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ROLE OF PASTURE IN THE GROWTH, INTAKE AND MEAT QUALITY OF SARDINIAN CATTLE BREEDS

dr. Marco Acciaro

Direttore della Scuola prof. Alba Pusino
Referente di Indirizzo prof. Nicola Macciotta
Docente Guida prof. Salvatore Pier Giacomo Rassu

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Introduction

1.1 The bovine species in Sardinia

The presence of bovine species in Sardinia is documented since the Neolithic age (2000-1800 b.C.) and it derives by the introduction in the Island of animals type macrocero, with horns directed backwards , attributable to ancestor *Bos macrocerus,* that probably was of North-Africa, Syrian or Iberian origin (Brandano et al., 1983).

These animals would have been improved through the introduction (900-400 b.C) of brachicero cattle with horns directed laterally, that was of Punic origin (Brandano et al., 1983).

Until the middle of the last century this bovine species (Sarda breed) was characterized by highly variable morphological, reproductive and productive features, at least from a phenotypic point of view; this variability has been reported as a consequence of the great variability of the environment where they are reared (i.e. altimetry, orography…).

The Sarda breed, characterized by hardness and resistance and well adapted to the different environments of the Island was raised mainly for milk production and demonstrates a poor attitude either to work in heavy soil plains (Campidano), because of its small size and under-developed chest, or to meat production because of the poor growth rate and the low dressing percentage.

Accordingly, to improve the size and the strength of Sarda breed and then the attitude to work, in the last century (1880) an importation of Modicana breed bulls began from Sicily to the Montiferru area (Oristano) mainly. These bulls crossed with Sarda breed, gave rise to Sardo-Modicana breed, best suited to work and to which belonged the "oxen working", in great demand until the advent of mechanization (40-50 years ago).

During the period of greatest expansion (1940-1950) the Sardo-Modicana breed represented 32.5% of total cattle in Sardinia.

To improve the milk and meat production of Sarda breed, in the second half of last century, Brown Swiss bulls were introduced in Sardinia, mainly in the north of the Island that, through the substitution crossing of Bruna breed bulls and Sarda cattle gave rise to an animal heavier and with best development of the hindquarters, the Bruno-Sarda breed.

In the best farming areas, where feeding resources available were able to meet the nutritional requirements of the animals, it results in a replacement of local population of cattle with the Brown characterized by an excellent ability to produce milk, whereas in disadvantaged areas this process gave rise to Bruno-Sarda breed reared for the production of calves characterized by a better conformation for the meat production.

This process of grading up associated with selective crossbreeding of native breed (Sarda) with Bruna and Modicana breed lasted until 50s with the exception of the less favourite areas of Island where the Sarda breed was reared as pure-bred.

In the years following the World War II, in irrigated lowland (mainly in Oristano area), there was the introduction of Friesian breed, initially of European origin then replaced by the North American one.

Afterwards, since the introduction of mechanization, the request of "oxen working" has stopped and in the lowlands the cosmopolitan specialised breeds for milk (Holstein Friesian and Brown) are preferred.

Actually in Sardinia the following breed are reared:

- The Sardo-Modicana in the Montiferru area mainly, whose consistency has been reduced in the last thirty years for the termination of the request for working animals and exploited for the calf production mainly.
- The Holstein Friesian and Brown in the lowland of the Island, where intensive milk production system is feasible.

- The Bruno-Sarda in the north and center of the Island mainly, in less favourite areas, to meat production.
- Tha Sarda in the less favoured hill and mountain regions of Island (Limbara, Gennargentu, Sette Fratelli-Serpeddì, Linas) where traditionally is used this small-frame cows that grazed upland pastures and forests all year around in suckler-cows system. This native breed is exploited for the calf production obtained by crossbreeding with bulls of the large-frame beef breed, mainly Charolais and Limousine, in order to improve meat production and carcass quality of calves.

1.2 Cattle livestock system in Sardinia

To date (BDN 2011) about 266.100 heads constitute the cattle livestock system in Sardinia. The 22.5% of the total heads (49.000) are dairy cows (Friesian and Brown) mainly localized in the western plains of the island.

About 217.200 heads form the beef production system, but it is noteworthy that only the 4.6% belongs to specialized beef breeds whereas the most of the animals comes from Sarda, Modicana, Bruno-Sarda breed and different cross-breeds (Table 1).

Table 1 - Consistency of cattle in Sardinia (BDN 2011)

	Heads (n°)
Cattle (total number)	266100
- Dairy cattle	49000
- Sarda breed	8900
- Sardo Bruna breed	20700
- Sardo Modicana breed	6800
- Charolais	4050
- Limousine	7880

Cattle meat production system in Sardinia is based mainly on suckler-cows system, on the use of different cattle breeds, from specialized to hardy breeds, and on

use of pasture; we are in the presence of an extensive livestock system also keeping in mind that 84% of the total number of farms has got fewer than 50 heads.

The total beef meat production per year in Sardinia is nearly 585.000 quintals equal as 134.4 million of euro with a self-supply of 48% (Rassu et al., 2011).

From slaughtering data (ERSAT 2005) follows that the beef meat production in Sardinia is mainly based on young bulls and heifers (42%) slaughtered at about 12 and 14 months old with live weights of 340-410 and 400-480 kg respectively. The calves represent 17.5% and the cows 20.2% of the total slaughtered cattle.

In the Island there isn't a traditional livestock system for beef cattle and farms use to cross bulls of specialized meat breed with native or non specialized breed cows (hereinafter referred to as hardy breed). Such technique makes it possible to combine the greater environmental adaptability of the hardy breed with the higher growth capacity of cross-bred calves than the pure ones.

Indeed the hardy breeds are often medium size and for this reason have limited maintenance requirements and an excellent ability to exploit the resources of the island pastures, often poor in quantity and quality; they are also characterized by a good maternal ability, being able to rear their calves excellently until weaning.

It should be noted that on this topic the Istituto Zootecnico e Caseario per la Sardegna (actually Department of research in animal production - DIRPA - of the Regional Agency for the Research in Agriculture -AGRIS) following 30 years of studies identified the Charolais and Limousine as the best breeds to use for crossbreeding with the Sardinian hardy breed. These 2 breeds, indeed, showe

d a good adaptability to the typical extensive farm conditions in Sardinia and also give rise to F1 calves characterized by an excellent growth rate (Vissac et al., 1976; Bibe et al., 1979; Casu et al., 1985; Casu et al., 1988) and for that reason they are now widely used.

In the Sardinian suckler-cows system, the calves follow their mothers at pasture until the weaning at about 6-7 months old, when they are brought to the fatness centers in the "Pianura Padania" or, rarely, finished in the farm and afterwards slaughtered. Such system, based on the selling of calves, characterized by a limited use of resources out of farm and sustainable from an economic point of view, suffered a severe crisis by the introduction of bluetongue in Sardinian.

The blue tongue is an infectious disease of ruminants transmitted by blood-sucking insects of the genus *Culicoides* and backed by a virus whose are known 24 serotypes; all species of ruminants are susceptible, but it is particularly damaging in sheep.

Of the 24 known serotypes, 5 are present in Sardinia and exactly 1, 2, 4, 8 and 16. This disease is included in the list of OIE (Office International des Epizooties) which determines the adoption of measures to restrict the movement and the trade of animals.

As a result of health constraints applied, only vaccinated cattle can leave the Island but the vaccination should be made after 90 days of age of the animal and however no later than April.

These regulations represent serious constraints on the movement of cattle towards the fatness centers because in April a large proportion of calves born in Sardinian farms does not have 90 days yet and can't be vaccinated.

The farms most damaged by these constrains were the ones that adopted the suckler-cows system, that have been forced to fatten calves on the farm, changing the production system and increasing the costs.

Indeed the animals are often confined in stable (when it is possible) or in small paddock and fed with large quantities of concentrates purchased out of the farms (Rassu et al., 2011).

Therefore, keeping in mind that Sardinia is the Italian region with the largest pasture area (Lucifero *et al*, 1973) with a grazing areas that exceeds 40% of land area (Pulina, personal communication), the use of pasture (alone or supplemented with concentrate) in the fattening of animals could represent a chance for Sardinian beef cattle livestock system.

The pasture-based system has different and positive aspects:

- Could help to reduce feeding costs. As French et al. 2001 "feed costs are a major proportion of total variable costs in most beef systems and efficiently managed grazed grass can be cheapest feedstuff in temperate climates".
- Could characterize the meat produced in Sardinia allowing to distinguish it on the market. As reported also by Priolo et al. (2002) the pasture represents one of the bases of the product's link with the production area and also it can give the product special features
- Several research suggest that grass-only diet can significantly alter the fatty acids composition and improve the overall antioxidant content of beef (Daley et al., 2010)
- Allows the animals to externalize their full behavioral repertoire, in environmental conditions close to those in which there are the wild species (Napolitano et al., 2005). The satisfaction of the physiological and ethological needs of animals, met by the grazing systems, is a prerequisite for obtaining quality productions.
- Contributes to the stability of the soil by reducing erosion and provide sustainable livestock production from an economic and environmental point of view, especially in hilly and mountainous areas (Braghieri et al., 2007).
- The grazing plays an active role in limiting fuel biomass helping to prevent fire danger (Franca et al., 2012).

Finally pasture-based systems are nowadays considered more environmentally friendly, providing also animal welfare, and hence begin to be socially more acceptable than more intensive systems. The grazing systems arouse now increased emphasis in the European Union Common Agricultural Policy (CAP) to enhance or maintain biodiversity in pastures, and by now it is generally accepted that grazing is an essential tool for to achieve nature conservation objectives in grassland (Tallowin *et al*., 2005)

Also the biodiversity of these habitats is influenced by grazing; in some cases a moderate stocking rate has also been shown to increase biodiversity (Adler et al., 2001 cited by Orr et al., 2012).

Unfortunately the seasonality of forage production in Sardinia does not meet the total requirements of growing and fattening animals (Rassu et al., 2004 and 2011).

Indeed, for meat ruminant production (but also for milk and wool) at pasture, a limited herbage intake is considered one of main constraints (Forbes, 1995 cited by Smith *et al*., 2005).

Therefore to successfully exploit the pasture for beef production, dry matter intake and *in vivo* digestibility should be quantified in order to estimate nutrient consumption by ruminant, to assess what growth rates it can provide and if it is necessary to integrate the animals, in order to optimize grazing ruminant productions (Decruyenaere et al., 2009).

With Reeves et al., 1996 "it is necessary to provide more accurate information on herbage intake and its relationship to performance of ruminant livestock".

In general terms nutrient intake is the major determinant of nutritional status and production performance in farm livestock; as Burns and Sollenberger (2002) "in grazing system dry matter intake (DMI) is generally the limiting factor to support high daily animal response. In general the animal productive response is a function of DMI X Digestibility, but changes in daily animal responses are influenced far more by

changes in daily DMI than changes in forage digestibility" (Noller, 1997 cited by Burns and Sollenberger, 2002).

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Chapter 2

Herbage intake measurement

2.1 Herbage intake measurement

There have been numerous estimates of herbage intake by grazing ruminants since their performance ultimately reflects the balance between theirs nutrient requirements and the nutrients that they are able to consume. This balance is very important to predict the performances of grazing animals; but while our understanding of the "demand side" of this balance has more increased, our understanding of the "supply side" still need more information (Dove and Mayes, 1991). Indeed, it is very difficult to estimate the voluntary intake of grazing ruminants easily and with a sufficient precision.

According to Illius et al (1996) intake can be considered as a "psychological" phenomenon, involving the integration of many signals and reflects the flexibility of a biological system evolved to cope with variability in food supply composition and animal states. More simply "herbage intake can be considered as the result of the interaction between plant, animal and environmental components."(Barrett et al., 2001;Decruyenaere et al., 2009).

The amount of herbage consumed by the grazing animal (Herbage Intake, HI) can be estimated through different methods classified into 2 categories: direct or indirect methods.

2.2 Direct methods

Direct methods are essentially based on the herbage mass measurement. Herbage intake is estimated very often by the method of difference (Macoon et al., 2003; Smith et al. 2005). The method implies the measurement of herbage mass before and after grazing (Walter and Evans, 1979). The herbage mass is usually

estimated by cutting and weighing the grass harvested on a defined area. A "sward height meter" or "rising plate meter" or "disk meter", measuring compressed sward surface height, may also be used to estimate grass density and quantity.

The difference method can give reliable results if grazing period is short (one or two days at the maximum) and stocking rate is high (ideally all grass of the grazing area must be consumed). If grazing period is longer the error of estimation due to the grass regrowth during the period is important. To evaluate the effect of grass regrowth, herbage mass and regrowth could be measured in cages that exclude grazing animals (exclosure cages). Grazing is simulated by successive cuttings and the herbage mass accumulation is measured. But the measured grass accumulation is often very different in grazed or non grazed area (Dove and Mayes 1991; Mayes and Dove 2000; Macoon et al. 2003) also because of the lack of defoliation and of urine and dung restitution in the latter case. The method of difference is mainly used to estimate the herbage intake refers to a herd/flock of animals (Reeves et al., 1996 cited by Smith et al. 2005).

Instantaneous intake can also be directly estimated through short-term changes in live weight (Penning and Hooper 1985; Coates et al., 2000 cited by Decruyenaere et al., 2009; Penning et al., 1995). Intake was determined as the difference between pre- and post-grazing live weight, (Barrett et al., 2001).

With this method it is possible to measure intake only over very short period, i.e. 1 hour. The accuracy of the measure is strongly dependent on the precision of the weight loss related to the dung and urine excretion during the period of measure and of insensible loss due to respiratory changes (the insensible weight loss).

The insensible weight loss is "the rate of reduction in live weight observed over a period of time, not associated with the loss of excreta but mainly caused by body moisture loss from respiration" (Dumont et al., 1994), and it is also affected by meteorological conditions (Gibb et al.,1995).

Another method of intake estimation is based on the knowledge of animal requirements and performance, and on the nutritive values of ingested diet. With beef cattle Minson et al., (1987) have estimated intake from live weight and growth rate with a good accuracy. The difficult of the method is the determination of the true herbivore requirements.

2.3 Indirect methods

Intake of grazing ruminants can be estimated by indirect methods such as, among others, the recording of animal feeding behaviour, the use of Near infrared Reflectance spectroscopy (NIRS) and the marker techniques.

2.3.1 Feeding behaviour recording

Intake can be indirectly estimated by studying the grazing behaviour. Indeed intake can be determined through the product of three parameters: grazing time, biting rate and bite mass (Decandia *et al*. 2000; Barrett *et al*. 2001). Grazing time and biting rate can be measured by visual observations (Rook et al., 2004). Whereas the presence of the observer can perturb animal behaviour at grazing, the animals should be the accustomed to the observer in order to avoid any behaviour modification (Agreil et al., 2004).

The recording of animal activities such as displacement, rumination, intake times have been largely tested and used to determine grazing time and biting rate (Laca et al., 2000).

These recording methodologies require expensive materials and the harnessing of the animal with recording apparatus that can disturb its behaviour.

The major source of error of the measure remains the determination of the biting mass which may be estimated trough the use of oesophageally fistulated animals (see below).

2.3.2 Near infrared Reflectance spectroscopy (NIRS)

One solution to estimate intake of grazing ruminants could be the use of NIRS (Near Infrared Reflectance Spectroscopy).

During last 20 years the potential of NIRS analysis to characterize the nutritive value of grazed grass has been widely used. This indirect method is based on the establishment of calibration databases linking a NIRS spectra to values such as chemical or biological composition obtained by reference measurement in laboratory.

The most developed NIRS calibrations allow to estimate firstly in vivo digestibility and secondly intake from the analysis of different organic substrates as forage, oesophageal extruda or faeces. Lyons and Stuth (1992) pioneered the application of NIRS technology to indirectly predict the quality of forage ingested by free-ranging animals via fecal scans. As diet chemistry changes, the by-products of digestion (plant residue, microbial bodies, secondary metabolites, slough tissue, etc.) also change. The behaviour of these secondary products in the faeces may be related to the characteristics of the primary product (i.e. ingested diet).

Different studies have shown that NIRS applied to faeces could be more or even accurate than classical method to predict diet characteristics of grazing ruminants. Indeed chemical composition of faeces can be detected by NIRS and linked to intake and digestibility. Garnsworthy et al. (2004) have concluded that direct prediction of dry matter intake of dairy cows by NIRS applied to faeces or by n-alkanes methods (see below) have similar accuracy. More recently Boval et al. (2004), Landau et al. (2004), Decandia et al. (2007), Keli et al. (2008) and Decruyenaere et al. (2009) have confirmed the interest of faecal NIRS to assess diet characteristics of cattle, dairy goats and sheep.

The main constraint of NIRS technique is the necessity to develop large reference databases that must be frequently updated to develop robust calibration covering the different field situations.

2.3.3 Methods based on the use of markers

Over the last 50 years the most successful and widely used method for estimating HI arise from separate estimates of faecal output (F) and the digestibility of the consumed diet (D) through their relationship with the intake of a diet (I):

$$I = F/(1-D) \qquad \text{eq.1}$$

Faecal output in grazing animals can be measured directly by total collection over a number of days using bags attached as harnesses, but under field conditions this is laborious and can disturb normal foraging behavior. Faecal output is therefore estimated from the dilution in a sample of faeces of an indigestible marker (external marker) administered orally to the animal daily or more often, over a period of 10-14 days (Mayes and Dove 2000) according to the equation:

$$\textbf{Faecal output (F)} = \frac{\textbf{Dj}}{\textbf{Fj}} \qquad \text{eq. 2}$$

Where Dj is the marker dose rate and Fj is the faecal marker concentration.

The faeces may be sampled over a 5-7 days period after the marker concentration has equilibrated in the faeces (Mayes and Dove, 2000)

Using markers, the accuracy of the estimated faecal excretion depends greatly on a representative collection of faecal samples (Whittington and Hansen, 1985 cited by Mahler et al., 1997), especially when there are distinct variations in the animal's diurnal patterns of feed intake and when the pasture shows heterogeneous species distribution.

A wide range of external marker has been used for the estimation of faecal output, but to date no one was found to have the characteristics of an ideal marker (Galyean 1987; Kotb and Luckey 1972 cit. by Dove and Mayes 1991) as:

- to be chemically discrete (easy of identification and analysis);
- to be indigestible in the digestive tract;

- to be inert and nontoxic to animals and microflora of the digestive tract;
- to be intimately associated with the material to be marked;
- should not influence the secretion, absorption and motility of the gastrointestinal tract .

Among others chromium sesquioxide (Cr_2O_3) has been shown to perform satisfactorily as a faecal output marker thanks to his high faecal recovery (Dove and Mayes 2005).

One of the main problems in using Cr_2O_3 is the diurnal variation in the faecal concentration of the marker. Diurnal variation can result from diurnal variation in intake, from the method of administration of marker and from events in the digestive tract that can influence the mixing marker-digesta and will result in samples of faeces no representative of the mean faecal marker concentration and a consequent in an error of the intake estimation.

Should also be noted that the Cr_2O_3 may represent a risk to human health.

Because of their high faecal recovery, rare earth salts as ytterbium (Yb) have been used as markers for faecal output estimation. Furthermore the development of mathematical modeling to describe marker kinetics has allowed the estimation of faecal output following a single oral dose of Yb (France et al 1988 cited by Mayes et al, 1995).

Contrary to Cr_2O_3, Yb not represents a risk of toxicity to humans or animals.

A critique of this element is to be found preferentially associated with smaller particles and that in some circumstances migrates from solid to liquid phase.

The rapid rate of passage combined with easy recovery suggests that small plastic particles (i.e. small polyamide particles) should be a suitable marker for the quantification of faecal excretion besides having the advantage that only simple laboratory equipment is necessary to measure them gravimetrically in faecal samples (Mahler et al., 1997).

Beyond the external markers, the internal markers, consisting of indigestible plant constituents, have been used.

An internal marker is an indicator that occurs naturally in a diet and that must be non absorbable and non-metabolizable in the gastrointestinal tract (Faichney 1975, Merchen 1993 cited by Sims et al., 2007).

The acid insoluble ash (AIA) (Van Keulen and Young 1977) have provided good results as internal marker in digestibility studies because of their high faecal recovery.

On the other hand considering that no enzymes for lignin degradation appear to exist in mammals or in anaerobic bacteria (Van Soest 1994), therefore, lignin is considered indigestible and has been evaluated as a potential internal marker.

Unfortunately Merchen (1993 cited by Sims et al., 2007) reported that underestimation of digestibility when using acid detergent lignin (ADL) as an internal marker resulted from incomplete recovery of lignin and that incomplete recovery is more problematic as dietary lignin concentration decreases.

Fahey and Yung (1983) have suggested a number of reasons to justify this incomplete recovery of lignin:

- true digestion
- apparent digestion linked to formation of complex carbohydrates-lignin
- partial destruction of the fractions of lignin present in the faeces by the common reagents used in analytical methods.

The other component of equation 1 is digestibility. Obtaining reliable estimates of the digestibility represents a problem greater than the measurement of faecal output, because errors in the estimation of digestibility can always cause a larger error in the intake estimate, especially when diet digestibility is high. A major difficulty in obtaining an accurate estimate of digestibility is to have a representative sample of the diet actually consumed by the grazing animal.

For this purpose estimates of digestibility have been made with oesophageal-fistulated animals obtaining samples representing the diet of the experimental animals. These samples are then used in *in vitro* estimates of the digestibility, simulating the digestive process through ruminal fluid with a buffer followed by incubation in pepsin (Tilley and Terry 1963).This technique must be calibrated against *in vivo* digestibility estimation. A major issue is that the samples obtained by the esophageal-fistulated animals can not represent the diet of the intact experimental animals because:

- esophageal extrusa samples are collected over a time-span of minutes, whereas the test animals may be graze for days or weeks. (This problem could be overcome by the use of a cannula fitted with a remote-control valve as pointed out by Raats *et al*., 1996).
- The esophageal-fistulated animals are surgically prepared and can be of different sex or physiological state and managed differently from the animals under-study and hence their diet may be different.
- Results of *in vitro* digestibility assays are often applied to animals which may differ markedly in sex, stage of growth, reproductive status, intake level and even species from the animals used in the original *in vitro/in vivo* calibration.
- The single *in vitro* estimate of digestibility is applied to all the experimental animals, despite that digestibility of a given diet differs among individual animals.
- The *in vitro* methods cannot accommodate possible interactions between forage and supplement, administered eventually when pasture quantity or quality is inadequate, during digestion.

To try to overcome some of these problems, indigestible diet components have been used as "internal markers" of digestibility which is then calculated as 1 – (concentration of internal marker in the diet/ concentration of internal marker in the faeces). Since the faeces come from individual experimental animals, digestibility estimates for each animal are obtained.

Beyond the markers indicated above for the estimation of faecal output, alkaline peroxide lignin, and indigestible acid detergent fiber have been used in digestibility trials (Sunvold and Cochran 1991).

The internal marker method allows the determination of whole-diet digestibility in animals fed mixed diets, if the concentration of the internal marker in complete diet can be estimated. When grazing animals are receiving feed supplements, the intake and the internal-marker concentration of the supplement must be known.

Despite the potential advantages of using an internal marker, as well as for external markers, many of the proposed markers have failed to meet the criteria required for an ideal marker (Kotb and Luckey 1972 cit. by Dove and Mayes 1991). In particular many of these have been characterized by inconsistent faecal recovery or were not chemically discrete compounds so there is uncertainty whether a component measured in the faeces is the same as that measured in the diet.

A number of the problems encountered with previous internal or external markers do not apply when using alkanes as markers.

2.3.3.1 Intake measurements using *n*-alkanes as markers

n-alkanes are the most common saturated hydrocarbons that are present in the waxes of most higher plants; they are present as mixtures with chain lengths ranging from 21 to 37 carbon atoms.

