**UNIVERSITA' CATTOLICA DEL SACRO CUORE**
**PIACENZA**

**Scuola di Dottorato per il Sistema Agro-alimentare**

**Doctoral School on the Agro-Food System**

**cycle XXII**

**S.S.D: AGR17, AGR15**

# STUDYING POST SLAUGHTER NEW TECHNOLOGY TO IMPROVE AUTOCTONAL BEEF MEAT QUALITY BY EXSTENSIVE REARING SYSTEM

**Candidate:** **Michela Contò**
**Matr. n.: 3580171**

**Academic Year 2008/2009**


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# 1 INTRODUCTION

## *1.1 Bovine livestock in Italy*

In Italy there are about 6 millions of live bovines 1 million of which are bulls, beefs and calves for meat production (Istat 2007/208). Most of them are in Northern Italy, (4 millions heads), in particular in Lombardia region (table 1). These animals are mainly calves and young beefs from milk breeds that come from French and North of Europe.
Only 149,000 of all live bovines belong to Italian breeds (Marchigiana, Chianina, Romagnola, Maremmana, Podolica and Piemontese) (ANABIC 2007). These Italian breeds are mainly reared in Central Italy, except for the Piemontese (in the North) and Podolica (in the South).

**Table 1.** Quantity of slaughtering animals per region and category (Istat 2007)

| Country | Calves <1 year | Beef > 1 year |
|---|---|---|
| **Piemonte** | 42,649 | 115,703 |
| **Valle d'Aosta** | 431 | 741 |
| **Lombardia** | 201,410 | 117,726 |
| **Trentino-Alto Adige** | 2,466 | 3,371 |
| **Bolzano** | 1,578 | 1,412 |
| **Trento** | 888 | 1,959 |
| **Veneto** | 134,289 | 221,599 |
| **Friuli-Venezia Giulia** | 2,356 | 8,890 |
| **Liguria** | 799 | 1,133 |
| **Emilia-Romagna** | 12,021 | 44,230 |
| **Toscana** | 9,727 | 12,659 |
| **Umbria** | 6,535 | 7,648 |
| **Marche** | 6,374 | 10,437 |
| **Lazio** | 10,962 | 13,721 |
| **Abruzzo** | 5,502 | 8,198 |
| **Molise** | 3,670 | 4,957 |
| **Campania** | 10,147 | 20,147 |
| **Puglia** | 6,457 | 8,452 |
| **Basilicata** | 4,811 | 5,321 |
| **Calabria** | 10,822 | 14,039 |
| **Sicilia** | 18,810 | 21,429 |
| **Sardegna** | 28,796 | 12,353 |
| **Italy** | **519,034** | **652,754** |

Previous regions are characterized by an intensive rearing system with:

- Higher mechanization;
- Higher head/UAA;
- Feed with higher protein and energy content (e.g. concentrated and maize silage);
- Breed with higher conversion and growth index (e.g. French breed Limousine and Charolaise, Simmenthal and/or crossbreeds);
- Specialized workers;
- Low time-consuming production processes;
- Farm management optimization;
- Contained areas (e.g. box on grilled and deep litter).

Although intensive system is very expensive due to the livestock management, since a lot of money is invested on structure, mechanization, workers and food and animals buying.

Furthermore, pollution is very high because a great deal of animals on a little area cause a high concentration of nitrate in the soil, (the 676 Act says that the maximum nitrate concentration is 170 kg/ha/year for the vulnerable zones and 340 kg/ha/year for non vulnerable zones, Nitrate Directive n. 676, (1991).

Whilst the North of Italy is characterized by an intensive system, in the South and in the Islands extensive system is common. This system is used in marginal lands that are characterized by wooded areas, hills and mountains.

Extensive system is characterized by:

- Closed productive cycle (cow-calf line), calves born in the farm;
- Pasture and semi-pasture areas;
- Low density head/UAA;
- Animal feeding based principally on pasture and hay

This rearing system is applicable to bovine characterized by high rusticity (e.g. good diseases and climate resistance capacity), good capacity of ingestion and utilization of low nutritional forage and some by-products, high fertility (1 calf/year), good motherly aptitude (i.e. good milk production), meat production aptitude, easy recovery of their strength after lactation stress.

In Italy, in the 2008, were slaughtered 3,833,459 heads (beefs and buffaloes), corresponding to about 1,059,314 tonnes of meat (fao.org). National production of beef

depends on quantity of calves import. In fact in our Country about 1.5 millions of heads are imported including 1.2 millions of heads from France.

The Italy meat productivity and the gains of breeding is so compromised by the high costs and the difficulty to found young animals for fattening from other Countries. Moreover, in the last year, the purchase of raw materials, like food for animals, is increased although the market of the meat has gone through a difficult period and it is still decreasing (graphic 1).

**Graphic 1**


FAO Statistics Division 2010 | 08 January 2010

## *1.2 Why the extensive rearing?*

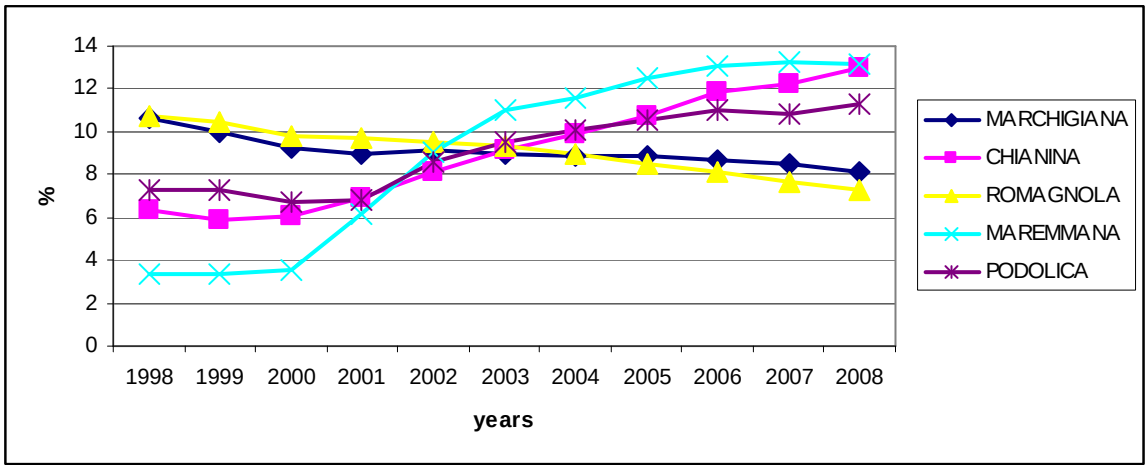
Although the extensive system presents some problems in the management it is useful for maintaining local ambient characteristics, for repopulating the marginal areas and for maintaining the pure line Italian breeds and the biodiversity.

In fact in the last ten years the number of Italian breed rearing farms has increased (graphic 2) of 14% in total (ANABIC), in particular we can see a large increase of farms in which Maremmana and Chianina are reared too. This trend is also confirmed by the increase of total animals registered in the ANABIC (the Italian National Bovine Meat Farmer Association).

This change is due to different factors. First of all the consumer's request, consumer prefer a quality product made in farms that respect animal welfare. Hence quality concept is strongly correlated to welfare.

Furthermore, the meat obtained in an extensive system has better organoleptic/sensorial characteristics (Giorgetti et al., 2009) and nutritional values than meat from intensive rearing system. This meat is characterized by a typical forages flavour derived from pasture; moreover, shows a higher quantity of CLA (conjugated linoleic acid, functional products that have benefits on human health, Kramer et al., 1998; Brown et al., 2002) a good ω3/ω6 ratio (Reline et al., 2004), and low saturated fatty acids. Meat of bovines fed pasture has a lower n-6 polyunsaturated fatty acids (PUFA) content when compared to the meat of intensive reared bovines (grain-based diet) (Enser, 2000; Yang et al., 2002). Unfortunately extensive rearing system has some limits that hinder its development. In fact it is more time-consuming than the intensive one because animal's growth is slow. There is also another problem about meat. In fact, even though the meat has usually a better flavour and it is also better from a nutritional point o view, it could be tougher and darker due to the bigger muscle activity during life.

**Graphic 2.** Italian breeds farm




## *1.3 Meat quality*

As previously mentioned, breeding system affects meat quality, but what is the meat quality, what are the factors that cause it and how is it changed?

Quality is not so easy to be described because quality covers inherent properties of meat decisive for suitability of the meat eating, further processing and storage including retail display (Henrik et al., 2004). Quality is defined by ISO 8402 (*International organization for standardization*) as "characteristics of product that satisfy consumers demand". Regarding meat quality, the safety, nutritional values, flavour, texture, water-holding capacity, colour, lipid content, lipid composition, oxidative stability and

uniformity are the main attributes that are required by consumers. However, high standards of quality affect also different aspects regarding environmental, ethical and animal welfare problems.

Anyway the first aspect that defines quality is tenderness, in fact consumers demand a more tender meat, and this aspect is often related to an extensive rearing system (Monsón et al., 2005).

To improve meat tenderness is important to understand what occurs during the ageing time, from slaughter to 8-21 days after, and in particular during the *rigor mortis*, when the muscle becomes meat, due to development of some biochemical processes.

The main subjects of tenderization process, are enzymes and structures of muscle: myofibrils and collagen. All this subjects have interacted one another during ageing time and on tenderization process.

### 1.3.1 Myofibrils

Muscle is a structure composed mostly of contractile fibres organized in fascicles by connective tissue. The quality of meat is therefore determined by the muscle architecture, attachment of fibres to connective tissue, and changes in these structures post-mortem; the effect of muscle fibre type on meat quality depends of fibre size and fibre type: glycolytic (white fibres), oxidative (red fibres) and mixed. The fibres are usually classified in type I (slow-contraction, red, small and oxidative metabolism); type IIA (fast-contraction, intermediate metabolism); type IIB (fast-contraction, white, large and glycolytic metabolism). Fibre type varies significantly among species, muscle types and regions within the same muscle. It is the variability and adaptability of fibre type that allows muscle to be a dynamic organ that responds to the needs of the animal.

### 1.3.2 Collagen content

Collagen is an elongated protein that forms extremely strong but very small fibrils (best seen with an electron microscope). Lot of these collagen fibrils are bound together to form collagen fibres that easily can be seen with a light microscope. Collagen is the most abundant protein in the animal body, and the collagen which occurs in meat may be an important source of meat toughness. Particularly with increase of age it forms a larger amount of insoluble collagen and so the meat of older cattle becomes tough.

Tropocollagen that constitutes collagen is a high molecular weight protein (300,000 Dalton) consisting of three polypeptide strands twisted into a triple helix. The triple helix is responsible for the stability of the molecule and for the property of self-assembly of molecules into micro fibrils. The flexible parts of each strand projecting beyond the triple helix (telopeptides) are responsible for the bonding between adjacent molecules that forms the cross links that bind tropocollagen molecules together. Different cross links between tropocollagen fibrils form different types of collagen. The various types of collagen of interest to understand the structure of meat are principally:

- Type I collagen forms striated fibres between 80 and 160 nm in diameter in blood vessel walls, tendon, bone, skin and meat.
- Type III collagen forms reticular fibres in tissues with some degree of elasticity, such as spleen, aorta and muscle.

Tropocollagen links from older animals are more resistant to heat degradation than those from younger animals.

### 1.3.3 Enzymes activity

Enzymes activity acts on some muscle structural in particular on myofibrils. Their actions are performed during the ageing time, period in which muscle becomes meat.
In this period there are two important classes of enzymes: glycolytic and proteolytic enzymes, that modify some meat aspects like pH and tenderness.

#### 1.3.3.1 Glycolytic enzymes

Glycolysis is an almost universal pathway for extraction of the energy available from carbohydrates. In aerobic organisms, considerably more energy can be harvested downstream from glycolysis in the citric acid cycle. Glycolysis produces energy in form of ATP and NADH.
If glycolysis continued indefinitely, all of $NAD^+$ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD+.
The method to convert NADH in NAD+ is the anaerobic respiration which produces pyruvate do the oxidation in a simpler way; in this process the pyruvate is converted into lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation.

This process occurs in animals slaughtered during the rigor mortis. After the death of the animals the blood can no longer perform its transport function in the Cori cycle and anaerobic respiration starts, so beginning an acid lactic store. This lactate accumulation determines a pH change from 7-7.2 in alive animal to 5.5 in death animals (Jensen et al., 2004).

#### 1.3.3.2 Proteolytic enzymes

In living muscles, intracellular protein degradation is mediated by a number of different endogenous proteolytic enzymes (Ouali 1990). Due to the many changes occurring in the course of meat tenderization are currently believed to be the result of proteolysis, every proteinase located inside muscle cells could be a potent contributor to meat ageing and must be considered in this context.

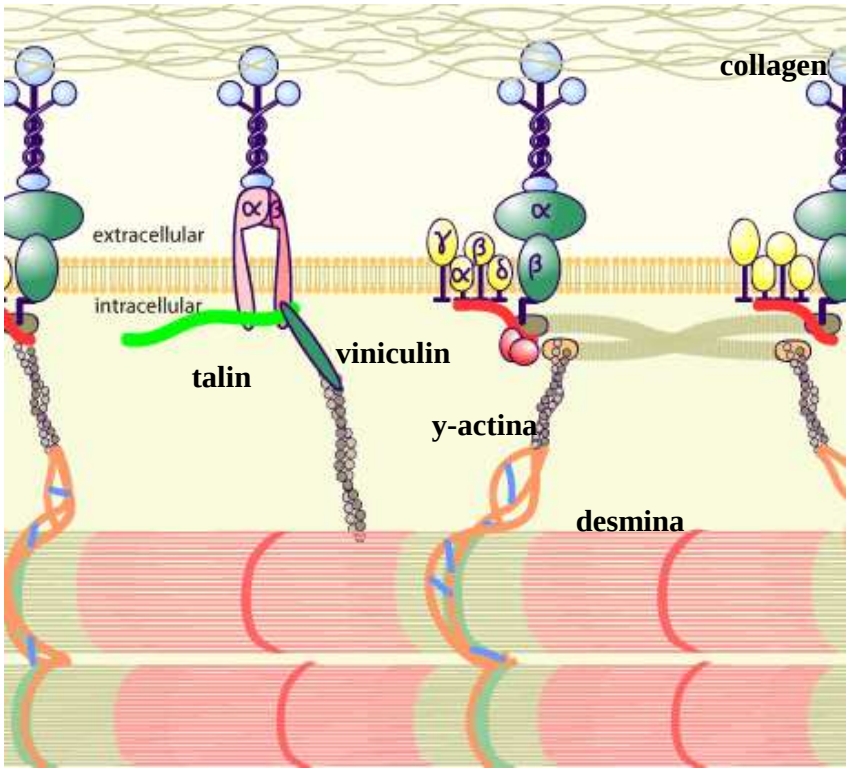
In the proteolytic systems detected in skeletal muscle, 2 proteinases have been noted that are able to degrade the myofibrillar proteins. These proteinases are calcium-dependent cysteine proteinases located in the cellular cytosol. They are named µ-calpain and m-calpain because activated at micromolar (50 to 70 µmol) and millimolar (1 to 5 mmol) concentration of $Ca^{2+}$ respectively (Hortòs et al., 1994). Their activity is optimal at pH 7.5 and has been shown to degrade Z-disk, troponin-T, and desmin, but it is inhibited by a specific calpain inhibitor called calpastatin (Koohmarie et al., 1987). The calpain proteolytic system also includes a tissue-specific calpain, calpain 3 (Suzuki et al., 1995). Other proteinases have been isolated, such as cathepsins B, H, and L, (Goll et al., 2002; Zeece et al., 1989), but their importance for meat tenderization is not completely clear (Johnson et al., 1990; Dransfield et al., 1992).

