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Nutrition and management strategies to improve health and meat quality in intensively reared ruminants

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CHAPTER 1

Foreword

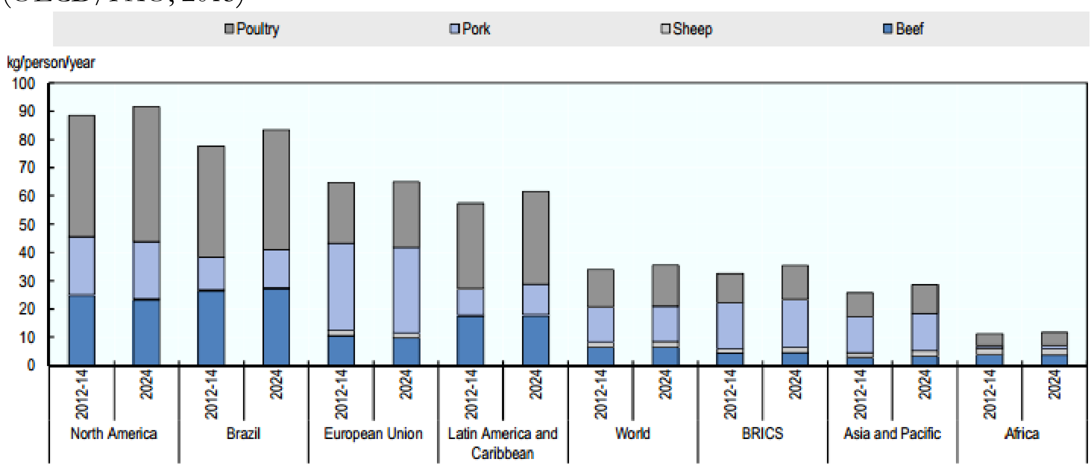
1. Foreword

1.1. Quality today: the critical aspects in ruminant's meat production chain

Sustainability is the capacity of a system to endure, and durability of meat industry is determined by consumers' demand and profitability. The United Nations "Our Common Future" report (Brundtlan et al., 1987), stated that a sustainable development is the development "that meets the needs of the present without compromising the ability of future generations to meet their own needs". Nowadays actors of meat supply chain need both to improve consumers' acceptability and reduce inefficiencies and losses of production, in order to ensure to the future generations of farmers, processors and retailers the opportunity to keep active the business.

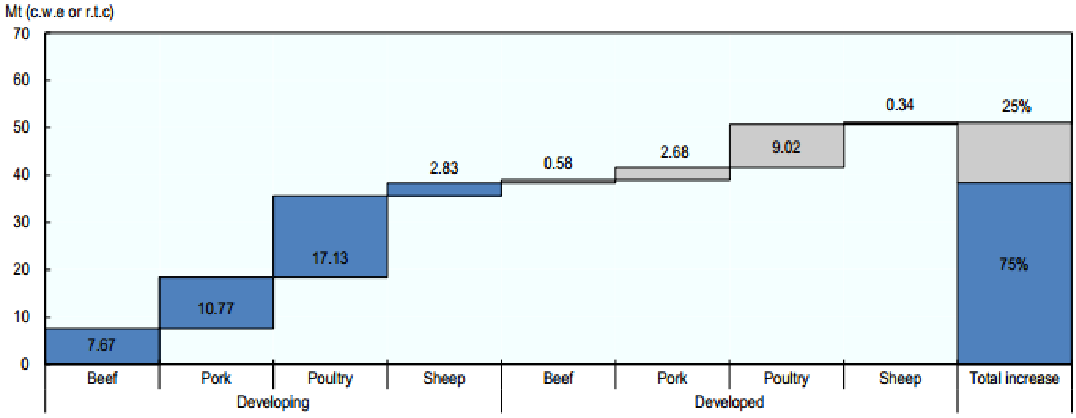
Beef and veal account for 22.1% (67 million ton of carcass weight equivalent) and lamb for the 4.6% (nearly 14 million ton of carcass weight equivalent) of world beef production and OECD/FAO (2015) forecasted a growth in bovine and lamb meat consumption in the next ten years (Fig. 1). Despite an overall increasing in the consumption of meat from ruminants, different trends are expected for developed and developing countries with a forecasted slightly reduction for the former and a rising for the latter. This scenario imply that bovine and lamb supply chains have both to find the way to buck the declining consumption in the developed countries and, on the other hand, improve production efficiency to fulfill the rising demand on developing ones.

Figure 1 Per capita meat consumed in the world. 2024 forecast vs. base period 2012-14 (OECD/FAO, 2015)



If, on overall, the scenario in the next ten years can be considered favorable for bovine and lamb meat industry, looking into the intensive farming system the future doesn't appear so bright. OECD/FAO (2015), indeed, forecasted only a slight increase in beef and lamb production in developed countries (Fig. 2), due to the limited resources, and the rising will be achievable only implementing production efficiency. Moreover, stagnant or declining demand in developed countries, represents a significant limiting factor because of it limits the opportunity to increase added value, as developing countries mainly demand for affordable meat.

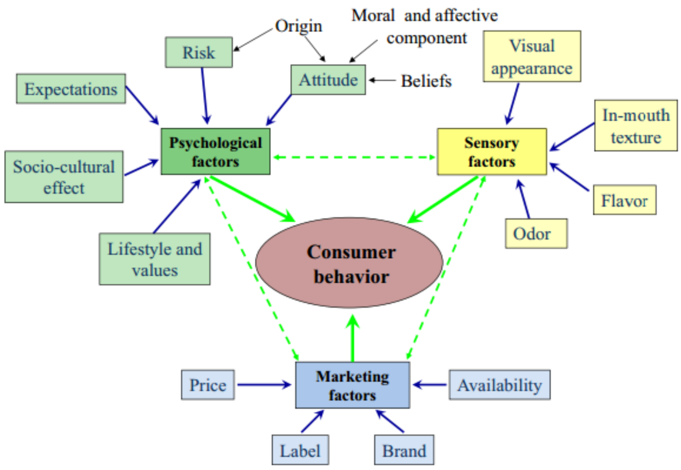
Figure 2 Growth of meat production by region and meat type. 2024 forecast vs. base period 2012-14 (OECD/FAO, 2015). (Mt=10^6 ton; c.w.e=carcass weight equivalent; r.t.c=ready to cook equivalent)



The concept of "meat quality" evolved and became more and more complex in the last decades. It moved from just "eating quality" to include several aspects as safety (especially during '90 and '00), ethics and nutritional characteristics. Therefore, as reviewed by Font-i-Furnols and Guerrero (2014), perceived quality by the consumers is nowadays a multifactorial complex and all the involved factors affect purchase choice and willingness to pay for meat (Fig. 3), driving therefore meat demand and profitability along the supply chain.

The improvement of perceived quality is therefore fundamental, both to satisfy consumers' expectations and to guarantee profitability to all the operators of the supply chain and the related allied industries. As is possible to see from Fig. 3, in the building of a modern concept of quality, the canonical sensory quality is nowadays accompanied by extrinsic psychological and marketing factors, more connected with the production system instead of the meat intrinsic characteristics.

Figure 3 Multidisciplinary model of the main factors affecting consumer behavior in a food domain (Font-i-Furnols and Guerrero, 2014)



Intensive farms are characterized by high production efficiency and large scale units can guarantee economy of scale with consequent increase in net return. For instance, in 2013 US produced nearly 11.7 million of ton of carcass weight equivalent of beef with a cattle population of nearly 90 million of heads, while Brazil produced 9.7 million of ton with a cattle population of 211 million of heads (FAOSTAT, 2013). Given that most of the beef cattle in US are produced in intensive feedlots, while in Brazil they are mostly extensively reared it gives an idea about the efficiency of intensive farms. Given the expected population growth to 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations, 2015), with consequent rising of food demand, meat from ruminants included, an increase of production efficiency is critical to guarantee food security. In this scenario, Smith et al. (2010) concluded that "Given the need to feed 9 billion people by the middle of this century, and increasing competition for land to deliver non-food ecosystem services, it is clear that per-area agricultural productivity needs to be maintained where it is already close to optimal, or increased in the large proportion of the world where it is suboptimal". Therefore, a farming intensification will be necessary in the future. Considering intensive farming system, the main factors impairing perceived quality are represented by: environmental impact, animal welfare, antibiotics utilization and sensory and nutritional quality of meat, while the improvement over the last decade enhanced consumers' confidence in meat safety, not anymore a major concern (Verbeke et al., 2010).

An improvement in these aspects will help to buck the declining trend in beef, veal and lamb consumption in western countries, mainly driven by ethical and nutritional reasons, and, contemporary, to satisfy the demand from developing countries because it will lead to an improvement of production efficiency too.

1.1.1. Environmental impact

In the latest years there is a growing concern about the environmental impact of meat production, especially from ruminants, even if one of the most impacting report in the bulding of this perception was clearly biased. As reviewed by DiLorenzo et al. (2014) the FAO report of 2006 (Steinfeld et al., 2006) overestimated by 5 times the livestock contribution to the overall greenhouse gasses emission (GHG). Indeed, the 2014 report from US EPA (Environmental Protection Agency) estimated a 3.3% contribution of the livestock sector to the total US GHG emissions (EPA, 2014).

Ruminants release methane through rumen fermentation, however, approaching to this topic is necessary to consider that they are the only capable to extensively convert pasture and agro-industrial wastes into high value protein, such as milk and meat.

Methane emissions can be reduced through dietary strategies (Hirstov et al., 2013a) and specific manure (Montes et al., 2013) and livestock management (Hisrtov et al., 2013b).

Environmental impact represents one of the extrinsic factors affecting consumer perception of meat quality, as it is related with ethical aspects. A reduction of GHG emissions is beneficial both from a production efficiency and a market points of view. Indeed, White and Brady (2014) demonstrated that, across different continents, on average consumers were willing to pay 14.8% more for meat that has been specifically certified for low environmental impact (i.e., reduced water use). Always from the reported study, and in order to enforce the concept that frequently there is a mistaken idea within the consumer about the environmental impact, higher willingness to pay was evident for unspecific environmental certification (e.g. grass fed, organic…), production systems that are generally less efficient than conventional meat production (Capper, 2012).

1.1.2. Animal welfare

Animal welfare is a growing concern in western population. For instance, 65 to 87% of EU consumers declared themselves interested or very interested in farm animal welfare (Miele, 2010), while in US 95% of the participants to a national survey declared to be "very concerned" about the welfare of farm animals (American Humane Association, 2014) and 65% of the interviewed in a survey conducted by Kansas State University declared to be concerned about the welfare of beef cattle (McKendree et al., 2015). A lack of knowledge about contemporary farm practices, animal conditions and welfare legislation is widespread within the consumers and intensive and large scale farming is commonly associated with poor animal welfare, even by rural population (Miele, 2010; Vogt et al., 2011; Di Pasquale et al., 2014). Moreover, the few knowledge about farm practices is mainly driven

from mass media, generally involved only during real or perceived "scandals" (BSE, avian influenza, dioxin…). In the interviewed by Fox and Ward (2008), concern about animal welfare was the main reason for a shift to a vegetarian diet and several surveys demonstrated that a significant proportion of consumers seems willing to pay more (5 to 10%) for food from animals raised with high welfare standard (Martelli, 2009; Napolitano et al., 2010; Di Pasquale et al., 2014; Animal Welfare Institute, 2015).
Given these evidences, a clear and trustable certification of animal welfare at retail level represents an interesting marketing opportunity even for beef, veal and lamb industry. Although only 18 % of the interviewed identify beef cattle, 14% identified veal calves and 6% identified sheep answering to the question "In your opinion, from the following list, for which three farm animals should the current level of welfare/protection be improved the most?" in the 2005 EU survey (EC, 2005), more recently Di Pasquale et al. (2014) reported that only 14% of the interviewed in a regional survey in Italy attributed a very good level of welfare to beef cattle and veal calves. Therefore, "animal-friendly" seems to represent a good market opportunity for meat for ruminants as well. Welfare certified and animal-friendly labelled products, although still to a limited extend, are already available and European Economic and Social Committee (EESC) (2011) stated that "A labelling scheme is needed that gives consumers objective information to enable them to choose animal products that exceed EU minimum animal welfare requirements". Welfare certification systems around EU are or will be developed starting from WelfareQuality® assessing protocol, in which animal based measures are predominantly. Moreover, a significant proportion of consumers believe that animal friendly products are healthier and safer (Martelli et al., 2009; Di Pasquale et al., 2014) and "absence of disease" has been recognized by the consumers as the most important factors in determining animal welfare in the survey of Di Pasquale et al. (2014).
The reported evidences highlight how fundamental is, even from a quality perspective, the reduction of pathologies in order to guarantee high welfare standard, with the consequent positive impact from a perceived quality point of view. Of course, a reduction of pathologies will also improve production efficiency.