These compounds are synthesized during the cell wall building-up period (mature cells) and they seem to play a role in reducing the water loss for transpiration. Over 90% of *n*-alkanes have odd-numbers of carbon atoms with C_{29}, C_{31} and C_{33} alkanes dominants in most pasture species. There are also marked species differences in the pattern of alkanes concentration: C_{31} and C_{33} are usually the dominant alkanes in grasses and C_{29} is the dominant in legumes. The main factors that affect the concentration of the alkanes within a forage species are the phenological stage, the growing stage and the part of the plant. Plant wax *n*-alkanes were originally suggested as internal markers for estimating digestibility (Mayes and Lamb, 1984) because their

relative inertness and ease of analysis. Afterwards Mayes et al (1986) developed an alkane method for estimating intake that can be regarded as double-marker method.

In this approach, animals are dosed with known quantities of an even chain alkane and intake is estimated from the daily dose rate and the dietary and faecal concentration of the dosed, even-chain alkane and of the natural, odd-chain alkane adjacent (see below) in chain length.

The classical double-marker approach is based on the following equations

$$F = \left(\frac{Dj}{Fj}\right) \times RRj \qquad \text{eq. 3}$$

Where F is faecal daily output, Dj is the amount of external marker dosed, Fj is the concentration of the external marker in the faeces, RRj is the recovery rate of the marker.

$$D = 1 - \frac{Hi}{\frac{Fi}{RRi}} \qquad \text{eq. 4}$$

Where D is the digestibility, Hi is the internal marker concentration in the herbage, Fi is the concentration of the internal marker in the faeces and RRi its corresponding recovery rate.

Then the intake is calculated by eq. 1 from results of equation 3 and 4; the equation for alkane estimation of herbage intake (without any supplementation) is:

$$Intake = \big((Fi/Fj)Dj\big)/(Hi - (Fi/Fj)Hj) \qquad \text{eq. 5}$$

However for a given alkane pair (plant odd-chain alkane *i* and dosed even-chain alkane *j*) intake is calculated directly from herbage alkane concentrations (Hi and Hj) faecal concentrations (Fi and Fj) and the dose rate of alkane Dj.

With regard to this method Mayes et al. (1986) suggested that "incomplete faecal recoveries would not matter provided the method employed a pair of alkanes which were similar in recovery". Note that in the faeces it is only the ratio of the alkane concentrations which is important and that alkanes adjacent in chain length have similar recoveries.

Many validation studies with sheep (Dove and Mayes, 1991), goats (Mayes et al., 1995) and cattle (Mayes et al., 1986; Hameleers and Mayes 1998) have shown that the most reliable intake estimates have been obtained using C_{32} and C_{33} as respective dosed and dietary alkanes.

Moreover the hexatriacontane (C_{36}) could be used to determine faecal output, since it has high faecal recovery (approximately 0.95, Mayes et al 1986); if animals were dosed with both C_{32} and C_{36} both intake and faecal output, and hence digestibility, could be determined using a single procedure.

The oral administration of synthetic even-chain alkanes to animal has been carried out using once or twice-daily dosing with either pellets or alkanes-impregnated shredded paper or paper stoppers, and gelatin capsules containing alkanes suspended on powdered cellulose.

Procedures for preparing pellets and capsules and for analyzing alkanes in diet and faeces have been discussed in several works (Dove and Mayes 1991, Mayes et al. 1986, Vulich et al. 1991 and 1995, cited by Dove and Mayes 1996).

Studies with both paper pellets and gelatin capsules for delivering alkanes have indicated that, in sheep, the faecal concentration of dosed alkane takes 5-6 days to reach equilibrium (Mayes et al. 1986, Dove et al. 1989, Dove et al. 1991 cited by Dove and Mayes 1991). However, in general, the equilibrium concentrations of the marker in faeces is reached 5-7 days from the beginning of alkane dosing.

It is noteworthy that with the alkane procedure, important consideration is whether there is diurnal variation in the ratio of the faecal concentrations of herbage and dosed alkanes (Fi/Fj in the eq. 2). As Dove et al. 1991 have reported, it is possible to have temporal variability in absolute faecal concentrations of both alkanes but a markedly constant ratio of their faecal concentration. Significant diurnal variation in faecal alkane levels in dairy cows has been reported after both once-daily and twice-daily dosing (Stakelum and Dillon 1990).

Several authors have investigated whether diurnal variation is a problem with the alkane procedure, and the results, although not definitive, suggests that temporal variation in alkane concentrations and ratios could not be a problem in the grazing animals (Dove and Mayes, 1991).

The main advantage of the alkane as compared with the classical double-marker methods is that the former method is not biased by the recovery of the markers in the faeces; indeed it is important the ratio of the faecal alkane concentrations not their absolute concentrations provided that the method employed a pair of alkanes which were similar in recovery. The faecal recovery of longer alkanes adjacent in chain length is very similar so that intake can be estimated accurately using, for example, dosed C_{32} alkane and either C_{31} or C_{33} alkane from the plant.

To reduce the labor required for daily or more frequent dosing of animals with alkanes, an intraruminal, alkane Controlled Release Device (CRD) has been developed. The use of CRD that allows a steady release of the marker in the rumen, helps to overcome the problems arising from diurnal variation in the faecal concentration of the marker and allows to reduce disturbance to the animals. They have been shown to give accurate estimates of intake in sheep (Molle et al., 1998) and cattle.

The main precaution required in the use of the method is to ensure that the diet sample, in terms of alkanes concentrations, is representative of that consumed by the experimental animals. Unfortunately under conditions in which animals can feed on complex vegetation communities, it may be extremely difficult to obtain feed samples having alkanes concentrations representative of those in the diets of individual animals.

Animals select for some plant species or plant parts that have a different n-alkane content than the average field sample. For the n-alkanes the fact that plant organs and plant species present different n-alkanes profile (Cortes et al., 2005) is an important source of error in the estimation of intake and the sampling of the herbage

actually grazed is probably the most limiting step of the marker-based methodology, alkanes included.

In general all factors that increase sward heterogeneity tend to decrease the reliability of herbage sampling; this difficulty can be circumvented through the observations of animals at pasture, and collecting herbage samples mimicking their selective behaviour (Berry et al., 2002, Estermann et al., 2003, Leiber et al., 2006).

This "hand plucking" method, that simulates the biting of the herbivore, may be used to sampling grazed grass. The precision of the measure is linked to the calibration between animals and operator observations. Such calibration appears easier to set up with cattle than with sheep and goats that have a more selective grazing behaviour (Wallis DeWries, 1995).

Another possibility would be the use of oesophageal-fistulated animals but this method, beyond the limits seen before, it is not respectful of animal welfare and can modify herbivore behaviour, as reported in several studies (Jones et al., 1992).

The faeces sampling can be done from:

- Total daily output, very accurate but not practical under grazing conditions.
- The dung collected from the ground.
- The rectum (grab samples) taken at different times during the day.

The grab-sampling technique is usually used for grazing animals and the samples are taken from day 5-7 after the beginning of dosing. The grab-samples can be individually analysed or pooled over the day or the measurement period; as general rule pooling over the measurement period is the best choice (Vulich et al.1995).

The alkane method for estimating intake have a number of advantages (Dove and Mayes, 2005):

- Intakes estimates are "individual" if the sample of vegetation analysed for alkanes is representative of the actual diet consumed.

- If, in addition to being dosed with an even-chain alkane to allow the estimation of intake, the animals are dosed with another external marker, faecal output can be estimated and from this, whole-diet digestibility. C_{36} alkane with a consistently high faecal recovery (~95%) can be used in this regard
- If the alkane concentration of the supplement and the supplement intake are known, the method can be used with animals which are also receiving supplementary feeds, something that happens frequently with grazing animals.
- The alkane concentration in plant, faeces and external marker are determined at the same time with the same analytical method that allows a reduction of analytical error and bias.

In spite of validation studies have shown that the alkane procedure for estimating dietary intake is reliable (Mayes et al., 1986) and that it is a good estimator of DM intake during grazing (Malossini *et al*. 1994, Smith *et al*. 2005), absolute validation of the method with grazing or browsing animals is impossible to achieve because alternative methods with which to compare the technique may be no more reliable or inferior (Dove and Mayes 1996). However as Smith *et al.* (2005) "for herbage intake estimations of individual grazing animals, the n-alkanes technique is the best technique to use"

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Chapter 3
Beef meat quality

3.1 Beef meat quality

The meat quality is an evolving concept that changes with the evolution of culture, consumer expectations, scientific knowledge and accuracy of analytical methods (Nardone et al. 1998). The different definitions ranging from "a whole of characteristics able to satisfy the explicit or indefinite consumer's requirements (ISO 9000, 2000)" to "more objective assessment through the relevant parameters such pH, meat color, tenderness, chemical composition, fatty acid composition" (Karlsson, 1993 Destefanis and Barge 1990).

The quality refers to a foodstuff is a concept dependent on a large number of variables, many of which are subjective and related to ethnic tradition, or even family (Sanudo et al., 1996, Morrissey et al., 1998).

Furthermore the moments of quality evaluation and thus the points of view may be different: for the breeder it is important that an animal will grow quickly, for the butcher is important the carcass yield, for the consumer the aspect of the cut of meat, flavor, tenderness, nutritional value, safety and hygiene.

For this reason to talk about beef quality "sensu lato" is of little significance. Among the different aspects of beef quality here will be taken into account the following:

- Chemical and nutritional quality, that concern aspects detectable by proximate analyses and involve classical components (moisture, protein, ether-extractable fat, vitamins, cholesterol,) (Leheska et al. 2008).
- Technological quality, that concerns the ability of the meat to the conservation and transformation (Monin 1991), i.e. pH and Water Holding Capacity (WHC).

- Sensory quality, that concerns the quality perceived by the senses of the consumer (Monin 1991) as, color, tenderness and water holding capacity;

3.2 Chemical and nutritional quality of beef meat

Beef meat is considered to be a highly nutritious and valued food. The importance of meat as source of high biological value protein and micronutrients (for example vitamins A, D, E, zinc, selenium) is well recognized (Biesalki 2005, Williamson et al. 2005 cited by Scollan et al. 2006).

The average composition of beef meat in comparison with those of other livestock species is shown in Table 1.

Table 1. Composition (for 100 g) of meat of different livestock species

	Beef	Lamb	Kid	Pork
Moisture (%)	72	70.1	74.8	73
Protein (%)	22.5	20.3	19.2	21.8
Fat (%)	3.7	5.8	5	2.2
Carbohydrates (%)	0.1-0.5	0.3	-	-
Energy (Kcal)	162	159	122	119

Williamson et al.,(2005); INRAN, (2007) modified by Manca, (2010)

Meat from ruminant, especially from bovines, is also an important source of heminic iron (about 2 to 5 mg 100 mg^{-1} fresh tissue according to the type of muscle, which is respectively 3 to 4 times higher than that of pork and chicken meat). This heminic iron in turn is 5 to 6 times more absorbed than the non-heminic iron from plants (Geay et al., 2001). Moreover ruminant meat is also an important source of vitamins of the B group: B1, B2, B6, B12 and niacin. Vitamins B6 (0.3 to 0.4 mg.100 mg^{-1} in the bovine) and B12 (1.5 to 2.5 mg.100 g^{-1}) in particular are virtually absent in plants but synthesized by microorganisms of the digestive tract of ruminants.

However, over the last 10–15 years, these positive attributes have often been underestimated and has been given more importance to several negative attributes.

The latter include the perception that beef contains high amounts of fat rich in saturated fatty acids often considered unhealthy.

3.2.1 Chemical composition

The chemical composition of muscles is characterised by a relatively constant level ofwater (about 75%), proteins (19 to 25%) minerals and carbohydrates (1–2%), whereas lipid composition is highly variable. The latter changes in the different muscles and butchery pieces depending on the relative proportions of inter-muscular and subcutaneous adipose tissues incorporated (Geay et al., 2001).

3.2.1.1 Proteins

Beef proteins are characterized by an high biological value, because of their complete amino-acid profile. The quality of meat proteins, in fact, is due primarily to the presence of the essential amino-acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) namely those that humans cannot synthesize despite being essential for its survival; and furthermore to their high digestibility (around 94%, Williams, 2007) thanks to "their amino-acid sequence that promotes gastric and intestinal proteolytic enzymes" (Manca, 2010).

An important meat protein is myoglobin, that is an iron- and oxygen-binding protein, related to hemoglobin. The myoglobin forms pigments that are the most responsible for meat color.

3.2.1.2 Lipids

Lipids, besides being one of the main components of the meat, supplies essential nutrients such as fat-soluble vitamins and essential fatty acids (EFA) and also affect flavor of foods (Williamson et al., 2005).

Fat content in beef varies largely depending on the cut and it is present as membrane fat (as phospholipid), intramuscular fat (IMF), intermuscular fat (between the muscles), and subcutaneous fat. The white strips of adipose tissue between the

bundles of muscle fibres represent the marbling fat, "closely linked to IMF content" (Scollan et al., 2006), the fat depot of most interest in relation to fatty acid composition and human health. In fact, because of its close link with the muscle fiber, it cannot be removed before consumption. Marbling fat is also an important meat quality trait in relation to juiciness, aroma and tenderness that are influenced also by him.

The main components of the intramuscular fat are:

- the polar lipids, mostly phospholipids, placed in cell membranes;
- the neutral lipids consisting mainly of triacylglycerols, an ester of three fatty acids and glycerol, contained in the adipocytes, .

The amount of triacylglycerols in intramuscular fat is affected by animal species, breed, nutrition, and age whereas the amount of phospholipid in intramuscular fat is relatively constant, and varies between 0.2 and 1% of muscle weight (DeSmet et al., 2004 cited by Manca, 2010).

Itis noteworthy that in ruminants, the fatty acids in triacylglycerols can be influenced by diet but to a much lesser extent than in monogastrics because of the biohydrogenation of dietary fatty acids that occurs in the rumen.

3.2.1.2.1. Fatty acid profile of IMF and relationships to human health

The fatty acids composition of intramuscular fat, in relation of the presence and position of the double bonds on fatty acid chain, consists on average (as % of total fatty acids) of (Scollan et al., 2006):

45–48% saturated fatty acids (SFA);

35–45% monounsaturated fatty acids (MUFA);

up to 5% polyunsatured fatty acids (PUFA).

Table 3 shows the fatty acid profile of intramuscular fat of meat from different livestock species. The most abundant fatty acid are oleic acid (C18:1 *cis*-9) and

palmitic acid (C16:0) in all species. In comparison with monogastric, ruminant meat is characterized by a higher percentage of SFA due to the biohydrogenation of dietary unsaturated fatty acid that occurs in rumen.

Table 3 - Fatty acid profile of muscle of different livestock species (% of total fatty acids).

Fatty Acid	Lamb	Suckling Lamb	Beef	Pork
C14:0	4.52	4.11	1.70	1.04
C16:0	22.58	19.33	21.75	22.31
C18:0	15.30	12.02	15.16	12.88
C18:1 cis - 9	27.63	29.28	37.69	32.62
C18:2 n-6 (LA)	5.90	10.97	9.52	16.97
C18:3n-3 (ALA)	1.70	1.95	0.22	0.47
C20:4 n-6 (AA)	2.53	6.89	3.08	4.00
C20:5n-3 (EPA)	1.33	1.65	0.33	0.17
C22:6n-3(DHA)	0.53	1.25	0.09	0.23
SFA	43.53	37.73	43.97	36.77
MUFA	35.98	34.89	40.00	40.05
PUFA	15.18	27.38	16.22	23.2
PUFA/SFA	0.35	0.73	0.37	0.64

By Manca, (2010).

The predominant SFA are myristic acid (C 14:0), palmitic acid and stearic acid (C 18:0). SFA is positively related to the level of plasma cholesterol, though 18:0 is considered as neutral in this regard (Yu *et al*., 1995 cited by Scollan *et al*., 2006).

Oleic acid (C 18:1 n-9) is the most predominant MUFA and the main PUFA are linoleic (LA, C18:2 n-6) and α-linolenic (ALA, C18:3 n-3) acids .

A large parts of linoleic and α-linolenic acid, their longer chain derivatives such arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docasahexaenoic acid (DHA; 22:6n-3), and long chain n-3 and n-6 PUFA (LC-PUFA) are esterified predominantly in phospholipid fraction, while the largest part of the fatty acids in triacylglycerols of intramuscular fat consists of SFA and MUFA, predominantly C16:0 and C18:1 cis-9 (Wood et al., 2008).

The phospholipid fatty acids are less influenced by diet, but for example, pasture could increase the n-3 fatty acids in both triacylglycerols and phospholipids (Dannenberger et al., 2004).

Beef also contains small amounts of the long chain C20/22 PUFA, n-3 PUFA and recent research has demonstrated that red meat is an important source of these fatty acids for human nutrition (Howe et al., 2006).

Beef meat is also a great source of conjugated linoleic acid (CLA) and in general the ruminant products are the richest natural source of this molecule (Cabiddu et al., 2006). The acronym CLA refers to a family of positional and geometric isomers of linoleic acid with two conjugated unsaturated double bonds which arises, but not only, from microbial hydrogenation of dietary linoleic acid in the rumen (Ha et al., 1990 cited by Realini et al. 2004). In addition, CLA is synthesized from trans-11 octadecenoic acid by Δ9 desaturase in adipose tissue (Bauman et al., 1999). Dannenberger *et al*. (2004) reported 10 isomers of CLA in beef. In Table 4 is reported the CLA isomeric profile in beef and lamb meat; in particular the isomer *cis*-9, *trans*-11 or Rumenic acid (RA), represents approximately 70% of total CLA isomers and the second most abundant isomer is the *trans*-7, *cis*-9 CLA while the isomer *trans*-10, *cis*-12 CLA, which may have harmful effects on human health (Clément *et al*., 2002; Alfaia et al., 2006; Wahle *et al*., 2004 cited by Manca, 2010), is less represented.

Table 4 - Isomers of CLA in meat of beef in different feeding systems and in lambs (% of total CLA).

	Beef (intensive)	Beef (extensive)	Lamb
cis-9, cis-11	1.08	1.42	-
cis-9, *trans*-11	59.89	78.35	77.31
cis-11, *trans*-13	1.10	0.72	0.15
cis-12, *trans*-14	1.21	1.35	0.64
trans-7, cis-9	12.09	9.17	8.04
trans-10, cis-12	3.79	2.12	0.22
trans-11, cis-13	1.26	1.22	6.86
trans-6, *trans*-8	-	0.23	0.04
trans-7, *trans*-9	15.03	0.81	0.56
trans-8, *trans*-10	0.37	0.38	0.37
trans-9, *trans*-11	1.16	2.14	2.17
trans-10, *trans*-12	1.04	0.59	0.74
trans-11, *trans*-13	0.57	1.03	1.80
trans-12, trans-14	0.55	0.48	1.10

by Manca, 2010

Fatty acids in ruminants are also characterised by nutraceutical properties that are summarized as follows:

- MUFA , reduce the level of blood cholesterol without reducing the level of high density lipoprotein (HDL) (Ulbricht and Southgate, 1991);

- oleic acid (C18:1 cis-9) can reduce the incidence of cardiovascular disease (Massaro et al., 1999 cited by Manca 2010);

- linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3) are essential fatty acids (EFA) as precursors of PUFA that are not synthesized by the human body and should be taken with diet. In addition LA is part of the lipid membrane, and ALA can reduce the risk of neurological disorders, heart disease and cancer in adults and children;

- odd and branched chain fatty acid (OBCFA) are important for their anticarcinogenic effects on cancer cells. The highest activity was observed with *iso*-16:0, and the activity decreased with increase or decrease of

the chain-lengths from C16:0 (Wongtangtintharn et al., 2004 cited by Manca 2010.

- the isomer of CLA cis-9, trans-11 or Rumenic acid (RA) is credited to be beneficial to human health reducing carcinogenesis, atherosclerosis, onset of diabetes, and body fat mass (Lee et al., 1994; Parodi 1997; Ip et al., 1999; Pariza et al., 2001; Belury, 2002 cited by Manca, 2010; De la Torre et al., 2006 cited by Scollan et al., 2006).
- vaccenic acid (VA, C18:1 trans-11), metabolized to CLA, play anticarcinogenic activities (Banni et al., 2002 cited by Scollan et al., 2006).
- Linoleic acid (C18:2 n-6) is essential for human growth and reproduction while linolenic acid (C18:3 n-3) is important for brain and retina functions. Moreover, n-3 PUFA exert a positive influence in the prevention of cardiovascular diseases (De Lorgeril et al., 1994 cited by Geay et al., 2001). A too high value of the ratio of n-6 PUFA to n-3 PUFA is associated to an increased risk of atherosclerosis or coronary diseases (see below). In this aspect (n-6/n-3 ratio), ruminant meat (bovine, ovine) with values comprised between 1 to 2, is superior to pork one, which presents values of approximately 7 (Wood et al., 1999). This is due to the fact that linolenic acid, (ALA C18:3n-3) abundant in fresh forages (> 50% total fatty acids) (Bauchart et al., 1984 cited by Geay et al., 2001), is stored in significant amounts in ruminant tissues (Enser et al., 1999 cited by Geay et al., 2001).

Also it should be noted that the PUFA of the n-3 family (C18:3 n-3 and its derivates) and of the n-6 family (C18:2 n-6 and its derivates) are synthesized only by plants. No metabolic conversion between n-3 PUFA and n-6 PUFA is possible. These fatty acids have to be provided by the diet (Geay et al., 2001).

The relationships between dietary fat and incidence of lifestyle diseases, particularly coronary heart disease are well established and this has contributed

towards the development of specific guidelines from the World Health Organization in relation to fat in the human diet (WHO, 2003 cited by Scollan et al., 2006).

The contribution of different fatty acids to the diet, as % of total energy intake, according to the recommendations, should be (Table 5):

Table 5 - Nutritional advice for fatty acid in human diet.

	Recommendation	Reference
Total fat	<15–30%	World Health Organization (2003)
SFA	<10%	
trans FA	*<1%*	
PUFA	6 – 10%	
PUFA n-6	5 – 8 %	
PUFA n-3	1 – 2 %	
C18:2n-6 /C18:3 n-3	between5:1and 10:1	Food and Agricultural Organization/ World Health Organization (1994)

Modified by Manca, 2010

Reducing the intake of SFA (which are known to raise total and low-density lipoprotein (LDL) cholesterol) and increasing the intake of n-3 PUFA is particularly encouraged.

Among the n-3 PUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docasahexaenoic acid (DHA; 22:6n-3) have been demonstrated to help in reducing the risk of cardiovascular disease, play a key role for proper brain and visual development in the foetus, extend the maintenance of neural and visual tissues throughout life (Calder, 2004 and Leaf *et al*., 2003 cited by Scollan et al., 2006) and may have roles in reducing cancer and obesity/type-2 diabetes (WHO, 2003).

3.2.1.2.2. Feeding system as factor affecting fatty acid profile in meat

Due to the above, considerable attention has been placed on improving the nutritional value of beef meat and the development of products which are beneficial to human health and disease prevention. Many efforts have been devoted to study the lipid composition also keeping in mind that fat content and fatty acid composition may

be affected by animal production factors such as nutrition. Actually, in fact, the nutritional modulation of the fatty acid profile of ruminant edible fats is an important research topic (Hess *et al*., 2008). It is well known that the use of forages in the diet of the animals influences the fatty acid profile of meat (French et al., 2000; Scollan et al., 2001; Steen et al., 2003; Noci et al., 2005; Daley et al., 2010) (Table 6, 7, 8 and 9).