### 1.3.4 Ageing time process

The *rigor* process has been characterised by Bendall (1973) as consisting of a delay and a rapid phase period. For the delay period, the level of ATP is constant, the creatine phosphate (CP) falls rapidly, while there is a slow production of lactate and no onset of *rigor* development. When the CP is low enough, a rapid decline in the ATP (rapid phase) is initiated, accompanied by a shortening of muscle and the development of a force under isometric conditions (Tornberg, 1996). Shortening is explained by the release of calcium ions into the myofibrillar space at ATP concentrations sufficiently high for contraction (Honikel et al., 1983).

During *rigor* development, not only longitudinal but also lateral contraction occurs (Tornberg, 1996). Offer et al., (1989) have shown that a decrease of about 9% in the cross-section area of the myofibrils takes place during *rigor*. This decrease is suggested to be partly due to a fall in pH and partly due to the attachment of myosin heads to the actine at *rigor* onset. The consequence of this shrinkage of the myofibrils is that the fibres shrink and the water that is left behind accumulates, first along the perimysial network and later along the endomysial network, giving rise to two types of extracellular compartments (Offer et al., 1989). Other structural events occurring during *rigor* are based on the proteolytic action, which also continues after fully developed *rigor*, the being called ageing (Taylor et al., 1995). These proteins are involved in inter (e.g. desmin and vinculin) and intra-myofibril (e.g. titin, nebulin, and troponin T) linkages or linking myofibrils to the sarcolemma by costameres (e.g. vinculin, dystrophin) and the attachment of muscles cells to the basal lamina (Hattori et al., 1995) (figure 1). Degradation of these proteins would, therefore, cause weakening of myofibrils and thus, tenderization (Koohamarie 1996).

**Figure 1**. Costamere complex

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Since these early observations, a great deal of evidence has accumulated indicating that the calpains have an important role in postmortem tenderization, and many studies have

focused attention on this role (Goll et al., 1992; Boehm et al., 1993; Dransfield 1992; Koohmarie 1992). This evidence has led to the prevailing view that the calpains are responsible for 90% or more of the tenderization that occurs during postmortem storage, (Goll et al., 1992).

The enzymes involved in postmortem proteolysis have been the subject of much debate; however, it is now generally accepted that calpain I (also called µ-calpain) is the major enzyme involved (Koomaharie et al., 1986; Geesink et al., 1999). The calcium concentration in postmortem muscle could reach 150 µmol,51 which is sufficient to activate calpain I but insufficient for calpain II activity (Koomaharie, 1992). The activity of calpain I is mostly regulated by calpastatin, its endogenous inhibitor (Geesink et al., 1999). Levels of calpastatin in muscle vary considerably among species (Oali et al., 1990; Koomaharie et al., 1991), breeds (Whipple et al., 1990; Shackelford et al., 1991; Shackelford et al., 1994) and muscles (Koomaharie et al., 1988; Geesink et al., 1992).

## *1.4 Changing of some Qualitative parameters during the ageing time*

### 1.4.1 pH

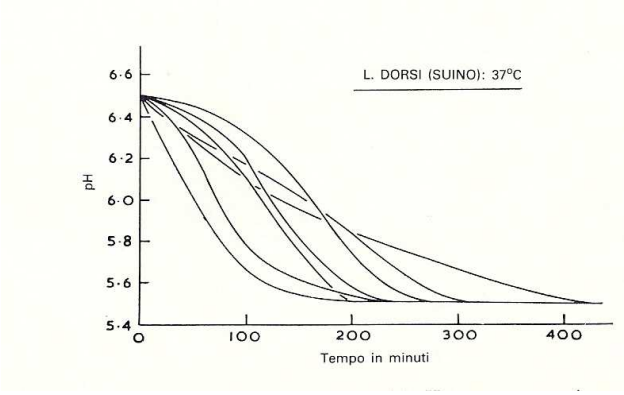
After animal is exsanguinated, the blood can no longer perform its transport function in the Cori cycle, and lactate accumulates in the musculature. This lactate accumulation causes a pH change, from 7-7.2 in live animal to 5.3-5.8 in death animal. pH falls due to formation of 0.1 mol $l^{-1}$ of lactic acid from glycogen by anaerobic glycolysis (Honikel, 2004). After all glycogen is used, by glycolytic enzymes, the lactic acid is not more produced hence the pH does not change and so is called final pH (Callow, 1937).

In bovine meat it takes 18-36 hours to reach the final pH (Honikel, 2004). This variability is due to different factors (Ouali, 1990). These factors depend on the animal (i.e. species, fibre types) (Hannula and Puolanne 2003) and environmental conditions (i.e. stress before slaughter, temperature) (Selye 1936). Temperature influence the glycolytic process, in fact more the temperature increase more the speed of the glycolytic process increases, on the other hand the glycolytic process rate, hence the pH, changes from muscle to muscle by the muscle position in the body and by the cooling method (Bendall 1978).

The rapidity of pH reductions in post mortem period affect the meat tenderness during ageing time. In general if we have a slow pH decline in the first 24 hours we will have a tough meat, but Marsh et al., (1987) reported a tenderness meat despite a slow pH decline.

Thompson (2002) was reported that there are two limits, one superior and one inferior, that causes tough meat, hence there is an ideal pH decline range (graphic 3).

**Graphic 3**. Variability of pH rate decline during post mortem phase in *L. dorsi* muscle.




Lawrie (1979)

### 1.4.2 Water content

Lean muscle contains approximately 75% of water. The other main components include proteins (approximately 20%), lipids or fat (approximately 5%), carbohydrates (approximately 1%) and vitamins and minerals (often analyzed as ash, approximately 1%) (Huff-Lonergan et al., 2005). The majority of water in muscle is held within the structure of the muscle and muscle cells. Specifically, within the muscle cell, water is found within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Offer & Cousins, 1992).

Water is a dipolar molecule and is attracted to charged species like proteins. In fact, some of the water in muscle cells is very closely bound to protein. By definition, *bound water* is water that exists in the vicinity of non-aqueous constituents (like proteins) and has reduced mobility, i.e. does not easily move to other compartments. This water is very resistant to freezing and to being driven off by conventional heating. True bound water is a very small fraction of the total water in muscle cells.

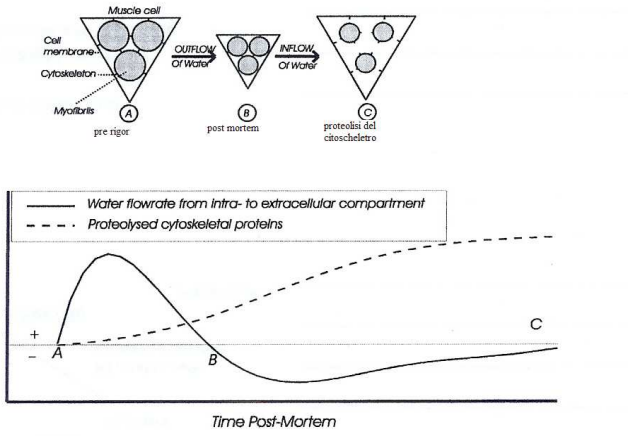
Another fraction of water that can be found in muscles and in meat is termed entrapped (also referred to as immobilized) water (Fennema, 1985). The water molecules in this fraction may be held either by steric (space) effects and/or by attraction to the bound water. This water is held within the structure of the muscle but is not bound with protein. In early *post-mortem* tissue, this water does not flow freely from the tissue, yet it can be removed by drying, and can be easily converted to ice during freezing. Entrapped or immobilized water is most affected by the *rigor* process and the conversion of muscle to meat. Upon alteration of muscle cell structure and lowering of the pH this water can also eventually escape as purge (Offer et al., 1988).

Free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat. Free water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found (Fennema, 1985).

The majority of the water that is affected by the process of converting muscle to meat is the entrapped (immobilized) water. Maintaining as much of this water as possible in meat is the goal of many processors. Some of the factors that can influence the retention of entrapped water include manipulation of the net charge of myofibrillar proteins and the structure of the muscle cell and its components (myofibrils, cytoskeletal linkages and membrane permeability) as well as the amount of extracellular space within the muscle itself.

During the conversion of muscle to meat, lactic acid builds up in the tissue leading to a reduction in pH. Once the pH has reached the isoelectric point (pI) of the major proteins, especially myosin (pI=5.4), the net charge of the protein is zero, meaning the numbers of positive and negative charges on the proteins are essentially equal. These positive and negative groups within the protein are attracted to each other and result in a reduction in the amount of water that can be attracted and held by that protein. Additionally, since charges repel, as the net charge of the proteins that make up the myofibril approaches zero (diminished net negative or positive charge) repulsion of structures within the myofibril is reduced allowing those structures to pack more closely together. At the end there is a reduction of space within the myofibril (graphic 4). Partial denaturation of the myosin head at low pH (especially if the temperature is still high) is also thought to be responsible for large part of the shrinkage in myofibrillar lattice spacing (Offer, 1991).

**Graphic 4.** Hypotesis of drip loss during ageing time




Kristensen and Purslow 2001

This amount of water, may subsequently be subject to considerable variation, due to the gains that occur during processing, or to losses through drip, evaporation or cooking. The juiciness and tenderness of meat and meat products depend on a great extent on their water content and excess drip produces an unattractive appearance (Offer et al., 1983).

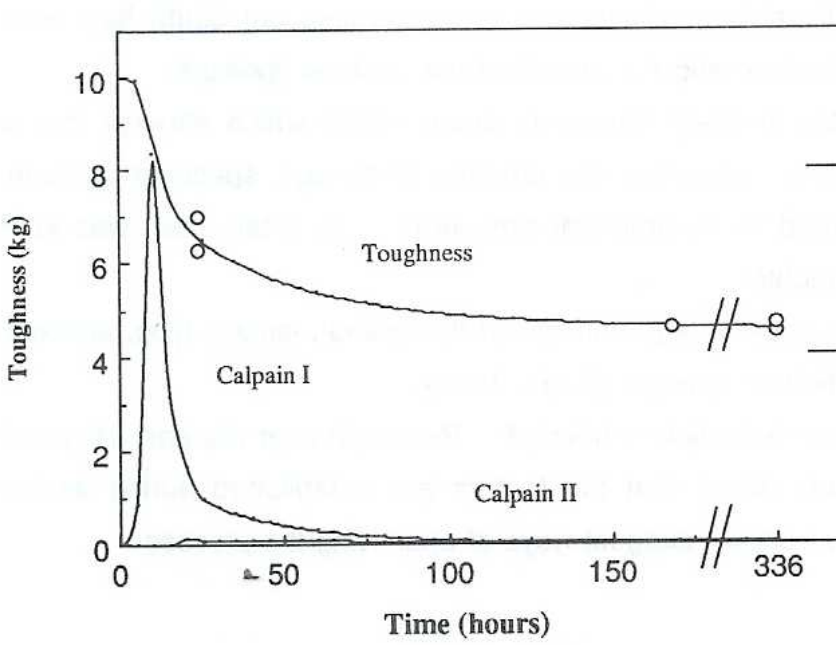
## *1.5 Tenderness*

Research has shown that significant improvement in tenderness can be achieved by controlling the physiological processes that affect tenderness and processes that are influenced by environmental factors. These factors include carcass chilling (May et al., 1992), electrical stimulation (Nour et al., 1994), time on feed (Van Koevering et al., 1995), post mortem ageing time (Huff-Lonergan et al., 1995), cooking method (Wulf et al., 1996a), and end-point temperature (Wheeler et al., 1999). The variation in tenderness is also due to the genetic variation, biological and physiological differences, biophysical changes during slaughter, and chemical differences created during post-mortem aging (Koohmaraie, 1996). In addition to these factors, the percentage of protein, fat, moisture, and collagen composition of meat may affect tenderness (Cross et al., 1973). In general, tenderness is affected by ageing, extent of rigor, sarcomere length, proteolytic activity, and a variety of physiological and chemical factors that occur during rigor mortis and post mortem (Pearson, 1987). Pre-*rigor* meat is tender but becomes progressively tougher as permanent cross-bridges form between myosin and actin (Pearson, 1987). Therefore, the variation of tenderness depends on the rate and extent of post-mortem tenderization. Tenderisation occurs as the structural proteins are degraded (i.e. proteolysis). Proteolysis can continue even in shortened muscle without

the meat-becoming tender (Locker et al, 1984; McDonagh, et al, 1999). If the muscle has been stretched, then it is more tender (Hopkins, et al, 2000b), but proteolysis may not have occurred to the same extent as non-stretched meat for the same shear force changes. Ageing is the process which leads meat to become tender and involves specific degradation of the structural proteins.

The processes affecting meat tenderness start at slaughter, but changes may not be significant at that time and also measurement of tenderness at this stage is meaningless. The endogenous enzymes responsible for tenderisation will be active throughout the *rigor* process (graphic 5).

**Graphic 5**. Toughness rate during ageing and enzymes activity.




Dramsfield (1992).