1.1.3. Antibiotics utilization

Antibiotics utilization in intensive rearing systems is strictly connected with morbidity and, therefore, animal welfare. Moreover, antibiotics utilization is a growing concern both for consumers, aware about residues in meat, and humane doctors. In the European Commission (EC) survey of 2006 (EC, 2006) consumers identified "residues of antibiotics and hormones" as one of the main warring risks associated to food intake.

Within intensively reared ruminants, veal calves represent the most critical production system, indeed a higher level of multiple bacterial resistance have been found in veal compared to conventional cattle (Pardon et al., 2014), and higher livestock associated methicillin-resistant Staphylococcus aureus (LA-MRSA) prevalence has been reported in veal producers compared to poultry, beef, dairy cattle and pig farmers (Vandendiressche et al., 2013). Intensive fattening of beef, veal and lambs is based on the purchase of young animals born in different farms and then transported, grouped to create homogeneous batches and then transported to the fattening units. Therefore, young animals are exposed to several stressors as transport, mixing with animals from different farms, feed restriction (especially for beef and lamb marketed from auction markets) and new diet and environment. All these stressors impair immune function and increase susceptibility to different diseases. Given these conditions, preventive strategies are needed to reduce the pathologies, thus improving animal welfare and limit antibiotics utilization in food producing animals.

1.1.4. Meat sensory and nutritional quality

Despite the highlighted role of psychological and ethical aspects, traditional indicators of meat quality as aspect and palatability still play a fundamental role in the building of consumer preference.

The main factors affecting consumers purchase intention based on visual appearance is represented by color (Dransfield et al., 1998;), likely because they relate it with freshness and wholesomeness (Mancini and Hunt, 2005). Generally, consumers associate a red–purple color with freshness while a brown color is associated with the opposite (Faustman and Cassens, 1990; Carpenter et al., 2001). Within red color, bright red seems to be preferred compared to pale (Grebitus, et al., 2013; Realini et al., 2014) or dark red (Killinger, et al., 2004). Banović et al. (2012) reported that, for regular beef consumers, color is used as an intrinsic cue to predict experienced eating quality, although Carpenter et al. (2001) found that eating satisfaction was not related with beef color.

Regarding lamb meat, color preference is affected by previous experience, as lighter lamb seems to be preferred in Spain (Bernués et al, 2012), while Khliji et al. (2010) showed that lamb color consumers' acceptability was related mainly with redness, and the reddest ($a^* \geq 9.5$) was the most preferred. These differences can be attributed to a different consumers' background, as Spanish lamb is mainly grain fed and slaughter younger than Australian prime lab, generally fed a pasture based diet and slaughter at 6 to 8 months of age.

If possible, color is even more important for veal calves, as it determines carcass value as white color is the more appreciated by the consumers,

while pink carcasses are discounted and mainly designed to ho.re.ca. market.
Taken together, these evidences underline that produce meat with a desirable color and improve its stability are crucial aspect to meet consumer satisfaction. Indeed, discount or mince portioned and packed meat at retail level because of a lack of color stability represent a significant economic loss both for beef (Smith et al., 2000) and lamb (Calnan et al. (2014) industry, while veal producers get a penalty for unacceptable red carcasses.
Other than color, fat content plays an important role in building consumer preference. On one side, a growing liking for lean meat was evident in these decades (Ngapo and Dransfield, 2006), likely due to the rising concern about fat in the diet, while on the other side some niche consumers prefer high marbled meat because of marbling degree is positively associated with tenderness and flavor liking in beef (Frank et al., 2014) and lamb (Hopkins et al., 2006). Preference for marbling varys between countries: European consumers tend to prefer less marbled beef (Ngapo and Dransfield, 2006; Realini et al., 2014), while highly marbled meat is particularly appreciated by US (Killinger et al., 2004) consumers.

Eating quality involves traits related with texture and taste. Focusing on meat from ruminants, it has been demonstrated that tenderness is the most important factors affecting consumer liking for beef (Dransfield et al., 1998; Reicks et al., 2011) and lamb (Thompson et al., 2005). In beef, veal and lamb tenderness is more critical compared to poultry and pork, because the latter two comes from younger animals (except for some special pork production, in which, however, most of the meat is processed and the older age is compensated by a higher marbling). Since it has been demonstrated that consumers are willing to pay more for tender meat (Dransfield et al., 1998; Feuz et al., 2004; Alfnes et al., 2005), increasing tenderness have to represent a priority target for beef veal and lamb industry in order to improve consumer satisfaction.
Other than texture traits, eating quality is also affected by aroma and flavor. If it is clear the relationship between tenderness and consumer liking, consumer response regarding aroma and flavor is more subjective. If we exclude taste defect as rancidity, bloody, livery or metallic flavor that, of course, negatively affect the acceptability, is hard to define the optimal aroma and flavor for meat from ruminants, while is more easy to stratify and create clusters of consumers based on their preference. Beef and lamb can be reared on feedlot or extensively on pasture, and these two different rearing systems lead to a different flavor and aroma. Looking into consumer preference, is evident that there is no a definitive predilection for one of the two tastes. Sañudo et al. (2007), in a study that involved consumers from six different European countries stated that consumers

can be divided into those who preferred "milk or concentrate taste" and those who preferred "grass taste." Similar distinction emerged from the study of Realini et al. (2009) In conclusion, these evidences underline that previous experience and familiarity with some different taste affect consumer preference in terms of aroma and flavor.

Other than aspect and palatability, nutritional quality represent an aspect that is acquiring growing importance in consumers' choice about food and there is a growing demand for food characterized by high nutritional value (Schmidt, 2000; European Commission, 2010). The importance of red meat in a balanced diet and its the nutritional value is well established. Briefly, red meat is a source of essential amino acids, unsaturated fatty acids, highly bioavailable minerals as iron, zinc, selenium, group B vitamins (especially B_{12}, not contained in vegetables) and numerous bioactive components as protein hydrolysate from collagen and myofibril digestion, ACE-inhibitory components from connective tissue hydrolysis, Nucleotides and nucleosides, phytanic acid and CLA (conjugated linoleic acid) and antioxidant (carnosine, vitamin E, glutathione…)(Young et al., 2013). Despite its fundamental role in human nutrition, recently red meat consumption has been associated ad risk factor for cardiovascular disease, colorectal and breast cancer (McAfee et al., 2010). However, Authors highlighted that most of the studies presented several biases, McNeill and Van Elswyk (2013) underline that the association between meat and animal fat consumption and colorectal cancer is doubtful and Micha et al. (2012) reported a lack of association between fresh red meat consumption and cardiovascular disease. Although that, there is a growing concern among the consumers about the healthiness of red meat.

Given the present scenario, an improvement of nutritional value of fresh red meat may represent a useful strategy to cope the growing believe that red meat is unhealthy. Moreover, given that meat from ruminants however represent an important amount of the total food intake, an improvement in essential nutrients content will determine an increasing intake of bioactive compounds without changing dietary habits (Olmedilla-Alonso et al., 2013).

1.2. Improve health and product quality of meat producing ruminants

An improvement of health and product quality of meat producing ruminants can be achieved through the application of specific nutritional and management strategies, thus leading to an improvement of production efficiency and perceived quality by the consumers.

As evident from the reported consumer surveys (Martelli, 2009; Miele, 2010; Napolitano et al., 2010), an improvement of perceived quality will rise, at the same time, consumers' expectation. Given the demonstrated role of the expectation on consumer satisfaction (Napolitano et al., 2010), high sensory quality has to be guarantee.

1.2.1. Preventive strategies to improve health of meat producing ruminants

In order to ameliorate perceived product quality and production efficiency, through the improvement of animal welfare and the reduction of antibiotics utilization, preventive strategies capable to boost the immune system and to early identify susceptible animals will help to reduce morbidity and severity of the pathologies. As previously reported, intensively reared beef cattle and veal calves are more susceptible to develop disease and health problem due to the characteristics of the rearing system, which, briefly, expose them to transport, mixing, high energy diet and high density conditions. However, in intensive farms, animals can be carefully inspected by operators and practitioners, while, on the contrary, pasture fed animals cannot be inspected and treated with the same frequency. Therefore, preventive strategies to improve animal health are necessary for both rearing systems.

1.2.1.1. Early identification of susceptible animals

The identification of susceptible animals or batches is fundamental in a management program aimed to reduce disease prevalence, minimizing at the same time antibiotics utilization due to the targeting of metaphylactic treatment only for high risk batches. Indeed, classify animals or batched based on the individual risk to counteract the most common disease will help to design specific management plans, optimizing production costs. As intensive fattening farms of meat producing ruminants receive young cattle, calves or lambs, their susceptibility to the most common diseases can be predicted measuring specific biomarkers at the arrival or based on the evaluation of individual characteristics and specific risk factors of the incoming animals (age, sex, market condition, management in the previous farm …). Several studies were conducted in the recent years to find reliable biomarkers to predict susceptibility to the most impacting disease, diarrhea for veal calves and BRD for both beef cattle and veal calves.

Regarding veal calves, is well known that an incomplete transfer of maternal immunity (failure passive transfer, FPT) increase susceptibility to both neonatal calf diarrhea and BRD (Virtala et al., 1996; Furman-Fratczak et al., 2011; Stilwell and Carvalho, 2011). To evaluate colostrum uptake within the first week of age the more specific test is represented by immunoglobulins G (IgG) quantification, with a cut-off value of 10 g/L (Weaver et al., 2000), although even cheap indirect screening tests, such as determination of total protein (TP) with a refractometer, performed reasonably well. US and Canada studies reported a prevalence of FPT of 19 to 38% (Tyler et al., 1996; Beam et al., 2009; Fliteau et al., 2003; Stilwell and Carvalho, 2011), range that can be reasonably expected even in the European dairy farms. Based on these evidences, Pardon et al. (2015) have recently carried out a study aimed to evaluate the association between immunoglobulins or any other protein determined by routine electrophoresis measure at arrival in the fattening unit (around 1 month old) and the occurrence of BRD and diarrhea in veal calves in the first weeks after arrival. Regarding diarrhea occurrence, an association has been found with alpha-2globulins, where for every increase in alpha-2 globulin by 1 g/L the diarrhea hazard increased by 17%. More interesting were the outcomes for BRD as the study demonstrated a clear effect of Ig concentration at arrival on BRD and ADG in the first 3 weeks, with a recommended cut-off 7.5 g/L. On the contrary, the other protein fractions determined by routine electrophoresis (albumin, alpha-1 globulins, alpha-2globulins, beta-globulins, gamma-globulins) and total proteins did not display any association of direct practical use. Moreover, calves seronegative for BCV and BRSV at arrival had an increased BRD risk in the first 3weeks of the production cycle. Based on these evidences Authors concluded that "assuring a seropositive status for these viruses either by vaccination on the farm of origin or by provision of colostrum from a vaccinated cow might be an appropriate approach to reduce the BRD risk and subsequently prudent antimicrobial use in the veal calf sector". Regarding beef cattle, instead, Richeson et al. (2013) reported an association between low eosinophil ($<0.108*10^3$ cell/μL) and high red blood cell count ($>11.2*10^6$ cell/μL) at arrival may represent a useful predictors of the risk to be affected by BRD in feedlot cattle. The reported studies showed promising results about the usefulness of some biomarkers in predicting the risk of intensively reared beef cattle and veal calves to be affected by the most impacting disease. However, before being routinely applied in beef and veal industry further studies have to be carried out to confirm the reported results and to evaluate the cost to benefit ratio of the different assessments. Another way to assess the risk to develop pathologies of the incoming batches of newly received animals can be represented by the evaluation of the intrinsic characteristics of the batch associated to the morbidity of the different pathologies.