Table 6. Comparison of mean saturated fatty acid composition (expressed as mg/g of fatty acid or as a % of total lipid) between grass-fed and grain-fed cattle

Treatment	Breed	C12:0 lauric	C14:0 myristic	C16:0 palmitic	C18:0 stearic	C20:0 arachidic	Total SFA (units as specified)	Total lipid (units as specified)	Reference
				g/100 g lipid					
Grass	Crossbred steers	0,05	1.24*	18.42*	17.54*	0.25*	38,76	9.76* mg/g muscle	Alfaia, et al. 2009,
Grain		0,06	1.84*	20.79*	14.96*	0.19*	39,27	13.03* mg/g muscle	
Grass	Mixed cattle	0,05	2.84*	26,9	17.0*	0.13*	48.8*	2.8* % of muscle	Leheska, et al. 2008,
Grain		0,07	3.45*	26,3	13.2*	0.08*	45.1*	4.4* % of muscle	
				% of total FA					
Grass	Angus crossbred steers	na	2,19	23,1	13.1*	na	38.4*	2.86* %IMF	Garcia et al. 2008,
Grain		na	2,44	22,1	10.8*	na	35.3*	3.85* %IMF	
				mg/100 g muscle tissue					
Grass	Angus steers	na	56.9*	508*	272,8	na	900*	2.12%* % of muscle	Ponnampalam, et al. 2006,
Grain		na	103.7*	899*	463,3	na	1568*	3.61%* % of muscle	
				% of total intramuscular fat reported as LSM					
Grass	Simmental bulls	0,04	1,82	22.56*	17.64*	na	43,91	1.51*% of muscle	Nuernberg, et al. 2005,
Grain		0,05	1,96	24.26*	16.80*	na	44,49	2.61* % of muscle	
				% of total FA					
Grass	Crossbred steers	na	2,2	22	19,1	na	42,8	2.7* %IMF	Descalzo, et al. 2005
Grain		na	2	25	18,2	na	45,5	4.7* %IMF	
				% fatty acid within intramuscular fat					
Grass	Hereford steers	na	1.64*	21.61*	17.74*	na	49,08	1.68* % of muscle	Realini, et al., 2004,
Grain		na	2.17*	24.26*	15.77*	na	47,62	3.18* % of muscle	

*Indicates a significant difference (at least $P < 0.05$) between feeding regimens was reported within each respective study. "na" indicates that the value was not reported in the original study. From Daley et al. 2010

Table 7. Comparison of mean polyunsatured fatty acid composition (expressed as mg/g of fatty acid or as a % of total lipid) between grass-fed and grain-fed cattle

Treatment	Breed	C18:1 t11 Vaccenic	C18:2 n-6 Linoleic	Total CLA	C18:3 n-3 Linolenic	C20:5n-3 EPA	C22:5n-3 DPA	C22:6n-3 DHA	Total PUFA	Total MUFA	Total n-6	Total n-3	n-6/n-3 ratio	Reference,
							g/100 g lipid							
Grass	Crossbred steers	1.35	12.55	5.14*	5.53*	2.13*	2.56*	0.20*	28.99*	24.69*	17.97*	10.41*	1.77*	Alfaia, et al. 2009
Grain		0.92	11.95	2.65*	0.48*	0.47*	0.91*	0.11*	19.06*	34.99*	17.08	1.97*	8.99*	
							g/100 g lipid							
Grass	Mixed cattle	2.95*	2.01	0.85*	0.71*	0.31	0.24*	na	3.41	42.5*	2.3	1.07*	2.78*	Leheska, et al., 2008
Grain		0.51*	2.38	0.48*	0.13*	0.19	0.06*	na	2.77	46.2*	2.58	0.19*	13.6*	
							% of total FAs							
Grass	Angus steers	3.22*	3.41	0.72*	1.30*	0.52*	0.70*	0.43*	7.95	37.7*	5.00*	2.95*	1.72*	Garcia et al., 2008
Grain		2.25*	3.93	0.58*	0.74*	0.12*	0.30*	0.14*	9.31	40.8*	8.05*	0.86*	10.38	
							% of total fatty acids							
Grass	Simmental bulls	na	6.56	0.87*	2.22*	0.94*	1.32*	0.17*	14.29*	56.09	9.8	4.70*	2.04*	Nuernberg, et al., 2005
Grain		na	5.22	0.72*	0.46*	0.08*	0.29*	0.05*	9.07*	55.51	7.73	0.90*	8.34*	
							% of total FAs							
Grass	Crossbred steers	4.2*	5.4	na	1.4*	tr	0.6	tr	10.31*	34.17*	7.4	2	3.72*	Descalzo et al., 2005
Grain		2.8*	4.7	na	0.7*	tr	0.4	tr	7.29*	37.83*	6.3	1.1	5.73*	
							% fatty acid within intramuscular fat							
Grass	Hereford steers	na	3.29*	0.53*	1.34*	0.69*	1.04*	0.09	9.96*	40.96*	na	na	1.44*	Realini, et al., 2004,
Grain		na	2.84*	0.25*	0.35*	0.30*	0.56*	0.09	6.02*	46.36*	na	na	3.00*	

* Indicates a significant difference (at least $P < 0.05$) between feeding regimens within each respective study reported. "na" indicates that the value was not reported in the original study. "tr" indicates trace amounts detected.
From Daley et al., 2010

Table 8. Influence of forage on the fatty acid composition (mg/100 g tissue) of beef longissimus muscle Grass v. concentrate (Adapted from Warren et al. 2003)

Fatty acids	Grass	Concentrate	SED	Significance*
Total	2581	1724	139.3	***
18:2n-6	62	146.9	6.68	***
18:3n-3	32	7.2	1.6	***
20:5n-3	17.7	4.5	1.05	***
22:5n-3	10.8	10.8	1.28	***
22:6n-3	5	1.3	0.3	***
n-6:n-3	1.2	8.9	0.24	***
P:S	0.09	0.24	0.01	***

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

Table 9. Influence of proportion of grass (g/kg DM) in the diet on the fatty acid composition (Adapted from French et al. 2000)

Fatty acids	Grass (g/kg DM)				SED	Significance*
	0	510	770	1000		
Total	3410	4490	4020	4360	650.5	NS
18:2n-6	120.5	105.8	94.4	85.9	6.05	**(linear)
18:3n-3	29.3	35.4	41.1	46	1.78	**(linear)
20:5n-3	4.9	11	9.8	9.4	1.32	*(quadratic)
n-6:n-3	4.15	2.86	2.47	2.33	0.197	**(linear)
P:S	0.09	0.1	0.11	0.13	0.01	**(linear)

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

In a recent review Daley et al. (2010) reported that grass finished cattle are typically lower in total fat as compared to grain-fed contemporaries but there is not consistent difference in total SFA content between these two feeding regimens (Table 6). As shown in the table 6 the content of myristic (C14:0) and palmitic acids (C16:0), considered to be more detrimental to serum cholesterol levels, were often higher in grain-fed beef as compared to grass-fed ones. On the other hand, grass finished meat contain elevated concentrations of stearic acid (C18:0), the only saturated fatty acid that does not seem to have impact on serum cholesterol. Thus, "grass finished beef tends to produce a more favorable SFA composition although little is known of how grass-finished beef would ultimately impact serum cholesterol levels in hyper-cholesterolemic patients as compared to a grain-fed beef" (Daley et al., 2010).

Grain-fed beef produces higher concentrations of MUFAs (which include oleic acid, C18:1 cis-9, the primary MUFA in beef) as compared to grass fed-beef.

Even so, grass-fed beef provides a higher concentration of TVA (C18:1 t11), an important acid for de novo synthesis of CLA (C18:2 c-9, t-11).

Rumen pH may help to explain the apparent differences in CLA content between grain and grass-finished meat products (see Table 7). Microbial biohydrogenation of LA and ALA by an anaerobic rumen bacterium, *Butyrivibrio fibrisolvens,* is a source of CLA origin and is highly dependent on rumen pH. Grain consumption decreases rumen pH, reducing *B. fibrisolven* activity, conversely grass-based diets provide for a more favorable rumen environment for subsequent bacterial synthesis. Indeed, while precursor can be found in both grains and green forages, grass-fed ruminants have been shown to produce 2-3 times more CLA than ruminants fed in confinement on high grain diet, "largely due to a more favorable rumen pH" as Daley et al., 2010. (Rule et al., 2002; French et al., 2000, Duckett et al., 1993; Mandell et al., 1997).

Compared to feeding concentrates, grazing results in higher proportions of n-3 PUFA, particularly C18:3 n-3, higher CLA, increased PUFA/SFA ratio and decreased n-6/n-3 FA ratio in intramuscular fat (French et al., 2000; Poulson et al., 2004; Nuernberg et al., 2005). This is because fresh pasture is a rich source of C18:3 n-3 (Dewhurst et al., 2001 and Boufaied et al., 2003 cited by Steinshamn et al. 2010) and has the ability to synthesize de novo ALA which is the building block of the n-3 series of essential fatty acids; moreover elongation and desaturation of C18:3 n-3 results in the synthesis of EPA and DHA.

The review cited above (Daley et al., 2010, Table 7) shows no significant changes to the overall concentration of n-6 FA between feeding regimens, although grass-fed beef show a higher concentrations of n-3 FA as compared to grain-fed contemporaries, creating a more favorable n-6/n-3 ratio with an overall average of 1.53 and 7.65 for grass-fed and grain-fed, respectively, for all studies reported in this review.

Also Scollan et al., (2006) reported that "feeding fresh grass or grass silage compared to concentrates, rich in 18:3n-3 and 18:2n-6, respectively, results in higher concentrations of n-3 PUFA in muscle lipids, both in the triacylglycerol and phospholipid fractions" (Table 8).

Studies in Ireland showed that both the proportion of grass in the diet (Table 9, French et al., 2000) but also length of time on grass were important in beef fatty acids composition.

In general these responses in both SFA and n-3 PUFA contribute towards beneficial increasing in PUFA/SFA and decreasing n-6/n-3 ratios.

The fatty acid composition of muscle affects its oxidative stability, as the polyunsatured fatty acids in phospholipid are more liable to oxidative breakdown. Oxidation of muscle lipids results in the production of primary and secondary products (hydroperoxides, free radicals, endo peroxides, malondialdehyde, epoxides, alkanes, alcohol and acids) that could be toxic to humans (Ladikos and Lougovois 1990 cited by Cifuni et al., 2004) and may also impair the health of animals (Durand et al., 2005 cited by Scollan et al., 2006). Moreover in muscle tissues they can promote myoglobin oxidation with consequences on colour (see below) and the formation of rancid odours and flavours.

Meat with more PUFA may be more oxidisable, but it is noteworthy that when these PUFA are derived from pasture feeding, they are associated with more antioxidant in the form of a-tocopherol, carotenoids and flavonoids (Wood and Enser, 1997), which stabilize the fatty acids (Gatellier et al., 2005; Richardson et al., 2004) and are also regarded as beneficial, improving the shelf life of meat (Simonne et al., 1996; Daly et al., 1999; Yang et al., 2002),

It is known that not only diet forage proportion (French et al., 2000) but also length of time on pasture (Noci et al., 2005) can affect the composition of fatty acids.

However, little is known about the effect of pasture type, e.g. botanical composition, on meat quality.

Lind *et al*. (2009 cited by Manca 2010) found a small but significant increase in PUFA content in mountain pasture compared to cultivated pasture (3.2% and 2.9%) and

suggests that pre-slaughter fattening on cultivated pastures could alter meat characteristics. Seasonal variation on forage quality, can also influence fatty acid profile of meat as reported by Mazzone et al. (2010) who found a 2-fold higher content in ALA and RA in autumn's lambs muscle compared to winter's lambs, due to the differences in diet through different season. The fact can be explained by a seasonal variation in ewe's milk fatty acid profile that reflect seasonal variation in fatty acid composition of forage (Cabiddu et al., 2005; Nudda et al., 2005) which in Mediterranean area shows a decrease in quality as pasture plants mature from the vegetative to the reproductive stage, as indicated by the decrease of more than 50% in ALA content (Nudda et al., 2003).

It has been found that cow's milk produced on high altitude grassland has higher content of C18:3n-3 than milk produced on lowland pastures; this has been explained by a reduced ruminal biohydrogenation of feed 18:3n-3 (Leiber et al., 2005), likely caused by properties of certain plant species of modular the ruminal biohydrogenation (Collomb et al., 2002 cited by Steinshamn *et al*. 2010; Cabiddu et al., 2010).

Meat from steers grazing semi-natural grassland in UK had a lower proportion of C16:0 and a higher proportion of PUFA than meat from steers grazing improved permanent pasture (Fraser et al., 2009). These studies indicate that there could be a pasture type effect on meat quality.

In Table 10 is reported the fatty acid composition of different forage species (Pulina et al., 2003 and Claphman et al., 2005 cited by Manca 2011).

The effects of pasture on CLA and PUFA n-3 contents in meat depend by the seasonal variation of quality forages, by the species composition of pasture and their fatty acid composition (Dewhurst et al., 2001 cited by Steinshamn et al. 2010); legumes for instance are richer in ALA compared to grasses (Cabiddu et al. 2005; Chiofalo et al. 2010) (Table 11).

Table 10 - Fatty acid composition in different forages (means of 3 harvests) (% of total lipid)

Forages	*C12*	*C14*	*C16*	*C16:1*	*C18*	*C18:1*	*LA*	*ALA*
Triticale	0.05	0.4	4.1	0.69	0.19	0.69	3.76	20.73
Perennial ryegrass	0.05	0.62	6.42	0.75	0.3	1.06	5.99	31
White clover	0.05	0.45	5.66	0.78	0.48	1.17	6.8	21.6
Chicory	0.02	0.41	6.02	0.9	0.23	0.66	7.58	28.83
Borage	0.01	0.3	5.42	0.5	0.58	1.4	5.07	24.8
Plantain	0	0.43	5.21	0.7	0.36	0.47	6.45	21.3
Natural pasture	-	-	12.92	-	1.03	2.05	10.57	60.36

Data from Clapham et al. (2005); Pulina et al., (2003) modified by Manca 2010

Table 11 - Content of LA and ALA in grasses and legumes (% of total fatty acid).

Specie		LA	ALA
Grasses	Lolium rigidum	11.57	62.45
	Lolium multiflorum	13.18	61.33
Legumes	Medicago polymorpha	14.88	63.92
	Hedysarum coronarium	9.04	63.52
	Trifolium subterraneum	14.16	72.3

Data from Cabiddu et al. (2005); Chiofalo et al. (2010) modified by Manca 2010

Generally grass-based systems increased concentrations of ALA and EPA in fat meat compared with indoor feeding system; conversely concentrates, rich in LA, lead to higher concentrations of LA and its longer chain derivatives such as arachidonic acid ARA. It is noteworthy that the main effects of grazing on fatty acid profile, are not removed by a short period of finishing indoor with concentrates. (Nuernberg et al., 2005; Scollan et al., 2006; Leheska et al., 2008; Garcia et al., 2008; Daley et al., 2010).

3.2.1.3 Vitamins

Beef is also a good source of vitamin E. (Table 12).

Table 12 - Vitamin content (for 100 g) of raw meat of different ruminant species.

	Mutton	Lamb	Beef	Veal
Tocopherol - Vit. E (mg)	0.2	0.44	0.63	0.5

Data from Williams (2007)

Vitamin E is a fat-soluble vitamin whose eight different isoforms are known with powerful antioxidant activity; they are all fat soluble and the most active is α-tocopherol.

Antioxidants such as vitamin E protect cells against the effects of free radicals. Free radicals are potentially damaging by-products of metabolism that may contribute to the development of chronic diseases such as cancer and cardiovascular diseases.

Numerous studies have shown that cattle finished on pasture produce higher levels of α-tocopherol in the final meat product than cattle fed high concentrate diets (De la Fuente et al., 2009; Realini et al., 2004; Descalzo et al., 2005; Descalzo et al., 2008).

The concentration of natural α-tocopherol found in grain-fed beef ranged between 0.75 to 2.92 μg/g of muscle, whereas pasture-fed beef ranged from 2.1 to 7.73 μg/g of tissue depending on the type of forage available to the animals (Daley et al., 2010).

Vitamin E acts post-mortem to delay oxidative deterioration of the meat; a process by which myoglobin is converted into brown metmyoglobin, producing a darkened, brown appearance to the meat. (see below).

In a study where grass-fed and grain-fed beef were directly compared, the meat bright red color associated with oxymyoglobin (perceived as good by the consumers) was retained longer in the retail display in the grass-fed group, even thought the grass-fed meat contained a higher concentration of more oxidizable n-3 PUFA. The authors concluded that "the antioxidants in grass probably caused higher tissue levels of vitamin E in grazing animals with benefits of lower lipid oxidation and better color retention despite the greater potential for lipid oxidation" (Yang et al., 2002 cited by Daley et al., 2010).

3.2.1.4 Cholesterol

Cholesterol, a substance present in all body cells, is a steroidal lipid. Feed of animal origin are high in cholesterol and this has implications on human health because elevated cholesterol levels are correlated with increased mortality due to cardiovascular disease (Chizzolini et al., 1999).

But the cholesterol also plays important biological functions as component of cell membranes, which regulates flow and permeability, as precursor of steroid hormones, of vitamin D and of bile salts.

The average contents of cholesterol in some kind of meat are presented in table 13, (Chizzolini et al., 1999).

Table 13. Cholesterol content of some representative types of meat and fat

Type of meat	Cholesterol (mg/100 g)
Beef (muscles)	60
Veal (muscles)	70
Pork (muscles)	65
Mutton (fllet)	70
Chicken (average)	81
Turkey (average)	74
Lamb (intermuscular fat)	75
Beef (intermuscular fat)	99
Pork (intermuscular fat)	93

Modified by Chizzolini et al., 1999

Although variations can be seen among different species, their magnitude is generally low significant and of not real use for dietary reductions of cholesterol intake. Interesting differences, instead have been reported in cholesterol content between muscle types (Wheeler et al., 1987; Lewis et al., 1993).

3.3 Technological quality of beef meat

This aspect of the quality concern the ability of the meat to the conservation and transformation (Monin 1991).

Despite the pH is a chemical characteristic, it is normally included between the technological qualities since its evolution determines the attitude to the conservation and transformation of meat.

The biochemical and structural events that happen in the first 24 h after the animal is slaughtered, that are the basis of the conversion of muscle to meat, influence the pH values. After exsanguinations (or bleeding), the various tissues continue their metabolism in response to specific tissue stimuli. To maintain the temperature and the integrity of the cells the ATP is consumed. Because of bleeding the input of O_2 to the muscle is prevented and the resynthesis of ATP is entrusted to anaerobic glycolysis. Within muscle cells, anaerobic glycolysis proceeds and produces lactic acid as end-product.

The increased acidity causes a loss in water binding ability and causes calcium release that is the trigger for muscle contraction and energy metabolism. As a result, cross-bridges are formed between myosin and actin.

The process of rigor mortis at the beginning is characterized by a decrease in pH and a formation of permanent cross-bridges, called actomyosin, between the actin and myosin filaments. Rigor begins in meat, in normal conditions, when the pH has fallen to an ultimate pH (pHu as measured at 24 h. *post mortem*) of about 5.4–5.7 (Maltin et al., 2003; Hannula and Puolanne, 2004 cited by Savell et al., 2004)

Ultimate pH is dependent on the:

- type of muscle, slow-twitch red muscles (see below), with lower glycogen contents, exhibit higher ultimate pH than fast-twitch white muscles (Geay et al., 2001);
- buffering capacity of muscle, which increases when glycolytic metabolism increases (Monin, 1991)

As Geay et al., (2001) "In anoxia conditions, acidification of muscle is provoked by the conversion of glycogen into lactic acid. Then, pH decreases from 7.0–7.2 to 5.4–5.8. The post-mortem pH fall rate depends on the contraction rate of the myofibres. As a consequence, it strongly varies from one muscle to the other."

During the first phase of rigor, the delay phase, the muscle is still extensible because there is still ATP available to bind with Mg^{2+} , which helps to disconnect the actin/myosin cross-bridges and in turn allows the muscles to relax. Creatine phosphate is depleted during this phase, inhibiting the phosphorylation of ADP into ATP.

This causes a decrease in ATP production, which is the signal of the start of the onset phase of rigor. Because of little ATP available to break down the actin and myosin bonds, muscles cannot relax and therefore become inextensible (Aberle et al., 2001 cited by Savell et al., 2004).

The pH decrease favors meat storage due to the slowering of microflora development and the pH value influence the water holding capacity (WHC i.e. meat ability to retain

inherent water) (Geay et al., 2001); in particular WHC increases with increasing pH due to the effect of pH on spatial organization of muscle myofibrils.

Lean muscle contains approximately 75% water.

The majority of water in muscle is held within the structure of the muscle and muscle cells. More specifically, 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).

Water is a dipolar molecule and it is attracted to charged species like proteins.

True bound water is a very small fraction of the total water in muscle cells, approximately 0.5 g of water per gram of protein, and it is very closely bound to protein.

Another fraction of water that can be found in muscles and in meat is termed entrapped (also referred to as immobilized) water. 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 per se to protein. Entrapped or immobilized water is most affected by the rigor process and the conversion of muscle to meat.

Free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat. (Huff-Lonergan and Lonergan 2005).

As well explained by Huff-Lonergan and Lonergan (2005) during the conversion of muscle to meat, lactic acid builds up in the tissue leading to a reduction in pH of the meat. 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 like 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. The end result of this is a reduction of space within the myofibril.

In parallel to pH decrease, osmotic pressure increases and reaches its maximum value after complete onset of rigor mortis (Ouali, 1990).

3.4 Sensory quality of meat

The sensory quality of meat concern what is perceived by the senses of the consumer (Monin 1991) as tenderness and color.

To better understand the sensory quality of meat is better to describe the structure of the muscle. The meat is the product of complex biochemical changes that take place into the muscle tissue after death of the animal and that determine the transformation of the muscle in meat. Indeed the determinants of meat eating quality are a multifactorial complex which are derived from the highly organized structure of the muscle and the properties of meat are determined at different levels from the molecular to the mechanical ones.

Striated muscle is made up of fiber bundles surrounded by a connective tissue network whose collagen is the main protein. Strands of fibers are grouped together in systems with connective tissue holding the system together. The connective tissue network is designed to combine and transmit the force of contraction to accomplish movement (Figure 1). Other cells, quantitatively less important, such as intramuscular adipocytes, are localised in the connective network.

The connective network has three levels of organisation: epimysium, perimysium and endomysium from the outer part to the inner part of the muscle.

The epimysium is the connective external envelope of the muscle; the perimysium surrounds each myofibre bundle and connects the bundles; the endomysium is a thin layer of the extracellular matrix which surrounds the sarcolemma of each myofibre.

The common characteristics of all collagens is the presence of one or several helical structures stabilized by intra-and inter-molecular bonds.

Muscle fibers are long polynuclear cells containing the contractile proteins (which myosin is the main), the enzymes for the storage and utilization of energy (carbohydrates and lipids) and the proteolytic enzymes involved in the *in vivo* protein metabolism but also in the degradation of proteins during meat ageing (Geay et al, 2001).

Three main myofiber types, which have an influence on meat quality, have been identified according to their contractile and metabolic activities: slow-twitch red oxidative (type I), fast-twitch red oxidative and glycolytic (type IIA) and fast-twitch white glycolytic (type IIB).

This structure of muscle and its changes ante e post-mortem influence the sensory quality of the meat. As Geay et al.,(2001) colour, flavour and texture (juiciness and tenderness) of meat are dependent not only on the structural and metabolic characteristics of muscle at slaughter but also on their modifications during rigor mortis and ageing".

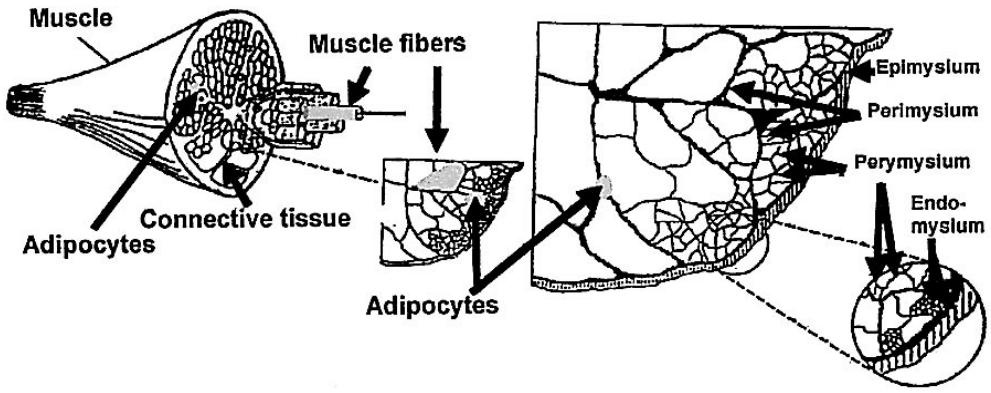

Figure 1. Muscle structure. By Geay et al., 2001

The four main components of sensory quality, according to consumer studies, are tenderness, colour, juciness and flavor; tenderness is the most important (Wood et al., 1999; Koohmaraie 1992; Monson et al., 2005)

3.4.1. Tenderness

Tenderness corresponds to the ease of mastication during meat consumption (Geay et al., 2001) or at the opposite to the force required to bite the meat (Monson et al*.,* 2005).