While proteolysis is taking place, significant tenderness changes are not evident until most of the muscle fibres are in *rigor* (Devine et al, 1995). Because an intact muscle is a collection of individual muscle fibres entering *rigor* in succession, intact muscle would appear to start to age (tenderize) before *rigor* is complete in all fibres. The development of *rigor* and the shortening of fibres would be expected to counter early proteolysis so that the expected peak in shear force is eventually negated by the cumulative post-*rigor* proteolysis. Once this reverses the rise in toughness resulting from *rigor* contractures the process of tenderisation occurs.

## 1.5.1 Tenderization techniques

Different techniques were tested on carcass by researchers for improving tenderness: electrical stimulation, injection of different elements as calcium or some natural compounds, temperature control such as very fast chilling, different carcass hanging.

### 1.5.1.1 Electrical stimulation

The historical reason of the development of electrical stimulation was the acceleration of post-mortem glycolysis so when muscle entered rigor it was prevented from shortening excessively (Swatland, 1981).

Electrical stimulation involves passing an electric current through the carcass of animals within a few hours from slaughtering. This electric current causes the contraction of the muscle increasing the rate of glycolysis so resulting an immediate fall in pH (Hwang et al., 2003).

The effects are a physical degradation of the myofibrillar matrix (Ho, et al, 1997) or the acceleration of proteolysis (Uytterhaegen et al, 1992). Electrical stimulation improves meat tenderness through its effects on physical alteration (i.e. prevention of cold shortening or causing physical disruption) and/ or the acceleration of energy turnover during and after the treatment. There is potentially a powerful association between physical disruption of the myofibrillar complex and increase in tenderness (Dutson, et al, 1980; Ho, Stromer et al, 1996).

Contracture bands are not a direct consequence of electrical current passing through the muscle, but rather due to the super contraction caused by localised excessive calcium ion release from the sarcoplasmic reticulum. It could be this extra calcium which also causes the tenderisation to proceed. This section will attempt to shed insight about the relative importance of ultra structural alteration for the effects seen as a result of electrical stimulation. Dutson, et al (1977) reported that electrical stimulation resulted in ultra structural changes in beef *longissimus* muscle.

However, there is not unanimity about which of these effects is most important in terms of reduction of the toughness of meat (Hwang et al., 2003). Furthermore, Devine et al. (2001) in a study found a wide variability in the tenderisation rate for sample held at 10° C after electrical stimulation, where differences in sarcomere length were not significant and there was no detectable difference in rate of tenderisation and final tenderisation compared to non-stimulated samples.

Electrical stimulation of carcasses after slaughter is a process that can have a significant effect on meat toughness, but it is not universally used and the reasons are not clear.

#### 1.5.1.2 Effect of cooling rate

The cooling system is usefully to reduce a microbial growth on the carcasses and for storage the product during marketing.

The meat industry has long been attracted by the concept of accelerating rate of carcass chilling but this has been inhibited by the risk that a rapid drop in muscle temperature can induce a condition known as cold-shortening which can result in unacceptably tough meat. To overcome this detriment effect on eating quality, criteria have been established for the combination of post-slaughter time, temperature and pH necessary to avoid cold-shortening. As a general rule, it has been recommended and widely accepted that temperature of beef should not fall below 10° C within 10 hours of slaughter. Experience has shown that, if these time/temperature conditions are observed, rigor mortis will have advanced sufficiently to avoid toughening the meat (Troy et al., 2001).

Furthermore, the cooling system could improve some quality characteristics of meat as tenderness. In fact the effects of calpains (proteolytic enzymes) and their inhibitors immediately post-mortem, depend on pH ant temperature and have an influence on the tenderness (Hannula, et al. 2004; Koohmaraie, 1996; Dransfield 1994).

(The cooling technique that some authors were studied was) One of the available cooling techniques is Very Fast Chilling (VFC) that means to reduce the core temperature of muscle to 0°C in 2-5 hours post mortem. Two mechanisms has been attributed to VFC: 1. the outer surface of the muscle may be frozen and act as a straitjacket to prevent cold shortening; 2. the intense cold release bound calcium so that the tenderising enzymes are stimulated. The VFC activity is different from animal to animal and from muscle to muscle of the same animal and its properties are influenced by time-temperature-pH combinations.

The VFC does not induce cold shortening because the cooling is initially fast but then slowed down to allow the pH to fall and rigor to set, the meat still is tender. This happens because during fast chilling, near 0°C, the ATP (adenosine triphosphate) is consumed by enzymatic glycolisys (Marsh, et al, 1956), and it won't more available for muscle contraction. Bendall (1973) found that ATPase activity decreases when temperature goes down from 38°C to 0°C; it has also been shown that the calcium bonding capacity of the sarcoplasmatic reticulum decreases strongly at temperature

below 10°C, and this occurs very early post mortem. Van Moeseke et al (2001) hypothesized that this early supply of free calcium, together with a high muscle pH, could result in an advanced and increased activation of calpains and therefore in an intense tenderisation, capable of overcoming toughness caused by cold shortening. An acceptable tenderness level for VFC beef was found after 7 days instead of 14 days of conventional chilling (Honikel, 1998).

#### 1.5.1.3 Pre-rigor Injections of Ionic Compounds

Both Webster's New World Dictionary of the American Language and Dorlands's Illustrated Medical Dictionary define infusion similarly as the introduction of a solution into the body, specifically into a vein, and perfusion as the act of pouring (such as a liquid) over or through an organ or a tissue (Polidori et al., 2000).

Infusion or perfusion of compounds to change the rate of glycolysis, rate and state of contraction, and rate of proteolysis appear to be feasible methods of manipulating the postmortem tenderization process in meat (Lee et al 2000; Koohmaraie et al 1990; Wheeler et al., 1991). It was reported in the mid1950s that infusion of bovine carcasses with salt solutions caused a considerable improvement in tenderness (Geesink et al 1994).

The results obtained in a recent study (Polidori et al., 2001) indicated that infusion of beef carcasses with CaCl2 accelerated post mortem tenderization. The direct action of calcium ions was described by Takahashi. Calcium ions have a dual function in post mortem muscle (Takahashi yet al., 1996); the rise of sarcoplasmic calcium ion concentrations to 3 to 5 mmol induces rigor contraction, and the further rise of the calcium ion concentration to 0.1 mmol weakens the structures of myofibrils, desmin intermediate filaments, and probably the endomysium and perimysium, thereby tenderizing the meat. The different ionic compounds injected before rigor in carcasses of meat animals in previous experiments were: calcium chloride, maltose, dextrose, polyphosphate and glycerine, sodium chloride, zinc chloride, sodium phosphate plus sodium chloride, sodium chloride and phosphate (Koohmaraie et al 1990; Farouk et al 1992; Koohmaraie, 1990; Lee et al 2000; Yancey et al 2002).

Other studies (Gonzalez et al 2001; Whipple et al, 1993; Taylor et al, 1991) have been performed to establish the conditions required to improve meat tenderness by using calcium chloride marination, and consequently to reduce the time required for the postmortem tenderization. The results obtained by Gonzalez et al (2001) showed that

calcium chloride marination was effective in increasing tenderness and in reducing the post mortem storage time necessary to achieve an acceptable level of tenderness in beef Cutaneus muscle. The results obtained by Whipple and Koohmaraie (1993) showed that the improvement in tenderness was due by the activation of calpain II, also called m-calpain.

#### 1.5.1.4 Hanging carcass

Based on the importance of cold shortening for determining tenderness of meat, several methods for restricting the contraction of pre-*rigor* muscles in intact carcasses or carcass sides were developed in the late 1960s and early 1970s. Herring, et al., (1965) compared a number of muscles from beef carcass sides which entered *rigor* either in a horizontal position with the limbs perpendicular to the vertebrae or in the common vertical position by the Achilles tendon suspension. Horizontally placed sides resulted in longer sarcomeres, lower fibre diameters and increased tenderness major muscles like *longissimus, gluteus medius, biceps femoris and semitendinosus.* Texas A & M Tenderstretch tenderizing method for beef carcass suspension from the eye of the aitch *obturator foramen*, also called Tenderstretch, was introduced by Hostetler, et al., (1970). These authors studied five different treatments for suspension positions and limb tying of beef carcass side (Hostetler, et al., 1972). The Tenderstretch was most efficient in improving sarcomere lengths and ultimately tenderness, in particular for the massive muscles of the loin and round. Smith et al., (1971) determined the tenderizing effects of different mechanical and physical on *longissimus* muscles in beef carcass sides, including Tenderstretch, skeleton severance in different position in the vertebrae, and use of weight loads attached to the neck. In addition, a method for attaching hook and a wire for making tension between the Achilles tendon and the vertebrae was tested. All these methods generally improved tenderness in many major muscles compared to Achilles tendon suspension.

The Tenderstretch is usually applied within 45 to 90 min after bleeding, while the muscles still are extensible and in the pre-*rigor* state. If the angle of the hind leg is much less than 90°, it indicates that the muscles have started to contract and that the is applied too late.

A number of studies support the positive effects of tender stretching on meat tenderness of beef, lamb and pork when being chilled rapidly and having a risk of cold shortening.

In beef, (Hostetler et al., 1970, 1972) increased the sarcomere lengths of *longissimus, semimembranosus* and *semitendinosus* muscles, but not the *psoas major* muscle, by using Tenderstretch. A study by Bouton et al., (1973) confirmed these findings of increased tenderness of *longissimus, semimembranous* and in addition *gluteus medius* muscles. The Tenderstretch gave tenderness values of non-aged meat equivalent to 21 days ageing. The muscles of the forequarter do not tenderize by using tender stretching, as no extra stretch or weight is applied to this part of the carcass.

# 2 OBJECT

Meat is an essential food for human nutrition, since it provides essential amino acids, fatty acids, protein, etc. and consumer, as mentioned in the Introduction, is always looking for more tender and flavourful meat that can satisfy its hedonistic need. Some Italian breeds and especially the rustic breeds have tougher meat than the specialized breeds reared in an intensive way, even if they show good nutritional characteristics and flavour. The knowledge acquired to date on the biochemical processes involved during the transformation of muscle into meat, i.e. during the period of meat maturation or ageing, allows us to plan and/or improved new technologies to enhance and it improves some very important qualitative traits as tenderness is.

The objects of this research are:

1. finding new techniques to improve meat tenderness in animals reared in an extensive system: by inducing increase of glycolytic potential of muscle; increase of calcium for raising the enzymes activity like μ e m calpaine that are calcium dependent enzymes; mechanical traction force exploitation on muscles by hanging bones;
2. studying ageing physical, chemical and biochemical process to modulate and drive the maturation process of meat to make tenderness process to be foreseeable and no more random.

Some tenderization techniques, developed to accelerate ageing time processes to reduce the time for meat's commercialization as descript previous will be topic of this experimental work.

The breeds used for this trial, are a typical rustic Italian breed (Maremmana) and the more popular milk breed of the world, also exploited for beef production, though as a sub product of milk farming (Holstein Fresian).

## *2.1 Experimental breeds*

1. Italian Holstein Fresian (figure 2), that is the most widespread breed in Italy with about 3 millions heads, and its rearing system is usually intensive.

**Figure 2**

www.cvm.msu.edu/.../ AP/bessie/breeds/breeds.htm

Holstein breed, largely used as dairy cattle originated in northern Holland and Friesland. Its chief characteristics are its large size and black and white spotted coat. Bull weight is about 900 – 1300 kg, high138 – 155 cm high. This cattle has probably been selected for dairy qualities long about 2,000 years. It has been widely distributed over the more fertile lowlands of continental Europe, where it is highly valued for its milk-producing ability.

2. Maremmana breed (figure 3). There are 9682 head in Italy (ANABIC 2008, graphic 4), always reared in extensively system, concentrated in the Centre of Italy and in marginal areas (Gigli et al.,2000a).

**Figure 3.**

www.agraria.org; www.britannica.com

This is a rustic breed and it is typical in an area, Tuscany, where once there were many swamps. It is very rustic, it can feed poor forages and it can live wild in marginal zone.
The Italian National Bovine Meat Farmer Association (A.N.A.B.I.C.) holds the genealogic tree of Italian White Breed (Romagnola, Marchigiana, Maremmana, Podolica) since 1966.
Maremmana breed has a grey coat and long horns. Its body is massive due to the growing of the skeleton, it also has a back highly developed. Its structure is made to be solid and sturdy.
This breed does not reach high weight but its maintenance is very easy because it does not need of high quality forages.
Sometimes are utilized cross-breeds between Maremmana and some typical meat breeds like Charolaise and Chianina in order to improve Maremmana's meat production.

# 3 MATHERIALS AND METHODS

## *3.1 Animals*

The experiment was carried out on 24 young bulls, 12 Maremmana and 12 Holstein. All the heads have been reared in Meat Production and Genetic Improvement Research Center (CRA-PCM) in Rome.

The Holstein bulls have been intensively reared in cages and they fed hay and maize silage *ad libitum* and 800g of concentrated/100kg of life weight in the finishing period (3 months before slaughter) with whilst the Maremmana ones, extensively reared, fed grass with an integration of 4/5 kg of hay during fattening period, while in the finishing period fed hay ad maize silage ad *libitum* and 800g of concentrated/100kg of life weight like Friesian bulls.

Animals were slaughtered at about 550 kg of life weight in the Slaughter House of CRA PCM, followed the CEE law.

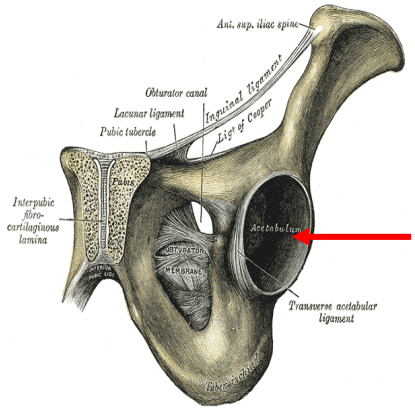
The work was divided into two typologies:

- technique operates on carcass, thesis I;
- technique operates on muscle, thesis II;

## *3.2 Sampling thesis I*

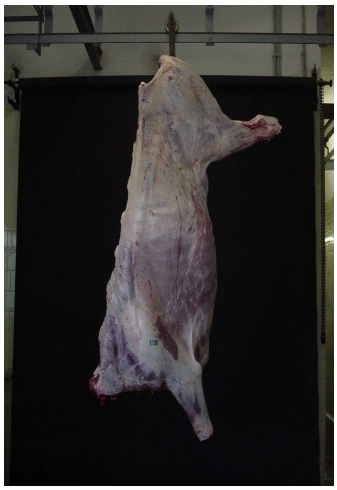
After slaughtering, the left side of carcass was suspended by the *foramen obturatum* of the aitch bone, (Pelvic hanging) for 24 hours (figure 4 and 5), while the right side normally by the Achilles Tendon.