For veal calves, Brscic et al. (2012) modelled the prevalence of BRD signs based on several risk factors. In the reported studies animals were inspected at the beginning, at half-way through, at the end of fattening period and at post-mortem inspection. Risk factors differed for the different stage and at the beginning of fattening cycle Authors found that calves heavier than 51 kg at arrival are less susceptible to show hampered respiration, although no differences have been found between the other categories of arrival weight (≤43, 44-47 and 48-51 kg). Animals received during autumn were more likely to show BRD signs even at the intermediate stage of fattening compared to those received in the other seasons. However, seasonality was ambiguous as post-mortem inspection showed that calves received during summer were more likely to show lungs with severe pneumonia signs and have the higher probability to show coughing 2 weeks before slaughter. Taken together these findings, seems that individual factors mainly affect the prevalence of BRD in the first stage of fattening but, with lighter animals that seems to be more at risk, likely because of younger and with a less mature immune system, or because of poor body condition or health status at arrival compared to heavier calves at the same age. On overall, however, none of the modelled prediction showed a high R^2 (0.16 to 0.46 for the different parameters), therefore highlighting that the considered risk factors were not able to explain a large proportion of the variation in BRD prevalence between batches. This result can be explained by the high individual variability within each batch (group of calves arrived and fattened together), as a batch contain animals that come from a lot of different farms, thus different for colostrum management, sanitary status, etc. Similar contradictorily results have been obtained by the same research groups considering gastrointestinal disorders (Brscic et al., 2011).

More conclusive, instead, seems the results of the studies undertaken on beef cattle regarding the association of intrinsic characteristics of the incoming animals and the susceptibility to BRD and locomotor apparatus disease (LAD). As reviewed by Taylor et al. (2010a) and Sgoifo Rossi et al. (2013), lighter and younger animals at arrival are more at risk to develop BRD due to the less mature immune system and the higher susceptibility to the transport stress. On the contrary, Compiani et al. (2014) reported that heavier animals are more susceptible to locomotor apparatus disease, likely due to the more intense social competitiveness during the adaptation period and to a higher protein and energy concentration of diets administered to older and heavier cattle during receiving period, thus increasing the risk of sub-acute ruminal acidosis, predisposing factor for lameness (Nocek, 1997). Increasing transport distance has been associated with increasing BRD morbidity (Sanderon et al., 2008; Cernicchiaro et al. (2012) and even position on the truck and the number of cattle per compartment seems to affect the probability to be treated for BRD, lower for animal transported in smaller groups (<15 head/compartment) and placed the forward (White et al., 2009). Breed and sex significantly affect both BRD and LAD risk. Entire males are more at risk to develop lameness due to the more intense competitive behaviour and to the higher dietary energy and protein content at European conditions (Compiani et al., 2013). As reviewed by Taylor et al. (2010a), some studies reported lower BRD prevalence for male compared with female while other Authors find the opposite situation. This inconsistency can be the results of different management, as in some studies in US and Canada animal have been castrated either before or after the arrival, while in other no distinction between entire and castrated male has been reported and castration after arrival increase BRD susceptibility. The breed effect on susceptibility to BRD seems to be more related to the production system, indeed Sgoifo Rossi et al. (2013) attributed the higher incidence of BRD in Limousine compared to Charolaise beef cattle due to the younger age at arrival in the Italian fattening units, while a specific breed effect can be found for double muscle cattle, more susceptible to develop respiratory disorder do to the lower lung weight (Fiems, 2012), while crossbreeds seems to be less susceptible due to higher rusticity. Considering British breed, contrasting results has been reported comparing Hereford and Angus, while *Bos indicus* seems o more susceptible than *Bos taurus* (Taylor et al., 2010a). A breed effect is evident for LAD as well with Charolaise that have been found more susceptible compared to limousine in the Italian intensive rearing system (Compiani et al., 2013). As hypothesized by the Authors difference attributed to the higher arrival weight, with consequent faster adaptation and more competitive behaviour, the administered diet higher in protein and energy content and because of Charolaise cattle generally come from flat pastures and in these circumstances joints are not adapted to intense solicitations or to sudden movements typically present on mountain pastures, from which, in example, Limousine or Aubrac come from. Higher BRD prevalence is reported in autumns and winter (Taylor et al., 2010a; Sgoifo Rossi et al., 2013) due to the cold weather and the high thermal excursion. For LAD the higher incidence found in spring in Italian feedlot by Compiani et al. (2013), has been attributed by the Authors to the increased risk of mycotoxicosis and the higher arrival weight of the cattle. Marketing conditions represents a significant source of variation for BRD susceptibility. Animals sourced from auction markets are more at risk to develop BRD compared to those purchase directly from farm (Taylor et al., 2010a) and conditioned animals (vaccinated, treated against parasites and fed a specific diet) are less susceptible to BRD (Duff and Galyean, 2007; Sgoifo Rossi et al., 2013). Moreover, calves that experienced FPT (Wittum and Perino, 1995) and calves that incurred in diarrhea (Pardon et al., 2013) at early stages of life are more susceptible to develop BRD on the subsequent stages.

From the literature review is evident that a rational on farm approach to sanitary treatment can be adopted using preventive strategies to early identify high risk batches, either based on batch characteristics or on specific biomarker assessment.

1.2.1.2. On farm strategies to reduce morbidity

In the previous paragraph inherent risk based on animal or batch characteristics has been discussed, but is necessary to take into account that on farm practices and structural characteristics can increase or reduce the risk to develop pathologies. In this paragraph strategies able to improve animal health or reduce the risk of the occurrence of pathologies will be discussed, with specific focus on intensive beef cattle and veal calves rearing system.

With the aim to reduce on farm morbidity, is important to improve, on one side immune status, and on the other side to limit the diseases transmission within the herd.

Nutrition and morbidity

Nutrition play a significant role in immune status and unbalanced or inadequate diets can lead to an increase in the incidence of pathologies. This aspect has been deeply investigated for the adaptation period of newly received beef cattle (first month at the fattening unit), in which BRD represent the most important issue (Duff and Galyean, 2007). Literature reviews suggest that a moderate energy and protein content in newly received beef cattle diets can help to reduce BRD morbidity (Galyean et al., 1999; Duff and Galyean, 2007). This seems to be in contrast with the rising of protein and energy requirement due to inflammatory status (Krehbiel et al., 2012), but, as mentioned, newly received beef cattle are exposed to a prolonged feed and water restriction that impair rumen function (Fluharty et al., 1996). In this condition the administration of high energy diets, which generally imply high level of starch, can induce sub-acute ruminal acidosis with consequent bacteria death (mainly gram negative) and increased liberation of lipopolysaccharide (LPS) that promote inflammatory status (Nagaraja and Titgemeyer, 2007). Concerning dietary crude protein (CP) Galyean et al. (1999) reported an increased BRD morbidity increasing dietary CP over 13% during receiving phase, although later on Authors stated that the reason at the basis of this finding still remain to be explained (Duff and Galyean, 2007).
Stress conditions impair immune function (Carrol and Forsberg, 2007) and, as summarized by Duff and Galyean (2007), micronutrients supplementation above the recommended level is necessary during stress because of the lower feed intake, the impaired rumen functionality and the increased requirement for immune response. Among micronutrients it was

demonstrated that copper, zinc and selenium improve immune function, although the effectiveness of an extra nutritional supplementation of these micronutrients on BRD morbidity during the receiving period is inconsistent (Duff and Galyean, 2007). Similarly, extra nutritional supplementation of B group, C, and A vitamins, although they play an important role in immune function, did not consistently determined an improvement in health and performance of newly received beef cattle (Duff and Galyean, 2007), while the results of the metanalysis of seven studies on newly received steers fed 0 to 2000 IU/head/d of synthetic vitamin E carried out by Elam (2007) reported that for every 100 IU increase in vitamin E intake per day, a 0.35% decrease in morbidity would be expected. Of course this prediction is valuable for the supplementation range reported above.
As reviewed by Duff and Galyean (2007), specific feed additives as direct-fed microbials (DFM), bacteria that beneficially modify gut microbiota, seems to be potentially able to reduce morbidity in feedlot cattle, and even if the exact mode of action remains to be determined, it has been proposed that the health improvement can be attributed to the competitive attachment of DFM vs. pathogens, as well as superior immune responses or greater gut permeability. However, effectiveness on beef cattle morbidity still have to be clearly demonstrated.

In veal calves, diarrhea account for a significant proportion of overall morbidity (Pardon et al., 2013) and feeding DFM has been extensively investigated as a preventive strategy to reduce diarrhea occurrence. Several studies reported a reduction of incidence and severity of diarrhea supplementing calves with DFM's containing *Lactobacillus spp.*, *Enterococcus faecium*, and *Bacillus spp.* (Abe et al., 1995; Timmerman et al., 2005; Wehnes et al., 2009 Jatkauskas and Vrotniakiene, 2010.; Nagashima et al., 2010; Signorini et al., 2012). Moreover, calves that were fed a probiotic that contained *E. coli* showed a reduction in the shedding of pathogenic enterohemorrhagic *Escherichia coli* in the feces (Tkalcic et al., 2003; Zhao et al., 2003). Other than probiotics, even prebiotics as mannan oligosaccharides have been investigated, but results are still contrasting, indeed some studies reported an improvement in fecal consistency or a reduction of diarrhea (Magalhães et al., 2008; Morrison et al., 2010) while some other reported a lack of significant effects (Terré et al., 2007; Hill et al., 2008). Regarding feeding systems in veal calves, Brscic et al., 2012 found that calves to which milk was provided with trough showed higher predicted prevalence of respiratory signs at early stage of fattening compared to those bucket fed or fed with an automatic system, while in the latest stages of fattening trough feeding seems to reduce the prevalence of BRD associated signs. Authors hypothesized that drinking from a common through increase pathogen spreading and also animal tend to drink faster from trough compared to bucket with higher risk of milk inhalation, while in the latest stages of fattening trough can guarantee higher hygiene standard compared to bucket. However, further studies are required to verify the link between feeding method and prevalence of respiratory disorder in calves.

Immunization against the most common pathogens

The reported evidences underline that through nutrition is possible to improve the immune function and that stressed animals have specific nutrient requirements in terms of macro and micro nutrients. However, a specific immunization can be achieved stimulating the of active immune system through the exposition of specific antigens with vaccination. In beef cattle vaccination is commonly applied especially against viruses involved in BRD, while cattle reared on soil ground are commonly vaccinated also against clostridia. As reviewed by Duff and Galyean (2007), BRD is a multifactorial syndrome in which aetiologiacl agents are represented by bacteria, such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, viral agents, including bovine herpes virus-1 (BHV-1), parainfluenza-3 (PI_3V), bovina viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine enteric coronavirus and mycoplasma as *Mycoplasma bovis*. As summarized by Taylor et al. (2010a), viruses damage respiratory clearance mechanisms and lung parenchyma, facilitating translocation of bacteria from the upper respiratory tract and the establishment of infection in compromised lung. Viral infection also interferes with the ability of the immune system to respond to bacterial infection. Based on the known etiology vaccines containing viral antigen, either from modified live or killed viruses, and bacterial toxoids has been developed. Commercially are available monovalent or multivalent vaccines or even vaccines that combine virus and bacterial antigens (Bowland and Shewen, 2000). The effectiveness of vaccination against respiratory disease is well recognized for feedlot cattle, and it has been recommended as part of a "preconditioning program" in cow calf farms after weaning and before the delivering to the feedlot by Duff and Galyean (2007) and Taylor et al. (2010b). Authors consider pre-shipment vaccination as the preferred option, however, if animals were not preconditioned or only a single dose of vaccine has been administered, vaccination in the first weeks after arrival has to be considered in order to improve the immunization. Effect of vaccination on newly received cattle as reviewed by Taylor et al. (2010b), contrasting results are reported in literature about the effectiveness of on-feedlot vaccination. Indeed, it can be limited by shipment stress, that can persist for several weeks after arrival (Purdy et al., 2000) and impairs immune reaction (Blecha et al., 1984; Loerch and Fluharty, 1999) and

concomitant infections. This seems to underline that vaccination should be postponed after arrival until a complete recover from shipment stress. Richeson et al. (2008) reported an improvement of ADG and IBR antibody titre delaying vaccination two weeks after arrival, however, in the study of Poe et al. (2013) vaccination on arrival increased bovine viral diarrhoea virus type 1a antibody concentrations compared with a two weeks delayed procedure and Sharon et al. (2013) did not found any effect of delaying vaccination on heifers performance and body temperature. Concerning the valence, higher effectiveness was reported for modified live virus against killed vaccines (Faber et al., 2000) and for multivalent compared to monovalent vaccines (Schunicht et al., 2003).
On overall, although the contrasting reported data, commercially on-feedlot vaccination is widely considered an effective strategy to limit BRD and enterotoxaemia caused by clostridia, as demonstrated by a recent US survey showing that in most of the feedlot vaccination against the reported viruses, *Pasteurellaceae*, *H. somni* and *Clostridia* is routinely applied, while vaccination against M. bovis is less common (USDA, 2013). From the report emerges also that in smaller feedlot (capacity 1000-7999 heads) all the entering cattle are vaccinated, while in larger units vaccination is applied based on same selection criteria. In this regard, targeted vaccination protocols, based on animals' inherent risk and pathogens circulation has been considered the preferred option in sanitary management of newly received beef cattle by Sgoifo Rossi et al. (2013).