There are two main components to meat tenderness, a connective tissue component and a myofibrillar (muscle) component.

3.4.1.1. The connective component

About the connective component, not only its content but also the thermal stability of connective tissue, the type and content of intermolecular cross-links, the diameter of the collagen fibres (a major component of connective tissue) and the isoforms of the collagen molecules contribute to meat toughness.

The collagen characteristics that influence the beef texture are determined by:

- Maturity. It is now widely accepted that there is a relationship between maturity and beef tenderness and that, as an animal matures, normally found an increase in toughness (Miller et al. 1983 cited by Muir et al., 1998). This is attributable to the stabilization and cross-linking of collagen molecules, into an insoluble heat resistant form which reduces the amount of collagen that can be solubilized during cooking, resulting in less tender meat.
- Animal growth rate. This is an important factor that play a role in determining collagen characteristics and beef texture. However, despite the numerous studies conducted to evaluate the influence of diet on meat quality and collagen properties, the results are contradictory. While some researchers have found small differences in tenderness and soluble collagen content between animals subjected to different planes of nutrition (French et al., 2001; Mandell et al.,1998), others have reported that high energy diets, which normally enhances growth rate, increase soluble heat-labile collagen due to an accelerated rate of connective tissue protein turnover (Aberle et al.,1981; Fishell et al., 1985; Wu et al., 1981) that positively affect beef texture. For this reason the modification of animal growth rates through the diet

has been a method used by researchers to improve beef tenderness (Fishell et al., 1985; Therkildsen et al., 2008).

3.4.1.2. The myofibrillar component

The myofibrillar component of tenderness appears to be a more important determinant of meat tenderness than connective tissue characteristics of the meat pre-slaughter. During rigor development, occurs the formation of a non-reversible actomyosin complex (Lawrie, 1974) and sarcomere shortening that determines the toughening of meat; indeed some research suggested that there is a strong negative relationship between sarcomere length and meat toughness. This process usually occurs within the first 24 h post-mortem (Koohmaraie *et al,*. 1996; Wheeler and Koohmaraie, 1999).

The tenderization process, due to the enzymatic activity during cooler storage, reduces the toughening effect and improves meat tenderness (Ouali, 1992). Post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins is responsible for tenderization.

Although some authors consider that meat tenderization during ageing depends on the dissociation of myofibrillar structures induced by the increase in cytosolic calcium after slaughter (Takahashi 1999 cited by Geay et al., 2001), this process essentially results from the endogenous proteolytic enzymes that play a major role in ageing within muscle tissue (Ouali, 1992).

Current evidence suggests that proteolysis of key myofibrillar and associated proteins (e.g. desmin, vinculin, titin, nebulin, vinculin, dystrophin, laminin and fibronectin) is the cause of meat tenderization. The function of these proteins is to maintain the structural integrity of myofibrils hence “the degradation of these proteins would cause weakening of myofibrils and, thus, tenderization.” (Koohmaraie 1994)

There are several endogenous proteolytic system in muscle including the cathepsin-lysosomal system, the calpain-calpastatin system, the ATP-dependent ubiquitin-proteasome system and the matrix metalloproteinases.

Cathepins and calpains are the two main enzymes involved in tenderization. Some evidences suggests that the calpain system is maybe the more important proteolytic enzyme in the post-mortem tenderizing process, especially in red meat (Koohmaraie, 1992; 0'Halloran et al. 1997) and studies over the last 20 years have suggested that tenderization is primarily a result of calpain-mediated degradation of myiofibrillar and cytoskeletal proteins (Koohmaraie, 1992 and 1996). The calpain system comprises 2 ubiquitously-expressed isoenzymes μ- and m-calpain (activated by Ca^{2+}) and the calpain-specific inhibitor calpastatin.

These enzymes specifically degrade "inter- and intra-myofibrillar cytoskeleton proteins such as desmin, nebulin and partly connectin" (Geay *et al*., 2001).

Indeed, it has been shown that the enzyme/inhibitor ratio influences the tenderization rate (Ouali and Talmant, 1990 cited by Geay *et al*., 2001). The μ-calpain/calpastatin ratio, equal to 1/4, 1/2.5 and 1/1.5 for beef, lamb and pork respectively (Koomaraie *et al*., 1991) can partly explain the lower tenderization rate in beef. Most recently Koohmaraie (1996) argued that this ratio is 1/2, 1/1.25 and 1/0.75 respectively.

Because of the pH dependency of the two enzyme systems involved in post-mortem tenderisation, the neutral calpains and the acidic lysosomal cathepsins, it would be expected that the pH early *post mortem* should influence the rate and extent of the tenderisation process especially in relation to proteolysis of the myofibril component of toughness. (O'Halloran *et al.,*1997).

The rate of tenderization varies with:

- temperature (Polidori et al, 2009); it becomes higher as the temperature rises and does not proceed in frozen state but it will continue during thawing. The range of temperature recommended is at 1 to 4 °C. This improves the tenderness of meat after cooking and also helps to slow down some bacteria growth ensuring the microbiological safety for consumers. In order to ensure effective ageing, the rapid chilling of carcasses before the completion of rigor mortis should be

prevented due to the fact that it causes cold shortening as well as leading to "frozen pre-rigor" and subsequently extremely tough meat. Locker and Hagyard (1963cited by Savell et al., 2005) defined cold shortening as a rapid decline in muscle temperature to less than 14ºC before the onset phase of rigor mortis. When carcasses are cooled quickly, they could be affected by cold-induced shortening. Fat thickness can play a significant role in the reduction of cold shortening during the chilling processes of beef. Increased thicknesses of subcutaneous fat can improve tenderness by allowing the carcass to chill more slowly and to increase enzyme activity (Savell et al., 2005). The rate and extent of post-mortem proteolysis is temperature (and pH) dependent (Koohmaraie 1992) and there is clear evidence that the rate at which carcasses cool after slaughter can influence meat tenderness in both cattle (Lochner *et al*. 1980 cited by Muir et al., 1998) and sheep (Smith et al. 1976 cited by Muir et al., 1998). In the literature there is a clear positive correlation between meat tenderness and carcass fatness (Bowling et al.1977). Bowling et al. (1977) assert that differences in cooling rate were only partly responsible for the difference in tenderness shown in grain- and forage-finished beef and Muir et al., 1998 assert that chilling rate is only partly responsible for the relationship between fatness and meat tenderness in cattle carcasses.

- Species; different species consisting of variable muscle composition differ in their rate of tenderization (Monson et al., 2004). Indeed the animals with high muscle development have greater number of muscle cells with a predominance of fast contracting white fibers, which have faster degradation and higher ageing rates. (Ouali, 1990 cited by Monson et al., 2004). The differences in tenderness between breeds could be due to the quantity, solubility and space organization of the collagen, fatness and calpain/calpastatin activity (Monson et al., 2005).
- Muscle: the large biological diversity of skeletal muscle can explain the high inconsistency in meat tenderness among different muscles. In fact every muscle is different structurally and metabolically; as a result large differences between muscles in the rate and the degree of post-mortem texture progression are expected. Some

researchers have suggested that different muscles may react differently upon the same stimulus (i.e. changes in growth rate) which may complicate the search for a method to increase tenderness in the entire carcass (Therkildsen et al., 2008).

Tenderness will improve with time of post-mortem ageing; according to Koohmaraie (1996) "to maximize the benefits of post-mortem storage on meat tenderness, beef should be stored for 10-14 days, lamb for 7-10 d, and pork for 5 d.

Meat ageing rate is also positively correlated to ATPase activity, which defines the contraction rate of muscle (Ouali 1990). Indeed, fast twitch glycolytic muscles have a faster ageing rate than slow-twitch oxidat*ive r*ed muscles. The rate of pH fall and pHu have important consequences for eating quality but the precise relationship between tenderness and pH is complex and not fully understood (Maltin et al., 2003). However meat with a high pHu (often >6.5) (darking cutting or dark, firm and dry DFD meat) occurs when animals have lower than normal glycogen levels at slaughter and as result lactate production is low. Glycogen content depends on the nutritional status of the animal, on the level of physical exercise and stress conditions of the animal during the period of time between the farm and slaughter (Geay et al., 2001). Some study shows that DFD meat may be more tender than normal (Dransfield 1981 cited by Maltin et al., 2003) because the reduction of glycolytic substrate availability causes more rapid ATP depletion and early rigor, reducing susceptibility to cold shortening and allows prolonged activity of protease. Other studies report that DFD beef is less tender than normal beef (Wulf et al., 2002 cited by Maltin et al., 2003). In contrast meat with low pHu is often of poorer eating quality; the enzymes responsible for post-mortem tenderization are inhibited by the acidification, and low pH is also associated with increased drip loss resulting in meat with lower overall acceptability (Maltin et al., 2003).

The activities of the proteinases are also determined by the the amount of active proteinases and inhibitors present (O'Halloran et al., 1997).

The concentrations of proteolytic enzymes is linked to growth rate: cattle grown rapidly prior to slaughter have been shown to produce more tender meat than their slower growing counterparts (Aberle et al. 1981;Fishell et al. 1985).

This has been attributed to increased protein turnover in rapidly growing cattle, resulting in higher concentrations of proteolytic enzymes in the carcass tissues at slaughter. Supporting evidence was provided by Shackelford *et al*. (1994) that measured the activity of the enzyme calpastatin, the endogenous inhibitor of calpains, and found that calpastatin activity was negatively associated with live weight gain.

Recent studies in cattle using growth rates at or above those used commercially showed that preslaughter growth rates do not affect tenderness (Moloney et al., 2001 and Sinclair et al., 2001 cited by Maltin et al., 2003). In contrast, where more extreme strategies are used to manipulate protein turnover, clear association between growth rates, turnover rates and the activity of the proteolytic systems can be seen. Although Oddy et al. (2001 cited by Maltin et al., 2003) suggest that nutritional history may impact on tenderness, the types of growth pattern described include nutritional restrictions of varying severity and duration. Commonly cattle in temperate regions are non subjected to such regimens of compromise and the main body of evidence concurs that normal variation in growth patterns do not account for the observed variation in eating quality. (Maltin et al., 2003). Consequently the comment made by Moloney *et al*., 2001, that "cattle management strategies during finishing, including feeding pattern and ration composition, generally have little impact on tenderness" seems correct.

Meat texture may be also influenced by various post-slaughter factors including protein denaturation, tenderstretch, hip suspension, electrical stimulation, temperature control during fresh meat storage, tenderization by natural processing (i.e. conditioning) or unnatural processing (i.e. addition of calcium salt), protein oxidation during storage and packaging methods. These factors will not be discussed here.

3.4.2. Meat colour

The first impression that consumers have of any meat product is its color and a possible

discoloration is seen as an indicator of freshness and wholesomeness.

Essentially, the color pigment of the muscle tissue is myoglobin whilst haemoglobin is the color pigment of blood. The level of haemoglobin in meat, depending on the degree of bleeding, is determined by the slaughtering process. According to Feiner (2006), the colour of raw meat depends for approximately 90 to 95% by myoglobin and around 2-5% by haemoglobin and other proteins that contribute in a small amount to the color of meat.

Hence, myoglobin is the principal contributor to meat color, especially in lean beef.

Myoglobin is a watersoluble protein bound to the external membrane of the mitochondria and sarcoplasmic reticulum, containing 8 α-helices (A–H) linked by short non-helical sections, that stores oxygen for aerobic metabolism in the muscle. Myoglobin also contains a prosthetic group, a heme ring with a centrally located iron atom that can form six bonds: four covalently attached to four nitrogen of the porphyrin ring structure, one attached to a globin protein and the 6th bond orbital is open, for formation of complexes with several compounds.

Oxidation state of iron and compounds (oxygen, water or nitric oxide) occupying the 6th orbital determine color of meat. Other heme proteins such as hemoglobin and cytochrome C may also play a role in beef, lamb, pork, and poultry meat color (Mancini and Hunt, 2005).

The myoglobin concentrations differs:

- Among animal species. For example, beef has considerably more myoglobin than pork or lamb, thus giving it a more intense color.
- Among muscles that differ greatly in activity and then in their oxygen demand. A greater myoglobin concentration yields a more intense color as in oxidative red muscle. (Geay et al., 2001)
- According to age: the level of myoglobin in muscles increases with the age of animal (Monin, 1991).

Immediately after cutting, meat color is deep purplish-red quite dark. As oxygen from the air comes into contact with the exposed meat surfaces it is absorbed and binds to the sixth

ligand (oxygenation); myoglobin is then oxygenated giving rise to the pigment called oxymyoglobin.

This pigment gives beef its bright cherry red color. It is the color that consumers associate with freshness. Oxygen must be available at a sufficient concentration in order to combine with the myoglobin to form oxymyoglobin.

Low temperature supports the formation of oxymyoglobin due to the enhancement of oxygen solubility (Mancini and Hunt, 2005; Feiner, 2006). Also, if temperature values are next to zero, enzymatic activity is minimal and oxygen penetration is favored. When the temperature rises the activity of respiratory enzymes increases and oxygen diffuses to a lesser extent in the muscle.

The change from myoglobin to oxymyoglobin and vice versa usually occurs quite readily. The oxygen partial pressure, pH and competition for oxygen by other respiratory processes also contribute to the depth of oxygen penetration and thickness of the oxymyoglobin layer (Mancini and Hunt, 2005).

Myoglobin and oxymyoglobin have the capacity to lose an electron (oxidation) giving rise to the metmyoglobin, produced when the central iron atom is oxidized to Fe^{3+} and H_20 is present on the 6th ligand and which turns the pigment to a brown color.

Thus, myoglobin can change from a dark purple color to a bright red color simply from oxygenation or to a brown color by losing electrons.

Similar to oxymyoglobin, metmyoglobin formation also depends on oxygen partial pressure, temperature, pH and microbial growth. Moreover the contamination of the meat would cause a chemical reaction resulting in the formation of the brown pigment metmyoglobin.

The reaction that produces the brown meat metmyoglobin occurs quite easily, but the reverse is more difficult.

Metmyoglobin is associated with chilled meat that has been stored too long (enzyme activity available to reduce metmyoglobin to myoglobin has been exhausted), but also appears

when partial pressure of oxygen is low. Oxygen partial pressure can be reduced when aerobic bacteria use the oxygen and it is unavailable to react with the myoglobin.

In raw meat there is a dynamic cycle and the pigments myoglobin, oxymyoglobin and metmyoglobin can be changed from one to the other, depending on the conditions at which the meat is stored. After cooking, a brown pigment called denatured metmyoglobin is formed, which normally cannot be changed to form another pigment.

Purplish-red or purplish- pink color typically associated with vacuum packaged product and muscle immediately after cutting arise from the deoxymyoglobin (heme iron is ferrous Fe2+ and no ligand at the 6th coordination site). To produce deoxymyoglobin a very low oxygen tension (<1.4 mm Hg; Brooks, 1935 cited by Mancini and Hunt, 2005) is required.

The rate and extent that muscle pH declines postmortem are both variable and have a great impact on the color of meat and meat products. The normal pH decline in muscles is from approximately 7.0-7.2 down to near pH 5.5-5.7 (pH ultimate, pHu) over about 24 hrs.

If the pH declines to the normal pH of 5.5-5.7 within 45 min or less, the muscle will appear very pale and soft (PSE Pale, Soft, Exudative). A very low ultimate pH (<5.4) will also result in a paler color.

If the pH does not drop much postmortem, the meat will be dark with a dull, dry surface (DFD Dark, Firm, Dry). As the ultimate pH increases, the meat gradually becomes darker. This darkening of color becomes more evident when the muscle pH exceeds 5.7.

The color changes observed with PSE and DFD meat however are mostly due to structural changes in muscle.

The pH affects the charge on the muscle proteins and thereafter alters the spacing between the fibers of the meat, and the change in structure affects how light is reflected and absorbed, and thus affects the visual appearance.

Indeed the muscle structure absorbs or reflects light and allows oxygen to penetrate influencing meat color (Geay et al 2001) i.e. higher light-scattering ability due to thin myofibrils results in pale color (Feiner, 2006).

Beef color is influenced by meat pH which in turn is related to stress prior to slaughter (Pearson,1966). High ultimate pH can affect the color of fresh meat because it affects enzyme activity and the rate of oxygenation. The high ultimate pH also determines a dry surface that hampers the penetration of oxygen into the meat and thus the oxygenation process.

3.4.2.1. Nutritional factors affecting meat color

There are several information in literature on the effect of feed type on beef meat color. Craig et al. (1959) have compared color reflectance and muscle pigment concentrations of pasture- and grain-fed steers and have found that animals with the greatest amount of fat within muscle (i.e., marbling) showed the brightest colored meat (highest reflectance values).

The authors also concluded that the differences in color of lean meat were caused by varying amounts of fat and moisture rather than a difference in the quantity of pigment. (Muir et al., 1998)

Muir et al. (1998) reviewed that meat colors were "brighter" in the more rapidly growing feedlot animals. Therefore, it is to be expected that younger animals will have lighter, brighter meat.

Recent work has attributed the effects of diet on muscle color to either altered glycogen storage, chilling rate, or antioxidant accumulation (Table 14) (Mancini and Hunt, 2005).

Table 14. Summary of research evaluating beef nutritional factors affecting meat color

Reference	Results/conclusions
French et al. (2000)	Grazing increases the yellowness of subcutaneous fat due to a greater amount of b-carotene in pasture than in concentrates. No dietary effects on longissimus color were reported
French et al. (2001)	When comparing finishing cattle on grass and concentrate, no diet effects on longissimus color will occur. Significant correlations between b* and carcass fat score (0.29) were noted
Vestergaard et al. (2000)	Forage-based, restricted diets promote oxidative, rather than anaerobic muscle metabolism. This would limit glycogen storage and result in a darker color compared with ad libitum concentrate diets
Lynch et al. (2002)	a-tocopherol levels in adipose tissue are higher in over-wintered than pastured heifers. This increases lipid stability, which could improve longissimus color life
Muramoto et al. (2003) cited by Mancini and Hunt	The yellowness of semimembranosus and longissimus muscles from Japanese black cattle are not affected by dietary b-carotene supplementation prior to slaughter (7500 mg/day for 28 days)
O'Sullivan et al. (2003c) cited by Mancini and Hunt	Although diet (high herbage versus ad libitum concentrate) has no effect on the color of over-wrapped longissimus steaks, herbage diets improve the color stability of steaks packaged in high-ox MAP
Baublits et al. (2004)	Supplementing forage-fed cattle with soyhulls improves muscle color without affecting fat color. Frame size (small, medium, and large) will have little influence on muscle or fat color
Bruce et al. (2004)	Compared with pastured steers, muscle from grain-finished steers is less dark and more red, which can be attributed to subcutaneous fat and slower postmortem chilling
Realini et al. (2004)	During a 21-day display, longissimus L*a*b* was greater for cattle finished on pasture versus finishing on concentrate. Adding vitamin C to ground beef improves color stability during display

By Mancini and Hunt, 2005

Changes in muscle lightness and yellowness were attributed to dietary effects on pre harvest glycogen and marbling levels. (Abril et al., 2001).

Muscle from pastured steers was darker than grain finished steers due to the dietary effects: more subcutaneous fat and slower postmortem chilling, which when combined with lower muscle pH should increase protein denaturation in grain finished animals relative to pasture finished animals (Bruce et al., 2004).

The protein denaturation would increase beef lightness L*, redness a* and yellowness b*

because muscle proteins degraded decrease their water holding capacity, thus increasing reflectance and cooking loss (Warriss and Brown, 1987 cited by Bruce et al., 2004).

Also housing system may affect beef color through changes in physical activity, which could influence muscle fiber type and metabolism.

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Objectives

The overall objective of the present thesis was to study the performances *in vitam*, the meat quality and the economic suitability of young bulls belonging to the Sardinian cattle breed fed at pasture in order to improve the knowledge on the use of pasture in finishing beef cattle.

Considering that the grazing areas in Sardinia exceeds 40% of land area the use of pasture (alone or supplemented with concentrate) in the fattening of animals could represent a chance for Sardinian beef cattle livestock system.

The specific experimental contribution were:

1. First experimental contribution: Role of pasture in the performance of Sarda young bulls: Live weight gain, intake of nutrients and dressing percentage

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2. Second experimental contribution: Role of pasture in the performance of Sarda young bulls: Meat quality

3. Third experimental contribution: Performance of Sardo-Bruna Beef with diurnal grazing

Chapter 4

Role of pasture in the performance of Sarda young bulls: live weight gain, intake of nutrients and dressing percentage

4.1 Introduction

The cattle livestock system in Sardinia, with the exception of the lowland areas, is based on suckler-cows system: the cattle normally grazed pastures of medium-low nutritive value, the suckled-calves follows their mothers at pasture until the weaning at about 6-7 months old, when they are brought mostly to the fatness centers in the Pianura Padania or, rarely, finished in the farm and afterwards slaughtered. Usually farmers prefer to sell calves at weaning because of the high costs of local fattening (Rassu et al., 2011).

This is one of the reason why Sardinia has a self-supply rate equal to only 48% of its beef meat consumption (Rassu et al., 2011).

The possibility of increasing the self-provision of beef meat is linked to the decrease of feeding costs of fattening through, for instance the use of grazing.

The pasture-based system has several positive aspects:

- The grazing could help to reduce feeding costs.
- The use of grazing can characterize the meat produced in Sardinia allowing to distinguish it on the market.
- The satisfaction of the physiological and ethological needs of animals, met by the grazing systems, is a prerequisite for obtaining quality productions.
- Research suggests that grass-only diet can significantly enhance the fatty acid composition so beneficial to human health and improve the overall antioxidant content of beef (Daley et al., 2010).
- Specially in hilly and mountainous areas the use of pastures contributes to the stability of the soil by reducing erosion and provides sustainable livestock production from an economic and environmental point of view (Braghieri et al., 2007).

- The grazing plays an active role in limiting fuel biomass helping to prevent fire danger (Franca et al., 2012).

Finally pasture-based systems are actually considered to be more environmentally friendly, provide animal welfare, and are socially more acceptable than more intensive systems. It is also generally accepted that grazing is an essential tool with which to achieve nature conservation objectives in grassland. (Tallowin et al. 2005).

Also the biodiversity of these habitats is influenced by grazing; in some cases a moderate stocking rate has also been shown to increase biodiversity (Adler et al., 2001 cited by Orr et al., 2012). In general terms the stocking intensity of the grazing livestock along with livestock breed (Rook et al., 2004), is "a key management variable influencing the structure and the composition of pastures" (Dumont et al., 2007).

The performance of ruminant animals on pasture are largely determined by their genetic capability and by digestible nutrient intake (Lippke, 2002). Quantifying herbage intake is necessary for the estimation of nutrient consumption by ruminant animals (Burns et al., 1994; Moore, 1996; Reeves et al., 1996), but "it is inherently difficult to quantify forage intake in grazing systems" (Macoon et al., 2003). Indeed, as Smit et al. (2005), "limited herbage intake is one the main constraints for ruminant production but the measurement of Dry Matter Intake (DMI) during grazing is still not very accurate"

Herbage intake could be accurately estimated with the n-alkane method (Dove and Mayes, 1991). The n-alkanes are long-chain (C_{25} to C_{35}) hydrocarbons present in the cuticular wax of plants that in combination with dosed alkanes, can be used as fecal markers to accurately estimate intake in grazing animals (Dove and Mayes, 1991; Berry et al., 2000; Lippke, 2002; Molina et al., 2004).