**Figure 4**




*Formen obturatum*

**Figure 5**



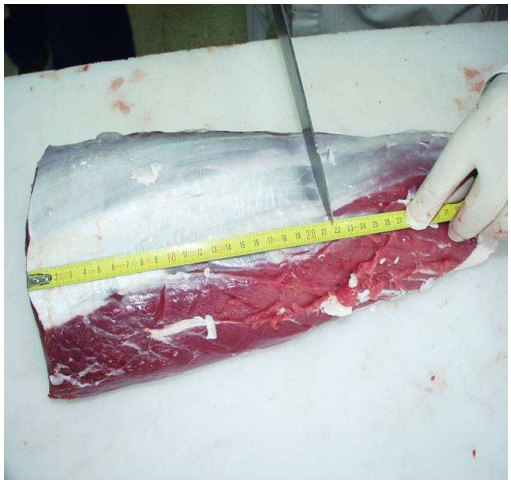
After 24 hours the left side of carcass was hung by Achilles Tendon. After 8 days from both side of carcasses the *Longissimus thoracis* (LD) and *Bicipite femoralis* (BF) muscles were carried out for evaluating the organoleptic, physical and biochemical aspects.

## *3.3 Sampling thesis II*

For the thesis II 30 minutes after slaughter a sample from right side of the *Longissimus thoracis* (between 8th and 13th rib) was taken and subdivided into three portions (figure 6) to obtain three different thesis:

- "Ca" ($CaCl_2$ infusion);
- "VFC" (Very Fast Chilling);
- "C" (Control).

**Figure 6**



On the first portion of Lt muscle, thesis Ca, a 300 mM $CaCl_2$ solution was injected inside muscle (Wulf et al., 1996 Weeler et al., 1997). The amount injected was evaluated as 5% of the weight of the sample. The sample was stored at 4°C in polyethylene bag.

In order to obtain the second thesis (VFC), another portion was stored into a freezer at -70° C until the core have reached 1°C of temperature. In this period every 15 minutes the pH and temperature were determinate to avoid the freezing of the interstitial water and checking the temperature fall; after 1 hour the sample was stored at 4°C in polyethylene bag.

In the third portion (C), no treatments were done. The sample was stored at 4°C in polyethylene bag.

## *3.4 Analyses*

### 3.4.1 Physical analyses

On each sample, the following physical analyses were performed at 8 days for thesis I and 3, 24 hours and 5, 8 days for thesis II:

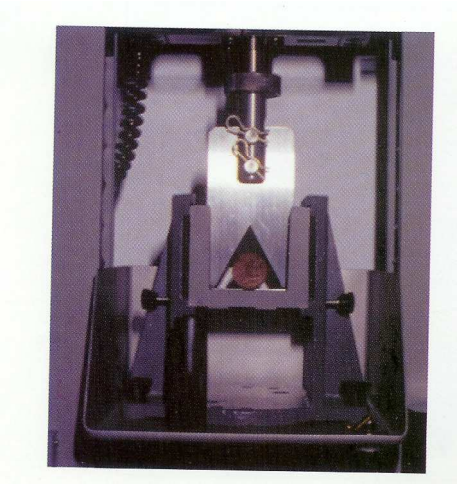
- **Temperature (T)** and **pH**, direct measurement was taken in four different point of samples by insertion of a probe, using an Hanna pHmeter instrument with recorder for temperature;

- **Drip loss** (**WHC**), a slice of muscles, 1.5 cm thick, were cut shortly after slaughter. All associated adipose tissue were removed. A slice of the muscle was weighed and a thin string was threaded through the top of the muscle sample. The sample was placed inside a plastic bag of standard weight and dimensions to be hanged. The string, tied to the muscle slice, protruded from the open side of the bag. The bag was then inflated and its opening was tied tightly by the end of the string coming out from the bag itself. The bagged slice was suspended from the string attached to the muscle in a refrigerator at 4°C long the analyses time (figure 7). At the end of the analyses period the sample was removed from the bags, gently blotted with paper towel and weighed. Drip was calculated as the weight loss of the muscle sample and was expressed as g/100g meat (Barton-Gade *et al*., 1993); this parameter was valuated only for thesis II;

**Figure 7**

- **Cooking loss** (**WHC cooking**), a slide of 2.5 cm was weighted by a precision balance and vacuum packed in a plastic bag. Samples were cooked in a bath at 80°C per 25 minutes, after cooking the samples were cooled in cold water for 20 minute. At the end the samples were removed from the bags, gently blotted with paper towel and weighed. Cooking loss was calculated as the weight loss of the sample and was expressed as g/100g meat;
- **Warner-Blatzer Shear Force** (**WBS**); Warner-Blatzer Shear force on cooked meat was determined in four 1 x 1cm cross section strips using an INSTRON 5543 texturometer, (figure 8), with 100 kg cut force and advance blade of 100 mm/min were used. The maximum load in breakpoint of the miofibrillar was expressed as kg; the resistance to cut was expresses in cm and the total energy necessary for cut in J.

**Figure 8**



- **Colour**: Minolta D3600 instrument with D65 illuminate was used, by CieL*a*b* (Cassens et al., 1995) method. It were determinate: lightness L*, red index a*, yellow index b*, chrome C and hue H. Before the analysis samples were exposed at the air for 1 h for promoting the blooming effect. With the same instrument was collected the visible spectrum of reflectance from 360 to 740 nm.

### 3.4.2 Chemical analyses

- Chemical percentage analyses by AOAC (1990) method:

  **dry matter (DM)** was performed to standardize the date obtained from ageing chemical analyses. Samples were put in a ceramic bowl, weighed, and placed in the oven at 101-102° C for 16-18 hours, after this time the samples were weighed and dry matter was calculated as difference of weight and express in percentage.

- **Glycolytic potential (PG),** was calculated in two step: first the glycogen content by kit Glucose Assay Sigma was calculated, and then the D-L lactic acid with kit D-L- lactic acid Method UV Biopharm were determined; at the end the glycolytic potential was obtained by

  (2*mM of glucose) + mM D-L lactic acids (Ylä-Ajos et al., 2006);

- **Myofibrillar index degradation** (**MFI**), by Culler et al., (1978) method. Samples were homogenized in buffer phosphate pH 6.8 and centrifuged 3000 rpm. The supernatant was discarded and the pellets were filtrated by 18 mesh filter. The filtered matter was assayed with Biureto for determination of total protein content and then the myofibrillar content that is 0.5 mg/ml of protein was calculated. The samples were read at 540 nm;

- **Total Collagen and Insolubile Collagen,** hydroxyproline was determined on 4 g of meat hydrolyzed at 110°C for 14 hours. A factor of 7.5 was utilized to convert hydroxyproline into collagen ISO (1978). Insoluble collagen was determined after cooking in a bath at 80°C for 2 hours in NaCl 0.9% solution. Collagen content was expressed as g of hydroxiprolin/100 g meat.

- **Calcium content,** mineral was determined by mineralization of sample in nitric acid 60% in a microwave oven. The solution was prepared with lanthanum chloride to prevent interference of phosphorus. Muscle calcium concentration were determined by atomic adsorption spectrometry (Perkin-Elmer instrument) at 422.7 nm, the calcium content was expressed as mg/100g meat.

### 3.4.3 Microscopically analysis

- **Sarcomer length**, 1 $cm^2$ of cross section strip was put in a test tube. It was submerged in glutaraldehyde 2.5% and kept for 1 hour. After this muscle fibres were carefully separated, put on a microscope glass slide adding one drop of distilled water, and covered with a covering glass slip. Samples were

observed with an immersion microscope measuring the length of ten sarcomeres and calculating the average in µm.

## *3.5 Statistical analysis*

Statistical analyses were performed using GLM procedure (SAS 1989), with three factorial model.

First thesis model is:

$Y = B_i + M_j + T_k + (B_i * M_j) + (M_j * T_k) + (B_i * T_k) + E_{ijk}$

Where B = breeds (Maremmana; Holstein);

M = muscles (BF; LD);

T = thesis (Achilles' tendon; Pelvic hanging)

and second:

$Y = B_i + AT_j + T_k + (B_i * M_j) + (M_j * T_k) + (B_i * T_k) + E_{ijk}$

Where B = breeds (Maremmana; Holstein);

AT = ageing time (24 hours; 5 and 8 days);

T = thesis (C, Ca, VFC).

Pearson correlation coefficient "r" was performed among physical and chemical parameters and among reflectance spectrum and all parameters.

For multivariate analyses the Principal Component Analysis (PCA) were obtained with Unscrambler program (Camo 2000). PCA was defined by load and score that define the size of the contribution of each original variable to the PCs., in addition, the loadings plot gives an overview of the importance of the original variables. The loading and score represent matrix, loading matrix (P) contains information about the variables: it is composed of a few vectors (Principal Components, PCs) which are (obtained as) lineal combinations of the original X-variables; whilst the score matrix (T) contains information about the objects. Each object is described in terms of its projections onto the PCs, (instead of the original variables).

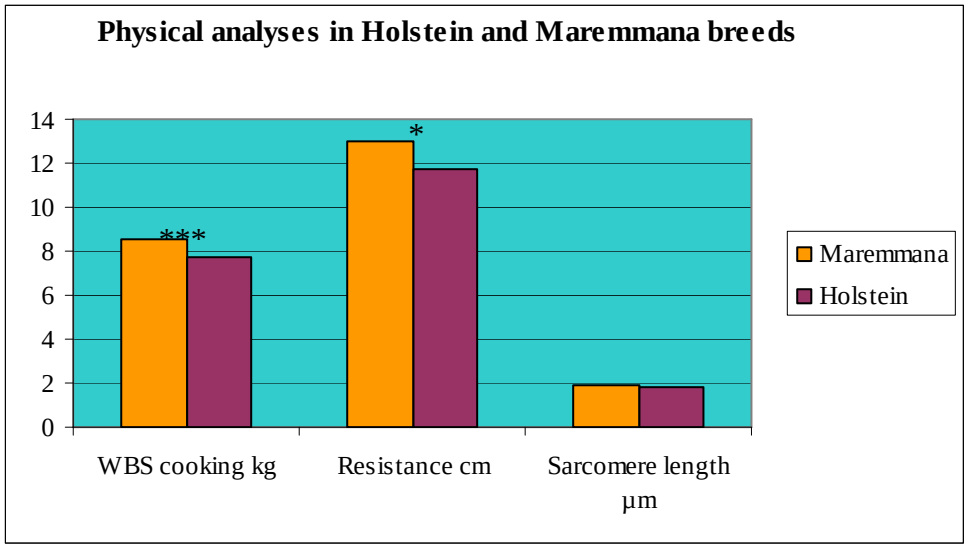
# 4 RESULTS AND DISCUSSION

## *4.1 Results in thesis I*

### 4.1.1 Physical analyses

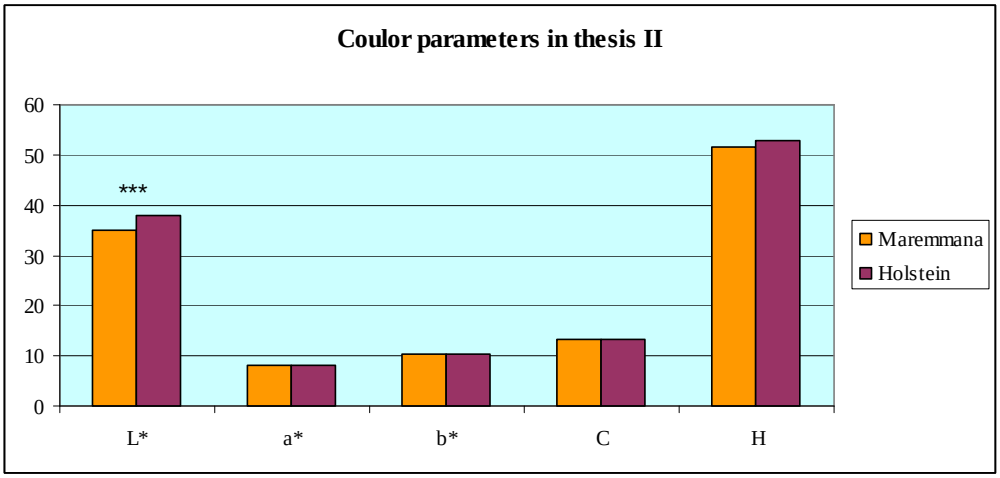
Graphic 6 shows the physical parameters and displays that Holstein was more tender than Maremmana, 7.72 vs 8.54 kg (P<0.002) respectively means of both muscles; similar differences was found for resistance and energy (11.73 vs 13.00 cm P<0.04 and 0.28 vs 0.31 J P<0.01); Maremmana in fact has a tough and dark (graphic 7) meat because is usually reared in extensive way, as reported also in Sargentini et al., (2004). In fact difference between breeds was found for L* where Holstein reported and higher value than Maremmana (37.87 vs 35.06), while the other colour parameters did not show significant differences between breeds, as no differences were found in WHC on cooking meat (26.80% in average for two breeds) and in sarcomere length (1.86 µm in average of both muscles).

**Graphic 6**




*means P<0.05

**Graphic 7**




* means P<0.05

The action of pelvic hanging was more evident, in WBS on cooked meat (table 2) that had reported mean values as 4.05 vs 5.34 kg (P< 0.001) in both muscles respectively for pelvic hanging and Achilles tendon, considering both the breeds, respectively for pelvic and Achilles tendon hanging, with a 24% decrease, similar trend was found by Ahnström et al., (2006) in *Semimembranosus* muscle with 21% decrease; same result was found in energy (0.18 vs 0.26 J P<0.05 respectively for two thesis) too; whilst resistance and WHC showed differences only between breeds, where Maremmana breed losses more liquid than Holstein. On the other hand Maremmana reported lower values, in both treatment, for resistance (14.77 cm vs 18.93 cm in average for the two treatments).

In both breeds the pelvic hanging caused higher value in sarcomere length than normal hanging (2.06 vs 1.70 µm average of breeds in Pelvic hanging and Achilles tendon). Any differences were not found between the breeds.

