualitativi misurati riguardano l'andamento della temperatura e del pH nella carcassa nelle 24h postmortem, la valutazione del colore nei due muscoli tramite Minolta Chroma Meter e la valutazione della tenerezza attraverso misurazione dello sforzo di taglio tramite sistema Warner-Bratzler in campioni frollati per 1, 4 e 7 giorni. Un gruppo di assaggiatori dell'università di Copenaghen ha effettuato una valutazione sensoriale dei campioni di carne frollati per 4 giorni e provenienti dai due muscoli dei due ibridi commerciali. I risultati del panel test sono stati successivamente elaborati con il programma statistico SAS per poter valutare una correlazione con i risultati ottenuti dalla valutazione dello sforzo di taglio attraverso il sistema (WBSF). Una valutazione sull'andamento ossidativo dei campioni di carne è stata, infine, effettuata tramite estrazione chimica e analisi spettrofotometrica sui TBARS utilizzando campioni conservati a 5±1°C e sottoposti a luce fluorescente (1000 LUX) per tre giorni in modo da simulare le condizioni standard di vendita al dettaglio.

## **Abstract**

The present study was carried out to establish the effect of two feeding strategies on some qualitative parameters of fresh pork. Regarding the feed strategies the first group was called NORMAL(Norm) with available the 100% of concentrate that they needed and RESTRICTIVE (Rest) with only the 60% of concentrate available. Two market hogs DLY and TLY are raised up at the organic research platform of Aarhus University, Denmark, from August to October 2012. The market hogs were 32 female pigs, born from the progeny F1 of Landrace x Yorkshire (LY) sows sired by Duroc (D) or Tamworth (T) boars. The pigs were reared outdoors in paddocks 1625 m<sup>2</sup> and slaughtered at about 90 days of age. All pigs at the time of slaughter, were weighed and from each carcass several samples were taken from two muscle beams (*biceps femoris* and *longissimus dorsi*). Quality parameters evaluated were, the trend of pH and temperature within 24h postmortem, the color in the two muscles by Minolta Chroma Meter, the development of tenderness by shear force measurement (WBSF) in the samples aging for 1, 4 and 7 days. A group of tasters from University of Copenhagen was involved for the sensory evaluation of sample of *longissimus dorsi* and *biceps femoris* from DLY and TLY respectively with 4 day of aging time. The result of the panel test has been elaborated with the statistical program SAS. The result of the shear force obtain from the system (WBSF) has been compared with the result from panel test by SAS for evaluated eventual correlations. An assessment of the development of oxidation in meat samples, was finally performed through chemical extraction and spectrophotometric analysis on TBARS, using samples stored at  $5 \pm 1$  ° C under fluorescent light (1000 LUX) for three days, in order to simulate the standard conditions of retail sale.

# 1. Introduction

## 1.1 Pig production

Pigs are mammal, belong to the genus *Sus*, of the Suidae family of even-toed ungulates. According with Cuvier, form the Asian wild boar, would be differentiated the common Eurasian wild boar (*Sus scrofa*), from which would be differentiated the domestic pig (*Sus scrofa domesticus* or *Sus domesticus*). The genetic difference between *Sus domesticus* and *Sus scrofa* is in the number of chromosomes, respectively 38 and 36. The earth, host about one billion pigs of which, over 100 mmt of pork to people for consumption (McGlone 2013). Actually pork, represent the 37% of overall meat eaten in the word. In 2011, only in China (50mmt) like swine carcass were produced, equivalent at half of the world pork meat production. The second largest producers is USA with a numbers of pigs (10 mmt) while the third was EU where only in Germany were product (5 mmt) of pigs. Over 80% of the world's pork is produced in Asia, in EU and North America. About 7.4 mmt of pork was traded in 2011 (6.7% of the total) thus most pigs are currently produced in the country in which they are consumed, although this may change with increases in labor and land costs in developed countries ( McGlone 2013).

Table - 1 Percentage of meat consumed (2012).

Meat	Meat consumed, 2012(mmt)	Percentage of meat (%)
Pork/Porcine	110,8	37,40%
Poultry	104,5	35,30%
Bovine	66,8	22,60%
Ovine	13,9	4,70%
Total common meats	296	100,00%

A reason for the increasing demand of pork (and other meats) is due at growth of population and could be also the increasing demand from the developing countries, than at the opening of new market. Moreover with the change lifestyle, the increase in the proportion of single person households, the limited time available for cooking and shopping, that have lead to change the eating habits,

with an increasing presence of products of animal origin on the ours kind of diet. Nevertheless, the trend is not expected to be the same for all types of meat. Escalating feed prices and slowing meat production growth, have pushed up international meat prices. Animal feed costs, are large proportion of the total cost of production around 70%, competing for resources (land, water, and fertilizer) with human food and fuel production, urbanization, and nature. Combined with climate change (making production more volatile and harvests more insecure), this will increase feed prices and lead to a demand for livestock that can sustain productivity on diets with decreased nutrient density than before, which calls for improvement of animal resource efficiency. While pork production of 100 years ago was mixed and grazing, today all developed countries use the industrialized systems for the majority of pigs and pork produced.

### **1.1.1 The main Breeds**

The principal breeds raise-up in the word are Large White ( Yorkshire in USA), Landrace, Duroc and Meishan (come from the region of lakes and valleys in China where is mainly grown), in US are common also the breeds like Berkshire, Chester White, Hampshire, Poland China and Spotted Swine, while the breeds Pietrain and Tamworth are common in UE.

Large White: they are long-bodied with excellent hams and fine white hair and, as their name suggests, erect ears, slightly dished faces and they are characterised by large size. While the Large White was originally developed as an active and outdoor breed, they do very well in intensive production systems. They and their descendants, the Yorkshire, are to be found in practically all crossbreeding and rotational breeding programmes using two or more breeds. Purebreed sows have an enviable reputation as dams and form the foundation of the classic Fl hybrid gilt. Modern breeding programmes have developed separate sire and dam lines to produce purebred Large White terminal sires that excel in growth rate and lean meat percentage and are incorporated in most terminal sire breeding programmes.

They can definitely stamp uniformity and quality on a pen of pigs from almost any breed or type of dam;

Landrace : they are medium to large breed that has a distinct physical appearance. Most of their characteristics are possible find to other breeds, where they have contributed a major portion of the foundation stock. They are white in colour, have a rather fine hair coat, long snouts and heavy drooping ears. They have long bodies and deep smooth sides, truly appear to be a breed developed very systematically and carefully to produce some of the world's greatest pork products for exacting home and export markets. The carcass meat ranges between 62.2 to 64.7 percent. The Landrace is a long and lean breed known for its very high fertility and excellent motherhood, and excellent ham development, some strain still occurs in PSS.

Duroc: the Duroc breed of hogs had its origin in the eastern United States and in the Corn Belt. Durocs have considerable variation in colour. An acceptable colour may range from a very light golden, almost yellow colour, to a very dark red that approaches mahogany. The red is a very practical colour that suits pork producers, and since it is a solid colour there is not concern about fancy points of proper markings. Durocs have a medium length and slight dish of the face. The ears should be drooping and should not be held erect.

Tamworth: is an English breed of hog that was of distinctly bacon-type. The body type, colouring, and general temperament of the Tamworth suggest that it is more a direct descendant of the old English hog than any of the other breeds of English origin. The ham is muscular and firm although it lacks the size and bulk found in most other breeds. On the other hand, no one can fail to admire the breed's smoothness and quality as shown by the firm, trim jowl, firm underline, and firm fleshing. The depth of side is also most commendable. The sows are excellent mothers and do a good job of suckling their litters. The Tamworth has the reputation of producing the best bacon of any of our breeds and is uniform in type.

### 1.1.1. Market hog

Commercial breeding goals have to be adapted on a regular basis: the relative value of traits changes over time when there are changes in production conditions, market requirements, and societal developments; insight into the biological background of traits improves; and technology development leads to novel options for selection so that improvement of novel traits becomes feasible. In the previous chapter was discussed how the increasing price of the feed is related with the meat price. The food–feed–fuel competition makes arable land an increasingly scarce resource. Productive, reproductive, feed conversion ratio (FCR) and environmental efficiency will then become ever more important. Productivity and efficiency traits (the core objective for food security) will thus have to form a steady element in breeding goals, not only for food security reasons, but also because of environmental interests. In the pork production the farmers generally use a crossbreeds whose propose is to improve the productive, reproductive and environmental efficiency in relation with the market requirements, in fig are shown the increasing complexity of the characteristics sought. The production of market hogs, is characterized by pyramidal organization, from the top to the bottom there are, the great-grand-parents (GGP), grand-parents (GP), parents (P)

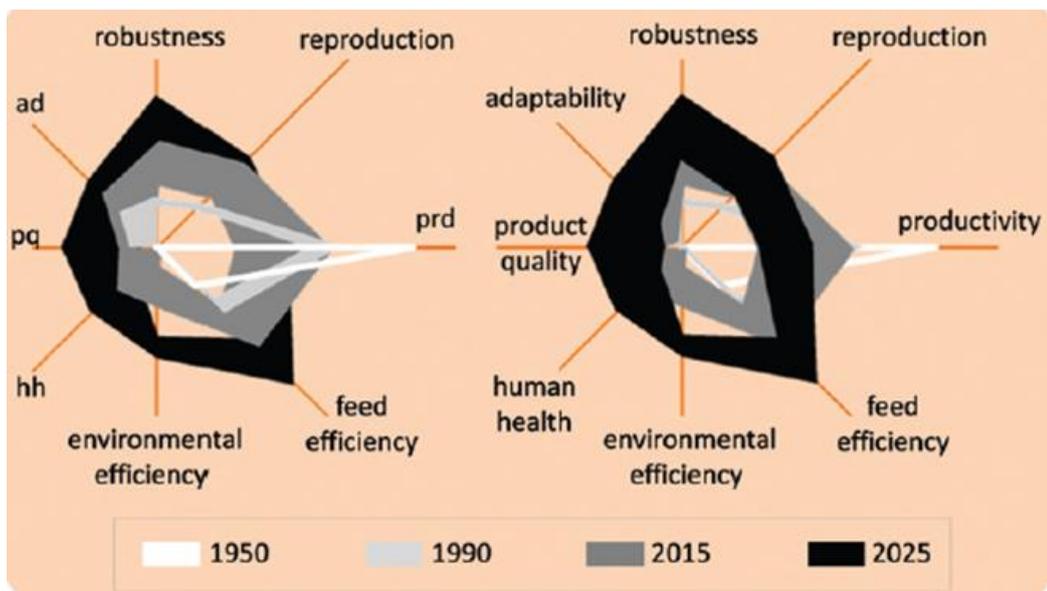


Figure 1 sought characteristics in the market hog, different of selection from 1950 to 2025

and in the end the markets hogs. GGP are pure line selected for a particular character. In fig.2. there is reported an example of market hogs production D x [LY] ( Duroc x Landrace Yorkshire). Crossbred pigs have some advantages over purebred pigs due by genetic phenomenon called heterosis (also known as hybrid vigor). Heterosis is a positive effect of the crossbreed in animals born from parents of different breeds or crossbreed obtained with other forms of intersection (as alternate or triple) features valuable livestock (growth, early maturity, fecundity, adaptive capacity), compared with the animals of pure breed. is defined heterosis because it is linked heterozygosity of crossbreeding, does not occur in all cases of intersection and tends to decrease quickly if they reproduce hybrids between them (hybridisation). The genetic mechanisms at the basis of heterosis are dominance and overdominance of genes of growth, fertility, vigor, resistance to adverse environmental causes, which cumulatively in (P).

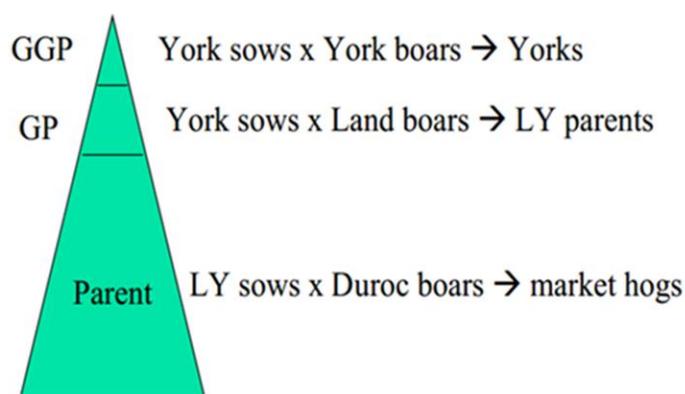


Figure 2 pyramidal structure for the crossbreeds improvement

### 1.1.3 Pig Market

In Europe is calculated per capita pig meat consumption around 40.4 kg taking the balance between pig meat production, imports and exports (Eurostat). More than half of the pig meat is consumed in further processed form in a wide range of products, while fresh pork is normally consumed in a lesser quantities. According with Verbeke et al., (2011) although pork occupies a very high share in the total meat consumption of many people, pork's image among consumers is not

univocally positive. In general consumers choose, to buy pork, because cheap and convenient for daily use, but they also perceived it as fattest and least healthy meat compared to poultry and beef. A different consumption patterns are present in EU, reflecting cultural differences and differences in traditional eating habits. Pork consumption frequency differed significantly among countries, ranging 9,4 times per week in Belgium, 13,9 times per week in Poland, 10,8 in Denmark and 11,6 in Germany. Trade of pig meat in EU, where distances are relatively short is traded mainly in chilled form. Chilled pork has a shorter shelf life than chilled beef. World trade in chilled and frozen pork is generally less sophisticated in terms of cuts and specifications than trade in chilled and frozen beef, with the main exception being the EU and North America. In the importing countries, most frozen pork is used in further processing for products such as smoked or cooked sausage. Pig meat sausages are the most important product, followed by preparations of pig meat, especially canned preparations. Trade in bacon and ham is also important.

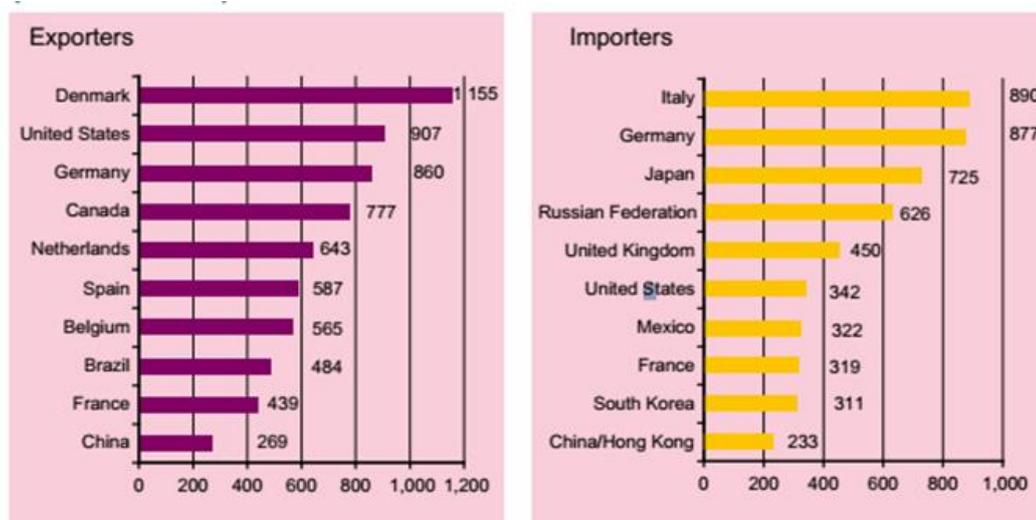


Figure 3 Top ten chilled/frozen pork trading countries. (FAO 2009)

The main import of the EU countries is pork for further processing plus limited quantities of pork for the fresh market. Almost all of the pork imported by EU countries is imported from other EU countries. Japan imports chilled pork from North America for the fresh meat market and frozen pork for further processing in

Japan. The Russian Federation imports lower-quality frozen pork for further processing. EU exporters dominate trade in the different types of further processed pig meat products, with Denmark in the lead, supplying other EU countries but also exporting to non-EU markets. The only other major exporter is China. Outside of the EU, the main importers of further processed pig meat products are Japan and China.

## 1.2. Muscles

One of the main sources of protein in the human diet is meat, that is the product resulting from the transformation of muscle tissue after stopping the bloodstream and then the inflow of oxygen (Gambacorta et al., 1995). The muscular apparatus is constituted by the association of about 600 muscles, of different shape and diffused on the surface and in depth, more or less involved in the fulfillment of movements. Muscle tissue can be distinguished in striated or voluntary (directly or indirectly associated with the movement of the skeleton and under control of the central nervous system), and smooth or involuntary (associated with intestine, glands, blood vessels and other organs). The only exception of this distinction is the heart which is a striated muscle but involuntary. The striated muscle is formed by the association in muscle

tissue of a large number of muscle fibers in association with fat cells, connective tissue, capillaries and nerves. The muscle fibers which are arranged in parallel to each other and linked together by a

connective network, vary

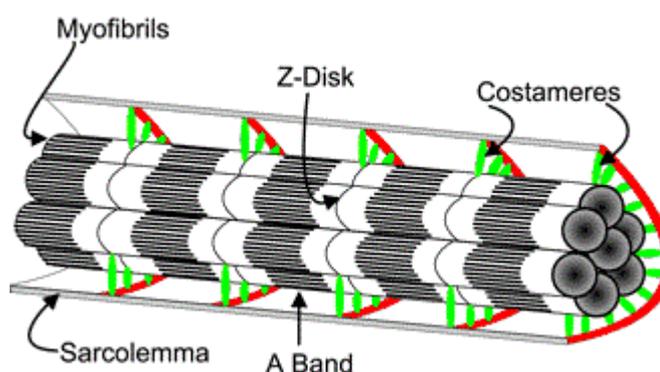


Figure 4 Structure of a muscle fiber.

according to species, breed, sex, age, muscle considered, nutrition, exercise, postnatal body weight, growth promoters (substances  $\beta$ -agonists, hormones, etc.). The fiber is bounded by a membrane, the sarcolemma. Within the sarcolemma, immersed in the sarcoplasm, there are the contractile structures i.e. the (myofibrils). The structural filaments that link the myofibrils to the sarcolemma, with the Z disk of peripheral myofibril, are called costamere proteins (fig.4), within which find proteins such as  $\gamma$ -actin vinculina, P-spectrin, talin and intermediate filaments such as desmin located along the myofibrils in the direction of the band I. Vinculin and desmin from the costamere structure extend to surround Z-disk. Other the structural function has been suggested that costamere protein may minimize the stress experienced by the sarcolemmal bilayer during forceful muscle contraction or stretch. (Ervasti, 2003). In the cytoplasm, are also present other cellular organelles such as mitochondria responsible for the production of energy substrate (ATP) used by muscles for movement.