The Sarda breed is a small-frame cow well adapted to Sardinia environment and able to exploit through grazing the marginal areas of the Island. These cattle are particularly hardy and may provide a viable option for areas of Sardinia of poor nutritive value. Furthermore, keeping in mind that in situations where the herbage of pastures is very heterogeneous (Malossini et al., 1995) selective grazing is probably essential for animals to meet their nutritional requirements. Aimed at improving our knowledge on performance of Sarda cattle

and assessing whether diet based on pasture can be a valid chance for Sardinian cattle livestock system, a study has been carried out to evaluate the effect of different feeding systems (pasture- vs hay-concentrate- based diets) on nutrient intake and growth of Sarda young bulls.

4.2 Materials and methods

4.2.1 Experimental site and experiment duration

The present study was conducted during 2010 (19/05 – 19/07) at the experimental farm of the Department of Animal Production Research (Agricultural Research Agency of Sardinia, AGRIS Sardegna) located in Foresta Burgos, in the centre of Sardinia (latitude 40°25'N, longitude 8°55'E, altitude 850 m above sea level).

The site has a Mediterranean climate, characterized by minimum and maximum mean temperatures of 1.7 °C (January) and 28.0 °C (July), respectively; the average annual precipitation is 905 mm, mostly from November to March.

4.2.2 Animals

The experiment was performed with 28 Sarda young bulls divided into 4 groups (7 heads each) homogeneous for live weight (LW 288.7±29.0 kg mean ± s.d..), Body Condition Score BCS (2.69±0.18) and age (355±25 days). One group was fed 24 h at pasture in a 7-ha paddock (group PAS) and did not receive any supplementation, the other three groups (FC groups) were kept in barns and fed daily with natural pasture hay (*ad libitum*) and concentrate: 2.5 kg/head (group FC1), 3.3 kg/head (group FC2) and *ad libitum* (group FC3).

The aim of the 3 levels of intake of the hay-concentrate diets was to produce a response relationship to the level of energy intake per kg of Live Weight (NELW) in order to compare the *infra vitam* performance and meat quality (see Chapter 6) of the animals fed with hay and concentrate with those of the animal fed at pasture, at an equal level of NELW.

Prior to allocation to treatments the animals were treated for internal and external parasites.

All animals were slaughtered at the fixed age (416±25 days) in an authorized slaughterhouse according to EU legislation.

The experiment was conducted in accordance with the Italian guidelines on animal welfare (DL No. 116, 27/01/1992).

4.2.3 Pasture and feed

The natural pasture used in the present study was a low hilly area of 7 ha with a large number of trees in particular *Quercus pubescens* L.. On the basis of the orographic characteristic of the pasture area, three subplots (microhabitat) were identified: Low Hill (LH), Upper Hill (UH) and Underwood (UW). The pasture did not receive any fertilizer and the animals of PAS group had free access to the different subplots.

The feed used in FC groups were meadow hay and a commercial concentrate.

4.2.4 Measurements

During the experiment, a census of all plant families and species present in the pasture and selected by the animals (see below herbage intake estimation) was realized.

On 2 occasions, at the beginning of the experiment and during the herbage intake estimation period (see below herbage intake estimation), for each subplot identified in the pasture, dry matter herbage biomass offered (HO) as well as chemical composition of herbage on offer were determined. HO was measured by clipping at ground level 6 samples (1m × 0.5m) in each subplot. The samples divided per plant families were weighted and dried at 60 °C till constant weight and analyzed for chemical composition.

Animal selectivity coefficients of the most representative chemical parameters were then calculated as ratio between their proportion in herbage selected (HS) by the animals (see below herbage intake estimation) and that offered (Estermann *et al*. 2001).

In groups FC1, FC2, FC3, dry matter intake (DMI) and *in vivo* DM digestibility (DMD, acid insoluble ash marker method) of hay-concentrate diets, as group average, were detected.

The digestibility in the acid insoluble ash (AIA) method is calculated from the relation between the nutrients and the indicator substance in the feed and in the faeces, by the following equation:

$$DMD\ (Dry\ Matter\ Digestibility) = 100 - (100\frac{\%indicator\ in\ feed\ DM}{\%\ indicator\ in\ faecal\ DM}) \qquad \text{eq.1}$$

(Khan et al., 2003).

The concentrate offered to the animals were sampled every 20 days whereas for the hay a sample of each round bale used was collected.

In one occasion during the experiment, herbage dry matter intake, faecal output and *in vivo* digestibility was estimated in PAS group by the n-alkane method (Dove and Mayes, 1991).

For this purpose during 13 days (from 07-06-2010 to 19-06-10) the animals were orally dosed every morning (at 8 a.m.) with known amount of C_{32} and C_{36} through hand-manufactured pellets, made of paper strip embedded with synthetic n-alkanes as external markers according to Mayes et al. (1986). Pellets contained 475 mg and 461 mg of C_{32} and C_{36} respectively. C_{36} was used to determine fecal output, since it has a relatively high fecal recovery of approximately 0.95 (Mayes et al., 1995). At the 9th day of alkane dosing, the collection of faeces started. The faeces were grab sampled in the morning over 5 days (Malossini et al., 1996; Berry et al., 2000; Estermann et al., 2001). The faeces grab-samples were pooled for each animal over the measurement period as suggested by Vulich and Hanrahn (1995).

During the same 5 days hand-plucked herbage samples were collected on 3 alternate days. Herbage sampling was done try to mimicking animal behavior (Berry *et al*. 2002) in order to obtain herbage samples which ideally reflect the herbage consumed by the animals. This fact is important because plant species and parts differ not only in nutrient but also in odd chain alkane concentrations (Dove and Mayes 1996; Mayer et al., 2003).

For that reason during the herbage sampling sessions the selection of the plant was based on the following criteria as suggested by Berry et al (2002):

- to select plant species and plant parts by picking close to animals or observing plants hanging from their mouths;
- to pick when cattle moved to a different plant community;
- to circulate between group members to minimize any influence of individual differences;
- to simulate actual bite size by tearing similarly at the sward and adapting the size of the sample to the intensity of feeding;
- to note the sheared plants directly after animals has eaten.

Every three weeks live body weight and BCS were recorded in all animals in the morning at about 08:00 h. Average daily gain (ADG) was then calculated as the coefficient of the linear regression of live weight on time.

The body weight at slaughtering, carcass weight, cold carcass weight (CCW, body weight minus blood, skin, viscera, feet, tail) after 24 h of storage at 4 °C and the dressing percentages were measured.

4.2.5 Chemical analysis

Feedstuff samples (concentrate, hay, and hand-plucked pasture herbage), were immediately frozen at −20 °C, until freeze-dried then ground through a 1-mm screen and analysed to determine chemical composition. Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ether extracts (EE) were determined (AOAC, 1990).The faeces of hay-concentrate fed animals together with hay and concentrate used in the experiment were analyzed in duplicate for acid insoluble ash (AIA) content by ashing 5 g at 450°C overnight, boiling in 100 ml of 2 ***N*** HCl for 5 min. filtering, and re-ashing samples at 450°C overnight (Van Keulen and Young, 1977).

The hand-plucked herbage samples (namely as selected herbage) and the grab samples were analysed for the alkane pattern. Alkanes were analysed by direct saponification with some minor variations to the method outlined by Dove (1992). We used 0.5 g of freeze-dried

faeces and 1 g of either concentrate or oven-dried forage with a replicate extraction for each sample. Quantification of the alkanes was performed using a GC Varian 3400 equipped with a flame ionisation detector (FID).

In this procedure duplicate samples are extracted using direct saponification and the alkane content of the extracts is determined by gas chromatography using C_{34} as the internal standard (Mayes et al., 1986)

4.2.6 Calculations

On the basis of alkane content of herbage and faeces samples corrected by their recovery rates (Berry et al., 2000) herbage DM intake (HI), faecal output (FO) and in vivo DM digestibility (DMD) were then calculated using the following formulae given by Mayes et al. (1986):

$$HI\left(\frac{kg}{day}DM\right) = \left[\left(\frac{Fi}{Fj}\right) X\, Dj\right] / \left[\left(Hi - \left(\frac{Fi}{Fj}\right) X\, Hj\right]\right. \quad \text{eq. 2}$$

$$FO\ \left(\frac{kg}{dayDM}\right) = [(Hi\, X\, Hj) + Dj/Fj] \quad \text{eq. 3}$$

$$DMD\ (\%) = (HI - FO)/HI \quad \text{eq. 4}$$

where Fi and Hi are the respective concentrations of natural odd-chain alkane (mg/kg DM) in faeces and herbage, Fj and Hj are the corresponding concentrations (mg/kg DM) of C_{32}, and Dj is the amount of even alkane dosed.

The energy content (EN, Mcal/kg DM) of the feed used in the experiment was calculated using the equations proposed by Van Soest (Licitra et al., 1997; Van Soest 1994; Van Soest and Fox 1992). The energy value of food is estimated from their levels of TDNm (Total Digestible Nutrient at maintenance) and of NDF (Neutral Detergent Fibre).

$$NEl3m\ \left(\frac{Mcal}{kg}DM\right) = 0.01TDNm[2.86 - (35.5/(100 - NDF))] \quad \text{eq.5}$$

Where NEl_{3m} is expressed as net energy for lactation at 3 times maintenance level, TDNm and NDF as % of DM (Cannas et al., 2002).

Since the animals in FC groups were fed at different nutrition level, the NEl_{3m} has to be corrected with Mertens equation (eq. 2.2) (Mertens 1983 cited by Cannas et al., 2002),

$$D\ (\%) = 0.033 + 0.132NDF - 0.033TDNm \quad \text{eq. 6}$$

This equation allows to estimate the discount, that represents the percentage of which NEl3m should be lowered or raised for each difference in nutrition level (Cannas et al., 2001).

The energy intake as Mcal ENl kg Live Weight^{-1} (NELW) and Feed Conversion Ratio (FCR) were then calculated.

4.2.7 Statistical analysis

Data of botanical and chemical composition of herbage on offer were analyzed with GLM procedure of SAS (1989) using subplot, period and their interaction as fixed effect. Data of chemical composition of herbage on offer and selected were analyzed with GLM procedure of SAS using type of herbage as fixed effect.

Data of live weight, average daily gain (ADG), BCS, carcass weight, cold carcass weight and dressing percentage were analyzed with GLM procedure of SAS (1989) using a mono-factorial model with diet as fixed effect.

In order to compare the data between PAS and FC animals, the performance *infra vitam* and dressing percentage data were adjusted to an equal energy intake. The procedure used was as follows:

1. A least squares linear regression relationship between ADG and NELW was produced for the animals on the three concentrate treatments. Tests were also made for curvilinear relationships, but these proved non significant, and so the linear relationships were used.
2. From this the NELW of the hay-concentrate diet required to sustain a carcass gain equivalent to that obtained for the grazing treatment was estimated.
3. By means of a correlation matrix were identified the parameters correlated with NELW.
4. Further relationships were developed between each of the parameters and NELW for the three concentrate treatments.
5. From these, values for the various parameters were predicted for the concentrate-fed animals at the estimated NELW from (2) above.

6. Differences between these predicted values for the concentrate-fed animals and the actual values for the pastured animals were then tested for statistical significance using a paired *t*-test.

Comparison between the pasture and concentrate treatments using the adjusted values gave a much stronger statistical comparison than comparison of the pasture treatment with individual concentrate treatments. (Steen et al., 2003).

P-values < 0.05 were considered significant unless indicated otherwise

4.3 Results and discussions

Inside the experimental pasture a total of 143 plant species belonging to 35 families were recorded. The most represented families were *Fabaceae* (19.6%), *Asteraceae* (16%) and *Poaceae* (12.6%).

At the beginning of the grazing period, HO was not different between the three subplots (4,8 t DM ha^{-1} ±1.9, means ± standard error, Table. 1). The herbage DM allowance, expressed as kg of DM per kg live weight was then equal to 7 (kg DM LW^{-1}) that could be considered high as found also by Machado et al. (2006) in similar condition.

After one month of cattle grazing the HO resulted higher in LH and UH whereas decreased in UW. A partial explanation may be that the animals have attended more the UW subplot, the most shaded areas. The quality of the herbage offered at pasture was higher in UW (higher CP and EE) than in LH and UH (Table 1).

A sharp decline of the quality on offer was observed in UH. Both LH and UW still offered a medium quality pasture with about 10% of CP . (Table 1)

The comparison between chemical composition of herbage selected and HO shows that animals choose a higher quality diet than that offered (Table 2). This is further highlighted by the coefficient of selectivity which shows that animals selected a diet with higher dry matter, CP and EE and less fiber component, except for ADL (Table 2).

Nevertheless the herbage selected by the animals showed a medium-low quality (CP content 12.6%, table 2), as usual in Mediterranean natural pasture (Molle et al., 1998; Molle et al., 2002), but still adequate to meet the requirements of this kind of animals (NRC 1996)

Animals selected in their diet 34 different species (24% of the total census species) belonging to 14 families, among which *Fabaceae* (35.3%), *Asteraceae* (11.8%), *Poaceae, Umbelliferae* and *Rosaceae* (8.8% each) were the most represented. The animals have selected legumes, probably because of their higher quality (10.6 vs 8.2 CP; 60.3 vs 67.8 NDF, for legumes and grasses respectively, data not given in table), and, as a consequence of the higher ADL content of legumes respect to grasses (7.8 vs 5.2 %), the coefficient of selectivity for ADL resulted higher than 1.

These results are not in agreement with Estermann et al. (2001) who found either in dairy cows or in beef cows grazing alpine pasture a higher selection for grasses than legumes.

Cattle are able to select for feed of a high digestibility and energy content (Christen et al., 1996), which might partly explain the preference for alpine grasses observed, which can be assumed to have lower contents of indigestible fibre compared to legumes and herbs (Estermann et al., 2001), contrarily to the Sardinia natural pastures, where legumes have usually an higher quality than grasses.

However although the nutrient selection to cover energy requirements is the most important factor influencing ruminant foraging strategy, in some cases forage nitrogen content seems to be an important factor (Newman et al., 1994) and this could explain, at least in part, our results.

Table 1. Herbage on offer (HO, t DM ha^{-1}) and its nutrient content (g per kg DM) at the beginning (May) of the grazing period and one month after (June), in the 3 subplots of the experimental pasture. (means ± standard error)

	LH		UW		UH				
	May	June	May	June	May	June	Subplot	Time	SxT
HO (t DM ha^{-1})	4.37±0.34b	7.60±0.63a	4.87±0.40b	3.64±0.63b	5.09±0.40b	7.03±0.63 a	**	**	***
DM, (g.kg^{-1} wet weight)	215.7±8 ab	241.8±15 a	202.6±9 b	227.6±15a b	199.2±9 b	244.2±15 a	ns	**	ns
EE (g)	22.7±0.5 a	18.2±0.9 bc	20.5±0.6b	23.2±0.9a	23.7±0.6a	16.1± 0.9c	ns	***	***
CP (g)	101.0±4.9b	112.8±9.0a b	117.5±5.9a	93.5±9.0 bc	108.1±5.5a b	69.8± 9.0c	*	**	**
NDF (g)	543.2±8 c	605.6±15 b	540.8±10 c	564.1±15bc	541.1±9 c	659.5±15 a	*	***	**
ADF (g)	331.2±3.6c	384.0±6.6b	322.0±4.4c	366.1±6.6b	333.5±4.1c	428.2±6.6 a	***	***	***
ADL (g)	43.2±3.0b	70.5±5.5a	44.9±3.6b	61.1±5.5a	48.3±3.4b	59.8±5.5a	ns	***	ns

Different letters following numbers in the same row indicate significant differences between means (P<0.05); *=P<0.05; **=P<0.01; *** P<0.001.

Table 2. Nutrient content (g per kg DM) of herbage on offer (HO) and of herbage selected by the animals (HS) and calculated coefficient of selectivity of principal chemical parameters (means ± standard error),

	HO	HS	Coefficient of selectivity
DM (g. kg^{-1} wet weight)	238±13	272±13	1.14
CP (g)	$92±6_b$	$126±7_a$	1.36
EE (g)	$19±1_b$	$36±1_a$	1.89
NDF (g)	$609±13_a$	$554±15_b$	0.91
ADF (g)	$393±8_a$	$361±9_b$	0.91
ADL (g)	64±3	72±3	1.12

Different letters following numbers in the same row indicate significant differences between means ($P<0.05$)

The chemical composition of the feed used in the FC groups, summarized in table 3, showed a low quality of hay (CP content 7.1%) in accordance to what found by other authors in similar conditions (Casu et al., 1981; Ligios et al., 1997; Sulas et al., 1997),

Table 3 Chemical composition (on DM basis %) of the experimental feeds used in FC groups (means± SE).

	DM	Ash	NDF	ADF	ADL	EE	CP
Concentrate	88.0 ± 0.3	10.3± 1.6	33.1 ± 1.4	16.6 ± 0.1	4.0 ± 0.4	3.7 ± 0.7	19.5 ± 1.8
Natural pasture hay	86.4 ± 0.4	6.1 ± 0.2	68.5 ± 0.9	39.8 ± 0.5	4.5 ± 0.1	1.9 ± 0.1	7.1 ± 0.2

The concentrations of the n-alkanes (from C_{27} to C_{36}) in the herbage selected by the animals at pasture are presented in Table 4. The odd-chain n-alkanes represented on average 96.2% of the total. These values are in agreement with those reported elsewhere (Mayes et al., 1986; Malossini et al., 1990; Dove and Mayes 1991; Malossini et al., 1994; Nielsen et al., 2003). It has to be noted the low content of C_{33} alkane probably because of the major contribution of legumes in the diet of animals.

Table 4. Average n-alkanes content in hand-plucked herbage samples (mg kg^{-1} DM)

	C_{27}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Herbage selected	49±7	234±36	12±2	170±20	7±1	27±7	4±5	0±0

DM intake and nutritive characteristics of experimental diets is reported in Table 5. Herbage dry matter intake (HDMI) was estimated both by C_{31}/C_{32} and C_{33}/C_{32} ratio (Table 5).

Table 5. Individual herbage DM intake (HDMI) estimated by C_{31}/C_{32} and C_{33}/C_{32} alkane ratio in PAS group, average group concentrate, hay and total DM intake in FC groups, and nutritive characteristic of experimental diets (means±standard error).

	FC1	FC2	FC3	PAS
HDMI C_{31}/C_{32} (kg DM day^{-1} $head^{-1}$)				8.6±0.4
HDMI C_{33}/C_{32} (kg DM day^{-1} $head^{-1}$)				9.5±0.8
DM intake of concentrate (kg DM day^{-1} $head^{-1}$)	2,5±0.05	3,3±0.02	7,7±0.17	
DM intake of hay (kg DM day^{-1} $head^{-1}$)	3,8±0.09	3,6±0.05	2,5±0.08	
Total DM intake (kg DM day^{-1} $head^{-1}$)	6,2±0.1	6,9±0.07	10,1±0.2	
CP content (% DM)	12,6±0.06	13,4±0.04	16,7±0.09	12,6±0.6
NDF content (% DM)	59,0±0.2	55,6±0.1	43,8±0.4	55,4±0.7
Energy content (Mcal $head^{-1}$ day^{-1})	8,4±0.2	9,8±0.1	17,5±0.4	10,0±0.5

Since the C_{33} alkane content (Table 4) of herbage was lower than 50 mg kg^{-1},DM considered as minimum threshold value for the use of n-alkanes as marker (Malossini et al., 1996; Estermann et al., 2001), we will take into account the intake value estimated by C_{31}/C_{32} ratio.

The values of estimated herbage DM in young sarda bulls were comparable with other findings (Redmon et al., 1995; Aranda-Osorio et al., 1996; Dicker et al., 1998; Bodine et al., 2001; Difante et al., 2009) also if expressed as intake per 100 kg live weight (Dicker et al., 1998; Estermann et al., 2001; Difante et al., 2009) and per metabolic weight (Nielsen et al., 2003; Aharoni et al., 2009) (Table 6).

The herbage DMI kg 100 kg LW^{-1} estimated in the PAS group is in agreement with the results of Morris et al. (1993, cited by Realini et al., 1999) that in beef steers have found DMI of approximately 2.60 – 2.87 kg 100 kg LW^{-1}; with Aranda-Osorio et al. (1996) that in 14-month-old Angus X (Hereford X Friesian) and Friesian steers have registered herbage DM intakes of 2.31 kg 100 kg LW^{-1} and with Jamieson and Hodgson (1979 cited by Nielsen et al., 2003) that in British Friesian steers have found herbage DMI that ranged between 2.9 and 3.3 kg 100 kg LW^{-1}.

Similar estimation of herbage DM intake, expressed as kg per metabolic weight, to that found in the present study, have been shown by Berry et al. (2002). They found, in fact, with Scottish Highland cattle grazing alpine pastures, an herbage DM intake tha was included between 12 and 14.6 g kg MW.

Table 6. DM intake per kg live weight (HI/LW), per kg of metabolic weight (HI/MW) of the experimental diets, and energy intake (Mcal.) per kg live weight NELW (Lsmeans ±SE)..

	Intake		
Experimental groups	HI/LW	HI/MW	NELW
FC1	1.8±0.1 c	7.7±0.4 c	0.027 ± 0.001 b
FC2	2.1±0.1 c	8.7±0.4 c	0.030 ± 0.001 b
FC3	3.5±0.1 a	14.8±0.4 a	0.052 ± 0.001 a
PAS	2.9±0.1 b	12.3±0.4 b	0.030 ± 0.001 b

Different letters following numbers in the same column indicate significant differences between means (P<0.05)

As expected, the FC3 group showed the highest live weight at slaughtering (365±13 kg), growth rate (1.21 ±0.06 kg d^{-1}) and BCS (3.01±0.07) followed by PAS, FC2 and FC1 (Table 7).

In Figure 1 are reported the trends of live weights of experimental groups: from June 29 the FC3 group have displayed the higher live weight (P< 0.001) respect to other groups.

Table 7. Live weights and BCS at the beginning of the experiment and at slaughtering, Average Daily Gain (ADG), and Feed Conversion Ratio (FCR as Mcal per kg ADG) of experimental groups (LSmeans ± SE).

	FC1	FC2	FC3	PAS
Initial live weight	289±12	288±12	289±12	289±12
Slaugthering live weight	329±13 c	336±13 bc	365±13 a	340±13 b
Average Daily Gain (ADG)	0.58±0.06 c	0.75±0.06 bc	1.21±0.06 a	0.78±0.06 b
Initial BCS	2.71±0.07	2.78±0.07	2.68±0.07	2.61±0.07
Slaughtering BCS	2.45±0.07 c	2.57±0.07 bc	3.01±0.07 a	2.67±0.07 b
Feed Conversion Ratio (FCR)	15.4 ± 1.3	13.3 ± 1.3	14.7 ± 1.3	13.9 ± 1.3

Different letters following numbers in the same row indicate significant differences between means (P<0.05)

The average daily gain of Sarda young bulls in FC3 group is not so different from what occurs in the more specialized fattening centers of the north Italy (1.38 kg head^{-1} day^{-1}, SMEA, 2011) with beef breeds as Charolais and Limousin, and similar to those detected in Podolian young bulls (Ragni et al., 2007). The ADG in PAS group was in agreement with results of Nielsen et al. (2003), Realini et al. (1999) and Boland and Scaglia (2011) with dairy breed steers, beef steers and beef calves respectively. Also Dawson and Steen (1998) reported ADG similar to those of the animals fed at pasture in this trial.

It is noteworthy the worse FCR value of FC3 group respect to FC2 and PAS group, despite not statistically significant. One of the reasons could be sought in an excessive intake of concentrate (administered *ad libitum*) by animals of FC3 group. This is confirmed by the low value of digestibility of FC3 diet (56.2%, AIA method), in comparison to digestibility values of FC1 (59.3%), FC2 (65.8%) and PAS diet (64.4%, n-alkanes method).