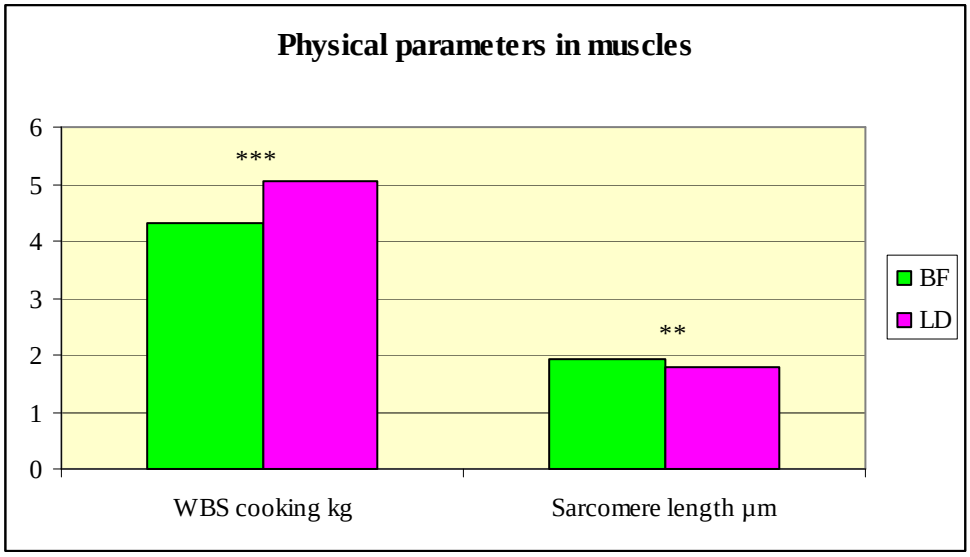
**Table 2**. Interaction among breeds and pelvic hanging treatment.

| WHC Cooking % | | | |
|---|---|---|---|
| | Achilles tendon | Pelvic hanging | Root MSE |
| Holstein | 25.04 B | 25.76 B | 3.192 |
| Maremmana | 30.71 A | 30.09A | |
| WBS cooking kg | | | |
| Holstein | 5.37 a | 4.04 b | 1.263 |
| Maremmana | 5.30 a | 4.06 b | |
| Resistance cm | | | |
| Holstein | 18.32 A | 19.53 A | 4.307 |
| Maremmana | 15.92 B | 13.61 B | |
| Energy J | | | |
| Holstein | 0.24 a | 0.17 b | 0.114 |
| Maremmana | 0.28 a | 0.18 b | |
| Sarcomere length μm | | | |
| Holstein | 1.67 b | 1.99 a | 0.242 |
| Maremmana | 1.73 b | 2.07 a | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

About muscles (graphic 8), was highlighted significant differences only in WBS on cooked meat where for BF muscle emerged a lower value than LD (4.31 vs 5.07 kg $P<0.001$ respectively), decreasing in shear force value reported by Hostetler et al., (1970) in LD muscle (6.26 vs 4.94 kg) whilst Shanks et al., (2002) did no find significative difference in WBS on BF muscle. This result means that the treatment influenced meat tenderness by the stretching effect. Sarcomere length was higher in BF muscle than in LD (1.93 vs 1.80 μm $P<0.01$ respectively) however both muscles showed a increasing of sarcomere length, this trend was found in Eikelenboom et al., (1997) and Ahnström et al., (2006) where the sarcomere length increased in LD muscle was from 1.75 to 2.11 and 1.6 to 2.9 μm for *Semimembranosus* for two authors respectively.

**Graphic 8**




* means P<0.05

Although the muscles have shown some differences only for WBS and sarcomere values their interaction highlights significative differences for all physical parameters, except for WHC cooking and resistance. Table 3 showed the positive treatment effect, in both muscles, even if Shanks et al., (2002) reported neither difference for sarcomere length on BF muscle.

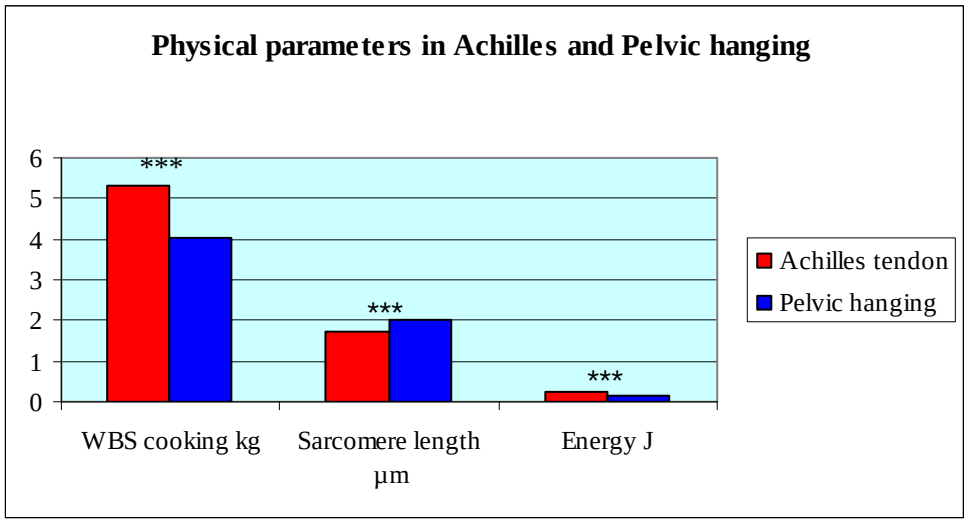
**Table 3**. Interaction among muscles and hanging treatment.

| WHC Cooking % | | | |
|---|---|---|---|
| | Achilles tendon | Pelvic hanging | Root MSE |
| BF | 27.77 | 27.65 | 3.192 |
| LD | 27.98 | 28.20 | |
| WBS cooking kg | | | |
| BF | 4.78 a B | 3.83 b | 1.263 |
| LD | 5.88 a A | 4.26 b | |
| Resistance cm | | | |
| BF | 17.23 | 16.60 | 4.307 |
| LD | 17.00 | 16.54 | |
| Energy J | | | |
| BF | 0.25 a | 0.17 b | 0.114 |
| LD | 0.27 a | 0.17 b | |
| Sarcomere length µ | | | |
| BF | 1.76 b | 2.10 a | 0.242 |
| LD | 1.64 b | 1.97 a | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

As a confirm of the hanging effect physical parameters (graphic 9, evaluated on Achilles tendon and pelvic hanging, reported a decreased toughness, so the WBS values on cooked samples were 5.33 vs 4.05 kg P<0.001 respectively for Achilles tendon and Pelvic hanging. WHC did not shown significative difference this trend was opposite in Bertram et al., (2007) article in which in pig muscle was reported the highest value in hanging pelvic than control.

**Graphic 9**



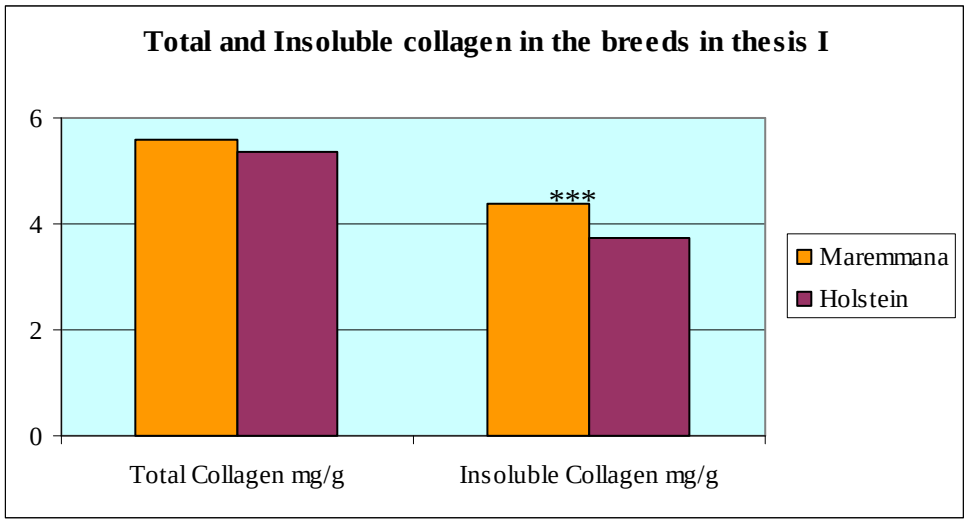

* means P<0.05

In pelvic suspension the hind leg hands vertically from the carcass reversing the effects on the muscles involved and the vertebral column is straightened and slightly separated as described by Hostetler et al. (1970). This reduces the possibility for the muscle to contract, which results in altered shape of the muscles compared to Achilles suspension. The hanging effect was shown in particular on fibrils length, in fact sarcomere in all analyses result higher (2.03 µm versus 1.70 µm per pelvic and Achilles tendon hanging P<0.001). The sarcomere elongation suggests that fibrils modify their respective distance because the position of carcass avoided a normal shrinkage and can be because the muscle enters in *rigor* in a more stretched state. Besides the effect on sarcomere length, Bouton et al. (1973) found that stretching significantly reduced adhesion between fibres, which implies a reduction in the connective tissue strength.

### 4.1.2 Chemical analyses

Graphic 10 showed the different between total and insoluble collagen in breeds; for insoluble collagen significant differences were found like in insoluble percentage collagen (3.72 vs 4.37 mg/g P<0.001 and 68.15 vs 77.88 % P<0.001 respectively for Holstein and Maremmana). No difference was reported for MFI.

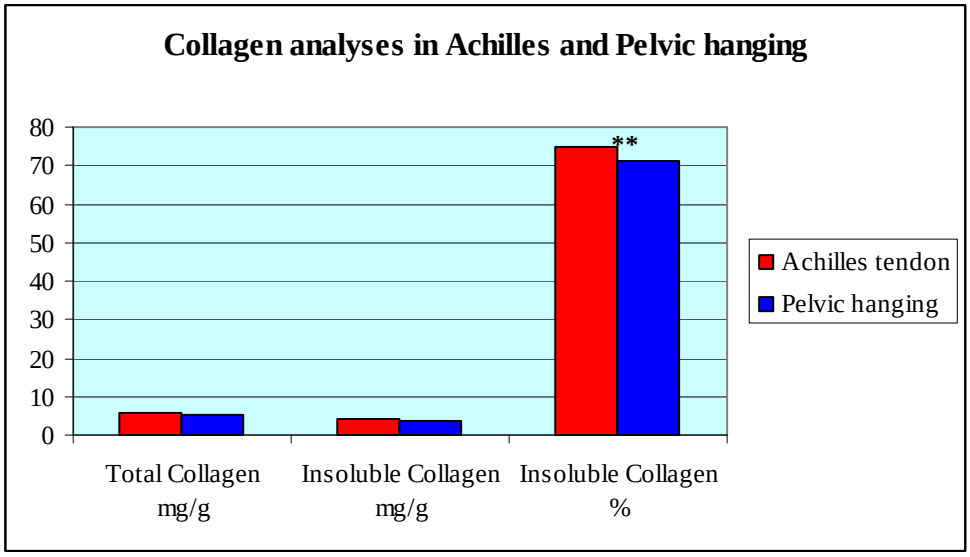
**Graphic 10**




* means P<0.05

In graphic 11 were reported the collagen analyses; significative difference was found only in insoluble collagen percentage that reported a lower value in pelvic hanging when compared with Achilles tendon (71.09% vs 74.93% P<0.01); whilst neither different were reported for MFI (59.31 average for two hanging) and for total and insoluble collagen.

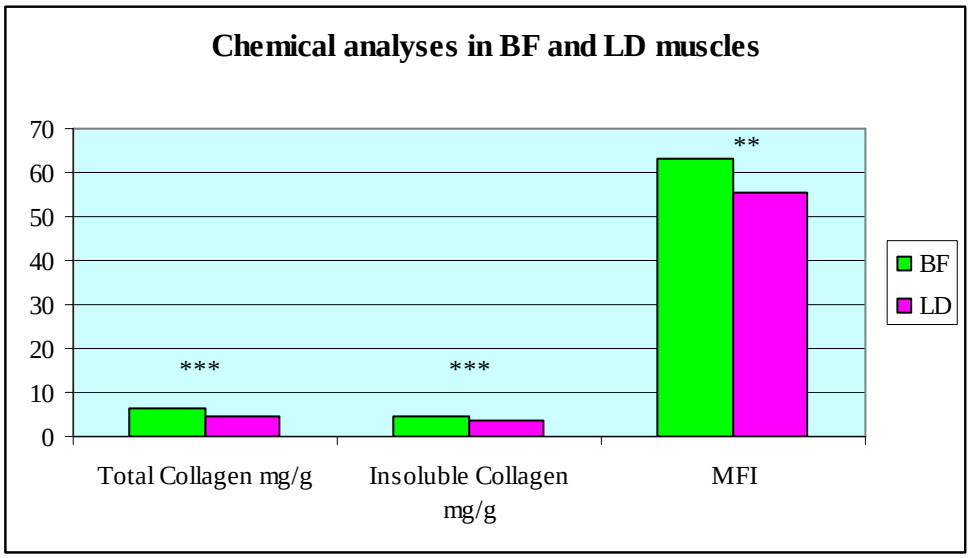
**Graphic 11**




* means P<0.05

The muscles (graphic 12) showed differences for all parameters except for insoluble collagen percentage; BF had higher MFI (63.34 vs 55.27 P<0.01), total collagen (6.21 vs 4.76 mg/g P<0.001), insoluble collagen (4.61 vs 3.48 mg/g P<0.001).

**Graphic 12**




* means P<0.05

The interactions (table 4) showed important differences for MFI only in Holstein where the pelvic hanging group had a lower value than Achilles tendon one (63.75 vs 54.22 P<0.04); Maremmana obtained the highest values for total and insoluble collagen in both hanging treatments.

**Table 4.** Interaction among breeds and treatment.

| MFI | | | |
|---|---|---|---|
| | Achilles tendon | Pelvic hanging | Root MSE |
| Holstein | 63.75 a | 54.22 b | 14.80 |
| Maremmana | 57.97 | 61.29 | |
| Total Collagen mg/g | | | |
| Holstein | 5.38 | 5.34 | 1.115 |
| Maremmana | 5.81 | 5.40 | |
| Insoluble collagen mg/g | | | |
| Holstein | 3.87 B | 3.57 B | 1.03 |
| Maremmana | 4.56 A | 4.18 A | |
| Insoluble Collagen % | | | |
| Holstein | 71.34 a B | 64.96 b B | 8.403 |
| Maremmana | 78.52 A | 77.21 A | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

No significative differences were reported in statistical analysis of interaction among muscles and treatment. The differences reported were just for the muscles, but we have to note that pelvic hanging in some case improved the difference between BF and LD as in MFI (table 5) or did not influence the existing difference like in total and insoluble collagen.