### **1.2.1. Fiber types**

The muscle volume is occupy from 75-90% of muscle fibers (Lee et al., 2010). The classification based on solubility, suggest that in the skeletal muscle there are three groups of protein: sarcoplasmatic, myofibrillar and stromal. Muscle contraction is attributed to myofibrillar proteins and one of the most representative myofibrillar proteins is myosin heavy chain (MHC) (Lee et al., 2010). Is possible distinguish the MHC isoform into four types that can coexisting in the same muscle, depending on the speed of contraction and the type of consumption of ATP (Ryu et al., 2011):

- Type I fiber (oxidative) at slow-twitch, with high aerobic and then low-capacity anaerobic. They are also called red fibers to the high content of myoglobin (a protein similar to hemoglobin that carries oxygen from it to the numerous and well-developed mitochondria).

- Fiber type II A(oxidative): fast fibers in mixed aerobic and anaerobic capacity.
- Fiber type IIB (glycolytic) fast fibers with low aerobic capacity and high glycolytic capacity. Fibers have rather thick, poor mitochondria with significant activity but easily fatiguable.
- Fiber type II X (intermediate) fast-twitch fibers with high glycolytic capacity and good aerobic capacity, they are intermediate to type IIA and IIB fibers in regard to fiber size and oxidative potential (Delp et al., 1996)

Their quantitative, depends of age, gender, hormones, breed and physical activity (Lee et al., 2010). They can modulate their metabolic capabilities in basic training as well as change from one to another fiber type in response to hormonal signals, altered functional demands or changes in neural input. The succinate dehydrogenase activity decreased in the rank order IIA > I > IIX > IIB (Tasić et al., 2011). In general the fibers of type I and II X are employed to efforts of long duration and intensity not high, while the fibers II A and II B to efforts of short duration and high intensity. These types of fibers are to be undifferentiated at birth for the presence of slow and fast myosin isoforms, which are then differentiated by the time of birth. Some studies have shown also the hypothesis that the IIB fibers are in preponderance in the small mammals and that preponderance tend to decrease with increasing size. The reason of this is explain for the greater need in the smaller animals to use the IIB fibers for their reliance on faster velocities of contraction during the locomotion (Delp et al., 1996).

### **1.2.2. Pre-natal myogenesis**

One of the major factor that influence of performance is the muscle growth rate that is correlated with the feeding strategy, gain to feed ratio , the size and the number of the myofibers. The latter is the main factor that influence the muscle mass (Oksbjerg et al., 2004). These values depend by a complex of pre- and

postnatal event. Skeletal muscle fiber formation occurs during prenatal development in different, though overlapping phases, embryonic and fetal development, perinatal development and postnatal growth. Myogenesis process lead at the formation of muscle tissue from the fusion of myocytes, formation of myoblasts and subsequent fusion into multinucleated fibers called myotubes. The process of formation of muscular fiber follow a precise sequence of phases, first of all, the paracrine factors induce myoblast to express MyoD protein, through activation of the transcription factors Pax3 and Myf 5 and migrate away from the dorsal region, the subsequent phases will then be, Proliferation, Determination and Differentiation. For the determination of the myoblast are mainly involved two family of regulatory protein, MyoD family and MEF2 (o MADS box). Myoblast lead to an increased number of cells and during the fusion release insulin-like growth factors that help the fusion of other myoblast (Polesskaya et al., 2007). In adult skeletal muscle myoblasts some residues persist in the form of quiescent stem cells (Kuang et al., 2006). Pig myogenesis as in many mammalian species is a biphasic phenomenon with the sequential formation of two generation of muscle fibers. A primary generation forms from 35 until 55 days of gestation (dg) and came from embryonic myoblast lead to increases the primary muscle fibers, followed by a second generation that comes from fetal myoblast and increases the secondary fibers (Oksbjerg et al., 2004; Lefaucheur et al., 1995). During the post natal phase, the increase of skeletal muscle is exclusively due to an increase in the size of muscle fibers formed in the prenatal stage (hypertrophy). Hypertrophy requires addition of new myonuclei that are procure from satellite cells that are stored on the surface of the skeletal muscle. These cells are activated by hormones, cytokines and molecules similar at hormones (White et al., 2010). The growth factors stimulate the division and differentiation cells and of most interest are IGF (insulin growth factor), FGF (fibroblast growth factor) and HGF (hepatocyte growth factor), these growth factor cooperate for the hypertrophy muscular.

### 1.2.3 Contraction

The striated muscle fibers have a longitudinal streak plus transverse striations. The first is formed by bundles of myofibrils. The myofibrils are transverse light bands and dark bands alternating with each other. The light bands are divided in the center by a dark line called "Z disks", while the dark bands have a clear zone called "streak H", divided in turn into two fields by a "A-band". The contractile unit of the myofibril is the sarcomere which is the portion between two Z disks. The contractile mechanism takes place between the light bands and is due to the reversible superposition of the two contractile proteins: myosin and actin. The mechanism of voluntary contraction is controlled by stimulation of the central nervous system through the endplates that activate the muscle fibers. The muscle contraction is due to the shortening of the sarcomere to overlap, due to the sliding centripetal of actin filaments and myosin. For contraction to begin it request an increase of intracellular calcium that lead to a change of the conformation of troponin and afterwards of the tropomyosin (responsible to hinder the formation of the bound actin-myosin). This change of the conformation, allow the formation of the bound actin-myosin. In this way, during the contraction, two Z lines contiguous approach. The sarcomere to shorten needs energy, which can be obtained by reductive cleavage of adenosine triphosphate (ATP) thanks to the activity of myosin ATPase or, in a faster manner, the actomyosin complex. The concentration of ATP available present in the muscle result to be enough only for a few contraction so for fulfill at this rapid energy demand in addition at mitochondrial oxidative metabolism act phosphocreatine, present in high concentration within the muscle and convert ADP in ATP and myokinase that catalyzes the conversion of ADP in AMP and ATP, these reaction together allow to enough the high demand of energy when oxygen is adequate during muscular activity (slow activity of the muscle) and keep constant the ATP concentration and maintain homeostasis. (Scheffler et al., 2007); Mechanism controlling pork quality development: The biochemistry controlling postmortem energy metabolism. When the contraction of the muscle, is more rapidly, the oxygen

result in deficit for the aerobic metabolism, than anaerobic glycolysis supply the required energy. In the muscular contraction

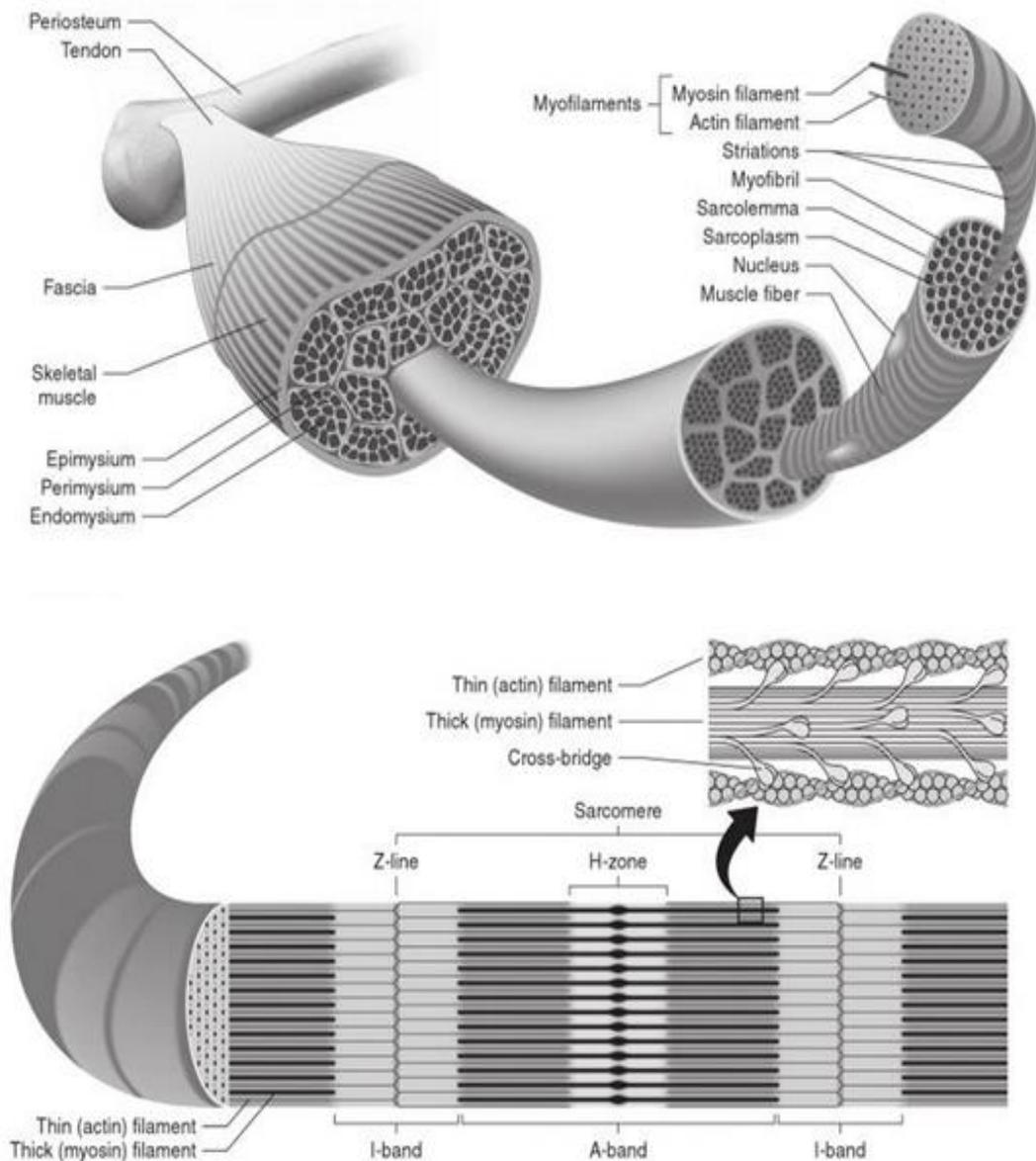


Figure 5A typical muscle comprises bundles (fascicles) of muscle fibres. Each muscle fibre, in turn, contains main myofibrils that are made of repeating series of sarcomeres. (Abernethy et al., 2013)

phase one molecule of myosin binds to ATP, forming the complex myosin-ATP that binds in turn to a molecule of actin. The complex actin-myosin-ATP can be

hydrolyzed, producing energy which is necessary for contraction, releasing adenosine and organic phosphate. This rigor that is established in the fabric remains until a new molecule of ATP binds to myosin, so as to allow the complex myosin-ATP to recommence the cycle, thereby presence of ATP appears to be extremely important for the contraction because: is hydrolyzed during each cycle of attack, displacement and detachment of cross bridges between actin and myosin; feeds on the membrane calcium pump, makes two important cycles biochemical, oxidative phosphorylation (aerobic process) and glycolysis (anaerobic process).

### **1.3 Conversion from muscle to meat / post mortem event**

The muscle after slaughter is not ready to be eat whereas shows a high value of toughness. In this phase the muscle structure change, detachment of sarcolemma from myofibrils, degradation of costamere attachment to sarcomeres, the fragmentation of muscle fiber with breakdown before of Z-lines and after of M-lines, leads to tenderness development (Christensen, 2002). The process that convert the muscle to meat involves biochemical reaction (that will be action on the meat tenderness) mainly in phase of post-mortem proteolysis, passing through three stage, that in order are *Pre rigor*, *Rigor mortis* and *Post rigor*. After slaughter the muscles remain functional and metabolically active for several days although depleted of the circulating blood and oxygen lead at change of the type of metabolism from aerobic glycolysis to anaerobic glycolysis. First of all the creatine kinase and myokinase supply ATP necessary to regenerate this last from ADP and AMP until 70% of PCr pool has been degraded, subsequently the concentration of ATP decreases rapidly. For supply energy, than muscle glycogen must be degraded in ATP metabolism switches from aerobic to anaerobic glycolysis to rephosphorylate ADP (Sheffler et al., 2007). When the rate of breakdown exceeds the rate of the ATP rephosphorylation, the formation of

actomyosin bonds leads to the rigor mortis. This result in heat, lactate,  $H^+$  which determines a decline of pH that decrease averagely from 7.4 to an ultimate value of approximately 5.5 when the generation of ATP is halted and correspond also to the end of *rigor mortis*. When the pH is still above 6 the concentration of free calcium ions within the sarcoplasm is kept low by transportation of calcium into the sarcoplasmic reticulum (Kristensen, 2003). With the progress of anaerobic glycolysis and pH drop the calcium pumps cease their activity leading a large increase of free calcium concentration that proceeds with the complex actin-myosin formation within the myofibrils and involving the muscle contraction (*rigor mortis*). The bound rupture actin-myosin is an energetic process that requires the use of ATP. This process consumes the remaining ATP that will not be enough for all the bound. The result is a decrease of the sarcomere length that leads an increase of the meat toughness witch reaches the maximum in the time of rigor.

### **1.3.1 Post mortem proteolysis and enzymes activity**

It is known that post mortem proteolysis improves meat tenderness (Pomponio et al., 2010). In order to consider involved in *post mortem* tenderization, proteases, must have access to the substrate, and must, be located inside the skeletal muscle and their activity should be reproduced in an *in vitro* system (Koochmaraie, 1994). The main groups of proteolytic enzymes involved in meat tenderization are, the calcium-dependent calpains which have an optimum activity at a pH range from 7 to 7,5 and the lysosomal cathepsins which are optimally active a pH lower 6 (Christensen , 2002). The calpains enzymes, have been divided on the basis of different request of calcium into  $\mu$ -calpain which requires 3 to 50  $\mu M Ca^{2+}$  for half-maximum activity and m-calpain wich requires 400 to 800  $\mu M Ca^{2+}$  for half-maximum activity (Koochmaraie, 1994). Both  $\mu$ -calpain and both m-calpain begin an autolysis process in presence of  $Ca^{2+}$  begin a autolysis process that leads to an

increase deeply of  $\text{Ca}^{2+}$  affinity (DeMartino, 1986). Goll et al (1992), in an *in vitro* experiment, showed as autolysis is multistep process that leads at the inactivation of the enzymes. *In vitro* studies put in evidence as in 10-15 minutes the muscular cells can destroy all myofibrillar Z-lines (Goll et al., 1995). This effect was not shown in *in vivo* studies, so to better understand the action of the calpain is necessary to include the interaction with the protein inhibitor, calpastatin (Pringle, 1994). The amount of calpastatin in *ante-mortem* tissue can predict the meat [tenderness], more high will be their contents, more low will be the meat tenderness (Woodward et al., 2000). The result of different studies have shown that the main role in the meat tenderization is played by  $\mu$ -calpain because the m-calpain activation requires high levels of  $\text{Ca}^{2+}$  that never are achieved in the cell so the role of cathepsin is not yet completely elucidated (Veiseth et al., 2001; Christensen, 2002). Almost all of the proteins costamere are degraded *in vitro* in the presence of calpains (Cottin et al., 1992), unlike of the  $\alpha$  actin and-actin. The structure costamere especially desmin, nebulin, vinculin and talin, is degraded within 72 hours of slaughter, and this process is strongly correlated with the distress. Furthermore the acidification of the muscle causes loss in water holding capacity (WHC). High levels of drip loss can occur in pig meat, sometimes up to 12% of carcass weight (Paredi et al., 2012). Other studies shown as pork meat tenderization is about twice as fast as beef, it been suggested that beef should be stored for a period of ten days and pork carcasses for four days (Dransfield, 1994).