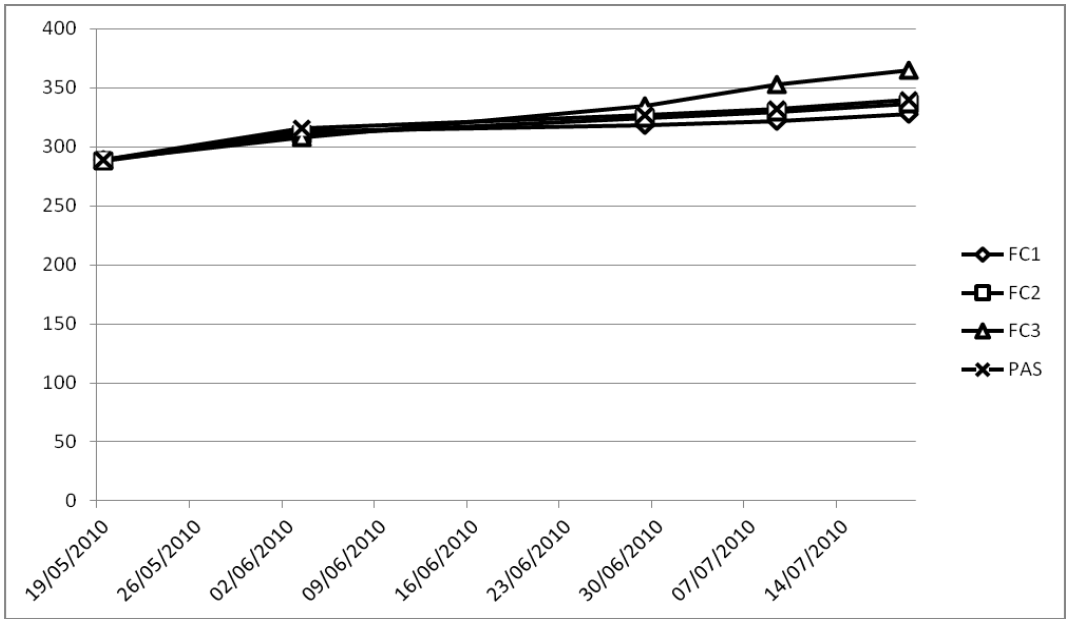

Figure 1. Live-weight of Sarda young bulls during the experimental period.

Table 8. Warm and Cold dressing percentage of experimental groups (LS means ±SE).

Experimental groups	Warm dressing percentage (%)	Cold dressing percentage (%)
FC1	49.5 ± 0.6 b	48.6 ±0.6 b
FC2	50.7 ±0.6 b	49.8 ±0.6 b
FC3	52.8 ±0.6 a	52.0 ±0.6 a
PAS	52.1 ±0.6 ab	51.2 ±0.6 ab

Different letters following numbers in the same column indicate significant differences between means ($P<0.05$)

The cold dressing percentage values calculated as cold carcass to slaughtering weight ratio % (Table 8) detected, were those expected for a not specialized breed and are in agreement with others trials (Serra et al., 2001; Cifuni et al., 2004) carried out in similar conditions. The low values obtained from the animals in FC1 and FC2 groups, together with the low ADG and the worsening of BCS values in these groups, confirm that the FC1 and FC2 diet did not fully meet the growth requirements of animals.

The PAS group animals showed similar performance to those of FC2 group even if the BCS at slaughtering of PAS group slightly improves compared with the initial BCS (contrary to FC2 group) and the dressing percentage of PAS group was higher than FC2 group.

These results could be explained by the greater DM intake of PAS group respect to FC2 group (Table 5 and 6).

Table 9. Effect of diet on dressing percentage at equal energy intake

	hay concentrate	pasture	s.e.	P
Cold dressing percentage (%)	50	51.2	0.76	0.17

The paired *t*-test (tab. 9), carried out to test the difference between the values of the hay-concentrate-fed animals and those of the pasture-fed animals at equal energy intake, showed that the dressing percentage (unique parameter related to NELW in the correlation matrix as) was not different between groups confirming what shown in table 8. In other words the pasture does not seem to have an effect "*per se*" (derived by the nutrient composition) on dressing percentage.

4.4 Conclusions

The results of this work confirm that a diet based on pasture for young bulls of Sarda breed seems to be a valid chance for Sardinian cattle livestock system. Indeed pasture-fed animals showed daily live gain and dressing percentage in agreement with other breeds, also more specialized than the Sarda for meat production, obtained in similar conditions.

The beef production in extensive systems in Sardinia is based on an efficient exploiting of Mediterranean pasture and the Sarda cattle breed seems to be able to do it, thanks to its selective grazing. Moreover the present paper gives a first insight useful to program the availability of pasture required to finish Sarda young bulls at pasture and to quantify the feeding costs saved with the grazing,

Suckler beef as a livestock system can utilize marginally profitable areas such as mediterranean hills. As Estermann et al. (2001) "This is assumed to be an ecologically and economically sound way of farming resulting in a high-priced meat as the ultimate product. The importance of suckler beef is currently growing whereas simultaneously the number of dairy cows is decreasing due to increasing milk yield at constant milk quota. This provides capacity for alternative livestock systems."

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Chapter 5

Role of pasture in the performance of Sarda young bulls: meat quality

5.1 Introduction

The meat quality is an evolving concept that does not have a unique meaning: the different definitions ranging from "a whole of characteristics able to satisfy the explicit or indefinite consumer's requirements (ISO 9000, 2000)" to "more objective assessment through the relevant parameters such as pH, meat color, tenderness, chemical composition, fatty acid composition" (Karlsson, 1993; Matassino et al., 1985; Destefanis and Barge 1990).

For this reason to talk about beef quality "sensu lato" is of little significance thus hereafter we will consider the following aspects of quality:

- Chemical and nutritional quality, that concern aspects detectable by proximate analyses and involve classical components (moisture, protein, ether-extractable fat, vitamins, cholesterol,)
- Technological quality, that concerns the ability of the meat to the conservation and transformation (Monin, 1991) like pH and Water Holding Capacity (WHC);
- Sensory quality, that concerns the characteristics perceived by the senses of the consumer (Monin, 1991) as, color and tenderness.

Meat quality in ruminants is influenced by different factors that can be divided into 2 categories:

- Factors directly linked with the animal (breed, age, sex, etc.)
- Factors external to the animal (feeding system, livestock system, diet, weather, slaughtering conditions, etc.) indicated by the generic expression" environmental" (Sarti, 1992; Priolo et al, 2001).

Among the external factors an important role is played by feeding, that can modify beef quality through its effect on the quantity of energy available to the animal (nutrition level) and through the nutrient composition of the feed (feed type) (Muir et al., 1998).

Different feed types indeed, vary in the amount of available energy as well as in nutrient composition. In the literature many researches on the effect of nutrition on beef quality have often compared forage-based diet with concentrate-based ones. In those studies the animals were usually slaughtered at different ages (same weight, but different growth rate) or at different weights (same age and again different growth rate). These trials are useful because they investigate real production situations, but it is more difficult to discriminate between the effects of the nutrition level on animal growth rate, thus indirectly on meat quality traits, and the direct effects of the nutrient composition on meat quality.

Two animals, fed at different nutrition level, show probably different growth rate; if we have to study some quality traits, we can decide to slaughter these animals at the same age (but at different weight) or at the same weight (but at different age); in both cases any difference in quality trait could be attributed to nutrition level (indirectly from the growth rate and hence from different carcass fatness that could lead to differences in several quality traits) or to some specific nutrient content in the two diets.

A large numbers of experiments have compared pasture and hay-concentrate diets but often pasture has sustained lower liveweight gains than those achieved with other diets (Bowling et al. 1977; Muir et al., 1998; Steen et al., 2003.) and the animal finished at pasture have produced carcasses with a low fat content. Consequently "it is difficult to ascertain the extent to which the low fat content has been due to lower growth rate or to forage rather than grain in the diet *per se*" (Steen et al., 2003). In other words "the specific effects of the dietary constituents on meat quality are not easy to evaluate." (Priolo et al. 2001), The animal growth rate is influenced by the feeding regime and it is difficult to establish if the meat characteristics are due to the growth rate or to dietary components for their intrinsic.

The use of pasture (alone or supplemented with concentrate) in the fattening of animals could represent a chance for Sardinian beef cattle livestock system as could help to

reduce feeding costs; moreover the meat fatty acid composition of pasture-fed cattle is more desirable in terms of its effect on human health (Sinclair and O'Dea, 1990 cited by Steen et al., 2003; Moreno et al., 2006).

The Sarda breed is a small-frame cow well adapted to Sardinia environment and able to exploit through grazing the marginal areas of the Island (Brandano et al., 1983). These cattle are particularly hardy and may provide a viable option for areas of Sardinia of poor nutritive value; however, to our knowledge, data on Sarda meat quality are scarce.

With the aim to improve our knowledge on meat quality parameters, chemical composition, fatty acid composition of intramuscular adipose tissue and water holding capacity (WHC) of Sarda meat and to evaluate the effects of pasture and concentrate diets for beef cattle on meat quality at equal growth rates an experiment was carried out.

5.2 Materials and methods

5.2.1 Experimental site and experiment duration

The present study was conducted during 2010 (19/05 – 19/07) at the experimental farm of the Department of Research in Animal Production (Agricultural Research Agency of Sardinia, AGRIS Sardegna) located in Foresta Burgos, in the centre of Sardinia (latitude 40°25'N, longitude 8°55'E, altitude 850 m above sea level).

5.2.2 Animals

The experiment was performed with 28 Sarda young bulls divided into 4 groups (7 heads each) homogeneous for live weight (LW, 288.7±29.0 kg mean ± standard deviation), Body Condition Score (BCS, 2.69±0.18) and age (355±25 days). One group was fed 24 h at pasture in a 7-ha paddock (group PAS) and did not receive any supplementation, the other three groups (FC) were kept in barns and fed daily with natural pasture hay (*ad libitum*) and concentrate: 2.5 kg/head (FC1), 3.3 kg/head (FC2) and *ad libitum* (FC3).

The aim of the 3 levels of intake of the hay-concentrate diets was to produce a response relationship to the level of energy intake per kg of Live Weight (NELW) so that the meat quality of the animals on the hay-concentrate diet and those at pasture could be compared at an equal level of animal performance. This approach was adopted rather than attempting to maintain equal growth rates for the 2 types of diet since to obtain the expected growth rates at pasture is aleatory, limiting the possibility of achieving equal rates of carcass gain for the 2 diets.

All animals were slaughtered at the fixed age (416±25 days) in an authorized slaughterhouse according to EU legislation.

Prior to allocation to treatments the animals were treated for internal and external parasites.

The experiment was conducted in accordance with the Italian guidelines on animal welfare (DL No. 116, 27/01/1992).

5.2.3 Pasture and feed

The natural pasture used in the present study was a low hilly area of 7 ha with a large number of trees in particular *Quercus pubescens* L. The feed used in FC groups were meadow hay and a commercial concentrate.

The chemical composition of herbage and feeds (AOAC 1990) was measured and the energy content (EN, Mcal/kg DM) of the feeds was calculated as Cannas et al. (2002) using the equations proposed by Van Soest (Licitra et al., 1997; Van Soest 1994; Van Soest and Fox 1992, see chapter 4).

5.2.4 Measurements

The young bulls were transported to a commercial abattoir and slaughtered within 1 h from arrival to minimize pre-slaughter stress. Young bulls were stunned by captive bolt pistol and dressed according to standard commercial practices. Immediately after slaughter, hot carcass weight were recorded. After 24 h chilling at 4 °C the carcass was weighted (cold

weight) to calculate dressing percentage calculated as cold carcass weight divided by slaughter live weight, measured the day before slaughter.

The pH value of longissimus thoracis (dorsi) muscle was measured 1 h after slaughter (pH 1h) and at 24 h post-mortem (pHu) by making a scalpel incision at the 6th/8th rib, using a Eutech pH 600 pH meter with a penetrating probe.

Meat color parameters at 24 hours post-mortem were detected on Longissimus dorsi (LD) at the sixth thoracic vertebra, at right angles to the sagittal plane surface. Color determinations were performed using the Chroma Meter CR-400 colorimeter of KONICA MINOLTA according to the CIE L* a* b* system.

Instrumental color measurements were recorded for L* (lightness; 0: black, 100: white), a* (redness/greenness; positive values: red, negative values: green), and b*(yellowness/blueness; positive values: yellow, negative values: blue), (Realini et al., 2004). Data were used to calculate Hue angle value (H°) and Chroma (C*)

Carcasses were chilled for 24 h at 4 °C. At 24 h post-mortem samples of Longissimus dorsi between sixth and seventh thoracic vertebrae from each left half-carcass were removed.

The caudal part of this sample was stored at – 20 °C until cooking loss measurement and Texture Profile Analysis (TPA) while the cranial part was ground, homogenized, vacuum-packaged and frozen at -20°C until chemical analysis.

5.2.4.1 Cooking loss measurement

To estimate water-holding capacity (WHC), weight losses were determined on samples of LD muscle: a 2.5 cm tick sub-sample from frozen samples of LD were allowed to thaw for 24 h at 4 °C and to bloom at room temperature for 4 h. After thawing, steaks were deboned, trimmed of subcutaneous fat and epimysium, weighed and grilled on a pre-heated Philips electric grill until internal temperature reached 71°C (AMSA, 1995). Cooked steaks were weighed and cooled to less than 10 °C. Cooking loss was determined by dividing the weight loss during cooking by the pre-cooked weight and reported as a percentage (Blanco et al, 2010).

5.2.4.2 Texture Profile Analisys (TPA)

Texture is a" kind of reflection of physiological stimulative on the tactile sensing when some organs touched with food; it is a set of physical parameters based on the food structure and belong to the fields of mechanics and rheology" (Chang et al., 2012). Meat texture is an organoleptic indexes and is related to the tenderness, mouth feels, edibility. TPA is a very important indicator of meat for determining the texture changes (Chang et al., 2012).

TPA test (Bourne, 1978) was performed using a Universal Testing Machine TAXT plus Texture Analyser (Stable Microsystems Ltd) with the Texture Exponent software (Vs.2.0.0.7). For this purpose cylindrical samples of meat with a 1.5 cm diameter and a height of 1.5 were obtained from LD section, parallel to muscle fibers.

A double compression cycle test was performed using an aluminium cylinder probe (P/75). Force–time deformation curves were obtained with a 5 kg load cell applied at a cross-head speed of 1 mm/s. The following parameters were evaluated: *hardness* (g, H), maximum force required to compress the sample; *chewiness* is the energy required to masticate a solid food product to a state ready for swallowing; *cohesiveness* (Co), extent to which the sample could be deformed prior to rupture (Chang et al., 2012).

5.2.4.3 Chemical analysis

Meat moisture, fat, protein and ash content was determined (AOAC Official Methods n. 950.46, 960.39, 981.10, 900.02, respectively; AOAC, 1990).

Intramuscular fat was determined according to modified Folch method (Christie, 1989).

The extracted lipids were subjected to acid transesterification (Chin *et al*., 1992, Stanton *et al*., 1997). The gaschromatographic separation of fatty acid methyl esters (FAME) was achieved, using a VARIAN 3900 GC, on capillary column Supelco SP 2560 (100 m length, 0.25 mm inner diameter, 0.2μm film thickness). Helium was used as carrier gas at a flow of 1 mL/min. The injection was performed in split mode and the split ratio was 1:50. One μL of FAME sample, was injected under the following GC conditions: the oven temperature was programmed at 45°C and held for 4 min, increased to 175°C at a rate of

13°C/min, held for 27 min, increased to 215°C at a rate of 4°C/min and held for 35 min. The injector and detector (Flame Ionization Detector) temperatures were set at 290°C. Individual FAMEs were identified by comparison to a standards mixture of 37 components (Matreya Inc., Pleasant Gap PA, USA). Conjugated Linoleic Acids (CLA) standards (CLA 9c, 11t; CLA 10t, 12c; CLA 9c, 11c; and CLA 9t, 11t, Matreya Inc., Pleasant Gap PA, USA) and published isomeric profiles (Kramer *et al*., 2004) were used to identify the CLA isomers. The quantitative measurements of each fatty acid methyl ester was performed through a calibration curve using the following internal standards C13:0 (C10:0–C17:0), and C19:0 (C18:0–C18:3). The concentration of each internal standard added to the fat sample was 170 mg/g of lipid. The data on fatty acid composition were processed to compute the content of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 PUFA (C18:3, C20:5 (EPA), C22:5 (DPA) and C22:6 (DHA)) and n-6 PUFA (C18:2, C20:3, C20:4). Moreover, ratios were calculated including the ratio of n-6/n-3, P/S (PUFA/SFA), P/S2 (PUFA/(SFA-C18:0)). Finally, the Atherogenicity index (AI as a dietary risk indicator for cardiovascular disease), Trombogenic index (TI as a sign of the potential aggregation of blood platelets) were calculated as follows:

AI = (C12:0+4*C14:0+C16:0)/(MUFA+PUFA),

TI = (C14:0+C16:0+C18:0)/[(0.5*n-6)+(0.5*MUFA)+(3*n-3)+(n-3/n-6)],

The α-tocoferol (Vitamin E) content and total cholesterol were determined following the methods proposed by Panfili et al. (1994) and Manzi et al. (1996).

5.3 Statistical analysis

Data on meat chemical composition, cooking loss measurements, TPA analysis and color parameters, were analyzed with GLM procedure of SAS (1989) using a mono-factorial model with diet as fixed effect.

In order to compare the data between PAS and FC animals, meat chemical composition data were adjusted to an equal energy intake kg live weight^{-1} (NELW see chapter 4). The procedure used was as follows:

- A least squares linear regression relationship between ADG and NELW was produced for the animals on the three concentrate treatments. Tests were also made for curvilinear relationships with NELW, but these proved non-significant, and so the linear relationships were used.
- From this the NELW of the hay-concentrate diet required to sustain a carcass gain equivalent to that obtained for the grazing treatment was estimated.
- By means of a correlation matrix were identified the parameters correlated with NELW.
- Further relationships were developed between each of the parameters and NELW for the three concentrate treatments.
- From these, values for the various parameters were predicted for the concentrate-fed animals at the estimated NELW from (2) above.
- Differences between these predicted values for the concentrate-fed animals and the actual values for the pastured animals were then tested for statistical significance using a paired *t*-test.

Comparison between the pasture and concentrate treatments using the adjusted values generally gave a much stronger statistical comparison than comparison of the pasture treatment with individual concentrate treatments (Steen et al., 2003),

P-values < 0.05 were considered significant

5.4 Results and discussion

The chemical composition of the feedstuff used in the experiment is reported in Chapter 4 Table 1 and Table 3.

Table 1. Ultimate pH (pHu) and color parameters of *Longissimus dorsi* at 6th/8th rib of Sarda young bulls (Lsmeans ± SE).

	FC1			FC2			FC3			PAS		
pHu	5.49	±	0.11	5.52	±	0.11	5.48	±	0.08	5.51	±	0.05
L meat	37.91	±	1.85	37.75	±	1.50	38.32	±	1.33	36.69	±	2.29
a meat	21.87	±	2.02	21.26	±	1.85	20.70	±	3.19	21.38	±	2.24
b meat	5.36	±	0.94	5.80	±	1.39	5.45	±	1.58	5.96	±	1.03
C meat	22.52	±	2.15	22.06	±	2.09	21.43	±	3.42	22.20	±	2.37
H meat	0.24	±	0.03	0.26	±	0.04	0.25	±	0.05	0.27	±	0.03

Different letters following numbers in the same row indicate significant differences between means (P<0.05).

Feeding treatments had no effect on ultimate pH, which ranged between 5.48 and 5.52, within the normal range (Table 1, Muir et al., 1998; Daly et al., 1999; Mazzette 2004; Sami et al., 2004), indicating no pre-slaughter stress (Ripoll et all., 2012).

The lack of feeding system effect on meat pHu recorded in this study agrees with what found by other authors (French et al., 2000; Priolo et al., 2001; and Leheska et al., 2008). The under-nutrition is the primary cause of high-ultimate pH in meat (Priolo et al., 2001), since animals do not have the possibility to accumulate sufficient glycogen reserve in their muscles and, as already known, the decrease in pH of the meat derives from the postmortem conversion of glycogen to lactate. But, provided that cattle are not stressed, the plane of nutrition appears to have little effect on ultimate muscle pH (Muir et al. 1998).

Moreover "a higher disposition to glycogen depletion response to pre-slaughter handling could be a risk factor for high ultimate pH" (Bowling et al. 1977; Priolo et al., 2001); animals raised and finished at pasture are nor generally accustomed to man handling.

However, in the current study, all young bulls were accustomed to the human presence and their performance (see chapter 4) show that the nutrition at pasture met their requirements so the pasture-finished animals had, probably, enough glycogen to lead to a normal ultimate pH.

The effect of feeding systems on color parameters of Sarda beef meat are reported in table 1. No difference was detected between the different feeding treatments in all color variables measured. These results seem to indicate that feeding system and pre-slaughter

growth rate do not affect muscle colour. These findings are in agreement with Davis et al. (1981 cited by Muir et al., 1998) who found that there were no differences in lean color when steers grain-fed grow at the same rate as pasture-fed ones. Also in a study of Leheska et al. (2008) there were no differences in color variables in beef obtained in grass and conventional feeding systems. On the other hand Miller et al. (1987) found that "plane of nutrition did not affect subjectively measured lean colour scores despite significant differences in carcass weight and marbling". It has also been suggested that muscle myoglobin concentrations are greater in free-ranging, grazing animals than those in feedlots as a result of differences in exercise (Varnam and Sutherland 1995 cited by Muir et al., 1998) resulting in darker beef caused by higher concentrations of oxidized metmyoglobin. However, Craig et al. (1966) found no differences in pigment concentration between grazing and feedlot animals. On the other hand in a review on meat color and flavour, Priolo et al. (2001) affirmed that "it is clear that meat from animal finished on pasture is darker than meat from animal finished on concentrate". Several factors seem to play a role to explain the darker meat in extensive production systems: intramuscular fat content because of fat is lighter in color than muscle and its precence could contribute to increase lightness value (Hedrick et al., 1983 cited by Priolo et al. 2001); age at slaughtering (Bidner et al., 1986); growth rate and compensatory growth (Barker et al., 1995 and Dufrasne et al., 1995 cited by Priolo et al., 2001); ultimate pH (Ledward et al., 1986 and Young et al., 1997 cited by Priolo et al.. 2001; Vestergaard et al.. 2000). In this experiment these factors (except intramuscular fat content see above) did not show differences among experimental groups, therefore, probably, none of them played a role on meat color.

About intramuscular fat content, conversely to what found by Priolo et al., (2001) in the aforementioned review and confirming the results of the current study, others (Cerdeño et al., 2006; French et al., 2001) reported that forage- and concentrate-fed cattle slaughtered at similar liveweight had similar L* and a* value, despite the different intramuscular fat content.

Table 2. Cooking loss measurements of Longissimus dorsi at $6^{th}/7^{th}$ rib of Sarda young bulls, (Lsmeans ±SE).

	FC1	FC2	FC3	PAS
Cooking loss (%)	0,31 ± 0,02	0,32 ± 0,02	0,31 ± 0,03	0,30 ± 0,02

In table 2 the WHC (cooking loss method) of *Longissimus dorsi* muscle is reported. The WHC was never influenced by the feeding system, as reported also by Sami et al. (2004) in Simmental young bulls, who found lower values than ours. Conversely Vestergaard et al. (2000 b cited by Cutrignelli et al., 2008) found "higher cooking loss values in extensively than in intensively fed bulls" without giving any explication for this result.

Maiorano et al. (2005) found on average higher cooking loss values than our results in Podolian cattle fed at pasture and in particular higher cooking loss in supplemented animals than un-supplemented ones. The authors explain these results with the higher intramuscular fat content in supplemented animals. In our work this aspect does not seem play a role.