**Table 5.** Interaction among muscles and treatment.

| MFI | | | |
|---|---|---|---|
| | Achilles tendon | Pelvic hanging | Root MSE |
| BF | 64.54 | 62.14 A | 14.80 |
| LD | 57.18 | 53.36 B | |
| Total Collagen mg/g | | | |
| BF | 6.40 A | 6.01 A | 1.115 |
| LD | 4.79 B | 4.72 B | |
| Insoluble collagen mg/g | | | |
| BF | 4.84 A | 4.38 A | 1.03 |
| LD | 3.58 B | 3.38 B | |
| Insoluble Collagen % | | | |
| BF | 75.87 | 72.56 | 8.403 |
| LD | 74.00 | 69.62 | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

## *4.2 Results in thesis II*

### 4.2.1 Physical analyses

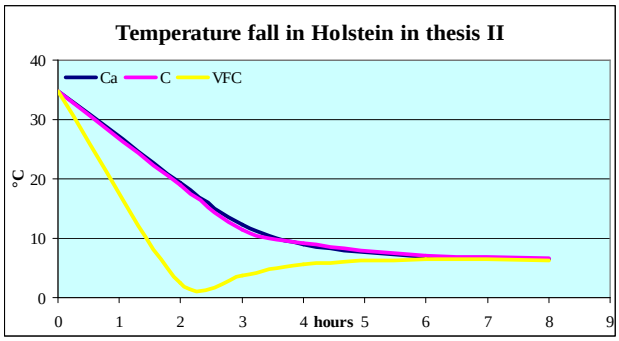
Because the different treatments in the three thesis the temperature and pH were very different among groups already in the first hours after treatments.

In fact in the VFC samples the temperature (graphics 13-14) reached a value near 0°C at 2 hours because the samples were maintained at -70 °C in this period, then the samples were stored at 4°C and they reached the final temperature within the 8 hours from slaughtered like the other thesis, that showed similar temperature trends for all experimental period.

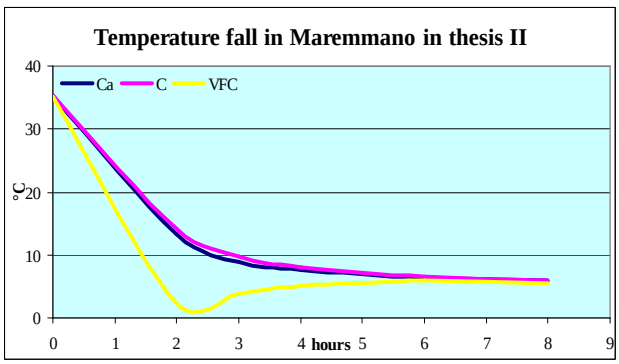
The pH fall (graphics 15-16) depend on treatment and temperature, in fact in VFC thesis the pH was constant, during the first hours when the temperature was very low, then rapidly fall reaching similar ultimate pH within the 24 hours (Trevisani et al., 1998). Ca treatment, because alteration in ionic strength and height calcium concentration that increase enzymatic activity, showed a high pH fall reaching the ultimate pH (about 5.6 for both breeds) already at 5 hours after slaughtering.

However the pH for the tree thesis showed different trend in the first 24 hours: higher for C group, low for Ca group and intermediate for VFC.

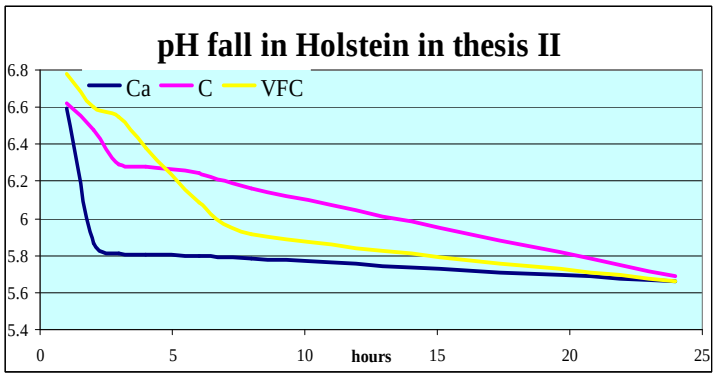
**Graphic 13**



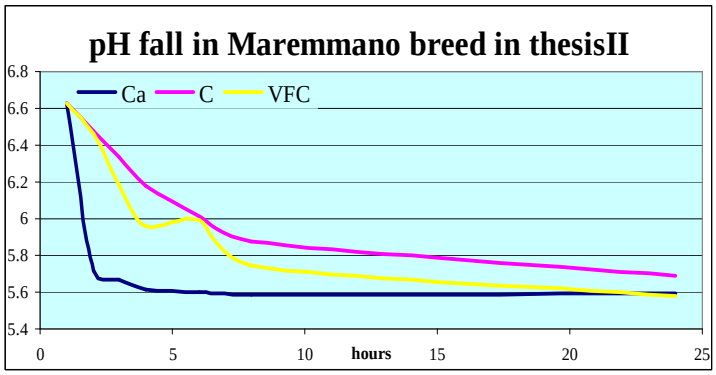

**Graphic 14**



**Graphic 15**




**Graphic 16**




All the physical analyses were performed after rigor, to estimate the different meat maturation process due to tenderness technologies tried.

In the first time the effect of principal factors (particularly thesis and times) on physical parameters were analyzed. Subsequently the interaction effects also between breeds to evaluate further differences were studies.

Analyzing all the times and breeds together to evaluate the effect of thesis, Ca showed the lowest value for WBS (table 6) and energy, whilst the control group showed the highest value (5.17 kg vs 10.48 kg and 0.25 J vs 0.34 J respectively for Ca and C); whereas VFC treatment placed in the middle position. No differences were found in resistance among thesis. Authors (Diles et al., 1994; Eilers et al., 1994; Gerelt et al., 2002) found a reductions in shear force from 10% to 30%, furthermore Jaturasitha et al., (2004) found of -51% in shear force and +52% in tenderness in meat treated with calcium infusion. Both treatments, that improve the tenderness, strangely produce a shrinkage effect evident on sarcomere length, in fact Ca and VFC groups reported the lower values than C.

The difference found in WHC for raw meat was been influenced by treatment, in fact Ca group lost more liquid than the other because infusion of liquid inside the samples and VFC produced more loss of water respect C group because crystal formation inside the muscle cells. Confirming this data many authors reported a reduced water holding capacity in CaCl2 injected meat because water purge (Dikeman et al., 2003; Koohmarae et al., 1990; Pringle et al., 1999; Wheeler et al., 1993). Similar trend was reported also in cooked meat with higher cooking loss in Ca group, lower in C group.

Lightness and yellowness did not show significant differences between groups, while redness was more evident in C group.

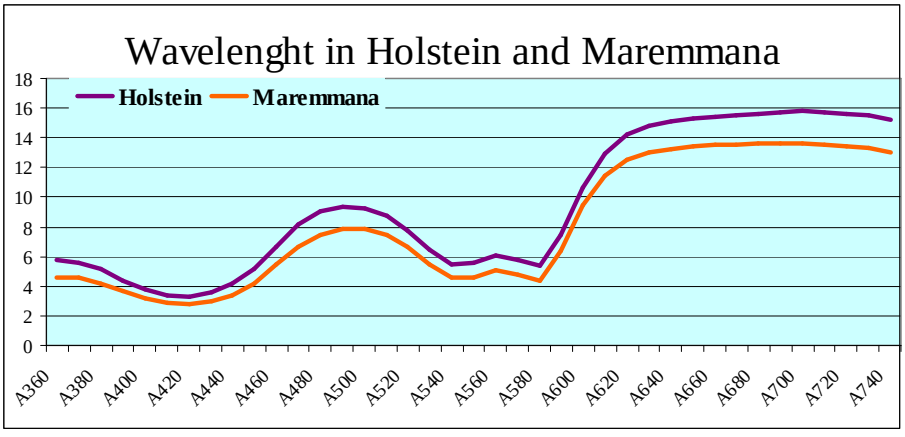
**Table 6**. Physical analyses into thesis II

| | C | Ca | VFC | Means | Root MSE |
|---|---|---|---|---|---|
| WBS Cooking kg | 10.48 a | 5.17 c | 8.75 b | 8.13 | 1.930 |
| Resistance cm | 12.04 | 12.64 | 12.43 | 12.37 | 4.498 |
| Energy J | 0.34 a | 0.25 c | 0.30 b | 0.30 | 0.083 |
| WHC raw % | 1.62 c | 2.75 a | 2.05 b | 2.14 | 0.766 |
| WHC cooking % | 26.18 b | 27.37 a | 26.79 ab | 26.78 | 3.570 |
| Sarcomere length µm | 1.73 a | 1.47 c | 1.58 b | 1.59 | 0.135 |
| L* | 36.71 | 36.28 | 36.40 | 36.44 | 2.758 |
| a* | 8.57 a | 7.72 b | 8.08 ab | 8.12 | 1.731 |
| b* | 10.68 | 10.19 | 10.36 | 10.41 | 1.529 |

Different letters means P<0.05

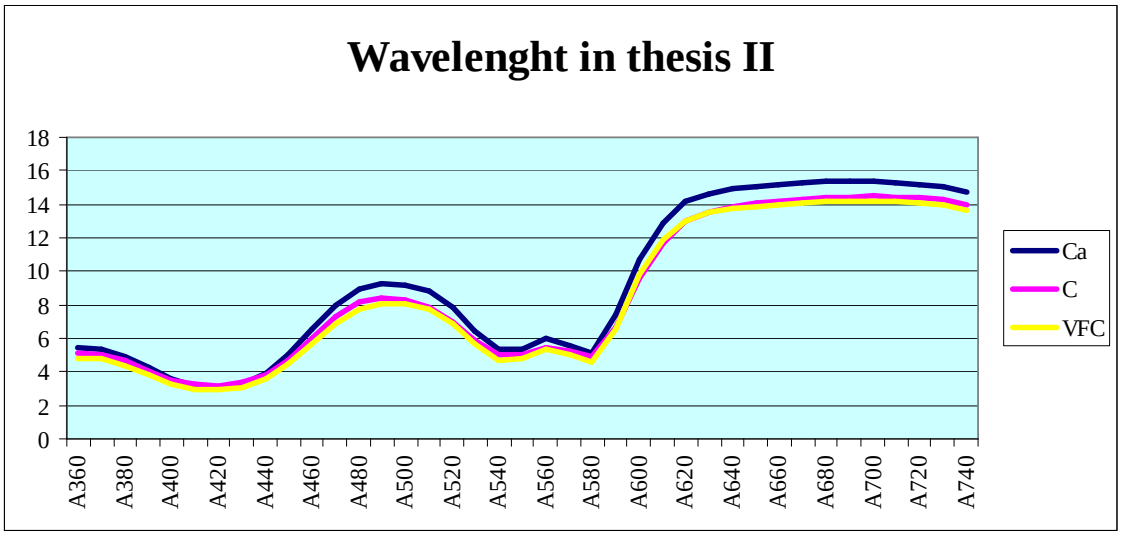
The reflectance spectra were different, in all visible spectrum (graphic 17), between breeds; Maremmana breed showed the minor reflectance values because meat of this breed is usually more dark.

**Graphic 17**



Ca showed major reflectance values (graphic 18), in particular between 460-550 nm and 620-740 nm, whiles VFC groups and C did not show significant differences. As reported in Failla et al., (2005) probably the growth of reflectance spectrum in this two wavelengths was due to a large protein degradation that occurs in Ca group as reported in Failla et al., (2005)

**Graphic 18**




The interaction among breeds and treatments (table 7) confirmed that calcium infusion showed lower values for WBS and energy in both breeds, probably for the higher enzymes activation (Dayton et al., 1976; Gerelt et al., 2002; Koohmarie et al., 1990; Pringle et al., 1999; Polidori et al., 2001; Wheeler et al., 1992). While for C group Maremmana breed showed higher value of WBS (11.34 vs 9.63 kg) and Energy (0.37 vs 0.31 J). This trend was agree with literature (Gigli et al., 2000b), but this difference disappeared in Ca and also in VFC group.

Some significant differences were found for WHC raw in VFC, where Maremmana reported the highest value (2.24 vs 1.86 %), whilst in the Holstein Ca group lost more liquid than the other.

Sarcomere length showed difference between breed only for C group (1.76 vs 1.69 μ for Holstein vs Maremmana breed), while similar trend was reported for both breeds in the experimental treatments.

**Table 7**. Interaction among breeds and C, Ca, VFC treatments on physical parameters.

| WBS cooking kg | | | | |
|---|---|---|---|---|
| | C | Ca | VFC | Root MSE |
| Holstein | 9.63 a B | 5.09 c | 8.46 b | 1.930 |
| Maremmana | 11.34 a A | 5.25 c | 9.04 b | |
| Resistance cm | | | | |
| Holstein | 11.60 | 12.66 | 10.95 B | 4.498 |
| Maremmana | 12.47 | 12.62 | 13.90 A | |
| Energy J | | | | |
| Holstein | 0.31 a B | 0.26 b | 0.29 ab | 0.083 |
| Maremmana | 0.37 a A | 0.25 c | 0.32 a | |
| WHC raw % | | | | |
| Holstein | 1.54 b | 2.73 a | 1.86 b B | 0.766 |
| Maremmana | 1.70 c | 2.76 a | 2.24 b A | |
| WHC cooking % | | | | |
| Holstein | 25.87 b | 27.60 a | 26.93 ab | 3.570 |
| Maremmana | 26.48 | 27.13 | 26.65 | |
| Sarcomere length μ | | | | |
| Holstein | 1.76 a A | 1.46 c | 1.60 b | 0.135 |
| Maremmana | 1.69 a B | 1.48 c | 1.59 b | |
| L* | | | | |
| Holstein | 38.01 A | 37.59 A | 38.01 A | 2.758 |
| Maremmana | 35.41 B | 35.00 B | 34.80 B | |
| a* | | | | |
| Holstein | 8.22 | 7.62 | 8.20 | 1.731 |
| Maremmana | 8.91 | 7.82 | 7.95 | |
| b* | | | | |
| Holstein | 10.69 | 10.14 | 10.70 | 1.529 |
| Maremmana | 10.66 | 10.24 | 10.03 | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

The Ca and VFC treatments did not influence colours parameters this trend was disagree from Lawrence et al., (2003) and Rees et al., (2002), in fact this Authors found the highest value in L* in Ca treatment than control.