Regarding veal calves, vaccination is not routinely applied in this kind of industry (Pardon et al, 2011 and 2013) and few studies have been recently performed to evaluate the effectiveness of vaccination against viruses involved in BRD on veal calves' health status and productive performance and contrasting results are available. As already discussed, given the young age at arrival, for this kind of animals passive immunity plays a key role in reduce morbidity for diarrhoea and BRD. Indeed, Pardon et al. (2015) reported that immunization against BRD viruses represented a protective factor reducing morbidity, however vaccination may be more critical in veal calves due to the possible interference of maternal antibodies (Thiry 2012).

<u>On-farm strategies to limit pathogen circulation</u>

A complete and effective preventive strategy not only has to be able to early identify high risk animals, develop targeted protocol based on risk assessment and improve immune function and immunization, but also it has to limit, as much as possible, pathogen circulation and disease transmission. Management choices and structural characteristics significantly affect pathogen transmission, thus animal welfare and production efficiency.

Stocking density and grouping management significantly affect morbidity. BRD mortality linearly decreased at the increasing of available air volume from 10-15 to >25 m^3/bullock (Beranger, 1986). Animals persistent infected by BVDV (PI) represent a risk factor for BRD due to the virus shedding (Duff and Galyean, 2007) and following the calculation of Callan and Garry (2002) is evident that reducing group numerousness reduce the probability of pathogen transmission: "if one estimates that 5% of calves born in a herd with bovine viral diarrhoea virus are persistently infected, then the probability of bovine viral diarrhoea virus exposure in a group of 10 calves is approximately 0.4 ($1–0.95^{10}$). If the stocking rate increases to 30 calves, the probability nearly doubles to 0.78 ($1–0.95^{30}$). In agreement with this calculation, (Svenson and Liberg, 2006) found that morbidity was higher in calves reared in group of 12 to 18 animals compared to those reared in smaller groups (6 to 9). Therefore, groups dimension has to be limited as much as possible. Air quality is another important factor to control, especially to limit respiratory disorder. Ammonia and hydrogen sulphide decreased mucociliary clearance and alveolar macrophage activity, and overall compromise to respiratory defence mechanisms. Carbon dioxide, carbon monoxide, and methane are asphyxiating gases, while dust particles come from both organic and inorganic sources, and particles less than 2 μm can penetrate to the alveolar spaces. Organic and inorganic dust particles can impair mucociliary clearance and overload alveolar macrophage phagocytic clearance. Most of the organic dust arises from faecal material, skin, and hair, containing therefore endotoxin and pathogen. Inhaled endotoxin can contribute to pulmonary compromise by initiating inflammatory reactions within the alveoli and alveolar vascular endothelium (Callan and Garry, 2002). The utilization of solid barrier between pens rather than mashed fens limit the opportunity to oronasal contact, thus reducing BRD morbidity, as demonstrated in dairy replacement (Lago et al., 2006). In agreement Brscic et al. (2012) reported a reduced occurrence of signs of BRD in animal housed in individual baby-boxes, that limit the contact with neighbourhoods. With the aim to assure good air quality in veal calves farm is necessary to take into account that calves are sensitive to air drafts, therefore ventilation should be designed to avoid excessive air circulation (Brscic et al., 2012).

1.2.2. Strategies to improve meat quality from intensively reared ruminants

As reported in the previous section, improve sensory quality of meat from ruminants is fundamental to satisfy consumer expectations, even higher if meat is marketed as premium product because of produced guaranteeing high welfare standard.

1.2.2.1. On farm strategies to improve meat quality

Ruminants nutrition and management significantly impact on product quality as they can improve nutritional value of the meat as well as sensory quality and affect the incidence of quality defect as dark cutting, for beef and lamb, or unacceptable red color for veal.

Improve nutritional quality

The achievable improvement of nutritional quality of meat from ruminants trough nutrition is essentially represented by the manipulation of labile lipid fraction and the enrichment with key micronutrients. Beef and lamb are fed a vegetable based, and in intensive fed ruminants linoleic acid (C18:2 n-6) is the most represented fatty acid, followed by linolenic acid (C18:3 n-3). However, at the rumen level 70 to 95% of linoleic acid and 85 to 100% of linolenic acid are biohydrogenated by rumen bacteria (Lock et al., 2006). This process lead to a partial or total saturation of the fatty acids, with the production of intermediate (linoleic and oleic acids and their isoforms) or completely saturated fatty acids as stearic acid (C18:0), determining thus the final fatty acid (FA) profile of meat from ruminants, in which monounsaturated fatty acids (MUFA, mainly C18:1) and saturated fatty acids (SFA, mainly C18:0) are the most represented FA's. Biohydrogenation is a protective mechanism, as UFA (unsaturated FA) are toxic for rumen bacteria because of the ability to uncoupling oxidative phosphorylation and the extent of biohydrogenation is faster increasing the number of insaturation, the retention in the rumen and the feed particle size (Gerson et al., 1998; Lock et al., 2006).

As reviewed by Mapyie et al. (2012), meat lipids are represented by triglycerides and phospholipids, originating both from dietary fats absorbed in the gut and from de novo synthesis. Palmitic acid (C16:0) produced from the latter pathway can be elongated to stearic acid (C18:0), and both FA can be subsequently converted to palmitoleic (C16:1) and oleic (C18:1) acids. Therefore, it is evident than meat PUFA comes from the feed. SFA are considered promoting factors for atherosclerosis and thrombosis and from a nutrional point of view are classified as atherogenics thromobogenic. Therefore, increasing unsaturated fatty acid content in meat from ruminants, reduce SFA:UFA ratio and improve n-3:n-6 ratio will improve the nutritional quality for the reasons explained in the next paragraphs. With this aim ruminants nutritionists are investigating feeding regimens capable to improve n-3 and essential fatty acids content in meat from ruminants without affecting both animal performance and meat sensory quality. In the recent years ruminant nutritionists and meat scientists mainly developed nutritional strategies aimed to improve meat n-3 FA, namely alpha linolenic acid (ALA) and the more bioactive eicosapentaenoic acid

(EPA, C20:5), docosapentaenoic acid (DPA C22:5) e docosahexaenoic acid (DHA, C22:6) and CLA content.

Conjugated linoleic acids (CLA) represent a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds (Bauman et al., 1999), and CLA in milk and meat fat of ruminants originate both from ruminal biohydrogenation of linoleic acid and tissues from trans-11 C18:1, another intermediate in the biohydrogenation of unsaturated fatty acids (Bauman et al., 1999). As reviewed by Benjamin and Spener (2009), CLA exerts several positive effects on human health, against obesity, anticancer, against atherosclerosis and diabetes, improve immune function and increased bone strength thanks to higher calcium absorption and lower mobilization from the bones and cis-9 trans-11 and trans-10 cis-12, alone or combined, have been recognized as the most bioactive isomers. In the reported beneficial effects an intake of 3.5 g/day of CLA is recommended (Mir et al., 2004). 80 to 90% of CLA in meat from ruminants is represented by cis-9 trans-11 isomers (Chin et al., 1992) and CLA content beef has been reported to varies from 1.2 to 12 mg/g of fat in beef (Mir et al., 2004) and from 0.6 to 15 mg/g of fat in meat for lamb (Khanal and Olson, 2004). The wide range highlight that nutrition plays a key role in meat CLA concentration. The improvement of CLA content in meat from intensively reared ruminants can be achieved increasing dietary inclusion of vegetable oils and oilseed rich in ALA (e.g. flaxseed and canola) or in linoleic acid (sunflower and soybean), in order to provide substrate to be converted by rumen bacteria into CLA (Khanal and Olson, 2004).
However, high level of vegetable oils depresses dry matter intake and digestibility (Allen, 2000) and also increase unsaturated fatty acids in meat, increasing susceptibility to lipid oxidation and consequent development of off odors (Wood et al., 2004) and reduced color stability (Faustman et al., 2010). Given this, in the last decade the administration of CLA supplements in intensively fed ruminants, in rumen protected form to avoid any further biohydrogenation, has been investigated (Gills et al., 2004 and 2007; Schiavon et al., 2010 and 2011; Schlegel et al., 2012). In the reported studies, although breed, supplied amount (4.1 to 55.8 g/CLA/d) and length of the dietary treatment varied consistently (32 to 330 days), a significant improvement in meat CLA content was evident. Form a label perspective, CLA are not mentioned in the EU Regulation 432/2012 concerning the permitted health claims on food, therefore, nowadays enriching meat with CLA does not represent yet an opportunity to improve perceived quality of meat from ruminants.

The positive effect of n-3 FA on human health are well established and is demonstrated a protective role against atherosclerosis, heart disease, stroke, hyperlipidemia and diabetes (Fares et al., 2014). These positive effects have to be attributed to the antinflammatory properties on n-3 FA, as they compete for Δ-6 desaturase with n-6 FA reducing the synthesis of arachidonic acid and improving those of EPA, from which antinflammatory cytokines are synthesized instead of proinflammatory prostaglandins produced from the former. Moreover, n-3 FA promote resolvins synthesis that inhibit synthesis and transport of proinflammatory mediators, as a ligand of PPAR's reduce pro-inflammatory genes expression, reduce adhesion and chemiotactic molecules and reactive oxygen species synthesis in monocyte, macrophages and endothelium (Calder, 2006). Currently investigated dietary strategies to improve n-3 content in meat from ruminants are represented by feeding sources rich in ALA or EPA and DHA.

Flaxseed represent the most suitable source of ALA, as it contains 35-45% of crude fat, nearly 60% represented by ALA coupled with a small proportion of SFA (DeClerque, 2006). Within the different forms of flaxseed, extruded flaxseed has to be preferred to raw flaxseed given the inactivation of anti-nutritional components and the partial protection of ALA at rumen level, while flaxseed oil is highly susceptible to lipid oxidation and can impair rumen function. Recently, calcium soaps from flaxseed oil has been developed, but considering the low rumen pH of ruminants for fattening, incomplete rumen bypass has to be expected. In most of the studies flaxseed has been included at 8-10% of the dietary DM, determining a significant improvement in meat n-3, although a different passage rate from feed to the muscle, likely affected by diet composition, duration of the supplementation and genetics was evident (Raes et al., 2004; Nassu et al., 2011; Juarez et al., 2012; Mapiye et al., 2013). The reported supplementation, however, significantly increase feeding cost. However, flaxseed effectively increased muscle n-3 content, from 8.9 mg/100g to 18.1 mg/100 g of muscle, even when supplied in a small amount (3.6% of DM, equals to 280 g/d) and for short time (Mach et al., 2006). As previously reported, increasing meat PUFA may increase susceptibility to lipid oxidation. When evaluated, meat sensory properties were not affected by flaxseed supplementation even at high dosage (Maddock et al., 2006; Juarez et al., 2012). Moreover, a triangle test demonstrated that nearly 75% of the consumers were not able to discriminate between animals fed (8.0 % of DM) or not with flaxseed. On the contrary, LaBrune et al., (2008), in beef from cattle fed 10% of flaxseed (920 g/head/d), found an impairment of color and oxidative stability and an increasing of off odors compared to those from animals fed corn and tallow. To overcome to the potentially negative effects of flaxseed supplementation on meat oxidative stability, a supplementation with vitamin E is recommended. In this regard, Juarez et al. (2011) reported an improvement of oxidative stability and a reduction of drip loss supplementing cattle fed flaxseed at 10% of DM with 1051 IU/α-tocopheryl acetate/head/d.