### **1.3.2 Tenderization Post mortem**

After *rigor mortis* the muscle tension decrease with *post mortem* storage through the proteolysis of the myofibrillar and loss of the muscle structural integrity. The tenderness value depends by a wide range of factors, like sarcomere length, quantity and quality of connective tissue, intramuscular fat, water holding capacity, rate of protein degradation in *post mortem*, that are in close correlation

with genotypes and breeds, management system, feeding strategy and diet composition, pre-slaughter handling and stunning, slaughter method, aging time, chilling and storage condition. Genotypes and breeds are closely related with the muscle structure (n° Myofibril, marbling fat and connective tissue), the number of muscle fibers and with the potential pig's growth. A high percentage of the oxidative fiber type and low percentage of the glycolytic fiber type were shown to be related to high pH value and to dark muscle color (Fielder et al., 2004). Management system is also correlated with tenderness and previous studies shown that conventional pork meat is often more tender than meat from organic pork production systems (Danielsen et al., 2000). It also acknowledged the importance of correct feeding strategies on growth rate, body composition and muscle development. Andersen et al. (2005) found that restricted feeding strategies determined a lower eating quality (tenderness) compared with *ad libitum* feeding in pigs and feeding strategies based on pasture may lead to lower eating quality such as lower tenderness and off-flavour ratings compared with grain-fed animals . During muscle growth protein turnover is increased with the increase of the rate of protein degradation although to a lesser extent than the rate of the protein synthesis. The rate of protein degradation seems to have a positive effect on postmortem proteolysis and therefore affect the meat tenderness (Therkildsen et al., 2004). It has been shown that it is possible to increase the protein turnover exploiting the compensatory growth and consequently improve the phenomenon of postmortem proteolysis and then the tenderness of the meat (Therkildsen et al., 2004). Regarding the feeding composition previous studies shown that there is a significant effect with the interaction with the breed thereby became necessary that protein and energy levels in pig diets are adjusted in relation to genotype (Wood et al. 2004 ).

## 1.4 Quality in pork meat

Pork meat quality has been variously defined, thus resulting in considerable confusion within the industry. A general perception includes those factors associated with quantitative yields, as well as those factors contributing to palatability. The meat scientist defines fresh meat quality as those factors associated with the palatability of fresh and cured products and economic losses during processing and distribution. The consumer perception of pork meat quality is tied to tenderness, juiciness and flavour of the cooked product. In carcasses from young swine, the factors most commonly associated with these traits are colour, texture and firmness of muscle and quantity of intramuscular fat (marbling). First of all to be suitable at the human consumption, the meat must have a content of fat and connective tissue not exceeding the value shown in table. The swine carcass is : "The body of a slaughtered pig, bled and eviscerated , whole or divided in half, without tongue , bristles, nails, genitals, peritoneal fat, kidneys, diaphragm, abdominal and thoracic organs . In the last two decades the concept of quality in pork meat, has become increasingly important, "The CEE regulations, provide that, in all EU slaughterhouses in which are slaughtered over 200 pigs per week, the commercial value of the carcass is determined by evaluation of lean meat and weight. May exempt from this assessment, slaughterhouses in which are slaughtered, from 11 to 200 pigs per week.

Table 2 Limit values of fat and connective tissue in the meat

Animal Species	Fat (%)	Connective tissue (%)
Pigs	30	25
Birds and rabbits	15	10
All other red meats and mixtures	25	25

The assessment of the lean is made through the use of proven methods based on measurement of one or more anatomical parts and are made with appropriate instruments like for example the Fat-o-Meat'er (FOM). According to EU guidelines, the lean meat content of the carcass is the result of the relationship between the weight of the red striated muscles obtained by total dissection of the carcass and the weight of the carcass. After the classification carcasses are labelled with capital letters indicating the category of weight (H = heavy, L = light) and the class of fleshiness (E, U, R, O, P) or, alternatively, with the letter indicating the category of weight followed by the percentage of lean meat. Each country has also the possibility to introduce the special class S in the case of the evaluation of carcasses from strains characterized by muscular hypertrophy, weighing less than 110 Kg. The benchmarks are as follows:

- S = % lean meat > 60
- E = % lean meat between 55 and 60
- U = % lean meat between 50 and 55
- R = % lean meat between 45 and 50
- O = % of lean meat in between 40 and 45
- P = % lean meat < 40

The pork meat industry give a multiple definition of high quality meat which is characterized by a combination of objective and subjective measurements which vary across national and international market. Some of the most common measurements used in determining meat pork quality are firmness, marbling, colour, pH and water holding capacity. Cantoni (2013) states that a quality product is due to the 50% to the farmer and the other 50% to slaughter and handling techniques. The breeders is responsible of the selection of genotype, quality of feeding, type of farming and transport of the animals to the slaughterhouse. The manufacturer assumes the responsibility to optimize the conditions before and after slaughter, the handling practices to ensure the quality optimum of the meat. The fundamental attributes of the raw material are:

- Juiciness, tenderness and flavour;
- WHC or drip-loss;
- Intramuscular fat;
- pH;
- Colour;

In practice, the parameters that are evaluated are

- colour;
- pH

Colour measurements are done using the Commission International De l' Eclairage (CIE) colour system (Commission International De l' Eclairage, 1976). Three fundamental colour coordinates are L\*, b\* and a\*. The L\* measures the lightness and is a measure of the light reflected (0 = black; 100 = white); a\* measures positive red, negative green and b\* measures positive yellow, negative blue (Commission International De l' Eclairage, 1976). Carcasses with L\* value 49–60 were classified as normal pork quality. Desirable lean quality in fresh pork is described as reddishpink in color, firm in texture and free of surface wateriness (non-exudative). Such high quality lean is identified as RFN. Quality variations from this ideal result in the less desirable quality extremes of pale, soft and exudative (PSE) lean, and dark, firm and dry (DFD) lean ( The measurement of color through the reflection of light (Chromameter Reflectance) to classify the quality of pork into four categories:

- RFN (red, firm, non-exudative) carcasses with pHu <6,0 and L\* value 49–60;
- RSE (red, soft, exudative) carcasses with pHu >5,5 and L\* colour score of <50;
- PSE (Pale, soft, exudative) carcasses with pHu <5,5 and L\* colour score of > 50;
- DFD (dark, firm, dry) carcasses with pHu >6,1 and L\* values from 42 to 48;

Fresh pork meat must have a good aspect for being an attractive to the consumer and meat colour makes the first impression. The assessment of the colour may be scored either objectively with a Minolta or other device, or visually by a trained person using a colour scale. The Minolta lightness ( $L^*$ ) score is produced by measuring light reflection from the surface of meat. Regarding pH, in a live muscle the value is of 7,0 to 7,2. As live muscle converts into meat, pH drops causing meat to become increasingly acidic. Both the rate of this change, and the final pH level are important in determining pork quality.

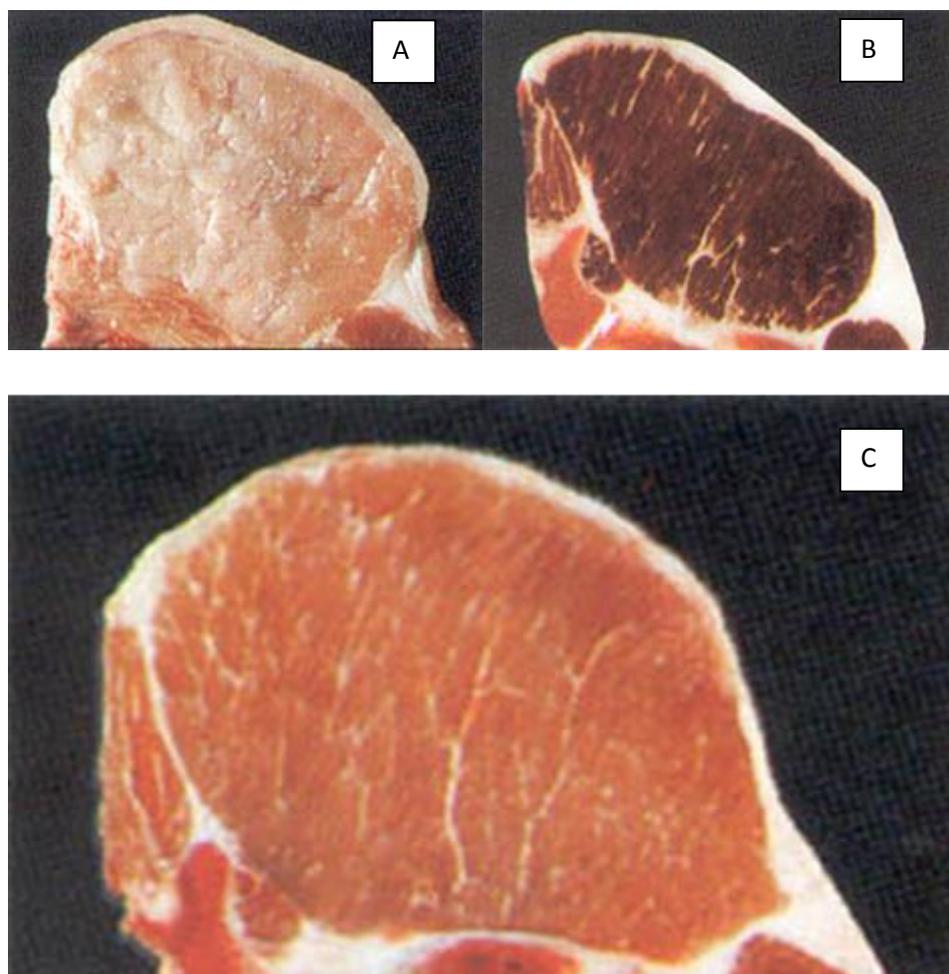


Figure 6 A) meat sample that present PSE, B) meat sample that present DFD and C) meat samples that present RFN.

The measured of pH is generally made within one hours of slaughter (initial pH) or within 24 hours (ultimate pH or pHu). According Rosenvold (2002) the evolution of early pH is more significant parameter for the forecast of the WHC in pork meat, than pHu. Generally, if initial pH is below 5,8, will be easy to find a PSE condition in the meat because pH dropped both too low and too quickly lead an ultimate pH most likely below 5,5. An opposite condition, meat with an ultimate pH above 6.1 may be classified as DFD, because pH did not drop to normal levels. A initial pH between 6.7 and 6.3, and pHu between 6.1 and 5.7 is considered the preferred range for a good quality meat. One of the traits that pH affects, is water holding capacity. This is a parameter that determines both drip loss from raw pork, and cooking loss during preparation. Meat which does not “hold water” is undesirable for further processing and fresh consumption. Drip loss above 5% and cooking loss above 25% indicate a quality problem. Whole loin package purge should not exceed 3%. Firmness is measured on a scale from 1 (very soft) to 5 (very firm). Marbling is measured on a similar scale of 1 (practically no marbling) to 10 (abundant marbling). Some export markets require a higher marbling score.

#### **1.4.1 PSS (porcine stress syndrome)**

Is a particular condition of the animal metabolism, that bring at abnormal biochemical postmortem activity and lead to a reduction of meat quality. The genetic influence on swine meat quality, include difference among animals within the same breed and is associated both a large number of genes with a small effect, knows as polygenic effects, and monogenetic effect which are knows as major gene (Rosenvold et al., 2003).

Major gene are:

- Halotane (HAL) gene is responsible of the Porcine Stress Syndrome. There are three halothane genotypes in pigs (NN, Nn and nn). Pigs

homozygous for halothane positive (genotype nn) are subject to deaths during transit to and during lairage at slaughter plants (Murray and Johnson, 1998; Fisher et al., 2000). Recent advance in the understanding of the regulation of the muscle contraction has led to the identification of a mutation in the ryanodine receptor in the sarcoplasmic reticulum calcium release channel that has been correlated with PSS and malignant hyperthermia. Stress-susceptible pigs respond to halothane anesthesia with limb muscle rigidity, increased body temperature and anaerobic metabolism (De Oliveira Band et al., 2005). In general pigs heterozygous and homozygous for HAL present, higher carcass yield, larger *longissimus dorsi* muscle area, and greater lean percentage. This positive effect are counterbalanced by a negative effect on colour and WHC (Zhang et al., 1992; Rosenvold, 2002). Pigs carriers of this gene are highly susceptible to stress and also following the correct transport procedures, in compliance with the animal welfare condition before the slaughter and the correct handling procedure of the carcass, the stress of the animals is enough to trigger a higher rate of *post mortem* glycolysis, that lead low pH values early *postmortem* that in combination with the simultaneously high temperatures, lead to the development of PSE meat characterised by high protein denaturation.

- Rendement Napole (RN) is a swine gene found to cause low ultimate pH and low WHC. The gene is also commonly called the "acid meat gene" or "Hampshire effect" due to the low ultimate meat pH that it causes and because this gene has primarily been observed in purebred or crossbred Hampshire populations. RN is associated to an extend pHu decline post-mortem and to greater glycogen store in the muscular cells. In the early post-mortem this gene have no effect its action takes place in pHu causing an inferior WHC and higher reflectance (lighter meat). The latest researches have shown like the decrease in WHC is due to a more pronounced proteolysis of sarcoplasmic protein and myosin tail. The technological yield is reduced by 5% in the meat that carries RN compared

with non-carrier with the consequence that processing industry has a strong interest to discriminate meat from the two genotypes.

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Polygenic effect:

- With the exception of the major gene the heritability of pork meat quality attribute is low or moderate, except for the intramuscular fat. An increase in intramuscular fat is normally associated with an improved eating quality. However, some discrepancy exists in the literature as reported by Fernandez et al., (1999). Although the heritability of intramuscular fat percentage and fat tissue is high the genetic correlation between them is low (Wood, 1990) . This suggest that the selection for high intramuscular fat in lean carcasses should be possible.

#### **1.4.2 PSE Meat**

One of the main economic losses in the pig industry is related to PSE syndrome, which originates from animal stressed and depends upon pig genetics and animal handling conditions before and during slaughtering. Fisher et al., (2000) have shown the correlation between PSE and PSS, respect the halotane genotype carriers pigs which shown PSE had the following genetic combination NN=8%, Nn=42% and nn=100% . After the discovery of the gene responsible of PSS which is correlated also to the high incidence of PSE, some countries like Denmark, The Netherlands, Switzerland and Sweden, eliminated in their selectionprograms (Rosenvold, 2002). PSE is characterized by an abnormal colour, consistency, and WHC, making the meat dry and unattractive to consumers. PSE is believed to be caused by metabolics impairs, after slaughter, energy stored in the muscles is rapidly depleted leading to rapid lowering of pH within the muscle fibers. Genetic predispositions and stress levels prior to slaughter are known to increase the incidence of PSE meat and several factors have been identified as playing a role in the occurrence of PSE. Genetics

(relative to the Porcine Stress Syndrome) and use of growth promotants (Paylean™) in swine diets have been extensively studied and their effects are well documented (Allison et al., 2005; Armstrong et al., 2004). Hambrecht et al. (2005) stated the greatest improvements in pork quality could be achieved by decreasing stress in the immediate pre-slaughter period. De Oliveira Band, et al., (2005) suggest that heavier pigs show a greater tendency to develop PSE meat. This due mainly from two factors, the first is tied to the larger volume/surface ratio that take longer to cool and the other is tied to the muscle glycogen content that is higher in heavier pig, leading to a greater decreases in muscle pH after slaughter.

### 1.4.3 DFD meat

The dark, firm, dry DFD (also known as dark cutting in beef), is related to acid production in pork muscle after slaughter. PSE develops, is due an accelerated lowering of pH while muscle temperature is still high, while the opposite happen in DFD that is the results of a deficient production of lactic acid in the muscle.

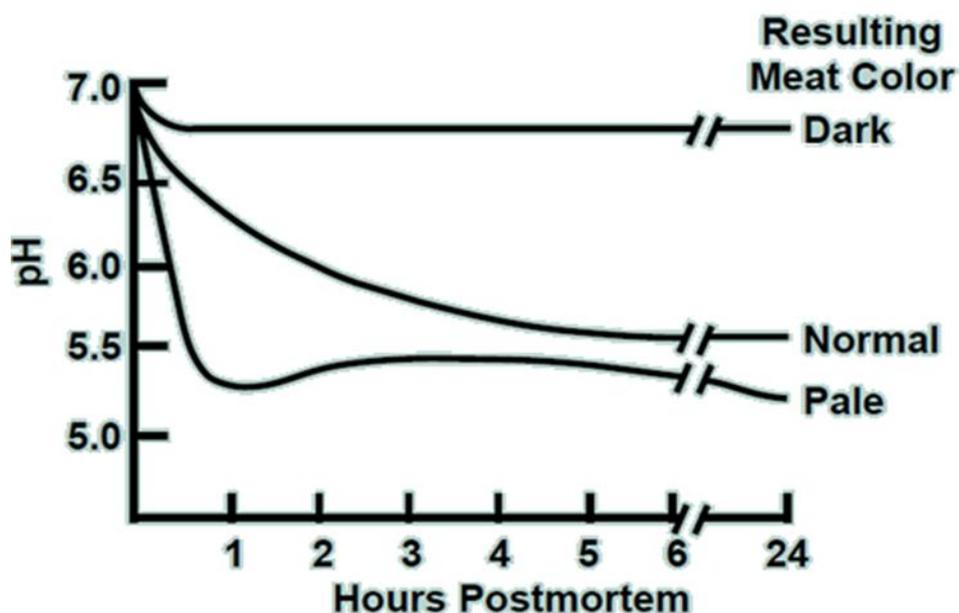


Figure 7 Trend of pH in the first 24h postmortem

DFD animals have low levels of muscular glycogen at slaughter with a low lactic acid production and limited pH fall.

While normal muscle has a final pH values around 5.5., DFD muscle has an ultimate pH above 6.0. The reduced acidity increases the water-holding ability in the lean, tightly binding water to muscle proteins, contributing to a firm texture. Muscle cells swollen with retained water and tightly packed together absorb more light (darker colour), and also restrict how deeply oxygen can penetrate into the tissue to "brighten" muscle pigment.