Table 3. TPA parameters of *Longissimus dorsi* at $6^{th}/7^{th}$ rib of Sarda young bulls. (Lsmeans±SE).

TPA parameters	FC1	FC2	FC3	PAS
Hardness	15.0 ± 5.6	13.9 ± 3.1	17.3 ± 4.3	12.9 ± 2.3
Chewiness	4.43 ± 1.17	3.88 ± 0.54	4.58 ± 1.60	3.47 ± 0.70
Cohesiviness	0.53 ± 0.02 a	0.53 ± 0.01 a	0.50 ± 0.02 b	0.52 ± 0.01 a

Different letters following numbers in the same row indicate significant differences between means ($P<0.05$)

Concerning TPA parameters the feeding treatments do not have determined differences on hardness and chewiness, while cohesiveness was lower in FC3 than the the counterparts ($P<0.05$, Table 3).

Despite several data indicate that grain-fed cattle produce meat more tender than forage-fed cattle (Bowling et al., 1977; Muir et al., 1998), other results show an opposite trend (Huffmand and Griffey, 1975 and Schupp et al., 1976 cited by Bowling et al., 1977; Bidner et al. 1981, Crouse et al. 1984, McIntyre and Ryan 1984 and Brennan et al. 1987 cited by Muir et al 1998).

These data indicate that weight and fatness of cattle are very important in determining tenderness; differences in tenderness may have resulted from the difference in fat cover and the subsequent effect on carcass chilling rate. Indeed, as Smith et al. (1976 cited by Bowling

et al. 1977), "increased carcass fatness decrease the rate of temperature decline, enhances the rate of duration of autolytic enzyme activity in muscle, lessens the extent of myofibrillar shortening and thereby increases tenderness".

It is therefore not surprising that when compared at equal fat cover grain feeding had no effect on tenderness.

However it seems likely that weight and fat differences in Sarda young bulls were not able to determine significant differences in hardness and chewiness, while the differences in cohesiveness appear unclear despite Wood et al (1990) stated that "fat cell expansionin the perimysial connective tissue forces muscle bundles apart, thus opening up the muscle structure".

Table 4. Proximate analysis, tocopherol and cholesterol content of *Longissimus dorsi* at $6^{th}/7^{th}$ rib of Sarda young bulls. (Lsmeans±SE).

	FC1	FC2	FC3	Pas
Dry matter	23.46 ± 0.49 b	23.51 ± 0.13 b	24.56 ± 0.29 a	23.59 ± 0.28 b
Ash (% as feed)	1.13 ± 0.02 b	1.15 ± 0.02 b	1.18 ± 0.03 a	1.15 ± 0.03 b
EE (% as feed)	0.52 ± 0.21 b	0.64 ± 0.19 b	0.98 ± 0.50 a	0.47 ± 0.14 b
Crude protein (% as feed)	21.50 ± 0.38	21.38 ± 0.37	22.31 ± 0.51	18.58 ± 5.21
Tocopherol (mg/kg of meat)	2.20 ± 0.31 b	2.11 ± 0.16 b	2.01 ± 0.31 b	3.95 ± 0.43 a
Cholesterol (mg/kg of meat)	473.82 ± 29.03 b	481.41 ± 23.13 b	467.16 ± 24.63 b	511.14 ± 26.30 a

Different letters following numbers in the same row indicate significant differences between means ($P<0.05$)

The feeding treatment affected dry matter, ash and intramuscular fat content in meat of Sarda young bulls, as reported in Table 4.

Dry matter (DM) content was highest in FC3 young bulls; this could be related to the muscle lipid concentration that was highest in FC3 group (Table 4) since there was a positive correlation between DM and lipid concentration in muscle ($r = 0.61$, $P < 0.001$ data not shown).

The highest content of intramuscular fat in FC3 animal was expected, considering the highest energy intake of this group (NELW see chapter 4), as provided by the experimental plan. This result is in agreement with Schroeder et al., (1980),

et al., (2000 a), Steen et al., (2003) Nuernberg et al., (2005) and Blanco et al., (2010), and the reason is to be found in the availability of net energy (and glucose) for fat synthesis during finishing as well argued by Scollan et al. (2006).

However, as stated by Muir et al. (1998) "the improvement in marbling (the term given to intramuscular fat and deposited between muscle bundles) may have been a result of the higher energy concentration in the grain diet rather than the fact that grain *per se* was fed". In the same review Muir et al. (1998) report the data of Davis et al. (1981) in which cattle fed a restricted grain diet grew at the same rate of those fed pasture and had similar levels of intramuscular fat.

Conversely Blanco et al. (2010) stated that "forage diets, that are extensively fermented in the rumen, promote acetate as the major source of carbon reducing lypogenesis (Pethick et al. 2004 cited by Blanco et al. 2010) whereas concentrates increase the amount of glucose entering the duodenum, which could increase lypogenesis (Davis, 1977 cited by Blanco et al., 2010). Thus, the low fat deposition could be related to the nature of the diet, the lower feeding level, greater mobility and lower growth rate prior to slaughter".

In order to distinguish the effects of feeding level from those of the nature of the diet we compared the intramuscular fat of hay-concentrate–fed animals and pasture-fed-animals at equal energy intake (NELW) (see chapter 4).

Table 5. Effect of diet on ether extract at equal energy intake kg live weight^{-1}

	hay concentrate	pasture	s.e.	P
ether extract	0.68	0.65	0.07	0.02

The data adjusted to equal energy intake showed (Table 5) a significant difference between the pasture- and hay-concentrate treatments in terms of intramuscular fat, therefore the lower intramuscular fat in the pastured-animals respect to FC3 group may be due to the pasture *per se.* An explanation is given by Pethick et al. (2004 cited by Blanco et al., 2010) stating that forage diets,"extensively fermented in the rumen, promote acetate as the major source of carbon reducing lypogenesis whereas concentrates increase the amount of glucose

entering the duodenum, which could increase lypogenesis" (Davis, 1977 cited by Blanco et al., 2010).

Data on the total cholesterol content in LD muscle of young Sarda bulls (mg/kg muscle) are depicted on table 4. The feeding systems has affected cholesterol content in *longissimus* muscle of Sarda young bulls and more precisely the pasture-fed animals (PAS group) showed the highest content value (511 mg kg^{-1}, table 4).

The cholesterol content in LD muscle of young Sarda bulls is in agreement with the values reported by Braghieri et al. (2005) in Podolian et Podolian X Limousine bulls, by Alfaia et al. (2006) in Carnalentejana-PDO beef and very close to those reported for Chianina breed (Poli et al. 1996 cited by Cutrignelli et al. 2008), while is lower than cholesterol content in young Marchigiana bulls. In general the values reported in the present work fall within the range indicated by Chizzolini et al. (1999). These authors, together with Kalač (2011), stated that "the differences sometimes detected in cholesterol content among some breed or between sexes or in relation with some feeding regimes are small; significant and interesting differences instead, have been reported in cholesterol content between muscle types". According to those authors Alfaia et al. (2006) affirm "the most likely reason for some of the differences in cholesterol content observed in different muscles might be variations in fibre type composition". This hypothesis results from the observation that oxidative muscles are known to be richer in phospholipids and that there is a direct correlation between the content of phospholipids and cholesterol, which is present mainly (60–80%) in the membrane component of the bovine muscle (Hoelscher et al., 1988). The direct relationship between phospholipids and cholesterol seems to be necessary to maintain membrane fluidity in a narrow range (Alasnier et al., 1996).

On the other hand it is known the positive relationship between the grazing and the type of muscle fibers. Vestergaard et al. (2000a) showed, indeed, that pasture feeding produces muscles with a higher proportion of oxidative fibers, and Hocquette et al. (2005) stated that pasture-fed bulls have a muscle metabolism more glycolytic than oxidative.

Therefore, if cholesterol content is higher in oxidative muscles, the reason for which the PAS group showed the highest cholesterol content seems to be based on the likely greater proportion of oxidative fibers, increased in the pasture-fed group.

On the other hand these results are not consistent across experiments: Duckett et al. (2009, with Angus-crossed steers) and Descalzo et al. (2005, in cross-bred steers, cited by Kalač, 2011) found that comparing pasture finishing systems with high concentrate ones not affect cholesterol content in LD muscle, and Garcia et al. (2008, cited by Kalač, 2011) reported significantly lower cholesterol content in pasture-finished beef than in the meat of concentrate-fed steers. Therefore the conclusions here reported must be treated with caution.

As reported in table 4 the feeding systems affected also the tocopherol content in LD muscle of young Sarda bulls, with the highest value in pastured animals. This result is in agreement with numerous studies confirming that cattle finished on pasture produce higher levels of a-tocopherol in the final meat product than cattle fed high concentrate diets. Daly et al. (1999) in Angus cross steers found more tocopherol content in LD muscle of pastured-animals than in grain fed animals and affirmed that tocopherol content is much higher in green leaf tissue than in grain or hay, so "the higher muscle concentration of tocopherol in meat from the pasture-fed animals reflect this". Mercier et al (2004 cited by Scollan et al. 2006) found that Charolais cows finished at pasture had higher vitamin E content in their meat than those fed with cereal concentrates. In the same review (Descalzo et al., 2000, Realini et al., 2004 cited by Scollan et al., 2006) is stated that "for production systems in Uruguay and Argentina beef finished off grass had higher vitamin E concentration and better lipid stability than those finished on concentrates.

De la Fuente et al. (2009), Descalzo et al. (2008) and Yang et al. (2002) reported a greater tocopherol content in grass-fed animals respect to grain-fed. The authors concluded that "it is the presence of the antioxidant in grass that probably caused higher tissue levels of vitamin E in grazed animals, with benefits of lower lipid oxidation and better color retention." Indeed vitamin E (its most active isoform α-tocopherol) delays the oxidative deterioration post-mortem of meat, reducing myoglobin oxidation (Geay et al., 2001), a process by which

“myoglobin is converted in brown metmyoglobin, producing a darkened appearance to the meat” (Daley et al., 2010), a color little appreciated by the consumers. In general Vitamin E helps to protect membrane phospholipids and cholesterol against oxidation.

The meat of PAS group showed α-tocopherol levels within the range needed to extend the shelf-life of retail beef (0.30-0.35 mg 100 g^{-1} of fresh meat, McDowell et al., 1996 cited by Geay et al., 2001).

Moreover it should be recalled that vitamin E may help prevent or delay coronary heart diseases, blocking the formation of nitrosamines (carcinogens formed in the stomach from nitrate consumed in the diet (Daley et al., 2010).

Table 6. The effect of feeding systems on individual fatty acid proportions in intra-muscular fat of *Longissimus dorsi* at $6^{th}/7^{th}$ rib of Sarda young bulls. (Lsmeans±SE).

Fatty acid (mg g total lipid^{-1})	FC1	FC 2	FC 3	PAS
C10:0	0.26 ± 0.03b	0.26 ± 0.03b	0.35 ± 0.03a	0.23 ± 0.03b
Short chain	0.26 ± 0.03b	0.26 ± 0.03b	0.35 ± 0.03a	0.23 ± 0.03b
C12:0	0.43 ± 0.03	0.35 ± 0.03	0.40 ± 0.03	0.36 ± 0.03
C14:0	10.91 ± 1.14	10.74 ± 1.06	12.38 ± 1.06	9.27 ± 1.06
C16:0	128.68 ± 7.51	128.48 ± 6.95	144.94 ± 6.95	119.06 ± 6.95
Medium chain	140.02 ± 8.64	139.57 ± 7.99	157.71 ± 7.99	128.70 ± 7.99
C18:0	132.72 ± 7.10	137.33 ± 6.57	142.82 ± 6.57	133.64 ± 6.57
C18:1 11t	16.18 ± 1.58	17.38 ± 1.46	13.69 ± 1.46	12.95 ± 1.46
C18:1 9c	155.35 ± 11.77ab	162.05 ± 10.89a	181.10 ± 10.89a	133.88 ± 10.89b
C18:2 9c,12c	78.92 ± 6.75	97.50 ± 6.25	83.45 ± 6.25	97.26 ± 6.25
C18:3 9c,12c,15c	9.12 ± 0.81bc	10.94 ± 0.75b	7.54 ± 0.75c	22.35 ± 0.75a
CLA 9c,11t	2.43 ± 0.32	3.02 ± 0.30	2.43 ± 0.30	1.99 ± 0.30
CLA 11t,13c	0.18 ± 0.04	0.23 ± 0.04	0.20 ± 0.04	0.22 ± 0.04
C20:4 5c,8c,11c,14c	14.57 ± 1.89ab	18.58 ± 1.75a	11.08 ± 1.75b	18.88 ± 1.75a
C20:5 5c,8c,11c,14c,17c EPA	0.27 ± 0.03b	0.26 ± 0.03b	0.18 ± 0.03b	0.49 ± 0.03a
C22:5 7c,10c,13c,16c,19c DPA	5.62 ± 0.70b	7.96 ± 0.64a	4.89 ± 0.64b	8.60 ± 0.64a
C22:6 4c,7c,10c,13c,16c,19c DHA	0.34 ± 0.06b	0.52 ± 0.06a	0.31 ± 0.06b	0.54 ± 0.06a
Long chain	415.69 ± 15.87	455.78 ± 14.70	447.68 ± 14.70	430.80 ± 14.70
total	555.97 ± 23.32	595.61 ± 21.60	605.75 ± 21.60	559.73 ± 21.60
SFA	272.99 ± 15.15	277.16 ± 14.03	300.89 ± 14.03	262.57 ± 14.03
MUFA	171.53 ± 12.98	179.43 ± 12.02	194.78 ± 12.02	146.83 ± 12.02
PUFA	111.44 ± 9.25b	139.02 ± 8.56a	110.07 ± 8.56b	150.33 ± 8.56a
UFA	282.97 ± 10.81	318.45 ± 10.01	304.86 ± 10.01	297.16 ± 10.01
Σn-3	15.35 ± 1.42c	19.68 ± 1.32b	12.91 ± 1.32c	31.97 ± 1.32a
Σn-6	93.49 ± 8.30	116.08 ± 7.68	94.53 ± 7.68	116.14 ± 7.68
Σn-6/Σn-3 ratio	6.45 ± 0.39ab	5.92 ± 0.36b	7.28 ± 0.36a	3.63 ± 0.36c
AI	0.61 ± 0.03ab	0.54 ± 0.03b	0.64 ± 0.03a	0.53 ± 0.03b
TI	1.55 ± 0.07a	1.34 ± 0.07b	1.64 ± 0.07a	1.15 ± 0.07b
PUFA/SFA	0.41 ± 0.06b	0.51 ± 0.06b	0.38 ± 0.06b	0.60 ± 0.06°

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; UFA: Unsaturated Fatty Acids.

Different letters following numbers in the same row indicate significant differences between means (P<0.05)

The fatty acid profile of intramuscular fat and some nutritional indexes of Sarda young bulls subjected to different feeding systems are reported in Table 6.

The predominant fatty acids in intramuscular fat were palmitic, (C16:0, 130.3 ± 5.4,, means±SE. mg g total lipid^{-1}) and stearic (C18:0, 136.6 ± 2.3) acids as SFA, oleic acid (C18:1 9c, 158.1 ± 9.7) as MUFA, linoleic (C18:2 9c,12c 89.3 ± 4.8) and arachidonic (C20:4n_15.8 ± 1.8) acids as PUFA. Similar results were reported previously for beef cattle

(e.g. Realini et al, 2004; Nuernberg et al., 2005; Alfaia et al., 2006;). Total SFA and MUFA were similar among group contrary to previous results found comparing concentrate-fed cattle with grazing-fed ones (French et al., 2000; Steen et al., 2003; Scollan et al., 2006). As stated by several authors, bovine feeding in pasture systems showed higher polyunsaturated fatty acids (PUFA) content in meat in comparison to bovines fed on grain-based diets (Realini et al., 2004; Alfaia et al., 2006; Enser, 2000 and Yang et al. 2002 cited by das Graças Padre et al., 2006). Lorenzen et al. (2007 cited by Alfaia et al., 2009) showed that meat from cattle finished on pasture had higher concentrations of PUFA than cattle finished on feedlot diets. In addition, the sum of n-3 PUFA is increased with the inclusion of grass to the diet, whereas the proportion of n-6 PUFA was unchanged for all treatments. According to Eriksson and Pickova (2007), this higher PUFA content in meat from pasture-fed bulls may be due to the higher protection from ruminal biohydrogenation of the fatty acids contained in fresh grass, relative to those in grain or silage. Moreover, the presence of secondary plant metabolites in the pastures might inhibit microbial biohydrogenation activity within the rumen (Lourenço et al., 2008 cited by Alfaia et al., 2009; Cabiddu et al., 2010). Saturated hypercholesterolemic fatty acids (C12:0, C14:0 and palmitic acid) were not affected by the dietary treatment, despite a tendency to increase with the increase of percentage of concentrate in the diet, showing the lowest values (not statistically significant) in pastured-animals (PAS group). In addition, meat from animals fed on concentrate diets had higher percentages of oleic acid than meat from grazing bulls. Concentrate diets are expected to increase the absorption of oleic acid: according to Duckett et al. (2009) the stearoyl-CoA desaturase (a major lipogenic enzyme that forms oleic acid from stearic acid, Wood et al., 2008) mRNA expression is 46-fold greater in subcutaneous adipose tissue of steers finished high concentrate diets compared with pasture-finished.

The content of trans 11 octadecenoate (C18:1 11t, vaccenic acid), one of the major intermediates formed during rumen biohydrogenation of C18 PUFA (Bessa et al., 2000 cited by Alfaia et al.,2006), was not statistically different among experimental groups (table 5).

Linoleic acid (C18:2 9c,12c) concentration did not differ among experimental groups as reported by others authors who have studied different finishing systems (Mitchell et al.,

1991 cited by Duckett et al., 2009; Mandell et al., 1998; Duckett et al. 2009). Conversely linolenic acid (C18:3 9c,12c,15c) content was the highest in pastured-animals, in agreement to Wood and Enser (1997) who claim that it is well known that green pastures are a good source of linolenic acid, in contrast to concentrate diets, and this explains the inverse relationship found in their work between the duration of finishing period on concentrate and the levels of linolenic acid.

Even Mandell et al. (1998) and Duckett et al. (1993 cited by Duckett et al., 2009) have shown that grain feeding reduce linolenic acid concentrations in LD muscle.

Arachidonic acid (C20:4 5c,8c,11c,14c) content was lower in FC3groups than FC2 and PAS groups contrary to Duckett et al. (2009) who found differences in arachidonic acid percentages between pastured animals and concentrate-fed animals.

Concentrations of n-3 fatty acids (DPA, DHA, derived by elongation and desaturation of linolenic acid) have followed the same trend: their content were greater for PAS and FC2 groups than the others. Duckett et al. (2009) found greater percentage of all n-3 fatty acids in animals at pasture than concentrate-fed animals.

Total n-6 PUFA content was unchanged between feeding systems while total n-3 PUFA was higher for PAS than other groups (table 6), likely due to an increase in all individual n-3 fatty acids in PAS group. According to our results, French et al. (2000) found higher linolenic and total n-3 fatty acid in LD muscle of steers fed only grass respect to various supplementation systems and any difference in linoleic and total n-6 fatty acid concentrations.

Also Nuernberg et al., (2005) reported an increase in the percentage of linolenic acid C18:3n-3, EPA C20:5n-3 and DHA C22:6n-3 in bulls fed a grass-based diet compared with those fed concentrate. Similar results for the percentage of C22:5n-3 (DPA) were also found by Alfaia et al. (2006) in grass-fed bulls when compared to concentrate-fed animals and by Realini et al., 2004. In the same work (Enser et al. 1998 cited by Realini et al., 2004) was reported that adipose tissues from pasture based diets had higher concentrations of n-3 PUFA in body tissues, while concentrate-based diets had higher concentrations of n-6 PUFA. These differences are a consequence of the fatty acid composition of the diet, α-linolenic acid

(C18:3, the n-3 series precursor) being the major fatty acid in grass lipids (constituting over 50% of total fatty acids in forage and its products, Scollan et al., 2006), and linoleic acid (C18:2, the n-6 series precursor) being a major component in grains (Marmer et al., 1984). However, according with Boufaied et al. (2003 cited by Blanco et al., 2010) we should be cautious in the comparison with other studies because forage species and conservation method may both affect fatty acid contents.

Since part of dietary linolenic acid escapes from ruminal hydrogenation, meat from forage-fed cattle has greater linolenic acid than that from concentrate-fed cattle (Mandell et al., 1998; Scollan et al.,

2006; Blanco et al., 2010).

The ratios of PUFA/SFA and n-6/n-3 are indexes widely used to evaluate the nutritional value of fat for human consumption.

Low ratios of PUFA/SFA (and high levels of cholesterol) have been considered as major risk factors of cardiovascular diseases, which are among the most important causes of human mortality in developed countries (Ganji et al., 2003 cited by Alfaia et al., 2006). Moreover a very high n-6/n-3 ratio favors the development of cardiovascular diseases, cancer, inflammatory and autoimmune diseases (Simopoulos, 2002 cited by Alfaia et al., 2006).

According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the n-6/n-3 ratio should be lower than 4, indicated as the most favorable by Committee on Medical Aspect of Food Policy (COMA) in order to prevent some cardiovascular diseases (British Department of Health, 1994 cited by Manca, 2011).

Both the n-6/n-3 ratio and the PUFA/SFA ratio were affected by feeding systems (table 6): the PAS group showed the lowest n-6/n-3 ratio and the highest PUFA/SFA ratio. Many authors have reported similar reductions in n-6/n-3 ratios for grass-fed beef compared with concentrate-finished ones (Wood and Enser, 1997; French et al., 2000; Duckett et al. 2003; Realini et al., 2004). Noci et al. (2005) found a linear decrease in n-6/n-3 ratios with increasing duration of grazing days.

Contrary to what happens for the other groups, the PUFA/SFA ratio and the n-6/n-3 ratio detected in PAS group were respectively above and below the recommended values for these nutritional indexes.

The other nutritional indexes (Atherogenicity index, AI and Trombogenic index, TI) have shown differences statistically significant among experimental groups (table 6), and the FC3 group was characterized (together with FC1 group) by the higher value of these indexes.

Considering that low values of AI and TI are recommended for a healthy diet, and that the values reported for PAS in our study are lower than those reported in Marchigiana young bulls (Cutrignelli et al., 2008; Badiani et al., 2002 and in Chianina young bulls (Poli et al., 1996), therefore, from a nutritional point of view, Sarda beef obtained at pasture seems to be very healthful.

The CLA 9c 11t content (rumenic acid, the major CLA isomer, Alfaia et al., 2006) did not differ among feeding strategies, contrary to results of several authors (French et al., 2000; Realini et al., 2004; Noci et al., 2005; Duckett et al. 2009) and showed lower value in comparison to those reported by others authors (Rule et al., 2002; Realini et al., 2004; Alfaia et al., 2006; Blanco et al., 2010 and Duckett et al., 2009).

The reason for the lack of different CLA cis-9, trans-11 content among experimental groups of Sarda young bulls remains unclear even if is in agreement with Blanco et al., 2010. In that study, as in ours, both vaccenic and linoleic acid contents were similar among different experimental groups. The authors stated that "the primary source of CLA found in beef is endogenous synthesis from vaccenic acid", (Enser et al., 1999; Daley et al., 2010). CLA is also formed during the biohydrogenation of linoleic acid in the rumen (Daley et al., 2010) but the synthesis from vaccenic acid in tissue is "quantitatively the most important contributor to tissue level (Wood et al., 2008). But the proportion of CLA deposited depends upon several factors as the basal diet, the breed, the age, sex and type of forage not only upon content of precursors (Scollan et al., 2006).