Considering the interaction between aging times and treatments (table 8) WBS trend highlights the tenderization process due to treatments, in particular Ca group, at all the times, showed a lower value compared to C group, that was the toughest, and VFC had a median value (5.17 kg Ca vs 10.48 kg C vs 8.75 kg VFC).

**Table 8**. Interaction among times and C, Ca, VFC treatments on physical analyses

| WBS cooking kg | | | | |
|---|---|---|---|---|
| | 24 hours | 5 days | 8 days | Root MSE |
| C | 12.82 a A | 10.42 b A | 8.20 c A | 1.930 |
| Ca | 7.23 a C | 4.86 b C | 3.41 c C | |
| VFC | 11.23 a B | 8.35 b B | 6.66 c B | |
| Resistance cm | | | | |
| C | 11.22 | 11.55 | 13.34 | 4.498 |
| Ca | 12.62 ab | 10.86 b | 14.44 a | |
| VFC | 12.41 | 12.01 | 12.86 | |
| Energy J | | | | |
| C | 0.36 A | 0.34 A | 0.32 A | 0.083 |
| Ca | 0.31 a B | 0.23 b C | 0.21 b C | |
| VFC | 0.35 a AB | 0.29 b B | 0.26 b B | |
| WHC raw % | | | | |
| C | 0.70 b B | 2.00 a B | 2.17 a B | 0.766 |
| Ca | 2.30 b A | 3.01 a A | 2.93 a A | |
| VFC | 1.09 b B | 2.39 a B | 2.67 a A | |
| WHC cooking % | | | | |
| C | 23.80 c AB | 26.08 b | 28.65 a | 3.570 |
| Ca | 25.44 b A | 26.82 b | 29.85 a | |
| VFC | 23.18 c B | 26.86 b | 30.34 a | |
| Sarcomere length μ | | | | |
| C | 1.68 b A | 1.71 b A | 1.79 a A | 0.135 |
| Ca | 1.38 b C | 1.45 b C | 1.58 a B | |
| VFC | 1.52 b B | 1.59 ab B | 1.65 a B | |
| L* | | | | |
| C | 35.99 | 37.25 | 36.89 | 2.758 |
| Ca | 36.76 | 36.69 | 35.40 | |
| VFC | 35.84 | 36.90 | 36.47 | |
| a* | | | | |
| C | 8.21 | 8.95 A | 8.44 A | 1.731 |
| Ca | 8.44 a | 7.68 ab B | 7.03 b B | |
| VFC | 7.88 | 8.56 AB | 7.81 AB | |
| b* | | | | |
| C | 10.46 AB | 10.91 A | 10.68 | 1.529 |
| Ca | 11.12 a A | 10.00 b B | 9.45 b | |
| VFC | 10.26 B | 10.70 AB | 10.14 | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

Ca group showed a good tenderization of meat already at 24 hours after slaughtered in fact reached lower WBS compared to the value at 8 days of C group (7.23kg vs 8.20 kg), however all the groups showed significant degree of values with aging times. Resistance was significant only for Ca group and only at 5 days vs 8 days. On the contrary Energy values for Ca and VFC reached the minimum value already at 5 days of aging, while in C group the value did not improve during times. This data agree with

numerous previous studies that reported a reduction in shear force values following CaCl2 treatment (Lawrence et al., 2003; Kerth et al., 1995; Pringle et al., 1999; Wheeler et al., 1997).

WHC on raw and cooked meat showed a higher loss of liquid at 8 days *post mortem* than other times, because loss of electrolytic potential of proteins with their degradation, this was agree with literature (Rees et al., 2002).

Time of ageing did not interfere with existing differences between treatments for WHC on raw and on cooked meat, although on raw samples Ca group had the highest loss of liquid for all the times, while on cooked meat its value is significant only 24 hours *post mortem* (25.44 % for Ca vs 23.48 % in average for the other groups).

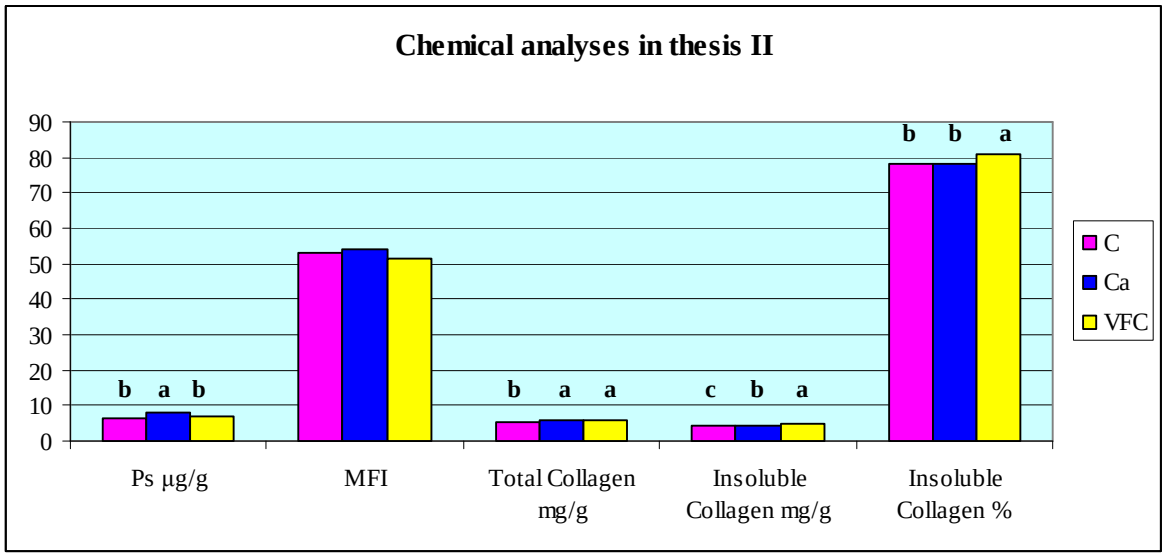
In any case, for both the theses, sarcomere length explains this fact; perhaps sarcomere length showed the stretch activity, due to Ca and VFC treatments; in fact at every times, except at 8 days, calcium infusion seemed to cause a reduction of this value, Morgan et al., (1991) found same trend about sarcomere for CaCl2 infusion, while King et al., (2003) confirmed that VFC caused contraction, reducing sarcomere length as cold shortening, but without to influence tenderization process.

Lightness was not influenced by time and treatment maintaining constant values, while redness a* index and yellowness b* index showed differences along times only in Ca groups in fact meat was more red and more yellow at 24 hours after slaughter. Ca group showed low redness value compared to C group at 8 days of aging (7.03 vs 8.44) while Ca group showed more yellowness index at 24 hours. Van Moeseke et al., (2001) reported not pronounced difference on VFC treatment and they found darker meat, related probably with muscle shortening.

### 4.2.2 Chemical analyses

The effect of different treatments was evaluated analyzing the chemical and biochemical parameters that change in different way for each treatment during times. Analyzing the effect of treatments (graphic 19) VFC and Ca group showed the highest value for total collagen probably height percentage of water loss produce concentration of structural protein, increasing all parameter expressed on fresh meat quantity. In fact the insoluble collagen as quantity and as percentage, obtained on cooked meat explained better the effect of treatments and showing shrinkage effect of VFC treatment that produce link between collagen fibres and a low solubility. Some Authors reported, in calcium infusion, some direct effect on connective tissue (Akataş et al., 2001; Gerelt et al., 2002), but the implication for connective tissue stability remain unclear from these studies. Other claim that there is a reduction in sarcomere length in calcium treatment which could favourably alter the connective tissue matrix (Jaturasitha et al., 2004, Morgan et al., 1991) even if Polidori et al., (2002) found no effect on sarcomere length. MFI did not show significant differences, this contrast with result reported by Beekman et al., 1994 where calcium chloride produced a large increase in myofibrillar fragmentation index values.

**Graphic 19**

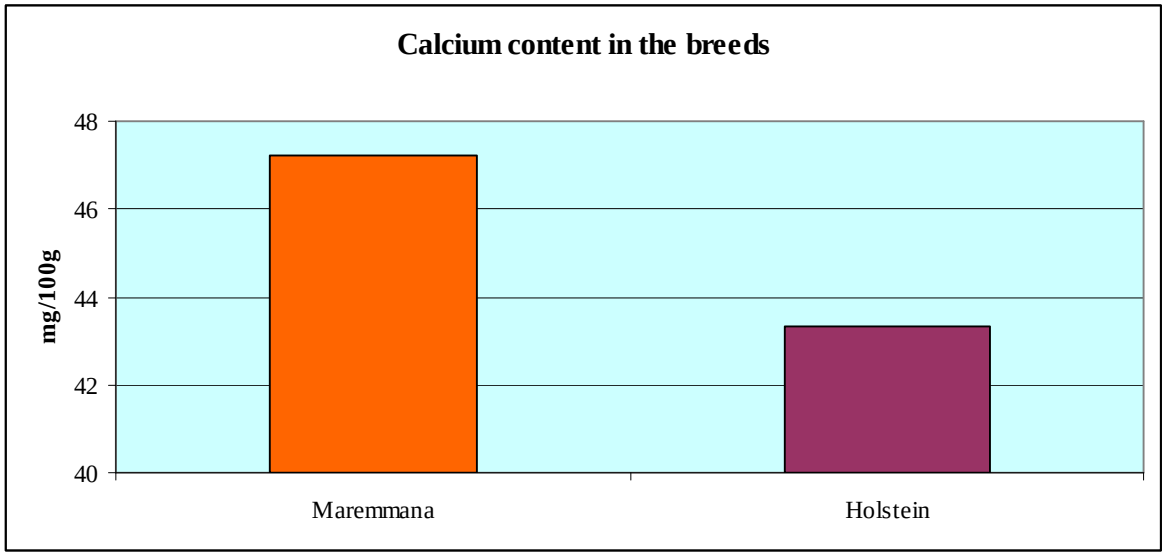



Different letters means P<0.05

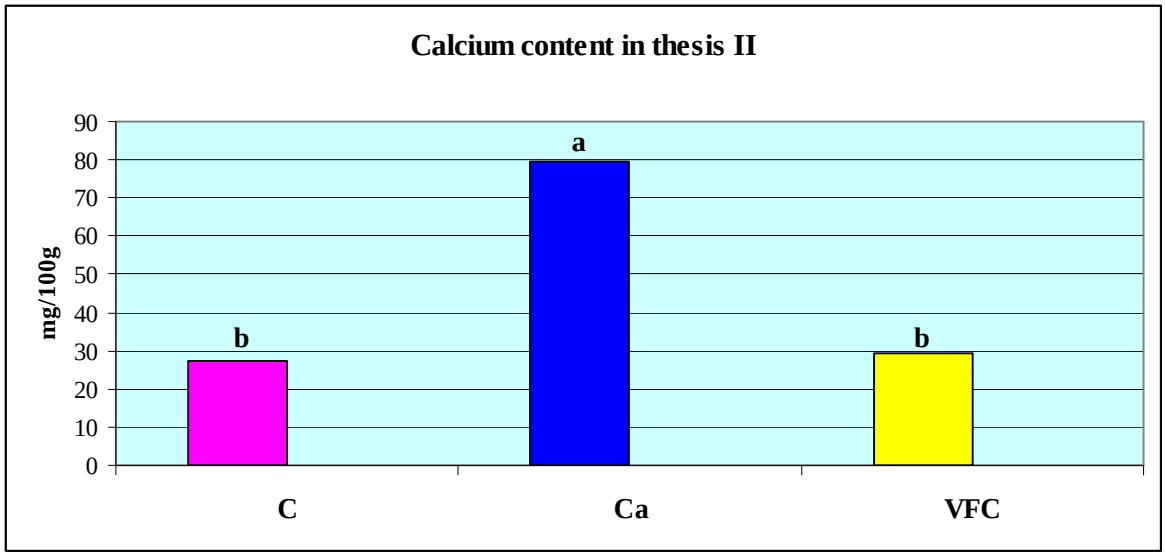
No significant differences were found between breeds in calcium content (graphics 20). Considering the treatments (graphic 21) the Ca group showed the highest value as we

expected because the infusion of CaCl2 (79.60 mg/100g vs 28.11 mg/100g per Ca and C, VFC in average).