#### 1.4.4 Intramuscular fat

While colour, firmness and exudation are important considerations in evaluating lean pork quality, marbling or intramuscular fat content of the lean, is an important factor especially for the improvement of the taste, than consumer satisfaction (Rincker et al., 2008). Marbling contributes to

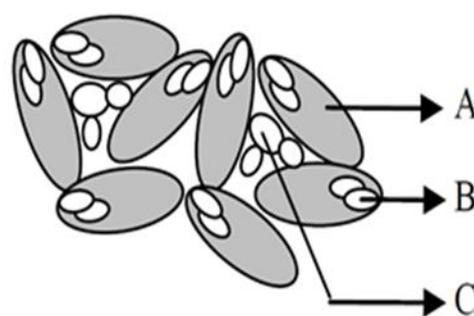


Figure 8 Illustrating of the difference between intramyocellular lipids (B) and extramyocellular lipids (C) within and between muscle fibers (A).

the juiciness and flavour of the meat, recent efforts made in the recent decades to obtain leaner pigs has generally reduced the marbling content of the lean, increasing the potential for less desirable eating satisfaction by consumers. In selecting pigs for desirable lean quality, marbling should be considered. Some genetic lines producing higher levels of marbling in the lean, have been used to niche market branded products valued for their high marbling content such as Berkshire Gold. As said, intramuscular fat (IMF) is a parameter of considerable importance in meat eating quality (Verbeke et al., 1999) and is a positive value that improve overall liking of pork meat and could improve also softness and tenderness. An IMF content below the recommended optimum range of 2,5-3%

diminishes eating quality while a higher IMF content does not further improve this parameter and will have adverse effect on consumer acceptability due to increased visibility of fat in the meat. It is important to said that the acceptability value IMF are different by country, for example in China or USA a value above 5% of IMF is habit in traditionally produced local pigs (Shi-Zheng et al., 2009). The IMF consist mainly of adipocytes (extramyocellular lipids, EMCLs) and myocytes (intramyocellular lipids, IMCLs). IMCLs are localized within muscle fiber cytoplasm and EMCLs are localized in adipocytes interlaced between muscle fibers. The lipids content in IMF can be subdivided in mono- and diacylglycerols, free fatty acids cholesterol and cholesteryl esters but the major constituent are phospholipids and triacylglycerols (TG) (Shi-Zheng et al., 2009). Phospholipids are constituents of cellular membranes so their contents is almost the same within the similar muscle and different between muscle (Shi-Zheng et al., 2009) and increases like TG from white glycolytic to red oxidative muscle types (Bonon et al., 2007). Triacylglycerols are stored in myocytes and adipocytes and represent a major form of energy storage for cellular metabolism (Vessby, 2000). Plasma lipid concentration play a role in the rate of uptake of free fatty acids (FFA) into muscle tissue. FFA can be converted in lipid droplets or to various signal molecules like fatty-acyl-CoA (involved in synthesis of sphingolipids, phospholipids, eicosanoids, etc.). An excess of these molecules can lead at lipid-mediated insulin desensitization. To be used as fuel by skeletal muscle, FFA must be converted in the cell to long-chain fatty and subjected to  $\beta$ -oxidation (Shi-Zheng et al., 2009). In fig. 6. is shown briefly how FFA can used for energy production through  $\beta$ -oxidation or undergo conversion to various signaling molecules. Is well known that the anatomical location of the fat deposition in pig carcass, are four: visceral, subcutaneous, intermuscular or intramuscular (Narv ez-Rivas, et al., 2009). Fat deposition is affect by the system of feeding, the anatomical location and the breeds. Ram rez et al.( 2007) showed how in rustics breeds the lipogenic enzyme activity is higher than conventional breeds.

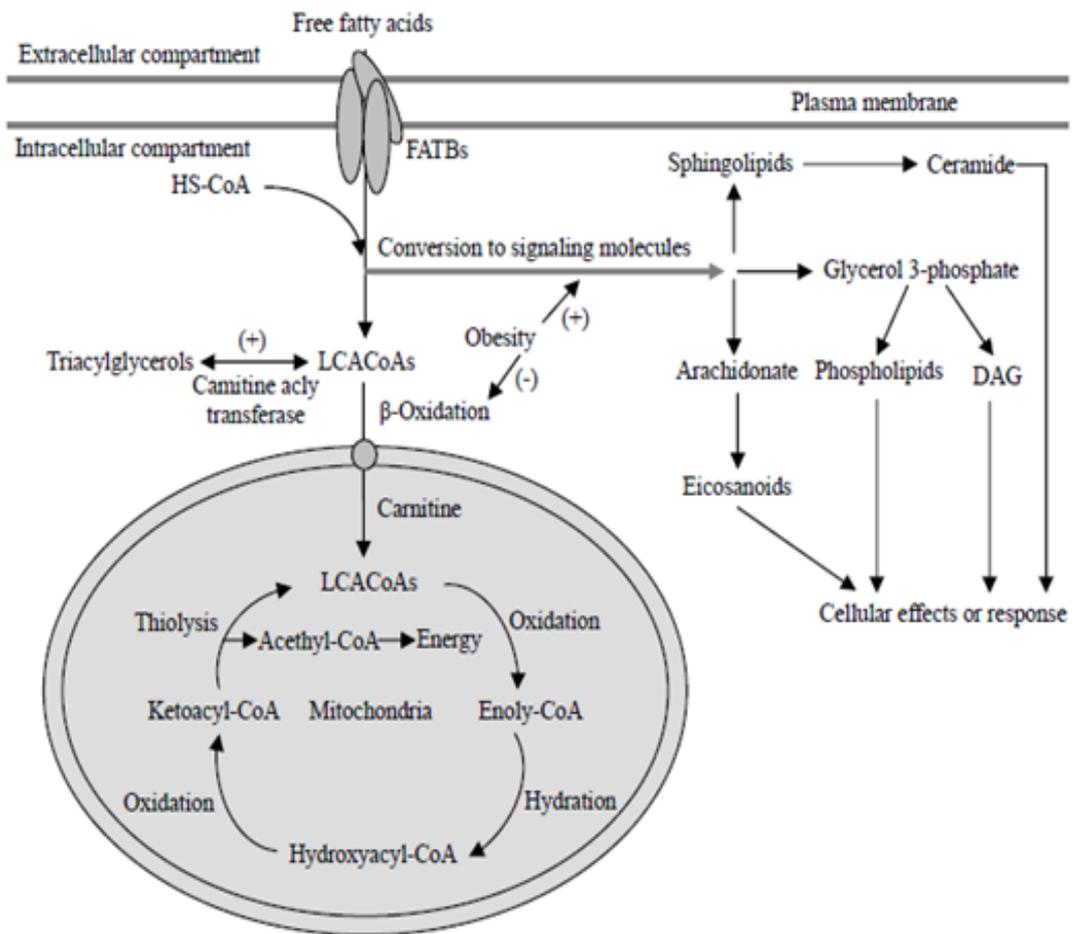


Figure 9 Metabolism of free fatty acids.

### 1.4.5 Lipid peroxidation

Lipid peroxidation (LP) is the oxidative deterioration of the unsaturated lipids. As said before the cellular membrane is rich of polyunsaturated fatty acids (PUFAs) that play an important role on the fluidity of the membrane from which depend the mobility of membrane proteins, electrical resistance and the phase properties (Devasagayam et.al., 2003). Fatty acids with more than one double bound are particularly susceptible to action of freed radicals which through a series of chain reaction leads to distrupts biological membranes and the production of a large number of by-products. Many of the latter are toxic and can be implicated in the pathogenesis of a number of disease and clinical condition, like diabetes, varius

chronic inflammatory conditions, fibrosis, cancers and many others diseases (Devasagayam et.al., 2003). The reaction of LP consist of three major steps: Initiation, Propagation and Termination.

- Initiation: is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as hydroxyl radical, ( $\bullet\text{HO}$ ) $\cdot$  and hydroperoxyl radical ( $\text{HO}_2$ ), which combines with a hydrogen atom to make water and a fatty acid radical.
- Propagation: The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxy-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way
- Termination: When a radical reacts with a non-radical, it always produces another radical. The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals.

The end product of LP are reactive aldehydes, of major toxicological interest are malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and various 2-alkenals. the LP may be affected directly by the degree of unsaturation of fatty acids, UV-rays, temperature and singlet oxygen or indirectly by the transition metals, heat and UV-rays. Living organisms have different molecules that speed up termination by catching free radicals and, therefore, protecting the cell membrane. One important such antioxidant is vitamin E and vitamin C (work mainly inside the cytoplasm). Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase. For the assessment of the by-products of LD, the most common method used is the estimation of aldehydic products by their ability to react with thiobarbituric acid (TBA) than yield “thiobarbituric acid

reactive substances” (TBARS), which can be easily measured by spectrophotometry.

#### **1.4.6 Tenderness**

Eating quality, usually defined as scores given by taste panelist for tenderness, juiciness and flavor is one of the most important factor appraised by the consumer, after the purchase (Wood et al., 2004; Andersen et al., 2005). In the last decades a lot of studies have been carried out to try to improve this parameters. Regarding tenderness, exist different operation that can improve its evolution. Some studies have shown the importance of the carcass suspension, changing the modality of the suspension from the Achilles tendon to the pelvic floor, improve significantly the sarcomere length, decrease Warner-Bratzler shear force, cooking loss, improve the WHC and improving technological yield (Fischer, 2000; Moller et al., 1986; Smuldres et al., 1990). Another factor that contributes to the performance of tenderness is the chilling rate of the carcass, that can influence technological and quality parameters of meat like weight loss, tenderness, WHC, colour (Savell, 2005). If muscle is cooled below about 10°C before the onset of rigor the subsequent meat is tough after cooking. The phenomenon is referred to as cold shortening. The mechanism of cold shortening is thought to be stimulation, by the low temperature, that lead to a massive release of calcium ions consequently to the break of calcium pumps. A different response by low temperature, seem to be between red (oxidative) muscles and withe (glycolytic) muscle. Red muscles seems to be more susceptible to the low temperature, showing a more intense shortening. In addition to the cold shortening the rate of chilling has significant effect also on the incidence of pH related-PSE meat (Offer, 1991). Conventional, spray and rapid/accelerated chilling system are commonly used for pork chilling in the commercial practice today (Tomović, et al., 2008). The use of different accelerated chilling system can be a method to reduce the incidence of pale, PSE in pork, on the other side if temperature

decreases too rapidly cold shortening could occur. In general white muscles have more rapid post-mortem glycolysis so are less susceptible to have cold shortening. The chilling process should be slow enough to avoid cold shortening and in the same time, rapid enough to avoid inhibition of ageing enzymes (Therkildsen, 2012) i.e. the carcass of pork not to chill below 10 °C in the first 5h., and not below 5 °C first that, pH values is not fell below 6 (Vladimir, 2008). The ultimate pH should be reached in pork within 6-9h post-mortem and the carcass not should be deboned before reaching 7°C in the deep leg (Honikel, 1999). Keep the temperature of the muscle at 14 °C until 1h after that pH had reached the value of 5.8 and after stored a 2 °C improve the rate of pH decline and consequently the meat tenderness (Rees, 2002). When using the electrical stimulation is possible to use more low chilling temperature, in this way the pH of carcass can reached value less than 6.0 in minor time and prior that the muscle temperature drop below 10 °C. It is known that pH and temperature of the carcass affect the rate of ageing. Is believed that protein denaturation occur when a rapid rate of pH decline occurs while muscle temperature is still high (Fisher, 1979). Regarding the aging time is well known that is one of the main factors that influence the variation of tenderness. The tenderness of the pork carcass increase rapidly in the first 48h post-mortem. In leg nearly 100% of the ageing occurs within 4 days post slaughter. In loin, 80 per cent of the total increase in tenderness occurs within 4 days, and 90 per cent within 6 days. Pork ageing enhances pork flavour and overall acceptability

- In loins pork flavour and overall liking increase with ageing and peak at about 9 days
- It improves the blooming potential of pork and increases the ability of vacuum-packed pork to bloom
- It has also been linked with improved water holding capacity and thus, juiciness

- Muscles that are rich in connective tissues, such as the silverside, do not tenderise as well as muscles low in connective tissue, such as the loin. This is because the connective tissue proteins are not broken down by the enzymes active post-slaughter

#### **1.4.7 Flavour and juiciness**

Flavor and juiciness are the other parameters that represent the eating quality, in this thesis will be briefly discussed. The study of meat flavour generally, is further complicated by the fact that the overall perception of flavour at the brain level is a result of the combined inputs from the taste-buds in the tongue and the aroma sensors in the nose. In addition, the concept of what constitutes an acceptable flavour may differ significantly among individuals. The latter problem can be overcome to some extent, using trained sensory panellists. The members of the panel group have been selected on the basis of their ability to detect small differences, and then trained to provide consistent descriptions of certain flavour “notes”. Sensory evaluation has been defined as “a scientific method used to evoke, measure, analyse, and interpret those responses to products as perceived through the sense of sight, smell, touch, taste and hearing” (Stone et al., 1993). The development of flavour depends of a multifactor combination, the strategic of feeding, weight of carcass, gender, breed, the aging time and the composition and the quantitative of fatty acid. A widely known sensory problem is the boar taint mainly caused by androstenone and skatole. Androstenone is a sexual pheromone produced in the Leydig cell in the testes. Skatole occurs during microbial degradation of the amino acid Tryptophan in the intestinal tract and is at relevant concentrations perceived as a faecal odour (Meier-Dinkela et al., 2013). According to Meier-Dinkela et al.,(2013), is interesting observe that the perception of androstenone depends from the olfactory receptor OR7D4 so there are some people that perceive it as an unpleasant urine-and sweat-like odour,

other find it pleasant i.e perfume like. Several studies have shown that there is less acceptance for pork from entire males with certain levels of androstenone and skatole compared to castrate or gilts. The juiciness is the feeling you get free juice during chewing. Juiciness is therefore of great importance for the overall eating experience, facilitates the chewing process as well as brings the flavour component in contact with the taste buds. The juiciness depends on the raw meat quality and on the cooking procedure. Some studies have shown a correlation with pHu, the centre temperature and the cooking procedure but still now it's far from understood as to juiciness is affected by the above parameter (Aaslyng, et al., 2003) .

### **1.5 Nutritional influences**

In past the principal concern of the farmer regarding the strategy of feeding, was the formulation of diets for growing-finishing pigs has largely been based on meeting the animal's requirements for energy and protein to optimize growth performance and carcass lean content and supplying sufficient minerals and vitamins to prevent deficiency symptoms. In the last decades there has been an increase in interest, to improving the pork quality also through a strategy of feeding even more focused to the improvement technological attributes such as WHC, muscle color, palability and trend of lipid oxidation (closely linked to the shelf life of the meat). Feeding strategy is became the management factor which is most actively used as quality control tool in the production of meat in relation to improve eating and technological quality. Through the feeding is possible regulate the protein turnover. Postnatal growth is determined by the difference between two dynamic processes, the rate of protein synthesis and the rate of protein degradation, during the growth of the animals a better growth performance is linked to high value of protein balance in the feeding. Furthermore the proteolytic potential in the muscle at the time of slaughter has long been regarded as an

important factor in the tenderization process in meat, which claim high muscle protein turnover at slaughter time. It is well known that during the compensatory growth both the rate of protein synthesis and degradation are elevated, consequently this practice could lead to improving tenderness. Moreover through the feeding it is possible to manipulate the postmortem muscle metabolism. It is well known that the store of glycogen plays a fundamental role in the change from muscle to meat, so high level of digestible carbohydrate sources, few days before the slaughter increase the muscle glycogen store lead a more low value of pHu (Andersen et al., 2005). At the same time, feeding can influence also the fatty acid composition and IMF. Regarding IMF if in the end of the finishing period of pigs they are fed with a lysine-deficient diet, will have an increased on IMF in the muscle. It is to highlight the importance of the finishing diet considered one of the main factor affecting the quality of the products (Narv ez-Rivas et al., 2009). When pigs are submitted at feed restriction (low protein and energy intakes) it is possible to observe a decreased IMF content and lipogenic capacity of muscle adipocytes. It is possible an increase of IMF also during feed restriction if the rate of protein intakes decrease of 20% and rate of energy 7% (Shi-Zheng et al., 2009). One of the causes that lead to meat deterioration are the processes of oxidation that as well as to reduce the shelf-life, lead to develop of off-flavour and a color change in the meat (Buckley et al., 1995). The changing of meat color, is due at the oxidation of muscle pigment that changes their chemical form. Myoglobin is converted in a first moment in Oxy-Myoglobin (cause a meat color change, from dark red to blood-red) and after met-Myoglobin producing a dull brown muscle color which is less attractive to the consumer. During the oxidation process it has been noted a reducing WHC it has supposed that this could depend from a loss of integrity of the cells membrane. The disorganization of cells membrane (as is well known rich in polyunsaturated fatty acids that are more susceptible to oxidation) is due at oxidation of phospholipids that are the main constituent of cells membrane. The lipid oxidation gives products that may change the aroma, flavor, color, texture and even the nutritive value of the food. It is well known, that dietary lipids, directly affect, the fatty acid and triacylglycerols profile of body fat in non-

ruminant animals. One approach to reducing the impact of oxidation is with the vacuum packaging, removing the air, or modified atmosphere packaging using for example CO<sub>2</sub>. Another approach to reducing oxidation in pork is to use antioxidant such as vitamin E as an ingredient in diets.  $\alpha$ -Tocopherol (vitamin E) is a membrane-associated antioxidant that can maintain cellular integrity and delay the lipid oxidation (Wang et al., 2012; Kerry et al., 1998). One of the methods most widely used for measuring the extent of lipid oxidation in muscle is the 2-thiobarbituric (TBA) test (Ulu, 2004).