Table 7. Effect of diet on meat quality parameters at equal energy intake kg live weight^{-1}

	hay concentrate	pasture	s.e.	P
C16:0	50	51,2	0,76	0,17
C18:1 9c	164.69	133.87	10.5	0.02
C18:3 9c12c15c	9.40	22.34	1.15	< 0.0001
C20:4 5c,8c,11c,14c	15.13	18.88	1.91	0.09
C20:5 5c,8c,11c,14c,17c EPA	0.24	0.49	6.28	0.0008
Σn-3	16.35	31.97	1.73	0.0001
TI	1.48	1.15	0.07	0.004
Cohesiviness	0.52	0.52	0.005	0.47

When the data were adjusted to an equal energy intake there was significant difference between the pasture- and hay-concentrate treatments in terms of the intramuscular fat (Table 5). The difference of oleic acid, linolenic acid, EPA, total n-3 content and of TI value, tested with paired *t*-test, are resulted statistically different (Table 7). In other words, the pasture has affected directly these parameters through its nutrient composition and not indirectly by means of different nutrition levels.

In particular the feeding system based on pasture has decreased the oleic acid, increased linoleic acid and EPA content in LD muscle of Sarda young bulls; morevover the pasture has lowered the TI value, helping to make the meat of Sarda young bulls more healthful.

5.5 Conclusions

The experimental work has allowed to improve the knowledge on meat traits of Sarda breed. Moreover the results of this study support the hypothesis that the pasture may be a chance for the cattle livestock system in Sardinia. The pasture-fed animals, in addition to have productive performance comparable to concentrate-fed animals (see chapter 5), have shown specific characteristics of meat, i.e. more content of n-3 PUFA, beneficial for the human health, better value of some nutritional indexes as PUFA/SFA ratio and n-6/n-3 ratio, that improve both nutritional characteristics and consumer perception of the meat produced by Sarda young bulls.

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Chapter 6

Performances of Sardo-Bruna Beef with diurnal grazing

6.1 Introduction

Sardinia, with about 250,000 cattle, of which 60,000 cows addressed to meat production, has a self-supply rate equal to only 48% of its beef meat consumption (Rassu et al., 2011). Usually farmers prefer to sell calves at weaning to fattening centers of other Regions, due to the high costs of local fattening. Therefore, the use of pasture (alone or supplemented with concentrate) in the fattening of animals could represent a chance for Sardinian beef cattle livestock system. The pasture-based system could help to reduce feeding costs, contribute to the stability of the soil by reducing erosion, play an active role in limiting fuel biomass helping to prevent fire danger (Franca et al., 2012) and provide sustainable livestock production from an economic and environmental point of view, specially in hilly and mountainous areas (Braghieri et al., 2007).

Finally pasture-based systems are nowadays considered more environmentally friendly, providing also animal welfare, and hence begin to be socially more acceptable than more intensive systems (Tallowin et al., 2005). Unfortunately the seasonality of forage production in Sardinia does not meet the total requirements of growing and fattening animals (Rassu et al., 2011) and pasture-based systems are usually limited in herbage availability and accessibility which has been considered as a major factor impacting animal productivity (Soder et al., 2009); this implies the need to often integrate animals with hay and concentrate.

The Sardo-Bruna breed originates by the introduction in Sardinia, in the second half of last century, of Brown Swiss breed bulls to improve the milk and meat production of Sarda breed. Through a substitution crossing has given rise to an animal heavier respect to Sarda breed and with best development of the hindquarters, the Bruno-Sarda breed.

Despite the Sardo-Bruna breed is the main cattle population used for meat production (as pure and/or as cross bred) in the Island, little or nothing is known about its production performance.

To evaluate productive and economic performance for meat production of Sardo-Bruna beef fattened with and without diurnal grazing a study was carried out from December to August 2011 in a private farm located in the centre of Sardinia.

6.2 Materials and methods

6.2.1 Experimental site

The present study was conducted at an private farm located in the centre of Sardinia, near Ghilarza (latitude 40°06' N, longitude 8°47' E, altitude 271 m above sea level).

6.2.2 Animals

After weaning (average age 8.5 months), 10 Sardo-Bruna young bulls were blocked in 2 homogeneous groups on the basis of live weight and submitted to the following feeding treatments:

- group MC (Male Concentrate)

5 Sardo-Bruna young bulls with a live weight of 291±26 kg (mean±s.d) were stall-fed with a diet composed by a mixture of barley and corn meal (50/502.6-5.5 kg head^{-1} day^{-1}), a complete mixed feed (1.2-1.5 kg head^{-1} day^{-1}, field peas (0.5-0.7 kg head^{-1} day^{-1}) and meadow hay (2.1-3.5 kg head^{-1} day^{-1}). The amounts of feed offered to the animals changed according to live weight and Average Daily Gain (ADG) detected

- group MP (Male Pasture)

5 Sardo-Bruna young bulls with a live weight of 287±26 kg were fed at pasture during daylight (from 9.00 to 17.00 a.m.) in a natural pasture with a stocking rate of 1.1 head ha^{-1}. During the rest of the day the animals were housed and received as supplementation a mixture of barley and corn meal (50/501.5-6.7 kg head^{-1} day^{-1}), a complete mixed feed (0.6-1.5 kg head^{-1} day^{-1}) and meadow hay (1-2 kg head^{-1} day^{-1}). The concentrates were administered before and after daily grazing whereas the hay was administered only in the evening. The amounts of feed offered to the animals

changed in accordance with the herbage quality of the pasture, with live weight and Average Daily Gain (ADG) detected

At the end of the experiment the animals were slaughtered at an EU-licensed abattoir at about 500 kg live weight, considered suitable for the males of Sardo-Bruna breed.

6.2.3 Measurements

Live weight (kg) of the animals were detected at the beginning of experimental period, every three months and at slaughtering. Average daily gains (g $head^{-1}$ day^{-1}) of the animals were then calculated.

On 3 occasions during the experiment samples of pasture herbage were hand-plucked. Samples of all feedstuff offered to the animals were also collected. The chemical composition of herbage and feeds (SS, CP, NDF, ADF, AOAC 1990) was measured.

After one month of the experiment beginning, and every two month until the slaughtering the total feed consumption was recorded and average group feed consumption was estimated.

72 hours after slaughtering the cold carcass weight (CCW) and from this the cold dressing percentage were measured (calculated as cold carcass to slaughter weight ratio X 100), and the pH value (pHu) at level of last rib of *Longissimus dorsi*, using Eutech pH 600 with a penetrating probe was detected.

Meat colour was measured after 72h from slaughtering, on M. *Longissimus dorsi* (LD) at level of last rib. Color measurements were made on the muscle surface using a Minolta CM-400 colorimeter (KonicaMinolta Sensing Inc., Tokyo, Japan) with C illuminant and 2° standard observer in the CIE L*, a*, and b* space (L* lightness is a measure of the light reflected (100=white; 0=black); a* redness, (measures positive red and negative green); and b* yellowness, (measures positive yellow, negative blue).

6.2.4 Economic evaluation

On the basis of feed cost and beef meat prices (expressed as kg of live weight) (personal communication of the farmer), an economic evaluation of the 2 experimental feeding regimen was calculated.

6.2.5 Statistical analysis

All data were analyzed by one way ANOVA procedure to evaluate differences between experimental groups. Significant differences are at $P < 0.05$ unless indicated otherwise.

6.3 Results and discussions

6.3.1 Feed composition and consumption

The chemical composition of the experimental feed is summarized in table 1. The quality of hay used in the experiment was low considering the level of CP (Table 1); this is unfortunately common in areas similar to that of the experiment as found by others authors (Casu et al., 1981; Ligios et al.,1997; Sulas et al.,1997).

Herbage quality showed a decay from December to June, typical of Mediterranean pastures (Molle et al., 1998; Molle et al. 2002).with a decrease of CP and an increase of NDF content (Table 1).

Table 1 Chemical composition[1] of the experimental feeds.

	SS	NDF	ADF	CP
Mixture of barley-corn meal	87.8	22.1	7.7	14.4
Complete mixed feed	86.6	26.7	12.5	17.1
Field peas	86.0	13.9	10.1	25.6
Meadow hay	89.5	67.4	46.3	7.9
Herbage december	10.8	44.9	24.0	30.3
Herbage may	18,7	48,4	30,0	15,2
Herbage june	38,9	68,6	44,4	7,5

[1] All nutrient concentrations are on a DM basis

Total feed consumption of the animals during the experimental period is reported in Table 2. As expected MC group showed an higher consumption of concentrates and hay

Table 2. Total food consumption of the experimental groups during the trial (kg head^{-1} as fed, means ± s.d.).

Feeds	MP	MC	St.dev.
Total concentrate	970b	1259a	68
Meadow hay	411b	620a	15

Different letters following numbers in the same row indicate significant differences between means ($P<0.05$)

6.3.2 Live weight and growth rate

The trend of the live weights is shown in figure 1. At slaughtering body weight was in accordance with that fixed as target (MP kg 536±17, MC kg 560±17 means±standard deviation).

As reported in other trials that compared concentrate- and grass-diet (Muir et al., 1998; Cutrignelli et al., 2008; Cruz et al., 2010), the group MC showed higher daily gain (Figure 2) than MP group. As a result the finishing period for MC and MP lasted 202 and 244 days respectively, the MC animals reaching the target weight for the slaughtering 42 days before the MP animals. These results are in line with previous research (Bidner *et al.*, 1986 cited by Cooke et al., 2004; Steen 1995; Fausto da Silva et al., 1998; Keane and Allen, 1998; Sami et al., 2004; Cutrignelli et al., 2008).

The average daily gain of Bruno-Sarda bulls in MC group (1.33 kg head^{-1} day^{-1} Figure 2) is similar to what occur in the more specialized fattening centers of the north Italy (1.38 kg head^{-1} day^{-1}, SMEA, 2011) with beef breed as Charolais and Limousin. ADG in MP group was in agreement with results of similar breeds in similar conditions (Keane, 1998; Raskin et al., 1998; Dawson and Steen. 1998; Estermann et al., 2001; Braghieri et al., 2007; Steinshamn et al., 2010; Boland and Scaglia, 2011).

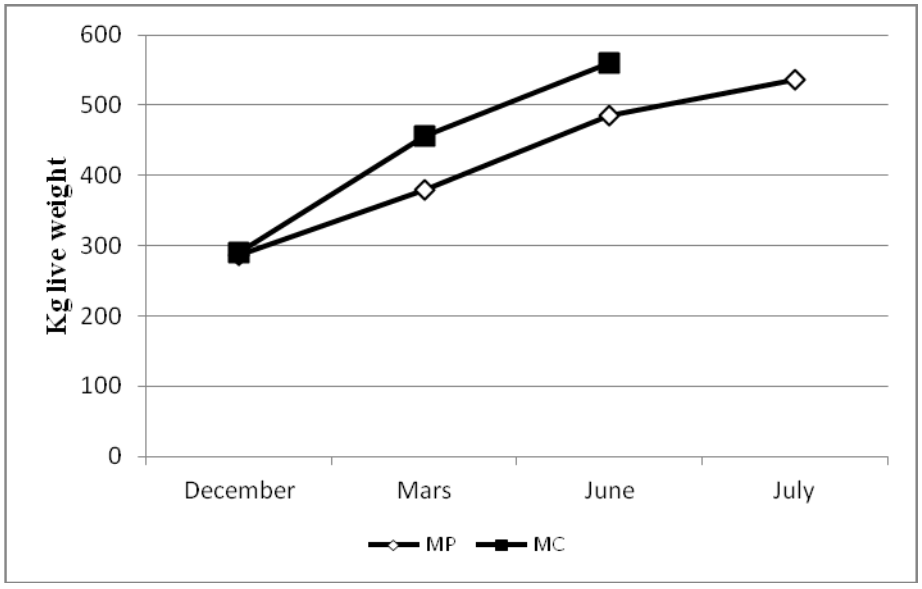

Figure 1. Live weight trend of experimental groups

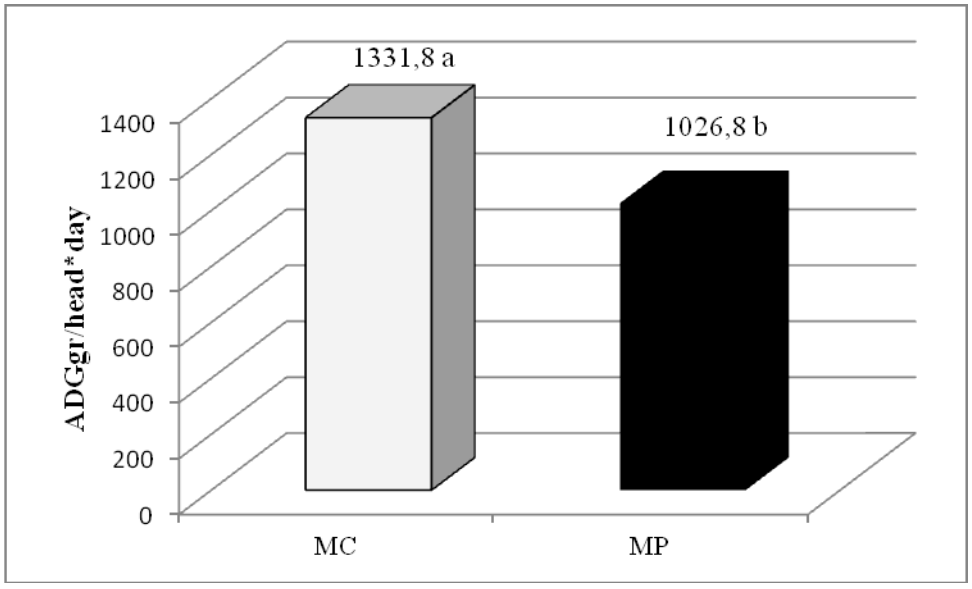

Figure 2. Average daily gain (ADG) of experimental groups

6.3.3 Slaughtering traits

The animals were slaughtered at a EU-licensed abattoir at live weight of 536±17 and 563±17 kg (means±standard deviation) for MP and MC respectively.

The cold dressing percentage values were those expected for a not specialized breed and are in agreement with others trials (Serra et al., 2001; Cifuni et al., 2004) carried out in similar conditions.

Table 3. Results at slaughter of experimental groups (means±standard deviation)

	MP	MC
Live weight (kg)	536±17	563±17
Warm carcass weight (kg)	289±30	295±15
Cold carcass weight (kg)	283±29	289±14
Cold dressing percentage (%)	52.8±1.4	51.2±1.0

The pHu values of MP and MC group were in the normal range (Table 4, Muir et al., 1998; Daly et al., 1999; Mazzette 2004; Sami et al., 2004).

The lack of feeding system effect on meat pHu in this study agreed with French et al. (2000). The decrease in pH of the meat derives from the postmortem conversion of glycogen to lactate and H^+, so the glycogen level at slaughter is inversely related to the ultimate pH.

Moreover the glycogen levels is linked to plane of nutrition; but plane of nutrition, even in terms of short-term fasting, appears to have little effect on ultimate muscle pH provided cattle are not stressed (Muir et al. 1998). Muscle glycogen concentrations are reduced in stressed animals, thus reducing the potential for pH decline in muscles post mortem. Bowling et al. (1977) suggested that grain-finished cattle are less susceptible to pre-slaughter stress because they become accustomed to people, pens, and confinement respect to cattle finished at pasture. Moreover lower muscle glycogen concentration of extensively fed animals is often associated with the lower dietary energy intake (Sami et al., 2004).

MP animals were housed during the night and received the supplementation, becoming accustomed to people and facilities and thus less susceptible to stress; furthermore their performances argue that the dietary energy intake was comparable to that of MC animals. In agreement with Muir et al. (1998) “when the confounding effects of grain-feeding on carcass weight are removed (i.e., carcasses are compared at the same weight and fat cover), there are not consistent effects of feed type on pH.”

No significant differences in meat color (Table 4) were detected between the MC and MP groups. The color parameters were in accordance with other authors (Daly et al.,1999; Vestergaard et al., 2000; Mazzette 2004; Sami et al., 2004). In a review on the effects of feeding systems on meat color, based on 35 experiments which report the effect of pasture (100%) vs concentrate finishing systems, Priolo et al., (2001), show that the meat from animals finished on pasture is darker than that from animals finished on concentrate. They also affirm that the effect of diet on meat color is considered rare because is also influenced by indirect effect as carcass fatness, pH, animal age and carcass weight.

In this trial the pasture did not represent the 100% of diet and furthermore pH value, animal age and carcass weight were not different between the experimental groups. This could explain why we have not detected significant differences in color measurement. Moreover, althoug we have not measured the carcass fatness, we agree with the statement of Priolo et al. (2001): "the effect of carcass fatness seems not to be extremely important for meat color". Regard to the fact that some authors consider the phsycal activity of grazing animals as a possible factor affecting meat colour, Coulon and Priolo (2002) in accordance with Vestergaard et al. (2000) state that the phsycal activity play an indirect role, changing the proportion among the different type of muscle fibres.

Unfortunately we have not investigated this aspect so the only thing we can say is that, in this work, the pasture did not affect the meat colour.

Table 4. pHu (72 h after slaughter) and color parameters (CIEL*a*b* system) measured on *Longissimus dorsi* muscle of MP and MC animals (means ± s.d.)

	MP	MC
pHu	5.50±0.08	5.45±0.05
L*	39.71±2.03	39.00±3.68
a*	18.72±2.50	20.09±2.34
b*	6.16±3.67	7.70±2.01

Overall the MP and MC groups present no differences in slaughtering traits, confirming that the use of pasture could help to adequately finish the Bruno-sarda young bulls.

6.3.4 Economic aspects

The cost of all feeds used in the period of the study as well as the price of beef meat is reported in Table 5.

Table 5 Feed costs and prices of beef meat *per* kg of live weight during the experimental period.

Feeds	€/kg
Mixture of barley-corn meal	0.20
Complete mixed feed	0.25
Field peas	0.25
Meadow hay	0.15
Beef meat	2.50

As reported in Table 2 the MP group has consumed 21% less concentrate and 34% less hay than MC group with lower feeding costs of about 25% (Table 4).

The daily cost of supplementation, was greater in MC than in MP 1.82±0.02 vs 1.08±0.02, (€ $head^{-1}$ day^{-1}, means±standard deviation; $P<0.001$) as a consequence of a higher average intake of concentrate food (MC 6.0±0.6, MP 3.9±0.6 kg $head^{-1}$ day^{-1}). If the cost is considered per kilo of growth no differences are detected between groups (Table 6) because of the higher ADG detected in MC group.

The cost per kilo of growth in MC was higher than what found in other experimental trials (1.11 €*kg of daily $gain^{-1}$; Lotti et al., 2011) that were indeed conducted using beef breed with a greater growth capacity, while the MP value was lesser, confirming the usefulness of grazing to reduce feeding costs.

Assuming a beef meat price of 2.5 € kg^{-1} live weight, the income during fattening — defined as difference between increase value (derived from live weight gain X beef meat price) and cost of supplementation — was higher (but not statistically different) in MP group than MC one (Table 7).

Table 6. Feeding cost of experimental trial

	MP	MC	St.dev.
Total cost of supplementation (€ head^{-1})	267b	368a	16
Daily cost of supplementation (€ head^{-1} day^{-1})	1.08b	1.82a	0.02
Cost of supplementation per kilo daily gain (€ kg^{-1})	1.09	1.37	0.1

Table 7. Economic results of MP and MC groups

	MP	MC	St.dev.
Increase value from live weight gain (€ head^{-1})	623b	672a	40
Total cost of supplementation (€ head^{-1})	267b	368a	16
Overall income during fattening[1] (€ head^{-1})	356	304	35

[1] Defined as difference between increase value and cost of supplementation

It is noteworthy that the feeding costs of experimental groups showed an opposite trends (Fig. 3); the MC feeding costs showed a first period (0-120 days) of strong increase followed by a second period (120 days-slaughtering) characterized by more moderate increases (Fig. 3). The trend of MP feeding costs showed conversely an opposite trend, with a first phase (0-120 days) to moderate increase of feeding costs and a second one (120-slaughtering) characterized by greater rise in costs.

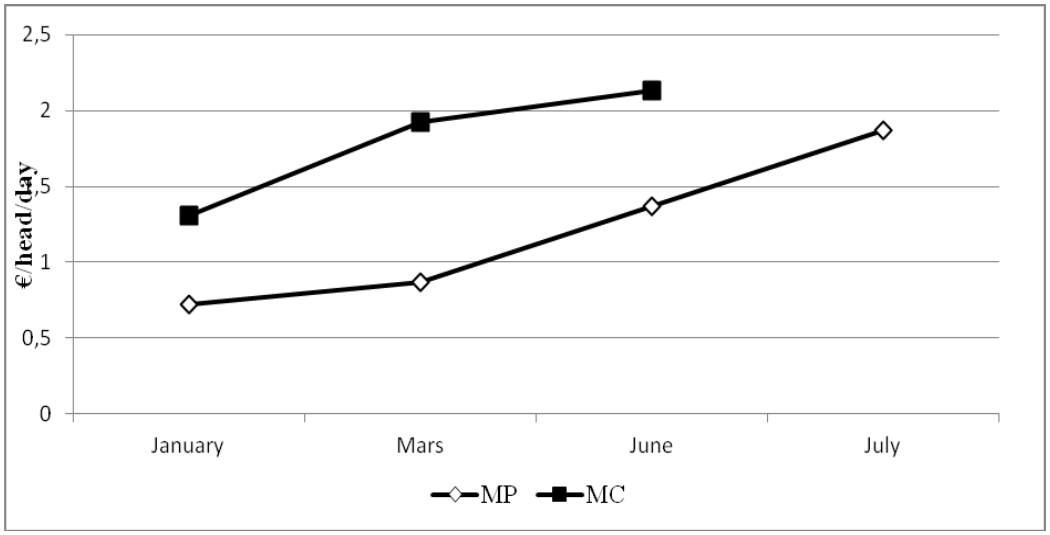

Figure 3. Trends of MC and MP feeding costs in the experimental period.

The pattern of MC feeding costs is probably due to the slowdown in the growth rate of animals, as expected in this breed after 12-14 months. In the first phase of growth (1-120

days) where the MC animals showed high ADG (1.45 kg head^{-1} day^{-1}), was necessary to increase the amount of concentrate administered to support this growth rate. Conversely the second phase, characterized by a reduced ADG (1.18 kg head^{-1} day^{-1}), resulted in lower increase of the concentrate administered.

The MP feeding costs trend is derived from the worsening of herbage quality (Table 1) in the second period which entailed an increase in the use of concentrate purchased out of the farm.

Therefore the difference between the MC and MP feeding costs, because of lowered growth rate of MC group and worsening of herbage quality, is tended to decrease (from 1.05 € head^{-1} day^{-1} in Mars to 0.76 € head^{-1} day^{-1} in June), making less attractive the grazing at least from an economic point of view.

The suggestions for the Sardinian farmers to avoid this problem could be:

- to slaughter the animals fed at pasture before the decay of grass quality. The risk is that the animals may not have reached yet the commercial maturity expected.

- To program the reproductive activity in order to anticipate the turned out to pasture of the weaned animals, exploiting also the autumn phase of the vegetative herbage growth. Actually only the 75% of the farms plan the reproductive activity (Rassu et al., 2004) and very few use artificial insemination (AI). On the other hand to have the weaning in autumn means to have the calving season in late spring, when the pasture could not meet the requirements of suckled cows (Rassu et al., 2004).

- To feed the animals in finishing period with barley or triticale silage. These winter cereals can be cultivated without irrigation and the silage, suitable to finishing animals, is able to reduce feeding costs.

6.4 Conclusions

The results of the study confirm the potential of Bruno-Sarda breed for meat production and the need of further studies for its better exploitation.

Food supplementation to grazing young bulls may be considered an efficient and economical rearing technique of Sardo-Bruna breed on Sardinia dry farms. About this topic, other studies are required to improve productive performance during first period of fattening without compromising economic ones.

If cattle fed on pasture and supplemented with concentrates and hay can achieve high growth rates, so that they achieve acceptable slaughter weights and degree of finish at a similar age to stall-fed cattle, it is likely that beef produced on pasture can be of comparable eating quality to that produced off a grain diet.

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