From a human health perspective EPA and DHA are more bioactive compared to ALA (Garti, 2008), therefore there is an increasing interesting in sustainable feed supplement (excluding therefore fish oil) able to provide a significant amount of these essential fatty acids. Algae is under investigation only in the recent years and its effectiveness in improve EPA and DHA in meat producing ruminants has been investigated in lambs. Supplementing feedlot finishing lambs for six weeks with 1.92% of algae Hopkins et al. (2014) found a 3 times increasing of loin EPA+DHA (43 vs 125 mg/kg of muscle). From a quality perspective, algae supplementation did not affect color stability but reduced vitamin E concentration and oxidative stability, similar to the findings of Ponnampalam et al. (2016). Both Authors concluded that, in order to limit the negative effects on oxidative stability, when lamb are supplemented with algae at nearly 2% of the diet, vitamin E have to be supplemented at supranutritional level to reach 2.95 mg/kg of muscle. Essential fatty acids content can be communicated on the label. However, it in EU, following the regulation 116/2010 a product can be claimed as "'source of omega-3 fatty acids" only if the product contains at least 0,3 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal and as "high polyunsaturated fat" if at least 45 % of the fatty acids present in the product derive from polyunsaturated fat under the condition that polyunsaturated fat provides more than 20 % of energy of the product.

Red meat is also a valuable source of minerals and other micronutrients. For instance, 100 g of beef provides 26% of the daily zinc requirement, while the average daily intake of a medium meat consumer (41–72 g/day in men and 24.2–45.5 g/day in female) is enough to fulfil the requirements of vitamin B_{12} and iron (Cardoso Pereira and Vicente, 2013). Nutritional value of meat from ruminants can be improved also by increasing the content of key minerals as Selenium (Se). This micronutrient, in form of Selenocysteine, is present at the active site of glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinase, seleno-phosphate synthetase 2, selenoprotein P and different kind of selenoproteins, being therefore involved in reduction of oxidized antioxidant, scavenging reactive oxygen species, synthesis of thyroid hormones, DNA and proteins protection from oxidation, redox signals and immune responses (Lu & Holmgren, 2009). Increasing meat Se content is an important outcome, as meat is one of the major contributors to Se intake in human diet and low Se status is linked with a higher risk of mortality, poor immune function and cognitive decline (Rayman, 2012). Moreover, in the majority of the EU countries, Se intake is lower than the recommended value of 55 μg/day (Rayman, 2004; Thomson, 2004). Se is also fundamental for animal health (Finch and Turner, 1996; Spears, 2000; Chauhan et al., 2014) and considering the soil deficiency in large areas of the world (Gissel-Nielsen, 1987; Cantor, 1997; McDowell, 1997; Oldfield, 2002), specific dietary Se supplementation is necessary if farm animals. Dietary Se can be supplemented trough inorganic or organic forms. The first are mainly represented by mineral salts as selenite or selenate of sodium or cobalt, while, among the latter selenium enriched yeast (Se-Y), in which the main selenocompund is represented by selenomethionine (SeMet), or SeMet itself (mainly produced from selenium enriched yeasts; Korhola et al., 1986) are the most common. Due to a different metabolism, inorganic forms are characterized by a lower bioavailability compared to the organic ones. Indeed, at rumen level, part of the dietary SeMet, the prevalent form in the provided Se-Y, is incorporated into bacterial protein, becoming thus part of metabolizable protein. The remaining amount is efficiently absorbed at gut level through methionine carrier. On the contrary, most of selenate is reduced to selenite and subsequently converted in low absorbable compounds or used by microbes to synthesized seleno-amino acids. The escape amount of selenate is actively absorbed via co-transport pathway along with sodium ions, while selenite is absorbed via passive transfer. After absorption, SeMet can be incorporated into proteins, in replacement of methionine or catabolized and Se utilized for SeCys synthesis. The absorbed selenite, instead, is reduced to selenide, utilized to synthesize SeCys, while the portion not immediately converted in SeCys is methylated and excreted (Weiss, 2005). Therefore, the different bioavailability underline that supplementation with organic forms is more effective in improve meat Se content when content compared to inorganic one at the same dosage (Juniper et al., 2008; Cozzi et al., 2011; Richards et al., 2011).

Improve tenderness and color stability

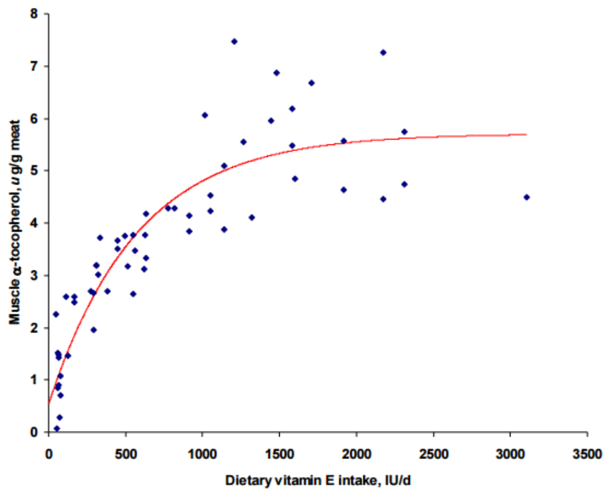
From an on-farm perspective, both management and, especially, nutrition affect meat tenderness. The two more important critical factors for meat tenderness that can be managed in an intensive production system are represented by age at slaughter and fat deposition, both marbling and subcutaneous. It is well established that, if characterized by a similar marbling and fat cover, meat from older animals is tougher than meat from younger ones. Most of the difference can be explained by the structural changes that affect muscle connective tissue as animal become older. Connective tissue is composed of collagen, elastin, and reticulin and, within them, collagen contributes to a greater extend to meat toughening, although it represents 1-15% of the total muscle (Nishimura, 2010). For instance, the amount of connective tissue is the variable highly correlated with the tenderness variation between the different muscle (Rhee et al., 2004) and several studies demonstrated the negative relationship between muscle collagen content and meat tenderness (Dransfield, 1977; Light et al., 1985; Nishiumi et al., 1995; Jeremiah et al., 2003). As animal become older collagen solubility decreases because of the increasing of cross-links within the collagen molecules (Boccard et al., 1979), with consequent reduction of collagen solubilisation during cooking and increasing in mechanical strength, which increase meat toughening (Nishimura, 2010). Crosslinks increased also with testosterone circulation and low intramuscular fat (Weston et al., 2002; Nishimura et al., 2010). Taken together these evidences highlight that, in order to limit collagen strength, is necessary to feed meat producing ruminants with high energy diet, in order to maximize growth rate and fat deposition. Increasing muscle growth determines high collagen remodelling, with consequent synthesis of new collagen characterized by a lower amount of crosslinks (Weston et al., 2002). High nutritional plane allow also to improve intramuscular fat (IMF) deposition, that happen mainly in the perimysium, between muscle fibre bundles. This causes the remodelling of extracellular matrix (ECM) and reduces the mechanical strength of intramuscular connective tissue (Nishimura, 2010). As reported, high energy diet is fundamental to guarantee a satisfactory IMF, which affects not only meat tenderness, due to the reported mechanism, but also flavour and drip loss. The minimum amount of IMF to achieve acceptable consumer satisfaction is about 3% to 4% for beef (Savell and Cross, 1986), and 5% for sheep meat (Hopkins et al., 2006). IMF is the major contributor to juiciness sensation and flavour, because of fat stimulate saliva production in mouth and most of the aromatic components are fat soluble (Hocquette et al., 2012). Increasing fat deposition is fundamental also to prevent an excessive chilling rate within the muscle, with consequent tenderness impairment, namely cold shortening (see next chapter) and to protect edible portion of the carcasses to excessive dehydration and oxidation.

During post-mortem, indeed, muscular proteins undergone to a marked oxidation that could impair meat tenderness, by decreasing protease activity and inducing miofibrillar protein cross-linking. Both μ- and m- calpains contains histidine and cysteine thiol-groups at their active site, which are highly susceptible to oxidation, with consequent inactivation. Moreover, myofibrillar proteins cross-linking reduce their susceptibility to degradation and improve the strength of the myofibrillar structure, increasing meat toughness (Huff Lonergan et al., 2010; Estevez, 2011; Lund et al., 2011). In this scenario, is possible to hypothesize that the increasing of muscular antioxidant activity could have been reduced the extent of enzymatic and myofibrillar protein oxidation. Rowe et al. (2004 a,b), reported that dietary

antioxidant supplementation in beef (synthetic vitamin E) reduced protein oxidation, positively promoting post mortem proteolysis and, consequently, beef tenderness. Therefore, even if limited evidences are available, high antioxidant supplementation seems to be able to improve meat tenderness.
Antioxidant supplementation, among which synthetic vitamin E is the most commonly employed, is particularly known to improve meat colour and oxidative stability by reducing susceptibility of muscle to lipid peroxidation (Liu et al., 1995; Faustman et al., 1998; Sales and Koukolová, 2011) (Fig. 4). Indeed, inadequate concentration of vitamin E was demonstrated to affect retail colour and oxidative stability of lamb (Ponnampalam et al., 2010).

Nutritional background is one of the main factors affecting muscle antioxidant status, and, thanks to the intake of forage rich in antioxidants, pasture fed ruminants are characterized by a higher level of muscle antioxidant when compared to those fed concentrate based diets (Daley et al., 2010; Zervas and Tsiplakou, 2011; Bekhit et al., 2013; Van Elswyk and MacNeill 2014). Therefore, in order to improve both antioxidant defences of live animals and meat quality vitamin E supplementation has to be provided to intensively reared ruminants.

Figure 4 Relationship between total dietary vitamin E intake and muscle α-tocopherol concentration in cattle



Vitamin E can be supplemented by administering either synthetic (all-rac α-tocopherol acetate) or natural (RRR α-tocopherol acetate) forms. Indeed, the conversion factor for international unit (IU) calculation from all-rac α-tocopheryl acetate to RRR α-tocopherol proposed by EFSA FEEDAP PANEL is 0.73 (EFSA, 2010) and higher bioavailablity has been confirmed in pork (Boler et al. 2009), turkey (Rey et al., 2015) and lamb (Kasapidou et al., 2009) muscle and in sheep (Gallardo et al., 2015) and cow milk (Weiss et al., 2009; Vagni et al., 2011). At the farm level, the inclusion of supranational levels of synthetic vitamin E is the most commonly employed

strategy to rise muscle vitamin E content, and it represent a useful strategy when sufficient availability of selenium is guarantee. Alternatively, antioxidant can be provided feeding high quality forages, such as lucerne, which contains vitamin E in natural form and other components exerting antioxidant activity, such as carotenoids, flavonoid and phenolic compounds (Ferreira et al., 2015).
Alternative to vitamin E, antioxidant components as plants extracts rich in polyphenols has been investigated with interesting results, varying depending on the tested plant extract (Brewer, 2008).
Another strategy to improve colour stability is to control pro-oxidant component in muscle. Iron and copper are the two main muscular pro-oxidant and Warner et al. (2010) reported that oxy/met ratio decreased, negatively affecting colour stability, as muscle iron and copper increased in lamb. As most of the diet administered to intensive fed ruminants contains mineral and vitamin mixes, it will be necessary to avoid excessive iron and copper supplementation especially during finishing and to take into account that low energy diets, characterized by a higher level of forages, naturally contains a higher level of iron due to soil contamination.

<u>On farm strategies to reduce dark cutting</u>

Dark Cutting (DC) or Dry Firm Dark (DFD) is a quality defect frequently determined by an incomplete post-mortem acidification and, consequently, final pH (pHu) above the recommended values (5.5-5.7). Generally, is described as DC meat of which pHu ranging from 5.8 to 6.1, while pHu of DFD meat is above 6.1, although is not currently available a uniform definition, either based on pHu and color. Briefly, the higher is the pHu, far from isoelectric point of muscle proteins, the more muscle proteins tend to interact with water and not with themselves. This determines lower light refraction and the higher susceptibility to oxidation, even because of the enzyme oxygen consumption rate is maximized at high pHu, and dry and firm aspect because of the enhanced water retention. DC and/or DFD affects ruminants' meat supply chain across different countries and production systems. Surveys conducted in the latest 15 years revealed a prevalence of DC/DFD in beef ranging from 1.3 to 13.9% in some of the most important producing countries, while, for sheep meat McPhail et al. (2014) found an incidence of DC carcasses (pH≥6.0) of 11.6%, close to 10.0% reported by Safari et al. (2002) in 909 loins at retail level in Australia, but higher than the 7.3% incidence found in an older work on 1536 New Zealand's carcasses (Devine and Chrystall, 1989). Above data underline that DC/DFD is still a concern for beef and lamb industry, with a great economic impact, despite this problem has been studied since lots of decades. The worldwide prevalence, despite the differences in rearing and marketing systems, climate conditions and slaughter plants characteristics across the different countries, highlights that several factors are implicated in the development of dark cutting. Despite is well established that DC meat is mainly affected by muscular final pH, closely related to muscular glycolytic potential at slaughter, some evidences highlight that DC meat can also be characterized by a glycolytic potential not significantly lower than those of carcasses that not exhibit a dark colour (Holdstock et al., 2014). Therefore, factors further than glycolytic potential can concur to DC occurrence in meat from ruminants.