## **2. AIM OF THE THESIS**

The aim of the research was to evaluate, how the feeding strategy and the genetic origin influenced the technology and sensorial characteristics of the fresh pork, such as pH and temperature post-mortem, color, tenderness and trend of lipid oxidation. In this experiment the samples were taken from two muscles, *longissimus dorsi* (LD) and *biceps femoris* (BF) of two market hogs (DLY and TLY). Furthermore, correlations between shear-force measurement (WBSF) and sensory analysis results were carried out to predict the sensorial characteristics of the meat.

### **3. Materials and Methods**

#### **3.1. Experimental design**

The experiment included, 32 female pigs from the progeny of Landrace x Yorkshire (LY) sows sired by Duroc (D) or Tamworth (T) boars. They were raised on the same conventional indoor pig farm until three weeks after which were included in the experiment that was carried out at the organic research platform of Aarhus University, Denmark, from August to October 2012. In the first time were introduced to two training paddocks, one for each breed combination, to familiarize with the electric fence. After one week there are moved to the experimental paddocks to become familiar with the paddocks and to be accustomed to the forage crops before introduction of two feeding strategies. Each paddock was in total 13x125 m and consisted of an area 13x107 m with a zone for drinking and wallowing, sown in autumn 2011 with a mixture containing 77% grass-clover and 23% herbs (the pasture was cut two weeks before the pigs were moved to the area) and an area 13x18m with root chicory where have been access in the last three weeks prior to slaughter. The paddock was divided in 4 zone, the first was immediately accessible, the second after 2 weeks, the third after 4 weeks and the fourth after 7 weeks. In the first three zone the fouraging crop was Grass-Clover the last was with Grass-clover + Chicory. For each crosses the pigs were divided in two groups and raised from an average of 50 day at two levels of concentrate feed each of this group was fed one time at day. Regarding the feed strategy the group was call NORMAL(Norm) with available the 100% of concentrate that they needed and RESTRICTIVE (Rest) with only the 60% of concentrate available. All pigs were slaughter at an abattoir and weight. For each carcass meat sample were taken from two muscle *longissimus dorsi* (LD) and *biceps femoris* (BF) was measured pH temperature and subjected at aging time for 1, 4 and 7 days in vacuum, after this stored at -20 °C.

## **2.2. pH and temperature measurement**

After slaughter, each carcass of pigs it was weighed, measured the temperature and pH respectively after 1, 3 and 22 hours from slaughter. pH and temperature was measured in LD and BF, 1 h, 3 h and 22 h post mortem, A Testo 901 thermometer (Testo, Lenzkirch, Germany) and a Metrohm pH meter (# 826, Metrohm, Switzerland) equipped with a Metrohm probe type glass electrode (Metrohm, Switzerland) were used. The pH meter was calibrated in buffers with pH 4.01 and 7.00 (Radiometer, Copenhagen, Denmark). The average of two measurements was used for both pH and temperature.

## **2.3. Drip loss and color measurement**

A 1½ cm thick muscle sample from LD and BF were used for determination of water-holding capacity measured as drip loss. Drip loss was measured over 48 h on approximately 90 g of muscle using the plastic bag method described by Honikel (1998). Color was measured on the LD and BF samples using a Minolta Chroma Meter CR-300 (Osaka, Japan) calibrated against a white tile ( $L^*=92.30$ ,  $a^*=0.32$  and  $b^*=0.33$ ). The aperture was 8 mm, and illuminant D65 and 10° Standard Observer were used. The samples were allowed to bloom for 1 hour at 4°C prior to the measurements. The three parameters  $L^*$ ,  $a^*$  and  $b^*$ , representing lightness, redness and yellowness, were measured on five sites of each sample, and the average of the five measurements was reported.

## **2.4. Shear force**

Shear force was measured on BF and LD aged 1, 4 and 7 days. On average twelve samples per day was evaluated and taken randomly from the freezer. The sample was weighted and subsequently thawed in cold water 4 °C for 4h. Once thawed,

the sample were removed from the plastic bag, dried, again weighed and then cut to obtain uniform samples (length 8cm high 4cm and thick 5). The samples were vacuum packed in a plastic bag and left to cool in water 4 °C for 10 minutes after which they were cooked for 50 minutes in hot water at 70 °C. Once cooked these were left in cold water until the next day after which they were removed from the plastic bag, dry and weight. For each sample were obtain eight strips of meat cut follow the direction of the myofibrils, thick 1cm and high 1cm. The shear force was measured with Stable Micro System Texture Analyser (Godalming, UK) equipped with a Warner-Bratzler shear blade with rectangular hole, 11 mm wide and 15mm high and a blade thickness of 1,2 mm (Honikel, 1998). The maximum shear force measured across the fibers direction, was recorded at a test speed of 50 mm/min according to the procedure described by Honikel (1998).

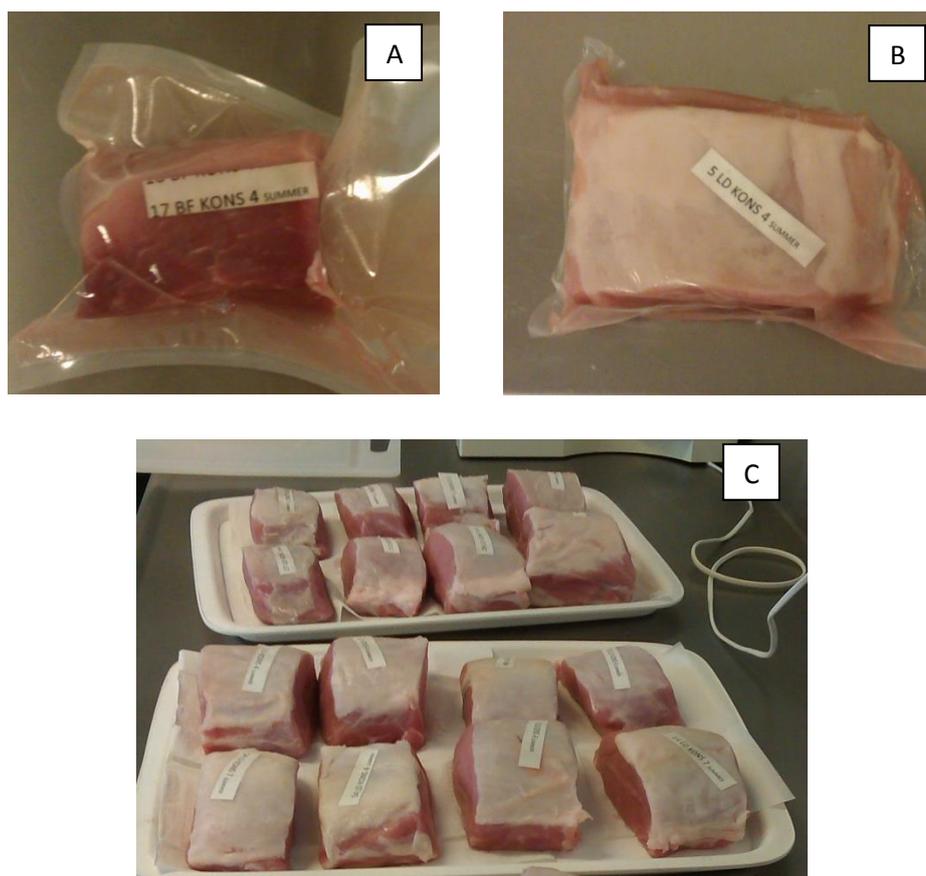


Figure 10 A) BF sample still frozen; B) LD sample still frozen; C) Meat samples thawed, dried and weighed..



Figure 11A) cutting procedures to obtain uniform samples; B) weighing uniform samples; C) cooking of the samples; D) weighing of the cooked samples; E) preparation of strips samples; F) Strips samples.

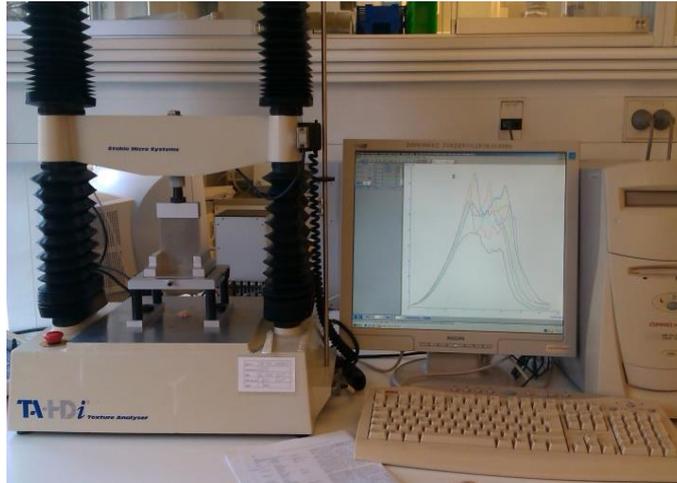


Figure 12 Shear-force assessment (WBS).

The result was reported in a graphic where in x-axis was reported the space in millimeters path from the blade to cut the strips of meat, while in the y-axis was reported the shear force express in Newton. From the graphic obtained (Fig.13) it was possible to obtain the following values:

- N\_top: represent the point beginning of the breakdown of the myofibril express in N;
- Dist\_top: is the distance among the 0 point and of the begin of breakdown of the myofibril express in mm;
- Slope : is the slope of the curve from the 0 point to the begin of breakdown of the myofibril;
- Area\_btop: is the area under the curve between the 0 point to the begin of breakdown of the myofibril express in mm<sup>2</sup>;
- Area\_atop: is the area under the curve between the begin of breakdown of the myofibrils and the end of the cut of meat express in mm<sup>2</sup>;
- Area\_cut: is the are under the curve between N\_top and N\_Con express in mm<sup>2</sup>;

- N\_Myo: represent the point of maximum resistance of the cut meat during the cutting express in N;
- Dist\_Myo: : represent the point of maximum resistance of the cut meat during the cutting express in mm;
- N\_Con : represent the end of breakdown of the connective tissue and then of the myofibrils express in N;
- Dist\_Con: represent the end of breakdown of the connective tissue and then of the myofibrils express in mm;
- Means : is the average of all points of breakdown of the myofibrils express in N;
- Els\_Con: is the distance between N\_top and N\_con express in mm;

The occur of a likely correlation among the result of shear force, the aging time, the interaction feeding\*breed, the type of the race and muscle was occurs by statistical program (SAS).

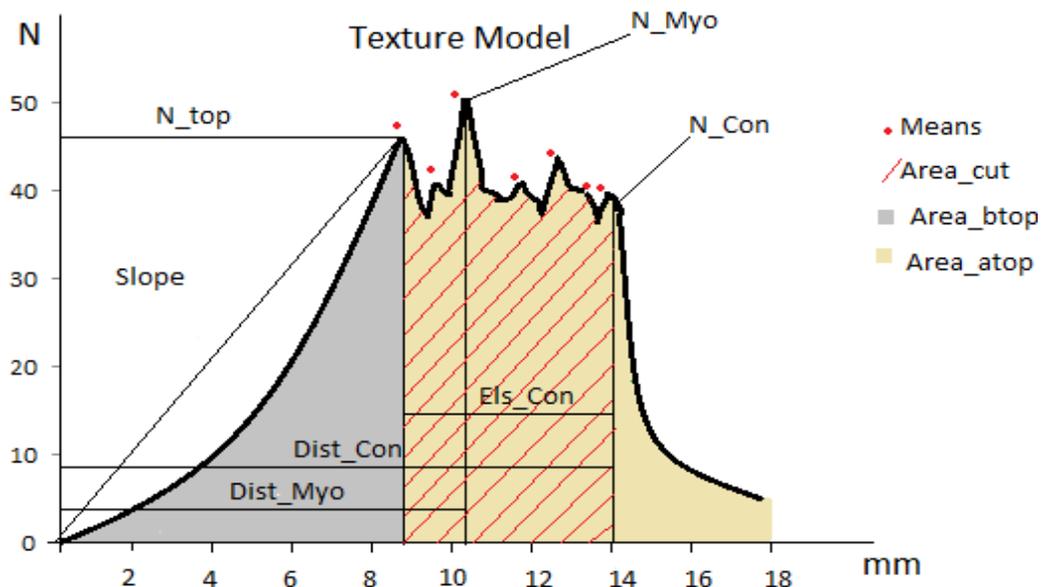


Figure 13 Texture model; x-axis represent the space in millimeters path from the blade to cut the strips of meat, y-axis represent the shear force express in Newton.

## 2.5. Panel Test

LD and BF were thawed for 22 h at 4°C before preparation. LD was prepared as chops and BF as roast. The chops were sliced into 2 cm slices and heated to 10 - 15°C before being fried on a frying pan with a temperature of 170°C (Pano Copter Fribergs 9200 cm, Göran Persson maskin AB, Göteborg, Sweden). The pan was wiped with a thin layer of grape seed oil before heating. The chops were turned every 2 minutes and fried to a core temperature of 65-66°C measured with a handheld probe (Testo 926, Testoterm Buhl and Bundsoe, Virum, Denmark). The roasts were heated in a convection oven (Combidamper, Elextroluxe A, O.C. Bjerregaard A/S, Denmark) at 100°C until the internal temperature reached 68-70°C measured with a handheld probe (Testo 926, Testoterm Buhl and Bundsoe, Virum, Denmark). The roasts rested for approximately 20 min covered with aluminium foil, before they were sliced into 10-mm thick slices. The meat samples were served on preheated plates as pieces of 4 x 3 cm in size. An 8-member, trained sensory panel evaluated each sample for tenderness, juiciness, aroma and flavor intensity on an unstructured scale from 0 to 15 (0 = extremely tough, dry, and bland; 15 = extremely tender, juicy, and intense, respectively).

A group of tasters from university of Copenhagen was involved for the sensory evaluation of sample of *longissimus dorsi* and *biceps femoris* from DLY and TLY respectively with 4 day of aging time. The result of the panel test has been elaborated with the statistical program SAS. The result of the shear force obtain from the system (WBSF) has been compared with the result from panel test by SAS for evaluated eventually correlation.

## 2.6. Lipid oxidation

One of the methods most widely used for measuring the extent of lipid oxidation in muscle is the 2-thiobarbituric (TBA) test (Hasret Ulu, 2004). Thiobarbituric Acid Reactive Substance (TBARS) are formed as a byproduct of lipid peroxidation. The reactive oxygen species (ROS) have a really short life, is

therefore easier measure the damage product form this species during oxidative stress like TBARS than measure directly. These lipids peroxidation can be measured by TBARS assay, using thiobarbituric acid as reagent, this test measured malondialdehyde (MDA) present in the sample like one of the several low-molecular-weight and products formed after decomposition of primary and secondary product of lipid peroxidation product. This test has been discussed in several literature regarding the specificity of TBARS on other compound than MDA but is still the most used test to determine the lipid peroxidation. The TBA test use a colorimetric evaluation based on the absorbance of a red chromogen formed on the reaction among TBA and MDA. For this assay can be used four method :

- 1) Assumes the heating of the sample and extraction of the colored complex followed by colorimetric test
- 2) Is picked a portion of the steam distillate from the food followed by colorimetric test
- 3) On aqueous or acid extraction of the sample
- 4) On lipids extracted from the sample

The TBA number expresses lipid oxidation in milligrams of MDA per kilogram of meat (1-2-3) and milligrams MDA per unit of lipid (4).For all of this method, the extraction for estimated MDA content is considered the best for ease and speed of execution of the method and because the meat is not exposed to heat.

Meat samples from each dietary treatment and breed still frozen were cut, in strips 0.5 cm thickness and 2 cm length, were, placed in a plastic tray, covered with a polythene film and placed in refrigerator ( $5\pm 1$  °C) under fluorescent light (~ 1000 LUX) for up to 6 days. For each sample were carried out four replicas respectively at 0, 1, 3 and 6 days. Each of these was whipped and 3 samples each of 300mg taken in microtubes. A solution of Thiobarbituric acid was prepared taken 0,65g of TBA and dissolved in 22,92ml H<sub>2</sub>O<sub>mq</sub> (Milli-Q) and 27,08 ml of

trichloroacetic 24%, this solution was left for 1 hours in heater at 60 °C to facilitate the dissolution of TBA. A solution of Tetraethoxypropan (96%) TEP was diluted at concentration of 1:1000, 1 : 100000, 1: 133000, 1:200000, 1:600000; for each solution of TEP 300 µl were taken and placed in microtubes and 600 µl of TBA previously prepared were added. Meat samples (300 mg each) were put in microtubes, vortexed at 1000 rpm and then left in oven at 60 °C for 1 hour. Each microtubes was centrifuged at 3000 rpm at 20 °C for 10 minutes. After that 300 µl of each solution was taken and placed in a 96-well plates and absorbance at 532 and 650 nm was measured by spectrophotometer. The spectrophotometer used was a Multi-mode Microplate Reader Synergy 2, (BiotTek Instruments Inc, USA). The development of MDA, shown in fig. 8 seems follow a linear trend, than for the experiment, it was considered appropriate evaluated for each sample the amount of MDA on days 0 and 3.