**Graphic 20**



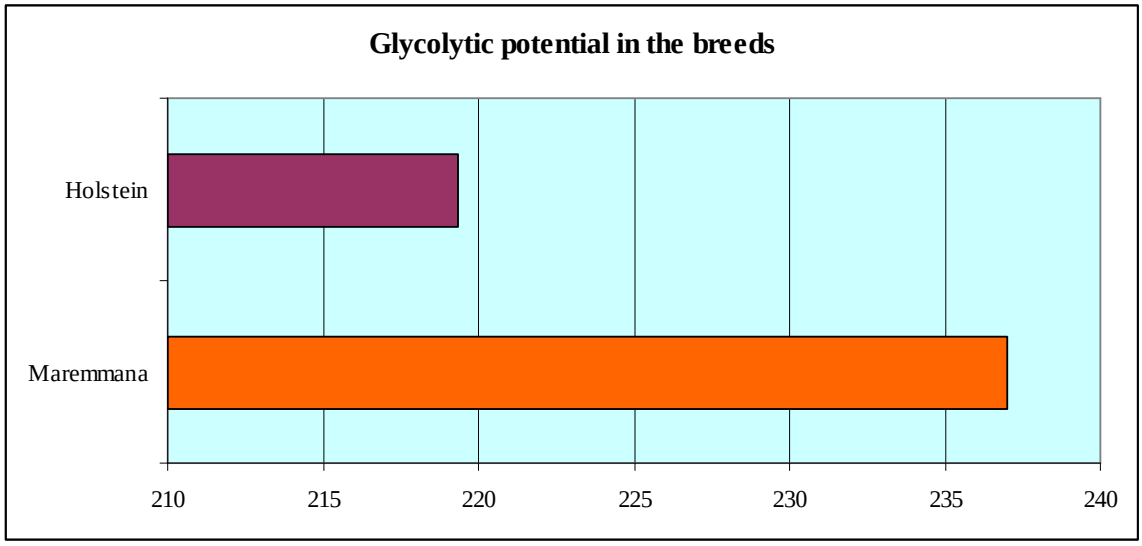

**Graphic 21**



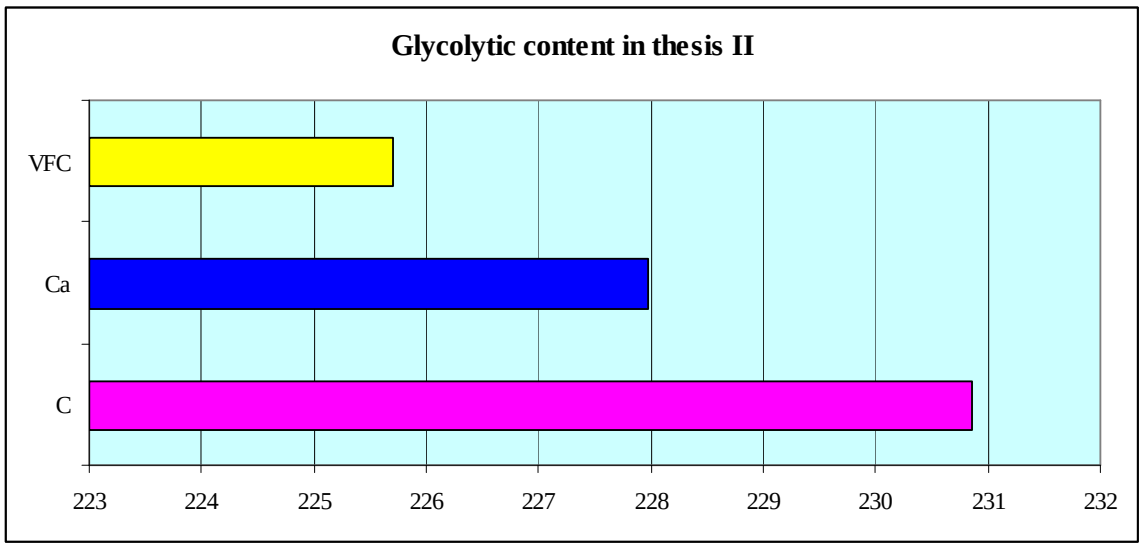

Glycolytic potential was not influenced by breeds or treatments (graphics 22 and 23) even if Maremmana showed a highest value. This trend was not clear in particular for calcium injection in fact several Authors reported that the increased calcium concentration results in an increased activity of calcium dependent ATPase thereby stimulating the rate of glycolysis due to the increased breakdown of ATP (Rees at al.,

2002), probably the fast fall of pH in Ca group produced by accelerate glycolytic activity and by quickly transformationt of muscular glycogen contributing to reduce the potential glycolytic before taking the meat sample, occurred at two 2 hours from slaughtered.

**Graphic 22**




**Graphic 23**




Considering the interaction between breeds and treatments (table 9) no significant difference was reported in MFI, while significant differences between breeds were reported in the two experimental groups, probably the higher quantity of water loss in Maremmana breed for Ca and VFC group produce a major concentration of total collagen, if the quantity was referred at fresh meat. Insolubility of collagen probably increased in VFC group for both breeds and also in Ca group for Maremmana breed

because shrinkage effect, that produce in the first hours after slaughtering higher formation of linkage between collagen fibres. The additive effects on total and insoluble collagen caused low difference among thesis and between breeds in percentage of insoluble collagen.

**Table 9.** Interaction of chemical parameters by breeds and N, Ca, VFC treatment.

| MFI | | | | |
|---|---|---|---|---|
| | C | Ca | VFC | Root MSE |
| Holstein | 52.35 | 56.63 | 53.66 | 14.88 |
| Maremmana | 53.96 | 51.84 | 49.37 | |
| Total Collagen mg/g | | | | |
| Holstein | 5.09 b | 5.41 ab B | 5.73 a B | 1.182 |
| Maremmana | 5.56 b | 6.21 a A | 6.38 a A | |
| Insoluble Collagen mg/g | | | | |
| Holstein | 4.01 b B | 4.16 b B | 4.64 a B | 0.976 |
| Maremmana | 4.28 c A | 4.91 b A | 5.16 a A | |
| Insoluble Collagen % | | | | |
| Holstein | 78.62 ab | 77.10 b | 81.74 a | 8.180 |
| Maremmana | 77.53 | 79.12 | 80.29 | |
| Calcium content mg/100g | | | | |
| Holstein | 24.13 b | 82.49 a | 23.37 b | 29.370 |
| Maremmana | 30.11 b | 76.70 a | 34.82 b | |
| Glycolytic potential | | | | |
| Holstein | 227.34 | 220.53 | 210.21 | 44.323 |
| Maremmana | 234.35 | 235.45 | 241.19 | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

The interaction between treatments and breeds did not show differences respect to the trend of principal factors.

**Table 10.** Interaction among times and C, Ca, VFC treatments on chemical analyses

| MFI | | | | |
|---|---|---|---|---|
| | 24 hours | 5 days | 8 days | Root MSE |
| C | 34.43 c | 51.01 b | 74.01 a | 14.110 |
| Ca | 34.32 c | 51.05 b | 77.34 a | |
| VFC | 33.50 c | 46.53 b | 74.51 a | |
| Total Collagen mg/g | | | | |
| C | 5.29 | 5.37 B | 5.31 B | 1.182 |
| Ca | 5.76 | 6.02 AB | 5.66 AB | |
| VFC | 6.06 | 6.10 A | 6.01 A | |
| Insoluble Collagen mg/g | | | | |
| C | 4.22 B | 4.10 B | 4.10 B | 0.976 |
| Ca | 4.62 AB | 4.60 AB | 4.37 AB | |
| VFC | 5.04 A | 4.94 A | 4.72 A | |
| Insoluble Collagen % | | | | |
| C | 80.23 | 76.47 | 77.52 | 8.180 |
| Ca | 80.28 | 76.79 | 77.27 | |
| VFC | 83.48 | 80.61 | 78.96 | |

Lowercase letter means in the same row differ significantly
Uppercase letter means in the same column different significantly

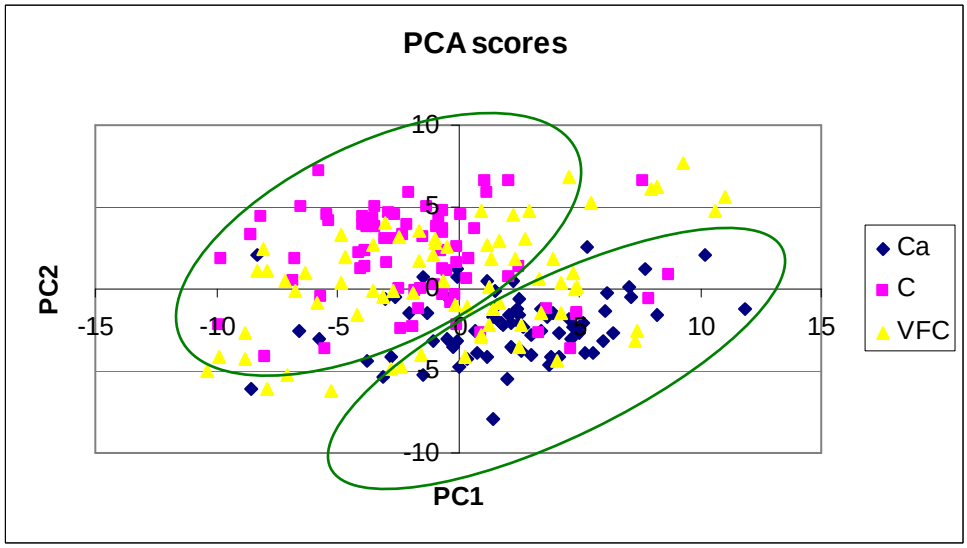
The trend of MFI depend exclusively by aging time and no differences were found in the different treatments.

As referred previously the difference on total collagen was evident after 5 days because more loss of water in the samples. In fact higher quantity was found in control group. Some trend was found in insoluble collagen that displayed a significant difference also at 24 hours when still water was held and this effect was probably due of shrinkage effect. No difference there was in percentage of insoluble collagen.

Ca quantity and glycolytic potential were no studied during aging because after 24 hours insignificant was the effect of Ca concentration and activity of glycolytic enzyme.

PCA explains 58% of variability. In PCA scores (graphic 24) was possible to distinguish calcium from control group, whilst VFC group was distributed in the whole plot. This trend means that calcium treatments had a bigger influence than VFC on the aforesaid parameters.

**Graphic 24**




PCA loading (graphic 25) showed WBS, WHC on cooked meat, total and insoluble collagen, to be the most important factors that explained principally the variance.

**Graphic 22**

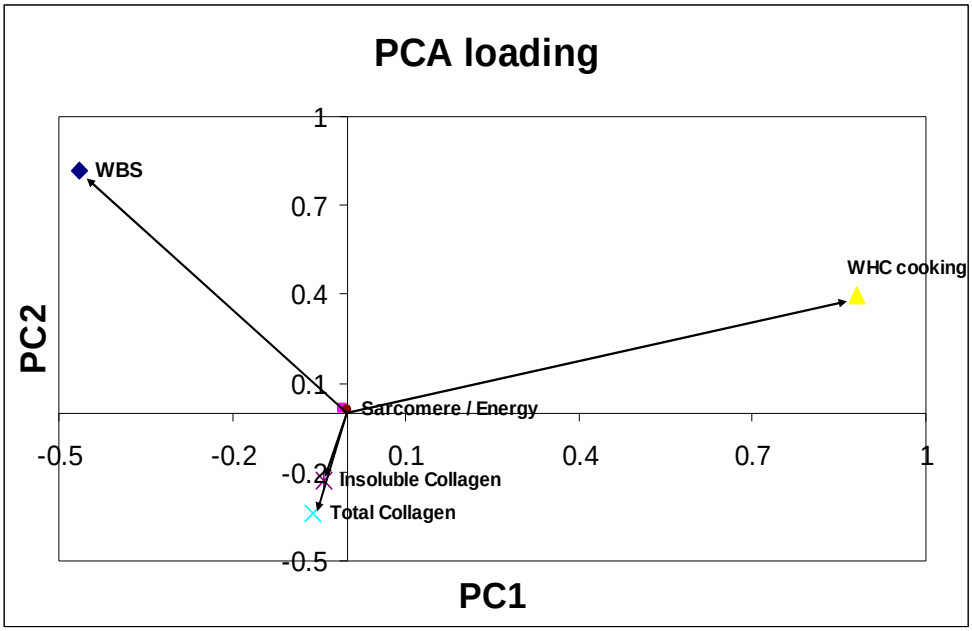



Table 11 showed the correlations between physical and chemical analyses. In fact some parameters are correlated one another because that parameters are subject of the same chemical process.

WBS on cooked meat was correlated with all the other parameters; in particular was positively correlated with energy, insoluble collagen percentage and sarcomere length

(P<0.01), whilst was negatively correlated with the rest of them (P<0.001), in particular with collagen and MFI because the cooking process improve the myofibrillar shrinkage and protein degradation (Bendall et al., 1983; Lawrie, 1994). Furthermore, also WHC on cooked and raw meat had a good correlation with MFI and insoluble collagen, this correlation was more important because these parameters defined the tenderization process (Honikel, 2004). It is worth to highline the correlation among total collagen and MFI and sarcomere length, which is strongly negative (P<0.01) and this clearly points out the existence of a wide range of interactions among parameters in such a complex matrix, like meat. Colour in this experiment did not show modification depending of treatment and did not result correlate with other parameters.

**Table 11**. Correlation among physical and chemical parameters.

| | WBS cooking kg/cm$^2$ | Resistance cm$^2$ | Energy J | WHC cooking % | WHC raw % | Total collagen mg/g | Insoluble collagen mg/g | Insoluble collagen % | MFI | Sarcomere µ |
|---|---|---|---|---|---|---|---|---|---|---|
| WBS cooking kg/cm$^2$ | | -0.23 *** | 0.66 *** | -0.31 *** | -0.53 *** | -0.22 *** | -0.18 ** | 0.21 *** | -0.29 *** | 0.15 ** |
| Resistance cm | | | ns | ns | 0.14 * | 0.54 *** | 0.50 *** | -0.34 *** | -0.18 ** | ns |
| Energy J | | | | -0.28 *** | -0.32 *** | ns | ns | ns | -0.27 *** | ns |
| WHC cooking % | | | | | 0.37 *** | -0.22 *** | -0.20 *** | 0.18 ** | 0.50 *** | ns |
| WHC raw % | | | | | | 0.18 ** | 0.16 ** | ns | 0.30 *** | -0.20 ** |
| Total collagen mg/g | | | | | | | 0.97 *** | -0.42 *** | -0.30 *** | -0.15 ** |
| Insoluble collagen mg/g | | | | | | | | | -0.32 *** | -0.16 *** |
| Insoluble collagen % | | | | | | | | | ns | ns |
| MFI | | | | | | | | | | 0.30 *** |

# 5 CONCLUSIONS

The analysis of the treatments to improve tenderness in animal rearing in extensive system produced very interesting results. In fact their action was visible in the experimental data coming out from our study, in particular on WBS, sarcomere length and insolubility of collagen.

In fact in thesis I sarcomere length was longer in pelvic suspension than in Achilles' tendon in both muscles (2.04 vs 1.7 μ respectively per BF and LD average) and in both breeds (2.03 vs 1.7 μ respectively per Holstein and Maremmana average) and this suggests that myofibrils were stretched more than in control group; sarcomere were probably influenced by the proximity of the individual muscle in relation to skeletal separation point and by muscle fibre orientation in relation to tension. This is a good starting point to improve meat tenderness when rearing animals in an extensive way, being suspension an easy and not destructive method to do on carcasses.

Also in thesis II there were interesting differences between the two treatments, in fact, how we do attend, calcium infusion has influenced positively meat tenderness and PCA highline this difference because C and Ca groups were well distinguished. Even if Ca group displayed an increase in collagen content also showed the lowest WBS on cooked meat. As for other parameters like myofibril degradation and soluble proteins, treatment did not show any difference from control.

The two theses proposed in this study showed that was possible to improve or at least decrease, until to reach the disappearance the significative differences in toughness between the animals rearing in extensive from animals rearing intensive. These suggest that could be possible to improve the tenderness and meat quality working on carcass and not on alive animals.

Furthermore some studies reported a strong correlation among some physical parameter (Lindahl et al., 2001), so is possible considerate the maturation of meat as a biochemical and physical complex and its variation must be considered together.

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