Physiological status and hormone implants

Other than pathologies, physiological status can be altered through the administration of metabolic modifiers, widely used in the main eat producing countries, in except for EU. Dikeman (2007) defined metabolic modifiers as "compounds that are either fed, injected, or implanted in animals to improve rate of gain, improve feed efficiency, increase dressing percent, increase carcass meat yield percentage, improve visual meat quality, extend shelf-life, improve meat's nutritional profile, or improve meat palatability". The effects of different metabolic modifiers on meat quality were extensively reviewed in the last decades and most of the available data for ruminants are relative to beef cattle. Scanga et al. (1998) pointed out that "aggressive" steroid implants, as androgen and oestrogen combination and/or multiple implants during fattening, especially if the last one is implanted less than 100 days before slaughter, increase the incidence of DC. Based on these data, Dikeman (2003) suggests to avoid aggressive (androgen/oestrogen with TBA) or moderate-aggressive implants within 70 days prior to slaughter. Steroid implants, indeed, increase stress sensitiveness of both male and female cattle (Scanga, 1998). Also β-agonists are widely employed, especially Zilpaterol and Ractopamine in beef cattle (e.g. in USA, Mexico, South Africa and Australia). The most recent reviews of Dikeman (2007) and Lean et al. (2014) report a lack of effects of β-agonists on meat final pH and colour. Moreover, no adverse effects of Ractopamine supplementation on cattle behaviour were reported by Baszczak et al. (2006). Regarding small ruminants, less data is available in literature. In the 3 studies in sheep (using cimaterol and L-644 969®) reviewed by Dunshea et al. (2005), an increasing in ultimate pH was evident, while in more recent works, according to the findings in beef cattle, nor or slightly effects on meat colour were reported after Zilpaterol administration in goats (López-Carlos et al., 2014) or unspecified β-agonists in lambs (Brand et al., 2013). Based on the available data, seems that β-agonists did not affect DC prevalence in meat from ruminants, although limited studies on sheep and goat are available.

Meat production from female ruminants (heifers, cows, ewes, goats…) account for a significant amount across the different countries and its quality can be affected by oestrous status. Kenny and Tarrant (1988) demonstrated that mounting behaviour during oestrus reduced muscular glycogen content and, consequently, promotes a higher incidence of high meat pHu. This data is confirmed by Scanga et al. (1998), which found a higher incidence of DC in entire compared with spayed heifers. Several strategies are reported to inhibit oestrus in fattening heifers, as e.g. ovariectomy and hysterectomy (not applicable due to the negative impact on animal welfare and health status), hormone implants (melengestrol acetate, trenbolone acetate-TBA, oestradiol-17β, oestradiol benzoate, progesterone; not applicable in EU) and immunization against Gn-RH (gonadotropin-releasing hormone) (Prendiville et al., 1995; SCVPH, 1999, Weddle-Schott and Meyer 2008, MLA 2011).

Age at slaughter

Approaching to the effect of age at slaughter on carcass quality, is necessary to consider that segregation of its effect in animal studies is difficult, especially in case of surveys or studies involving a big number of animals reared under commercial condition, as the different age at slaughter is often determined by factors as breed characteristics, growth rate, diet and rearing system.

It is well established that the more cattle are older at slaughter the more their meat appear darker, as confirmed by several studies carried out in different countries and rearing conditions (Shakelford et al., 1994; Troy et al. 2003; Dunne et al., 2004; Kelava et al. 2008; Węglarz 2010; Girard et al., 2012; Huges et al., 2014; Mpakama et al., 2014). At the same manner, a darkening effect at the increasing of slaughter age has been reported in lambs by Pethick et al. (2005b), Wiese et al. (2005), Hopkins et al. (2007) and Warner el al. (2007b) in Australia and by Vergara et al. (1999), Diaz et al. (2003), Martínez-Cerezo et al., (2005) in Europe.

The reported findings are ascribable to an increasing in muscle iron and pigment content, mostly myoglobin, as animal become older, as demonstrated in beef (Boccard et al., 1979) and sheep (Martínez-Cerezo et al., 2005; Gardner et al., 2007; Pannier et al., 2010). The rising in myoglobin content is explained by a shifting to a more oxidative metabolism with age (Greenwod et al., 2007; Mlynek et al., 2006; Girard et al., 2011). Concerning pHu, in some studies a higher pH in older compared to younger animals was found in lamb (Diaz et al., 2003 and Hopkins et al., 2007) and beef (Dunne et al., 2004; Sañudo et al., 2007; Kelava et al., 2008; Huges et al., 2014). As suggested by Hopkins et al. (2007), an increasing in pHu with age can be attributable to a rising of muscle adrenalin sensitivity, aspect that could have boosted glycogen depletion in older animals. This hypothesis is supported by the findings of Gardner et al. (2005), which reported increased adrenalin sensitivity on both Angus and Piemontese cattle at 36 vs 15 months of age. Although that, several works did not report any effect of slaughter age on pHu in both sheep (Wiese et al., 2005; Martínez-Cerezo et al., 2005) and beef (Troy et al. 2003; Warren et al., 2008; Girard et al., 2012; Mpakama et al. 2014).

Regarding DC incidence, an old survey of Tarrant (1981) conducted sending questionnaire to senior scientist in meat research laboratory from 19 countries across Europe, America, Oceania and Africa, reported a higher estimated incidence of DC in carcasses from cows (6-10%) compared to those from heifers (1-5%). Authors underline also that the reported value for cows could be underestimated, given the darker colour of old animals. This data has to be carefully taken, due to the fact that cows are mostly culled animals, with impaired health status and, often, undergone to a poor pre-slaughter management, especially in the past.

On overall, reported data suggest that older animal has darker meat due to higher pigment content, and could be more prone to DC given the higher adrenalin sensitivity.

Nutritional mamagement

In order to produce meat with a desirable final pH, at least 50-60 μmol/g of muscular glycogen at slaughter (Tarrant, 1989) or 100 μmol/g of glycolytic potential (2 × [glycogen + glucose + glucose- 6-phosphate] + lactate) (Wulf et al, 2002) seems to be necessary. From a nutritional point of view, diet energy density is a fundamental factor affecting muscle glycogen content. Concerning beef, Immonen et al. (2000b) reported an increasing in both muscular glycogen content and glycogen repletion after adrenalin challenge, coupled with a reduction of glycogen loss in cold season, in animal fed 90% corn + 10% hay vs animas fed 100% hay, although no differences were found in pHu. More interesting, Authors noted that switching from high to low energy diet minimally decreased muscular glycogen, while feeding high energy diet for 37 days to hay-fed cattle increased glycogen up to level typical of high energy-fed cattle. Short-term high energy diet administration has been demonstrated also an effective strategy to reduce pre-slaughter glycogen loss and improve pHu in both cold and warm season (Immonen et al., 2000a). Accordingly, Gardner et al. (2001b) reported a higher glycogen repletion rate in *semimbranosus* muscle (prevalent oxidative) after exercise in cattle fed cereal-based vs silage- or hay-based diet. Similarly, Knee et al. (2007) demonstrated that cattle supplemented with ad libitum high energy triticale-based feedstuff for 3 or 4 weeks exhibited higher muscle glycogen compared to only pasture-fed animals, concluding that the latter have a higher risk to develop DC. Similar effect of increasing dietary energy density or supplementing pasture has been achieved in sheep. As reviewed by Gardner et al. (2014), a linear increasing in muscle glycogen was found by Pethick and Rowe (1996) increasing energy level. An increasing in muscle glycogen concentration and repletion rate increasing dietary energy content has been reported in both lambs selected or not for high muscling (Martin et al., 2004), while Hopkins et al. (2005a) reported a lower pHu and a lighter colour in lambs fed high vs low nutritional plane. The reported data suggest that is necessary to provide a high energy diet to ruminants, in order to reduce DC susceptibility. A lower DC prevalence in beef carcasses with lower ossification score has been reported by McGilchrist et al. (2014), underlining that higher growth rate, achievable administering a high energy diet, reduce DC prevalence due to higher glycogen content. Furthermore, higher pHu and darker colour has been reported in beef carcasses with fact thickness lower than 0.76 cm by Page et al. (2001), while Mach et al. (2008) reported a higher DC incidence in cattle characterized by worsen conformation and fatness score Other than provide high energy diet or supplement, specific dietary strategies have been tested to reduce transport stress and reduce DC incidence. Magnesium oxide supplementation reduced glycogenolisis after exercise, promoted higher glycogen repletion, improving both glycogen at slaughter and muscle pHu in sheep (Gardner et al., 2001a), while it was ineffective in cattle up to 0.75% of the diet in the latest two weeks pre-slaughter (Bass et al. 2010).

Temperament and human-animal interaction on farm

Variability in DC incidence between farms rearing animals of the same breed, administering the same diet and that consign the animal to the same slaughterhouse can be essentially explained by two different factors: individual variability in temperament and stress susceptibility and quality of handling practices.

Operators play a crucial role in DC incidence in ruminants, indeed in the beef 9 feedlots surveyed by Scanga et al. (1998) DC prevalence varied between 0.05% and 0.64%. The effect of farmer behaviour and gentle handling is evident in the study of Lensink et al. (2001) in which calves (undergone to the same dietary and sanitary management) originated from farms with positive human-animal interaction (high frequency of gentle contacts as petting, touching, letting suck the fingers, or talking in a soft voice) exhibited a lighter meat colour and a lower pHu compared to those sourced from farms characterized by a negative human-animal interaction (high frequency of rough contacts, hitting, kicking or shouting). Authors concluded that this result is attributable to a lower fear reaction to handling before slaughter. A previous work demonstrated, indeed, that gently handled calves are less agitated and showed a higher muscular glycolytic potential (Lensink et al., 2000).

Individual variability in temperament is well known by operators and technicians and the relationship between on farm and pre-slaughter behaviour and DC prevalence has been widely investigated, especially in cattle, in the latest years. Several measurements have been employed to classify cattle temperament (behaviour during chute restrain, exit speed from the chute, pen behaviour interacting with humans, behaviour during loading and unloading etc.) and a positive relationship between agitate temperament and higher pHu and/or DC incidence has been reported in several studies (Voisinet et al., 1997; Wulf et al., 1997; Cafe et al., 2011; Hall et al., 2011). This underline that animals with more excitable temperament seems to be much more stress sensitives. Although that, other works reported a lack of association between on-farm temperament and meat quality (Fordayce et al., 1988; Petherick et al., 2002; Ferguson et al., 2006; King et al., 2006; del Campo et al., 2010; Coombes et al., 2014). Cited works are conducted across different species (*Bos Taurus* and *Bos indicus*) and management systems, factors that could have been affected the consistency across studies. From a general point of view, agitate animals seems to be more stress sensitive, due to that a more careful handling practices has to be taken with them in order to minimize the risk of DC.

Pre-slaughter phase

Pre-slaughter phase, from farm gate to stunning, imply several stressors, like human handling, transport, novel/unfamiliar environments, feed and water restriction, mixing with unfamiliar animals and changes in climate conditions (especially in case of long transport and/or prolonged lairage) as reviewed by Ferguson and Warner (2000). The intensity of these factors is essentially affected by: marketing conditions (direct consignment or saleyard/auction market), transport length, lairage time and management or voluntary fasting on farm.

Lambs and cattle can be direct consigned from farm to slaughterhouse or being sold through saleyard/auction market. A higher DC incidence in cattle sourced from saleyard compared to those directly consigned from farm to slaughterhouse has been reported by Shorthose et al. (1998), Warren et al. (2010) and Vimiso and Muchenje (2013), while Warner et al (1998) found a higher pHu in saleyard cattle, despite no differences in DC prevalence. On the contrary, Ferguson et al. (2007b) did not report any differences in meat colour between directly consigned or saleyard cattle and McPhail et al. (2014) did not found any marketing method effect (direct consignment or saleyard) on pHu in lambs. Although the contrasting results, likely due to confounding factors as genotype, dietary background, transport length and pre-slaughter management as underlined by Ferguson et al. (2001), sourcing animals from auction market can be considered a risk factors for DC due to the fact that they are exposed to more handling, mixing and feed restriction compared to those sourced directly from the farm (Ferguson et al., 2001; Ferguson and Warner, 2008).