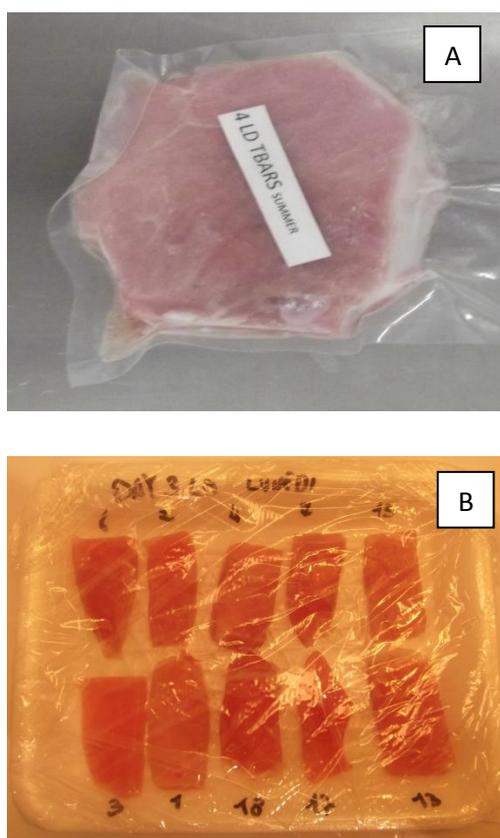


Figure 14 A) Frozen meat sample; B) Samples ready to be stored in refrigerator under fluorescent light.

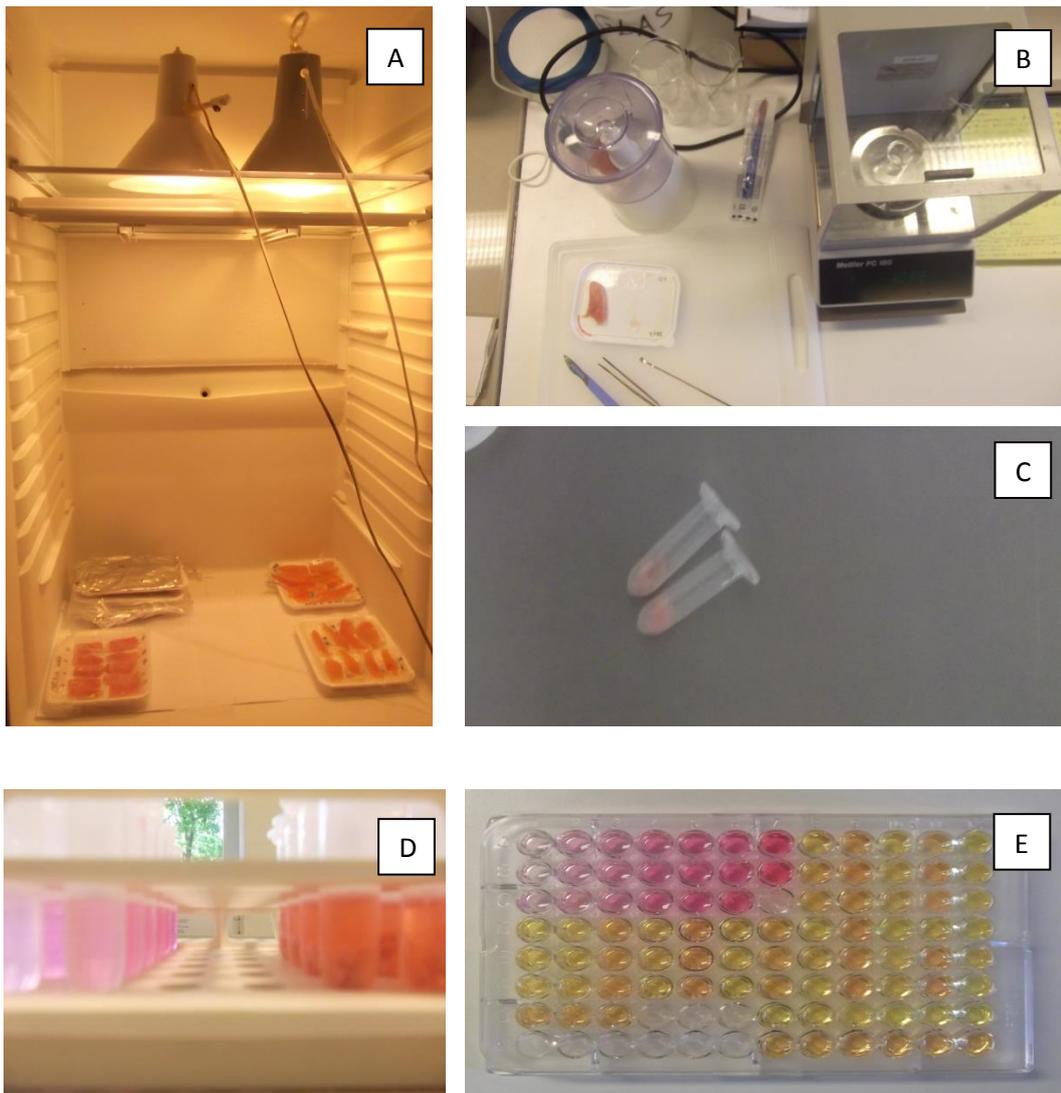


Figure 15 A) Refrigerator at  $5 \pm 1$  °C; B) mashed sample; C) Microtubes containing 300mg of mashed meat samples; D) Microtubes containing the diluted solution of TEP and TBA (left side of the picture), microtubes containing the meat samples and TBA (right side of the picture); E) 96-well plates containing the solution to be undergo at spectrophotometric reading.

### Processing data of standard curve

A standar curve was prepared with a solution of Tetraethoxypropan (96%) (TEP) diluted at concentration of 1:1000, 1:100000, 1:133000, 1:200000, 1:600000. From the result obtained by the spectrophotometer it is calculated the difference between the read at 534 and 650nm, after that the average between the three

results (3 repetition for each sample) it have been calculated. For each solution it have been calculated the n°mol of TEP present in the different diluted solution (  $n^{\circ} mol = \frac{\mu l TEP}{MM TEP}$  ). A standard curve was built using the average of the result from the spectrophotometer measured and the n°mol TEP (fig.16). The point 0 of the curve was a sample with only H<sub>2</sub>Omq. The n°mol of MDA of the meat sample was calculated using the equation of the linear regression, were x represent n°mol of TBA and y the value of absorbance obtain by spectrophotomric measure.

Table - 3 Value of absorbance of the solution of the spectrophotometer measured of the Tetraethoxypropan used for the standar curve

S. Curve	1	2	3	4	5	6	
<b>A</b>	0,137	0,614	0,57	1,143	1,347	1,934	Read 1:534
<b>B</b>	0,126	0,64	0,52	1,084	1,489	2,148	Read 1:534
<b>C</b>	0,144	0,674	0,581	1,079	1,541	1,977	Read 1:534

<b>A</b>	0,036	0,035	0,035	0,036	0,036	0,036	Read 1:650
<b>B</b>	0,035	0,035	0,035	0,035	0,035	0,035	Read 1:650
<b>C</b>	0,035	0,035	0,035	0,034	0,034	0,035	Read 1:650

<b>μl Tep</b>	0	1,66	2,5	5	7,5	10
<b>Avarage</b>	0,10033	0,522	0,60766	1,067	1,424	1,984333

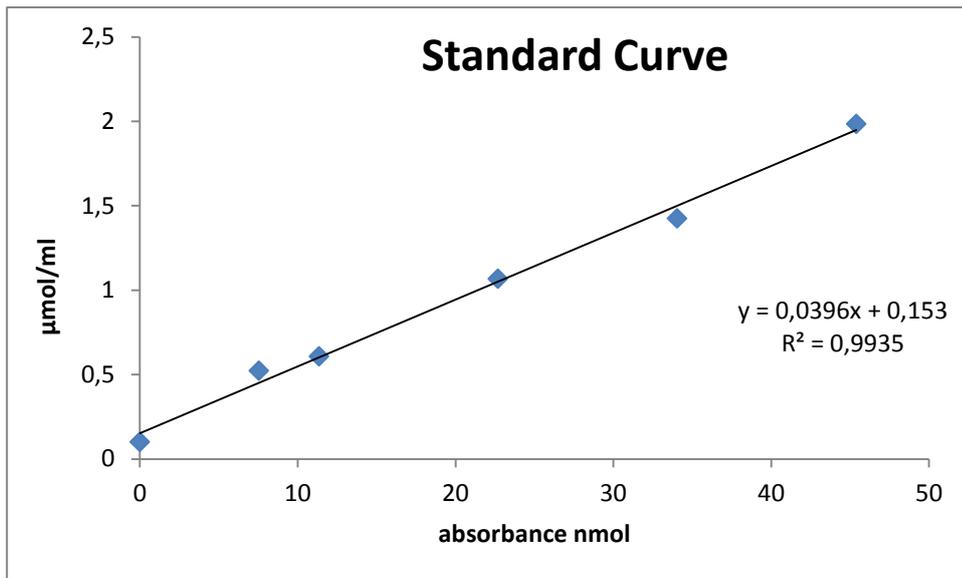


Figure 16 Standard curve; where in y-axis are reported  $\mu\text{mol}$  while in x-axis the value of absorbance.

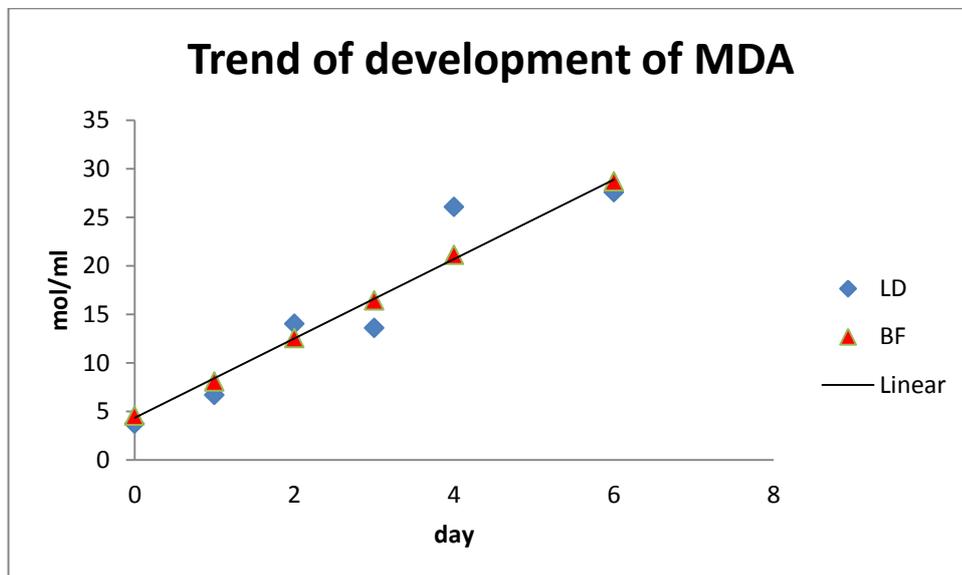


Figure 17 Trend of MDA (express in mol/ml) in meat sample stored for 0,1,2,3,4,5 and 6 days at the same condition of temperature and storage for 2 types of muscle, *longissimus dorsi* and *biceps femoris*.

## 4. RESULT AND DISCUSSION

### 4.1. Trend of postmortem pH and temperature

PH measurement after 1, 3 and 22 hours postmortem, in *longissimus dorsi* (LD) and *biceps femoris* (BF) muscles, showed a rapid decrease with some differences due to the feeding strategy and crossbreed. In LD (Fig. 18) pH dropped slowly in DLY pigs fed restricted diet with a value which was higher after 3 hours after slaughtering respect to the others groups ( $P<0.05$ ), while after 22 hours of ageing pH was higher in DLY restricted and TLY normally fed (5.68 and 5.66 vs 5.58, respectively:  $P<0.01$ ). In BF the pH behavior during ageing put in evidence a feeding strategy effect three hours after slaughter ( $P<0.01$ ) showing lower values in restricted fed groups, while no statistical differences were found after 22 hours of ageing. The terminal sire has a significant influence on pH1, the higher pH1 observed in meat from Duroc derived pigs is in agreement with Mason et al. (2005) who showed higher pH when compared with the values found in Landrace strains pigs, and with Latorre et al. (2003) and Affentranger et al. (1996) who found that Duroc derived pigs had higher initial pH than Pietrain or Large White derived pigs. Serrano et al. (1998) showed no statistical differences due to breed. Feeding restricted diet or diet partially characterized by grass to slaughter determine higher pH45 values in pork meat (Mason et al., 2005). Pre-slaughter restricted feeding could potentially reduce muscle glycogen content at slaughter, increasing the initial pH (Pettigrew and Esnaola, 2001). LD temperature trend during ageing was strongly affected by feeding strategy showing the lowest values after 3 hours from slaughter in restricted fed group, although after 22 hours temperature was similar in all groups. In BF temperature drop was different respect that observed in LD with a temperature which was around 20-25°C after 3 hours from slaughter, although a feeding strategy effect was also observed. Indeed, similarly to LD, meat from restricted fed animals showed lower temperature three hours after slaughtering compared to normal fed ones (Fig.20).

The lower drop in meat temperature during ageing is probably due to the higher fat deposition in normal fed animals

Table - 4 Effect of crossbreed and feeding strategy on pH and temperature values during storage.

<i>longissimus dorsi</i>	TLY	TLY	DLY	DLY	Breed*feeding	Breed	Feeding
	Norm	Rest	Norm	Rest			
PH1	6,30ab	6,13c	6,22b	6,39°	*	n.s.	n.s.
PH3	6,03b	6,03b	5,93c	6,30°	*	n.s.	*
PH22	5,66a	5,58b	5,58b	5,68°	**	n.s.	n.s.
temp1	36,62A	33,86C	37,16A	35,71B	n.s.	n.s.	**
temp3	7,56A	3,74B	8,40A	3,55B	n.s.	n.s.	***
temp22	4,51A	4,08B	4,27A	3,96B	n.s.	***	n.s.
<i>biceps femoris</i>							
PH1	6,30b	6,21c	6,38ab	6,44°	n.s.	*	n.s.
PH3	5,76ab	5,55c	5,94a	5,71b	n.s.	*	**
PH22	5,68	5,59	5,69	5,61	n.s.	n.s.	n.s.
temp1	39,04a	38,81a	38,55ab	38,16b	n.s.	*	n.s.
temp3	24,6A	20,03B	25,54A	20,95B	n.s.	n.s.	***
temp22	5,14	4,81	5,06	4,45	*	**	n.s.

\*P<0.05; \*\*P<0.01;

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

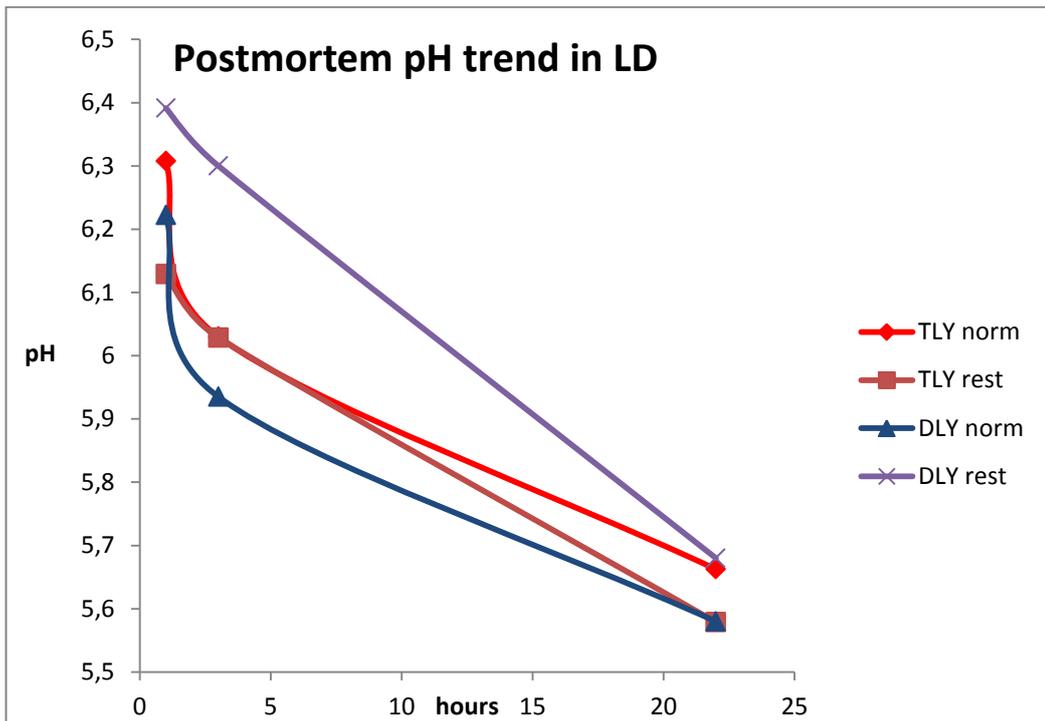


Figure 18 Effect of crossbreed and feeding strategy on postmortem pH decline in LD muscle.