Other than marketing system, stressful practices in the latest days before slaughter can predispose to DC development due to glycogen depletion. Munier et al. (2006) reported that cattle mixed in the latest stages of finishing phase had an increased DC incidence due to the stress caused by the fighting for establish a new dominance hierarchy. In case of stressful events in the latest days pre-slaughter, a recovering period, necessary to restore glycogen reserves, has to be guaranteed in order to minimize DC incidence. Warris et al. (1984), slaughtering bulls (fed high energy diet) after 0, 1, 2, 4, 7, 9, 10 days after been mixed overnight with unfamiliar, found that pHu reached nearly normal levels in animals allowed to recover from mixing stress for at least 2 days. Although that, Authors suggest that 4 to 7 days appeared necessary to fully recover and prevent negative effect on meat colour. As reported in the previous chapters, glycogen repletion is affected by several factors, and a longer recovering period should be guarantee if animals are kept on pasture. Devine et al. (2006) reported, indeed, that Merino weathers allowed recovering on pasture for 8 or 10 days after stressful handling (weighing and shearing) had lower DC prevalence than animals that recovered only 1 or 3 days. It is possible to conclude that stressful operations should be avoided in the latest days before slaughter and, if stressful events happen, a proper recovering period has to be guaranteed in order to reduce DC risk.

Identification of susceptible animals on farm

The identification of susceptible animals on farm plays a key role in reducing DC incidence, as can allow farmer to apply specific intervention or delay the slaughtering. The most important risk factors for DC identifiable on farm are represented by:

- Animals affected by clinical or subclinical disease
- Females on heat
- Animals implanted with aggressive implants especially during the latest 2-3 months prior to slaughter
- Susceptible breeds:
    - Sheep: Merino as more stress sensitive (Young et al., 1993; Hopkins and Fogarty, 1998; Gardner et al., 1999; Warner et al., 2006; Hopkins et al., 2007) and animals selecting for high muscularity (Warner et al., 2007a)
    - Beef: dairy breeds, due to the higher pigment content and double muscle animals (Shackelford et al., 1994; Fiems, 2012), because of the higher stress sensitiveness, the faster glycogen depletion and the slower glycogen repletion
- Older animals
- Entire animals

- Animals fed with low energy diet prior to slaughter
- Extreme weather conditions or weather variability in the days prior to slaughter
- Agitate animals or animals undergone to a stressful practice in the latest days before slaughter

Strategies to reduce DC prevalence on farm

As reported, castration and spaying can reduce DC prevalence thanks to a calmer temperament a lack of heat. Concerning breed, particular attention has to be paid when dealing with susceptible breeds. From a nutritional point of view, providing a high energy diet, especially during finishing phase, increase glycogen storage and, improving growth rate, allow to reduce the age at slaughter. High energy diet has to be proper balanced and prepared to avoid acidosis, as it impairs feed intake (Schwartzkopf-Genswein et al., 2003) and promote agitate and aggressive behaviour (Commun et al., 2011). Moreover, specific dietary supplements, as magnesium oxide or hyperglycaemic substances as sugars, glycerol and propylene glycol, can improve glycogen level and reduce the negative effect of pre-slaughter stress. Heat stress, able to promote DC frequency, can be reduced providing shade or, for animals kept in close barns, improving ventilation trough a proper barn design and with fans. Given the moderate heritability of temperament traits, they could be considered for genetic selection. Stressful practices should be avoided in the days prior to slaughter, in case of unavoidable stressful practices a proper recover period, longer if animals are fed with low energy diet, has to be guarantee.

Other than reported strategies, is necessary to underline that all the efforts have to be putted to reduce stress during animal handling. This goal is achievable trough proper handling strategies, design and maintenance of handling facilities (see Grandin 2008 for proper handling and handling facilities design)

Strategies to reduce DC prevalence off farm

Low stress conditions have to be guarantee also from farm gate to slaughtering in order to minimize DC incidence.

1.2.2.2. *Post farm strategies to enhance meat quality*

Produce high quality meat is a process toward all the production chian from animal birth to the retailers and even a single mistake in one of the several steps of the production chani can compromise the final quality. Here the most criticals aspects from farm gate to the processing will be briefly reviewed

Regarding meat from ruminants, the step from farm gate to the stunning is critical especially for DC or DFD.

Transport conditions

Transport is one of the most stressful step of beef and lamb meat production chain due to pre-transport management, noise, vibration, novelty, social regrouping, crowding, climatic factors (temperature, humidity and gases), restraint, loading and unloading, time of transit and feed and water deprivation (Swanson and Morrow-Tesch, 2000). Concerning these aspects, Tarrant (1990) stated that the most stressful aspect of the transport chain for cattle is confinement on a moving vehicle, while confinement on a stationary vehicle, loading/unloading and re-penning in a new environment are less stressful events. Author underline also that distress may be avoided by observing statutory rest periods on long journeys, good animal handling, considerate driving technique, and using correctly designed pens, loading ramps and stock crates. Factors affecting DC incidence related to transport, as loading management and facilities, truck layout and equipment, stoking density and mixing, transport length, driver experience and road characteristics. Several studies have been carried out to compare the effects of transport length on meat DC prevalence and contrasting results are available in literature. On overall, as reviewed by Ferguson et al. (2001), short transport (<400 km) seems to not affect meat pHu, while a small increase (0.1-0.2 pH units) has to expected for longer distances. Studies conducted subsequently of this review seems to confirm this assertion, as no effects on DC prevalence have been reported after short transport in cattle by María et al. (2003) (0 vs 3 and 6 hours); Ferreira et al. (2006) (2 vs 5 hours); Mach et al. (2008) (no differences between animals transported for less than 2 to more than 3.45 hours), while Gallo et al. (2003) reported an increasing in DC incidence in cattle transported for 16 hours compared to those transported for 3 hours. Although that, Jones and Tong (1989) reported a rising DC frequency as transport distance increased from < 100 km to more than 300 km, Vimiso and Muchenje (2013) reported a linear relationship between transport distance and pHu in South African cattle and Kadim et al. (2012) found an increasing in pHu in sheep transported for 75 min compared not transported ones. Moreover, Mounier et al. (2006) found that transport duration increased, unloading became more difficult. Concerning sheep and goat, Dalmau et al. (2014) did not reported any differences between lambs transported for 1 or 24 hours, while a season seems to play an important role, as short transport impairs meat pHu in both sheep (0 vs 3 hours; Kadim et al., 2009) and goats (0 vs 2 hours; Kadim et al., 2006) transported in hot conditions. Based on the reported data is possible to assume that, generally, short transport did not increase DC prevalence, while longer transport is more likely to increase the probability of DC, especially for beef. However, hot season may increase stress response even to short transport, predisposing a higher DC incidence. Regarding transport length, European Food Safety Authority (EFSA) recommended that "during journeys of 8 to 29 hours, cattle should be offered water during rest periods. This is especially important in hot conditions. Adult cattle should not be transported on a journey of longer than 29 hours, even when ventilation is good and space allowance adequate. After this time there should be a 24-hour recovery period with access to appropriate food and water", while no recommendations have given for journey shorted than 8 hours. Concerning sheep, European authority stated that "healthy adult sheep, transported under good conditions can tolerate transport durations and associated feed and water withdrawal periods of up to 48 h" (EFSA, 2011). Cattle transport legislation is different across countries, as reported by Schwartzkopf-Genswein et al. (2012) in Canada the maximum transport time is 52 h, higher than those allowed in EU (30 hours) and USA (28 hours). In case of long journey, however, Tarrant and Grandin (2000) suggest to avoid rest stops and animals unloading, as they represent only a useless prolonging of overall journey time, unless in presence of adequate resting facilities and animals are carefully handled. Mounier et al. (2006) reported that loading was easier in farm with corridors and ramps, while unloading was more difficult when bulls had been mixed immediately before loading. Loading and unloading have to be carefully conducted, as represented a stressor factors. Indeed, Werren et al. (2010) reported that agitate cattle at unloading had higher DC incidence than calm. In this regard, EFSA (2011) recommended that "cattle should be transported in vehicles fitted with partitions so that the animals can be transported, loaded and unloaded in small groups". Loading animals from different farm and/or of different sex should be avoided, as it was related to a higher DC frequency (Jones and Tong, 1989; Mach et al., 2008; Werren et al., 2010). In case of unavoidable mixing of sex or loading of animals from different farms, different groups have to be separated with appropriate fens. Werren et al. (2010) reported, indeed, a higher incidence of DC when mixed-sex trucks carried not-separated animals. Space allowance requirement are calculated by allometric equations (see FAWC, 1998; Broom 2003, 2007, 2008, and Petherick and Phillips, 2009) and, taken together the equation developed, recommended space allowance for polled/dehorned cattle is 1.20-1.60 m2/head for 550 kg animals or higher than 1.4-1.6 m2/head for >700 kg animals, while for sheep have been suggested 0.2-0.3 m2/head if lower than 55 kg or >0.3 m^2/head if heavier than 55 kg (EFSA, 2011). For horned animals, EFSA (2011) suggests to guarantee 7% more space for cattle and 0.1 m2/head more space for sheep. Regarding DC prevalence, Vimiso and Muchenje (2013) reported a linear relationship between stocking density and pHu, while Eldridge and Winfield (1988) did not found any differences in pHu comparing a space allowance of 0.89, 1.16 or

1.39 m2/cattle during a 320 km transport. At the same manner, no different DC frequency has been reported comparing animals transported with a density higher or lower than 0.86 cattle/ m2 (average 0.83±0.23, compliant with EU indications) in the survey of Mach et al. (2008). Regarding further factors that significantly affected DC prevalence, Warren et al. (2010) reported a higher DC prevalence in consignment in which truck driver didn't undertook a specific training for livestock transport, as well as driver experience reduced DC prevalence. In the same study was found that increasing ventilation during cold season increase DC frequency. Also road type may affect DC prevalence, as Miranda-de la Lama (2011) reported higher pHu and a lower L* in lambs transported through unpaved rural secondary road compared to those transported on paved road for 3 hours.

Taken toghether the reported evidences suggest that, in order to limit DC occourrence, animals have to be carefully handled during loading and downloading and both farms and slaughter plants have to adopt proper designed loading and unloading ramp, as it easy the operations. Loading ramps have to be properly designed, in order to facilitate animal's movement and avoid injuries. Moreover, loading animals of different sex and\or from different farms on the same truck should be avoided. In case of unavoidable mixing, animals have to be kept separated with proper partitions. Short transports have to be preferred. In case of long journey, rest stops should be avoided (if not mandatory) as only prolong the overall journey time and loading and downloading may act as stressors. Paved roads have to be preferred and truck drivers should undertake a specific training course for livestock transport.