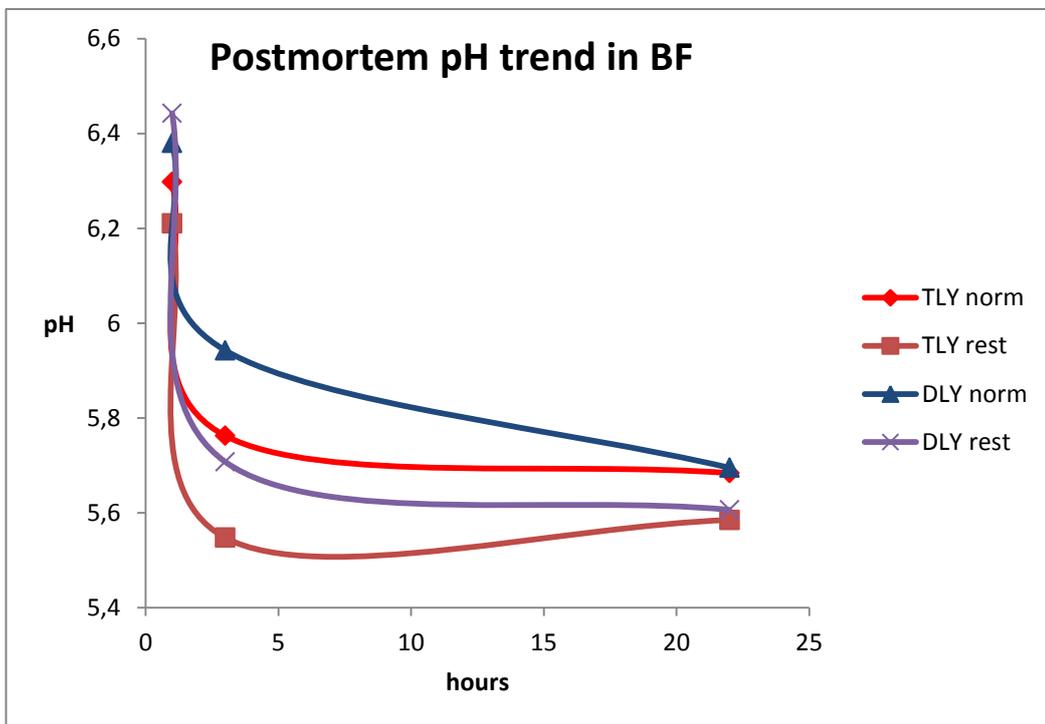


Figure 19 Effect of crossbreed and feeding strategy on postmortem pH decline in BF muscle.

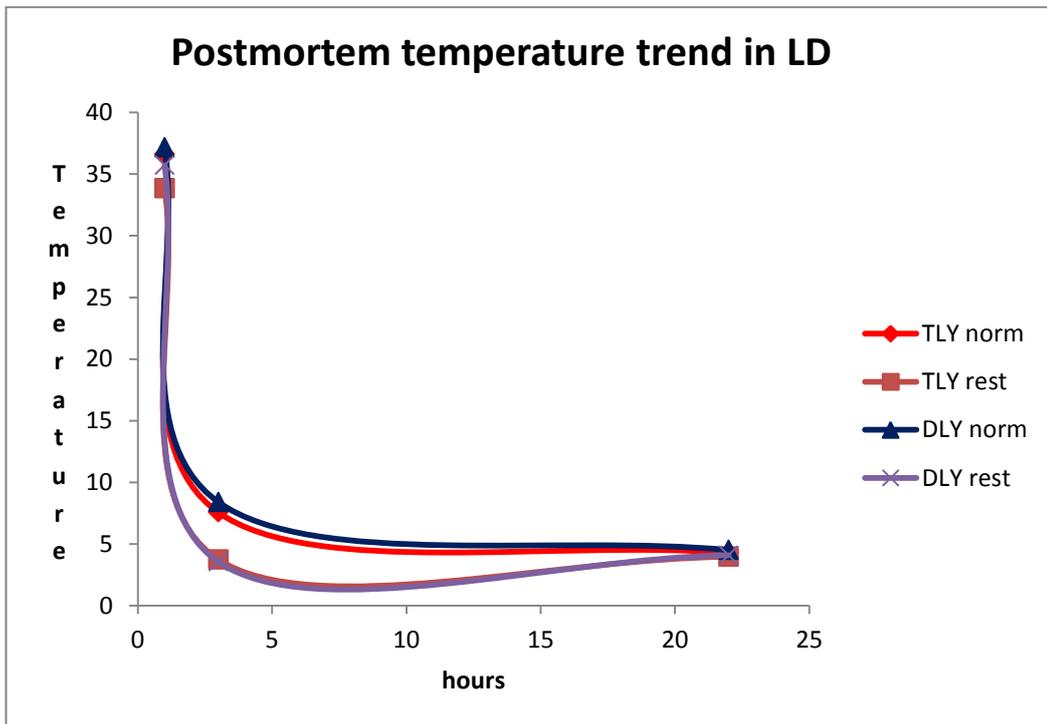


Figure 20 Effect of crossbreed and feeding strategy on postmortem temperature decline in LD muscle.

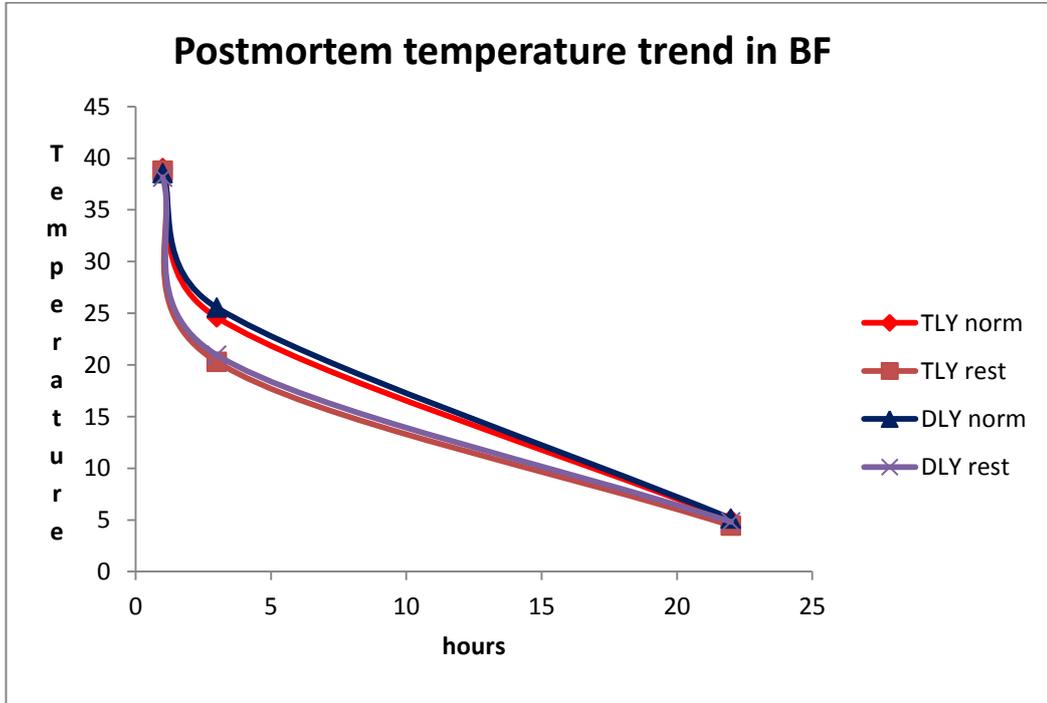


Figure 21 Effect of crossbreed and feeding strategy on postmortem temperature decline in BF muscle.

## 4.2 Lean meat yield

Slaughter weight resulted strongly influenced by crossbreed and feeding strategy putting in evidence higher slaughter weight in DLY and normal fed animals ( $P<0.01$ ). BF weight was strongly influenced by breed ( $P<0.01$ ), higher in DLY, while no statistical significant effect were due to feeding strategy and the interaction between breed and feeding strategy (Tab.5). Similar results were found for LD, which area was larger in DLY crossbreed ( $P<0.01$ ) and in normal fed animals. The results were similar to those found by Mason et al. (2005) who show as in outdoor restricted fed animal till slaughter, LD area was lower when compared to the ad libitum fed animals. Fat area was strongly affected by breed ( $P<0.01$ ) and in a lesser extent by feeding strategy ( $P<0.05$ ) and was larger in TLY breed and in normal fed animals. Tamworth breed as terminal sire determines a higher fat deposition and consequently lower LD area, although it is also strongly influenced by diet. Mason et al. (2005) in a similar trial showed as Landrace breed as terminal cross determines leaner carcass respect those obtained using Duroc breed, but in a previous work Wood et al. (2004) showed as Tamworth breed had higher fat depots area when compared with modern breed as Duroc.

Table - 5 Effect of crossbreed and feeding strategy on BF weight, LD area and fat area.

		DLY	DLY	TLY	TLY	breed	Feeding	breed*feeding
		Norm	Rest	Norm	Rest			
Sl. Weight		88,20A	81,04B	73,81C	66,88D	**	**	n.s.
W_BF	G	1535,7A	1518,68A	1240B	1223,8B	***	n.s.	n.s.
LD_area	cm <sup>2</sup>	54,7A	43B	38,6B	34,4C	**	**	n.s.
fat_area	cm <sup>2</sup>	20,43B	13,61C	35,3A	24,96B	***	*	n.s.

\* $P<0.05$ ; \*\* $P<0.01$ ;

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

### 4.3 Drip loss and colour

In BF, drip loss expressed as percentage was strongly influenced by feeding strategy ( $P<0.01$ ) and by the interaction between feeding strategy and breed ( $P<0.05$ ) showing higher values in restricted animals and in particular in restricted fed DLY (Tab.6). In LD drip loss was strongly affected by feeding strategy ( $P<0.01$ ) with higher values in restricted fed group without any effect of the interaction (Tab.6). About meat colour characteristics measured by CIElab\* (Tab.6) system in BF no significant differences were found for L parameter which was strongly affected by feeding strategy in LD showing higher L values in meat from restricted fed animals. In BF both redness, both yellowness were strongly influenced by breed showing the highest values in TLY while no statistical differences among the same parameters were found in LD. The obtained results are in agreement to those found by Mason (2005), McCarthy et al. (2001) and Mitumoto et al. (2002) who found like neither breed, neither feeding strategies influenced colour parameters before meat packaging. Similar results were found also by Serrano et al. (2009) in Iberian pigs fed a restricted although in indoor rearing systems.

Table - 6 Effect of breed or feeding strategies on drip loss and meat colour parameters in sampled muscles.

		TLY	TLY	DLY	DLY	Breed	Feeding	Breed*Feeding
		Norm	Rest	Norm	Rest			
<i>longissimus dorsi</i>								
Drip loss	%	6,55B	8,30A	5,22C	7,90A	n.s.	**	n.s.
L		46,42B	49,39A	46,28B	49,39A	n.s.	***	n.s.
A		12,20	12,46	14,45	12,96	n.s.	n.s.	n.s.
B		7,43	8,76	8,51	8,88	n.s.	n.s.	n.s.
<i>biceps femoris</i>								
Drip loss	%	2,15C	4,07A	2,33BC	2,57B	n.s.	**	*
L		53,04	51,06	51,45	50,88	n.s.	n.s.	n.s.
a		6,68B	5,64C	7,83B	9,13A	**	n.s.	n.s.
b		6,23B	4,93C	6,51B	7,34A	**	n.s.	*

\* $P<0.05$ ; \*\* $P<0.01$ ;  
A, B:  $P<0.01$ ; a, b:  $P<0.05$

#### 4.4 Shear force

For the shear force measurements, parameters that were chosen to be better analyzed, were those that showed a higher correlation with the results of the panel test: *N\_top*, *Mean\_S*, *N\_Myo* and *Slope* (see tab.7).

Table - 7 Correlation results among panel test results and shear force measurements.

LD muscle	<i>N_top</i>	<i>Mean_S</i>	<i>N_Myo</i>	<i>N_Con</i>	<i>Stope</i>
Bite resistance	*	*	**	n.s.	**
Crumble	***	***	***	**	***
Tenderness	**	n.s.	**	*	***
chewing time	**	**	**	n.s.	n.s.
BF muscle					
Bite resistance	*	*	*	n.s.	*
Crumble	*	*	n.s.	*	n.s.
Tenderness	*	*	*	*	*
chewing time	*	*	*	*	*

\*P<0.05; \*\*P<0.01;

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

The panel test evaluated the odour (*Meat, Acid, Piggy*), the flavour (*Meat, Piggy, Acid, Sweet, Metallic and Bitter*) and texture attributes (*Bite resistance, Crunchy, Juiciness, Fibrous, Tenderness, Crumble and Chewing time*) of the meat. The correlation between shear force and panel test was made only on texture attributes, and a directly correlation was found from *N\_top*, *Mean\_S*, *N\_myo* and *Slope* with *Bite resistance*, *Crumble* and *Chewing time*. On the other hand, inverse correlation was found with *Tenderness*. Regarding *N\_top*, *Mean\_S*, *N\_Myo* and *Slope*, it has been found that they were strongly affected by ageing time and feed strategy for LD and BF muscle, for the latter a high correlation was found also with the breed. In fig. (22 to 29) trend of *N\_top*, *Mean\_S*, *N\_Myo* and *Slope* during ageing time are reported. *Breed\*Feeding* interaction does not seem to influence the parameters of shear force, in any of the two examined muscles ( $P > 0,05$ ). BF tenderness parameters during ageing showed different behaviour at 1 day, 4 days and 7 days after slaughter. At 1 day of ageing tenderness parameters were strongly influenced by breed showing the highest values in DLY breed, while only *slope* parameter was slightly influenced also by feeding strategy ( $P<0.05$ ). At 4 days of ageing, in

BF, no significant differences were found among the measured parameters were found, while after 7 days of ageing only feeding systems seems to influence the tenderness ( $P<0.01$ ). All the measured parameters were higher in meat from restricted fed animals. LD tenderness parameters (Tab.8) from 1 day to 7 days of ageing are strongly influenced by feeding system ( $P<0.01$ ) showing the highest values of shear force in meat from restricted fed animals.

Table - 8 Effect of breed and feeding strategy on shear force parameters measured in *longissimus dorsi*.

	TLY	TLY	DLY	DLY	Breed*feeding	Breed	Feeding
1 Day	Norm	Rest	Norm	Rest			
N_top	32,62	43,18	38,21	44,29	n.s.	n.s.	**
Mean_S	26,43	36,12	31,43	38,01	n.s.	n.s.	***
N_Myo	31,63	40,28	35,95	41,16	n.s.	n.s.	**
Slope	5,49	6,56	5,9	6,45	n.s.	n.s.	*
4 Day							
N_top	26,5	40,15	32,73	38,97	n.s.	n.s.	***
Mean_S	22,25	34,13	26,96	33,53	n.s.	n.s.	***
N_Myo	25,2	37,22	29,09	33,74	n.s.	n.s.	**
Slope	4,61	6,2	4,98	5,49	n.s.	n.s.	**
7 Day							
N_top	27,4691	35,3382	22	38	n.s.	***	n.s.
Mean_S	23,831	30,6542	18,2782	32,3379	n.s.	n.s.	***
N_Myo	23,5851	30,2233	20,1124	33,3327	n.s.	***	n.s.
Slope	4,1208	5,0248	3,7412	5,7011	n.s.	***	n.s.

\* $P<0.05$ ; \*\* $P<0.01$ ;  
A, B:  $P<0.01$ ; a, b: $P<0.05$

Table 9 - Effect of breed and feeding strategy on shear force parameters measured in *biceps femoris*.

	TLY	TLY	DLY	DLY	Breed*feeding	Breed	Feeding
1 Day	Norm	Rest	Norm	Rest			
N_top	40,84	45,24	53,08	55,05	n.s.	***	n.s.

Mean_S	34,62	37,97	46,19	47,11	n.s.	***	n.s.
N_Myo	37,6	42,48	45,25	46,44	n.s.	**	n.s.
Slope	5,71	6,31	6,43	6,73	n.s.	*	*
<b>4 Day</b>							
N_top	38,53	43,74	46,61	45,11	n.s.	n.s.	n.s.
Mean_S	32,47	36,65	40,23	38,87	n.s.	n.s.	n.s.
N_Myo	35,06	39,67	38,1	39,18	n.s.	n.s.	n.s.
Slope	5,37	6,06	5,68	5,95	n.s.	n.s.	n.s.
<b>7 Day</b>							
N_top	36,513	43,486	35,689	39,1702	n.s.	n.s.	**
Mean_S	30,8304	37,449	29,2334	33,331	n.s.	n.s.	***
N_Myo	29,1255	36,5998	27,7376	34,157	n.s.	n.s.	***
Slope	4,7062	5,5741	4,5358	5,2585	n.s.	n.s.	***

\*P<0.05; \*\*P<0.01;  
A, B: P<0.01; a, b:P<0.05

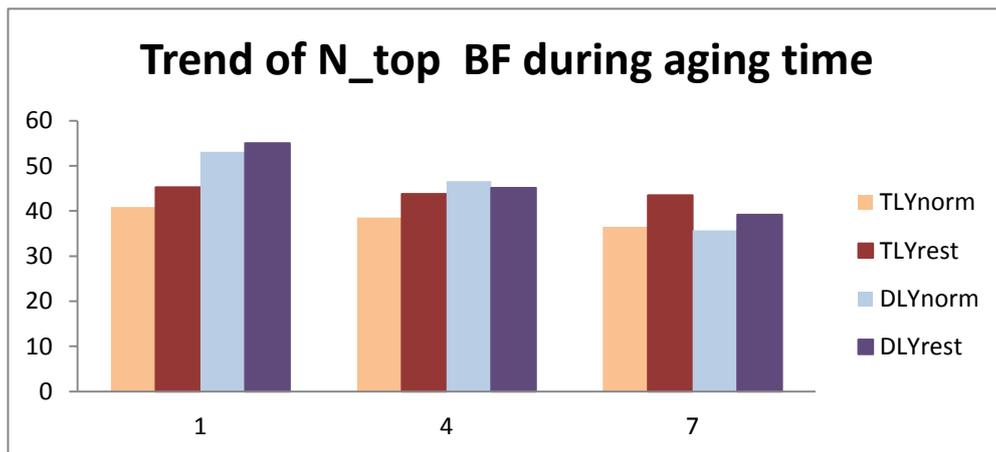


Figure 22 Effect of ageing, crossbreed and feeding strategy on shearforce (N\_top) decline in BF.

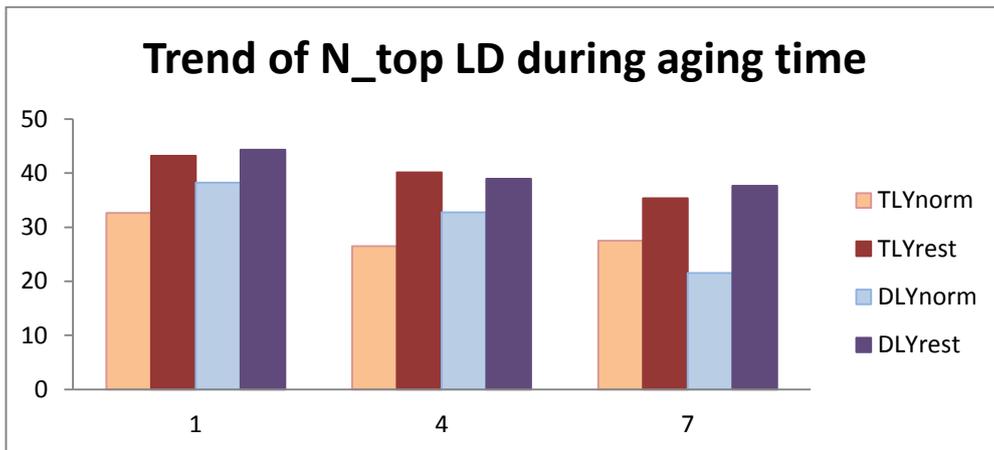


Figure 23 Effect of ageing, crossbreed and feeding strategy on shearforce (N\_top) decline in LD

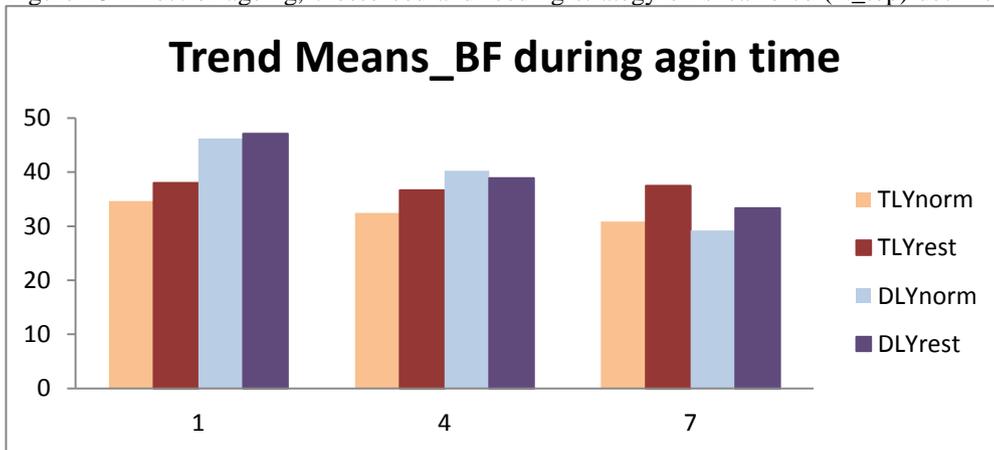


Figure 24 Effect of ageing, crossbreed and feeding strategy on shearforce (Means) decline in BF

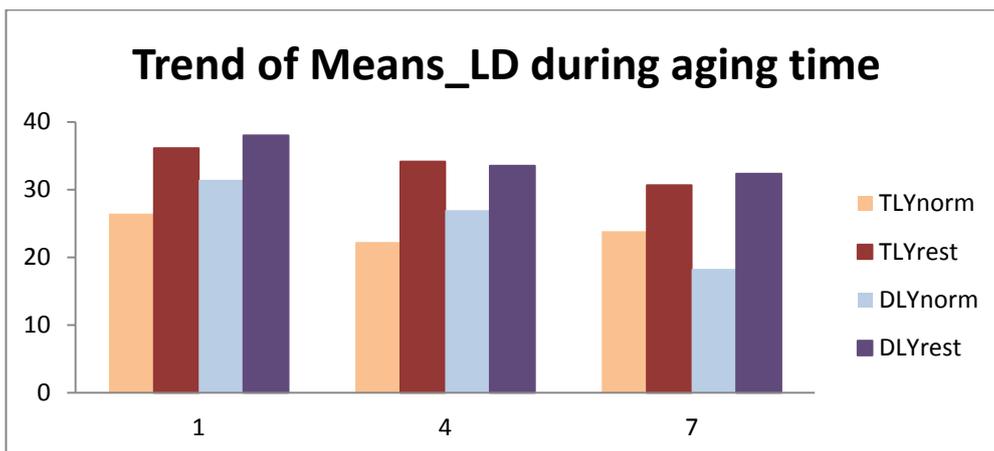


Figure 25 Effect of ageing, crossbreed and feeding strategy on shearforce (Means) decline in LD.

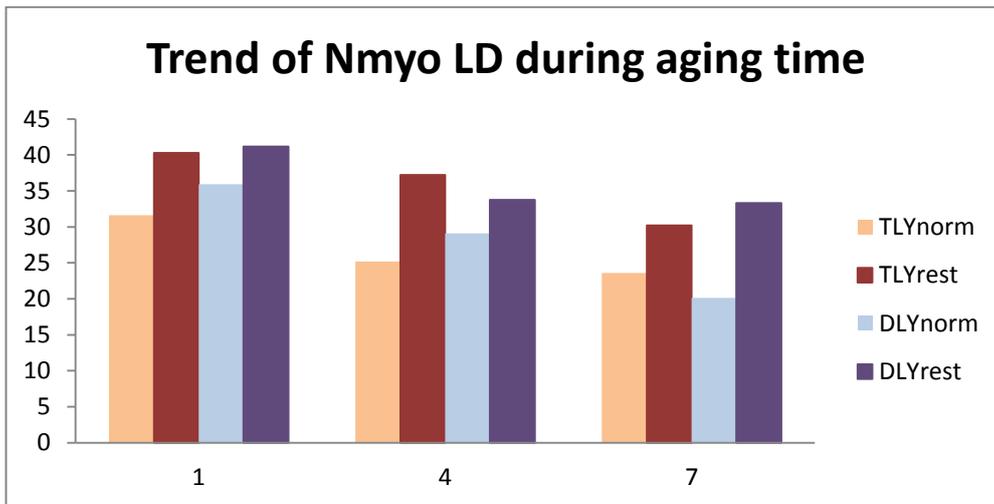


Figure 26 Effect of ageing, crossbreed and feeding strategy on shearforce (Nmyo) decline in LD.

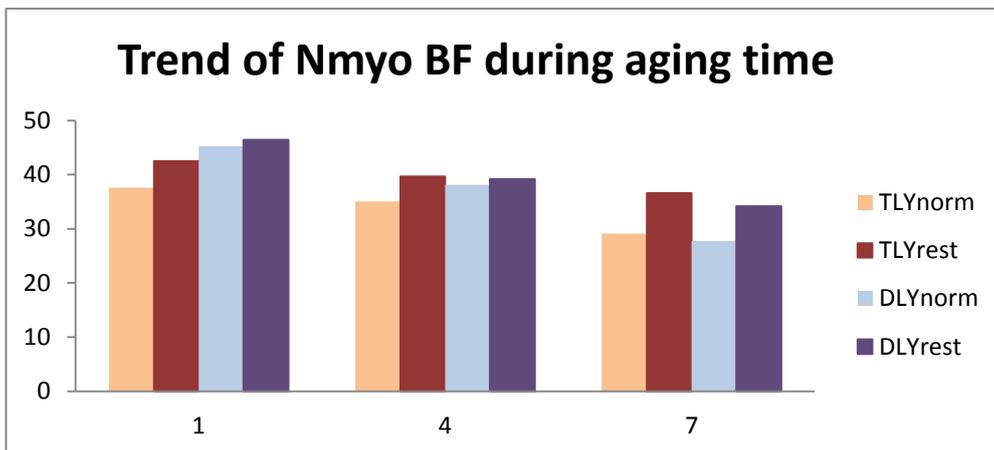


Figure 27 Effect of ageing, crossbreed and feeding strategy on shearforce (Nmyo) decline in BF.

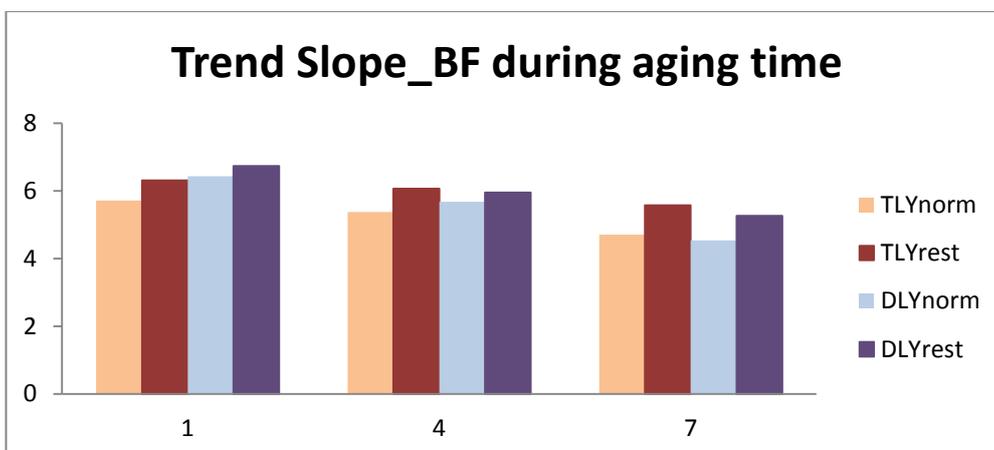


Figure 28 Effect of ageing, crossbreed and feeding strategy on shearforce (Slope) decline in BF.

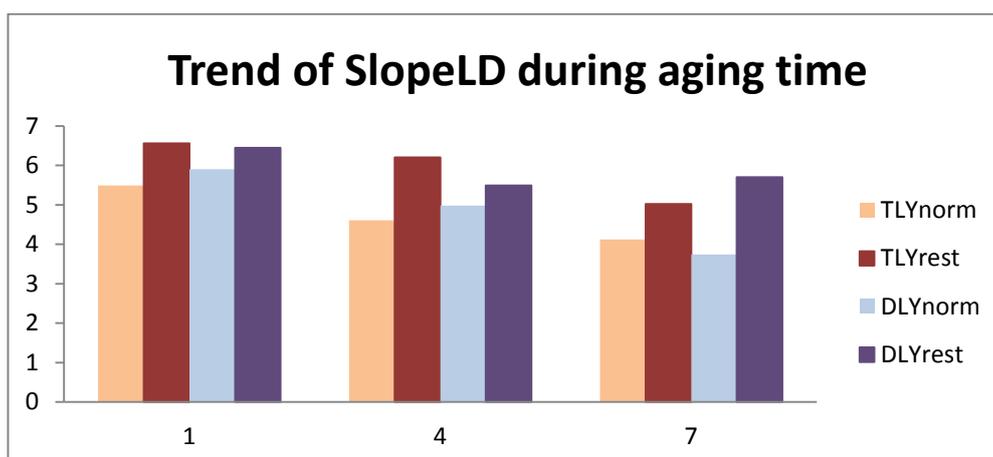


Figure 29 Effect of ageing, crossbreed and feeding strategy on shearforce (Slope) decline in LD.

#### 4.5 Lipid peroxidation

Table and Figures represent the trend of TBARs in BF muscle and LD muscle, respectively at time 0 and after three days of storage. In BF, lipid oxidative stability was slightly affected by the feeding system ( $P < 0.05$ ) at time 0 of conservation, but after 3 days, although there is an increase in TBARs values, neither feeding strategies, neither breed or their interaction affected this parameter. LD oxidative stability was affected by feeding system at time 0 whereas after 3 days, interaction between breed and feeding strategies was slightly effective ( $P < 0.05$ ) with a strong increase of TBARs value in meat from TLY normally fed and DLY restricted fed, while TLY restricted fed showed an intermediate level and DLY normally fed showed the lowest level. In a previous work, Mason et al. (2005) showed as, in similar rearing conditions, restricted fed animals showed similar levels of lipid oxidation to those found in control group. The results obtained in the present work suggest that the antioxidant vitamin ingested with the pasture in restricted fed animals are not able to guarantee a normal oxidative stability of the muscle during conservation.

Table 10 – Effect of breed, feeding system and their interaction on lipid oxidative stability.

*longissimus dorsi*      TLY      TLY      DLY      DLY

		Norm	Rest	norm	rest	Breed	Feeding	breed*feeding
Tbars_0	mgMDA/kg sample	1.28B	2.26A	1.33B	1.99A	n.s.	**	n.s.
Tbars_3	"	6.84a	5.43ab	3.42b	6.28a	n.s.	n.s.	*

<i>biceps femoris</i>								
		Norm	Rest	norm	rest	Breed	Feeding	breed*feeding
Tbars_0	mgMDA/kg sample	1.65ab	1.21b	2.37a	1.39b	n.s.	*	n.s.
Tbars_3	"	4.59	3.91	7.21	4.86	n.s.	n.s.	n.s.

\*P<0.05; \*\*P<0.01;  
A, B: P<0.01; a, b:P<0.05

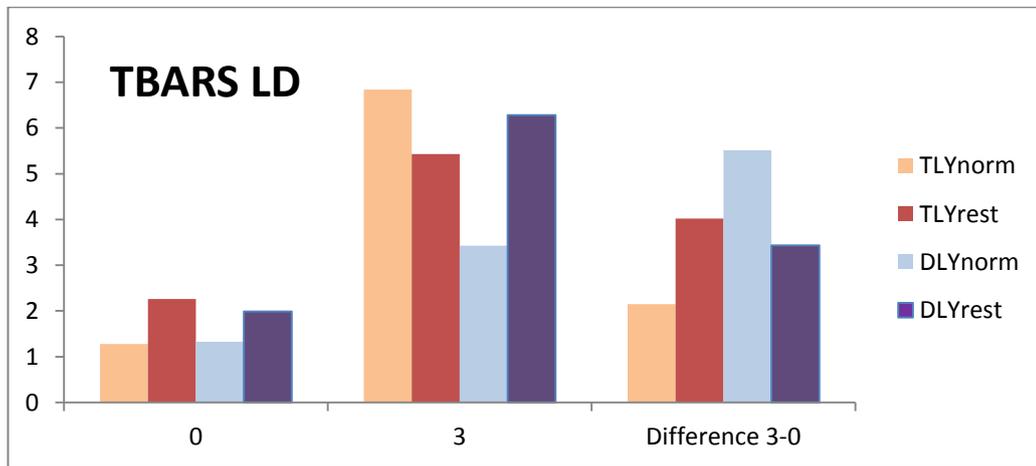


Figure 30 Effect of ageing, crossbreed and feeding strategy on TBARS trend in LD.

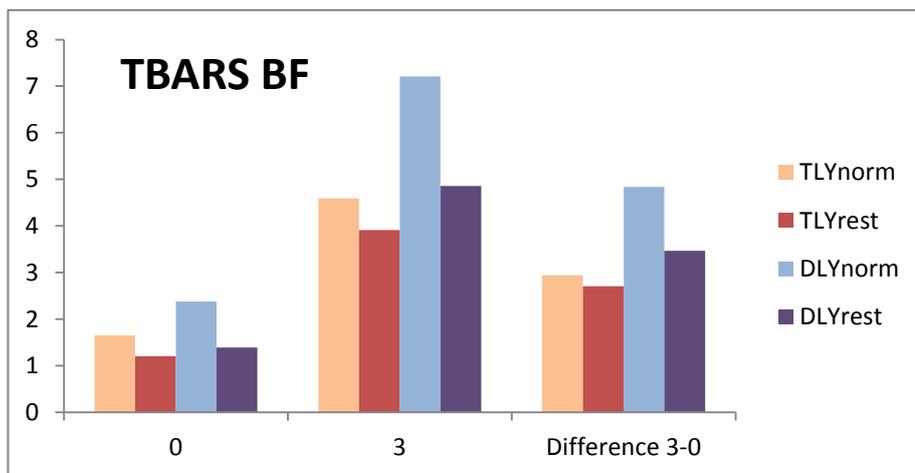


Figure 31 Effect of ageing, crossbreed and feeding strategy on TBARS trend in BF.

## **5.FINAL REMARKS**

Results from the present study show that the choice of breed and feeding strategy affect some qualitative aspects of fresh pork, to different extent between *longissimus dorsi* and *biceps femoris*, such as tenderness, drip loss, carcass weight, the amount of lean meat, oxidative stability, the postmortem pH and temperature trend. Between the two crossbreeds studied, DLY is the one it showed higher yields both in terms of weight of the carcass and lean meat. From the other side it is the one that requires more ageing days to achieve good levels of tenderness as well as be more affected by the strategies power, compared with TLY. The effect of the breeds like Tamworth makes more suitable the pigs both at range farming and at processed meat production, while breeds like Duroc makes pigs more suitable for fresh pork production and traditional farming. Finally, the choice of the crossbreeds and the feed strategy to be adopted should be a consequence of the type of production choice and the type of market targeted. Thereby, the breed and the strategy of feed can be a powerful means to optimize the quantity and quality of the meat production.

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