Lairage conditions

As previously highlighted for transport, contrasting data are present in literature concerning the link between pre-slaughter lairage duration and DC prevalence in ruminants. An increasing in DC frequency increasing lairage time has been widely reported in both surveys and controlled studies, across different countries. Llewelyn et al. (2002) reported an increasing in DC frequency comparing 12 to 24 hours versus 25 to 36 hours lairage in Australian cattle; in the survey of Mach et al. (2008) cattle undergone to a lairage longer than 15.8 hours exhibited a higher DC frequency in a Spanish abattoir, while Kreikemeier et al. (1998), in a US survey, found that cattle held at slaughter plant over weekend or holiday (36-84 h) showed higher DC frequency than those processed less than 12 h after consignment. Similarly, Warren et al. (2010) found that Canadian cattle held overnight at the plant tending to dark cut more often than cattle slaughtered on the day of delivery. Liotta et al. (2007), comparing a lairage time of 31 vs 57-59 hours (water and straw were provided) after 56 h journey, reported that the shorter time determined lower pHu and lighter meat, while Gallo et al. (2003), comparing lairage of, 3, 6, 12 or 24 h found that pHu increased by 0.013 units for each hour of lairage, both after 3 or 16 h transport; longer periods in lairage were also associated with an increase in DC frequency. Similar results have been reported in sheep by Toohey and Hopkins (2006), in which animals slaughtered after 2 days of lairage exhibited a higher pHu that those slaughter after 24 hours, while comparing the data reported by Jacob et al (2005b) across different consignment, a marked increasing of the frequency of carcasses with pHu >5.7 is evident in animals kept on lairage 2 vs 0 and 1 day, with smaller differences among the latter two lairage times. The negative effect of prolonged lairage can be attributed to a prolonged feed and water withdrawal and a longer exposure to stress, especially if lairage is not properly conducted as highlighted in the following paragraphs. On the other hand, lairage can allow animals to recover from transport stress and, if properly conducted and feed and water are available, to restore depleted glycogen reserves (Warriss et al., 1984), especially after a long and/or stressful journey. According to that, a positive effect increasing lairage time has been reported in different studies. Mounier et al. (2006) in a survey conducted in France reported a reduction of pHu in short transported cattle at the increasing of lairage time (from 20 min to 48 hours). At the same manner, del Campo et al. (2010) reported a lower pHu in cattle undergone to 15 hours of lairage overnight compared to those slaughtered after 3 hours in the day. Authors suggest that, in this case the effect the negative effect of shorter lairage probably occurred mainly because the time was not enough to allow animals to familiarize with the new environment and that waiting conditions were more stressful during the day. In sheep, similar results have been reported by Jacob et al. (2005b), in which both lambs and sheep slaughter at the arrival exhibited a higher pHu compared to those slaughtered after 1 or 2 days of lairage, according to Ekiz et al. (2012) that reported a higher pHu, after short transport, in lambs undergone to a only 30 min of lairage respect to those kept for 18 hours. A lack of effects of lairage time on meat color and pHu have been reported comparing 3 vs 18 hours of lairage in cattle by Ferguson et al. (2007a) and between lambs slaughtered at arrival or after 12 hours of lairage by Liste et al. (2011). This variability can be reasonably attributed to differences in lairage management, transport duration, dietary background, pre-slaughter stress and genotype across the reported studies. Lairage management, indeed, plays a key role in DC incidence. During lairage, mixing unfamiliar animals has to be avoided, as it increases DC frequency (Mohan Raj et al., 1992; Kreikemeier et al., 1998; Lahucky et al., 1998; Llewelyn et al. 2002) due the fight to establish a dominance hierarchy (Kenny and Tarrant, 1987).

Similarly, a high stocking density in lairage pen may promote fighting behaviour, especially in agitate animals; Mach et al. (2008) reported indeed a higher DC frequency increasing stoking density over 0.26 bulls/m2. Excessive physical activity can predispose to DC, as demonstrated by Warner et al. (2005), which reported that lambs subjected to acute exercise 15 min pre-slaughter resulted in higher pHu. On the other and, restrain and isolation from familiar animals, even for short time, increased DC incidence in both beef (Apple et al., 2005) and sheep (Apple et al., 1995). Provide feed and water during long lairage, if trough well designed facilities that minimize animal competitive behaviour, can be effective to promote glycogen restore, hydration and calm behaviour. The effectiveness of providing electrolytes, high sugar supplement or a mixture of electrolytes, sugars and amino acids during pre-slaughter and/or lairage has been reviewed and confirmed by Shaefer et al. (1997). In the same topic, Petchik et al. (1999), based on the results of several trials carried out to test both liquid and feed rich in carbohydrate and electrolyte, suggested that the most effective way is to administer them through water and during lairage, rather than on feed. Other than electrolyte, hyperglycaemic products has been tested by the same group and, even if a positive effect of 48-h glycogen repletion after exercise has been obtained feeding glycerol (at 3.5%) and propylene glycol (at 1.5%) in drinking water, it did not improve muscular glycogen content when tested in slaughter scenario (Gardner et al., 2014). The effectiveness in reducing DC incidence of other micronutrient supplementation, as tryptophan and tyrosine, able to alleviate stress response and glycogenolisis, has to be demonstrated (Schaefer et al., 2001). However, cases of failure or low feed and water intake during lairage, due to unfamiliarity with the new environment/trough, have been reported by Pethick et al. (1999) and Jacob et al. (2006). On overall, in order to reduce the risk of DC/DFD Sourcing animals directly from farm rather than from saleyard\auction markets has to be preferred, as they are exposed to a shorter fasting and overall pre-slaughter stress. Lairage time should be as short as possible for well managed and short transported animals, as it may increase pre-slaughter stress, while it can allow recovering and restoring glycogen after long transports. Based on the reviewed studies, however, lairage longer than 24 hours seems to exert a detrimental effect on DC frequency. Lairage can allow a recover after transport stress only if properly conducted. Undergone animals to a prolonged fasting can increase DC incidence as it represents a stressor and may impair muscular glycogen content, therefore, in case of prolonged lairage, feed and water have to be provided. EU Welfare Quality® group suggest that, for cattle kept in lairage overnight, available water and at least 2 kg of feed have to be provided (Velarde and Dalmau, 2012). Regarding feeding strategies, the supplementation of liquid feed rich in sugars, glucose precursors (e.g.

glycerol or propylene glycol) and electrolytes can help to counteract the effects of transport stress and reduce DC frequency. Mixing sex or unfamiliar animals has to be avoided during lairage, as it promotes competitive behaviour, as well as a high stocking density. On the other hand, animals have not to be isolated from familiars and restrained or tied. The installation of electrify overhead wire grid can effectively reduce mounting behaviour, with positive effect on muscle glycogen and DC frequency as reported by Kenny and Tarrant (1987).

Handling in slaughter plants

As is possible to understand from the previous chapters, stressful handling in slaughter plant may play a key role in increasing DC prevalence, therefore it must be as less stressful as possible. These aspects have been reviewed by Grandin (1996). Author stated that the main causes of welfare problems in slaughter plants are represented by: poor or improper design and maintenance of stunning and handling equipment and facilities; distraction which impede animal movement; lack of employee training and supervision and poor animal's conditions. In example, Author demonstrated that cattle will move easily through a curved race compared to a straight one, and that an animal should be able to see at least two or three body lengths before file curves. The most frequent distractions impede animal movement found in Canadian slaughterhouses survey were: light problems (animals tend to move from a darker to an illuminate place, but light must not shine directly in their eyes), air blowing towards approaching animals, object in movement or sparking reflection and noises. Other distraction or welfare problems can be represented by shadows, drain gates and changes of fences or flor type or slick floor. The most common mistake made by slaughterhouse employer is that they try to move too many animals at the same time and suggest that forcing pens should not be filled more than three-quarters full. Employers have also to remain calm, avoid sudden, jerky motions or yelling and minimize electric prods usage. It's important to underline that, as Author suggest, all the problems have to be addressed to maintain a high welfare standard. Based on Grandin's "Animal Welfare and Humane Slaughter Audits", the acceptable threshold for cattle falling during handling is 1% and no more than 25% of cattle have to be moved with electric prod. Pre-slaughter stress can also impair meat tenderness.

Post mortem tenderization and importance of ageing

After animal bleeding, tissues come into an ischemic anoxic state with a consequent drop of the redox potential (from +250 to -50 mV). Hypoxia induce the onset of apoptosis, the first step of the conversion of the fresh muscle into meat (Ouali et al., 2013). Apoptosis, also called "programmed death" is a physiological mechanism by which damaged, dangerous or useless cell are deleted (Green, 2005). Apoptosis process mainly involves caspase, proteases that degrading mitochondrial membrane determines the liberation of cytochrome C, which constitute, together with caspase 9 and other components, a complex activating effector caspases. Being the first proteolytic complex activated since cell death, caspases are considered as the initiators of the conversion of muscle into meat (Herrera-Mendez et al. 2006).

From an energetic point of view, after bleeding ATP is produced by creatine/phosphocreatine complex, which synthesize ATP from ADP+Pi. When phosphocreatine reserves are exhausted and not regenerated, the main source of ATP is represented by the anaerobic degradation of glycogen. Anaerobic degradation of glucose determines the accumulation of catabolites as CO_2, HCO_3^-, NH_4^+ and lactic acid, that cannot be transported to the liver, with consequent cytosolic pH drop from 7 to 5.4-5.7 in optimal conditions (Lawrie and Ledward, 2006). The progressive pH and temperature drop after death reduce the functionality of the Ca-ATPase in RE membranes, with consequent rising of cytosolic Ca (from 1 μM in vivo to nearly 250 μM post mortem) (Ji and Takahashi, 2006), until glycogen exhaustion, with consequent *rigor mortis* due to the lack of ATP (necessary to dissolve the contraction and start a new contraction cycle). The speed of glycogen degradation is function of muscle fiber type and genetic variability, however it is reduced by the drop of temperature and pH (Herrera-Mendez et al., 2006).

Muscle protein degradation is operated by proteases, activated by the rising of sarcoplasmic Ca or the reduction of pH.

As reviewed by Kemp et al. (2010) calpains are the most important enzymatic complex involved in postmortem proteolysis of muscle fibers. Calpains are cytosolic protease Ca-dependent, with optimal pH of 7 cleaving both sarcomere and cytoskeletal proteins. Two isoforms are involved in postmortem tenderization, μ- and m-calpains. The former active at micro molar Ca concentration (3-30 μM), exerting therefore protease activity during the first hours postmortem, while the rising of Ca concentration determines the autolysis. The latter required millimolar Ca to be active (0.2-0.6 mM) and anltough their activity remain constant from 2 to 7 days postmortem, its role in meat tenderization seems to be maginal due to the fact that the postmortem calcium concentration is suboptimal for their activity (Hui, 2012).

Calpains activity is antagonized by calpastatin, Ca-dependent specifi endogenous inhibitor (Dayton et al., 1976). Its muscle concentration is specie-specific, with the calpastatin/μ-calpain ratio of nearly 3.5:1 in beef

and 2.5:1 in lamb (Ouali e Talmat, 1990). This ratio is also breed-dependent, and it is lower for double muscle breeds (Fiems, 2012). As for μ-calpains, calpastatin are active only in the first 2-3 days post mortem (Hwang and Thompson,, 2001).
Cathepsins are another protein complex involved in muscle tenderization. They are involved in apoptosis process and its activity is maximized at low pH, from 3 to 9 days postmortem. During ageing the main target is represented by actine and myosin, although some isoforms are active against collagen proteins. Cathepsines activity is antagonized by the cytosolic inibithor cystatine (Kemp et al., 2010; Lomiwes et al., 2014).
Proteasomes are a multicatalytic complex involved in cytosolic and nuclear protein degradation (Coux et al., 1996). Their activity, ubiquitin- and ATP-dependent, is mainly directed against miofibrillar proteins (actine, myosin, nebulin and tropomyosin) and is is maintained during the post-mortem, with substantial activity still detectable at a7 days post-mortem and at pH levels of less than 6 (Kemp et al., 2010).
Within tenderization process, as ATP-dependent, caspases are active untill the exhaustion of the ATP reserves, and their activity is mainly targeted against desmin, troponin and tropomyosin. Caspases degrade also calpastatin, inderctly improving meat tenderness. Caspases activity is inhibited dy SERPINS (serine protease inhibitors) and they are considered a reliable predictor of meat tenderness (Ouali et al., 2013).

As reported, muscle collagen content is negatively related with perceived tenderness. Although the effect of ageing on collagen degradation is still controversial, as reviewed by Purslow (2005) post mortem ageing improves collagen solubility and reduces its structural integrity and mechanical strength and degradation of collagen matrix is mainly operated by metallopeptidase sand β-glucoronidase. Collagen degradation is slower than myofibrillar degradation as Nishimura et al. (1998) reported a linear reduction in collagen strength only starting from 10 days postmortem, being halved at 35 days. Most of the studies reporting an improvement of collagen degradation during postmortem ageing were conducted in vitro or in ex-vivo, while focusin on cooked meat Purslow (2005) stated that the reduction of intramuscular collagen strength during ageing on raw meat seems to not affect collagen stability after cooking.

On overall, the postmortem tenderization mechanism explains why increasing ageing improved perceived tenderness. Therefore, in order to improve meat acceptability a proper ageing period has to be guarantee. Moreover, protein degradation determines also the production of volatile and aromatics peptides (Spanier et al., 1990). Meat can be aged bone-in (dry ageing) or vacuum packed (wet ageing). Comparing these two differents methods, Jeremiah and Gibson (2003) reported higher aceptability for wet aged meat, although prolonged wet ageing improved the occourrence of defects as livery and methallic taste. On the contrary, Warren and Kanster (1992) riported that bone-in ageing improved desirable flavors.

Is known since decades that tenderness is minimized at intermediate pHu (5.8-6.1) (Lawrie and Ledward, 2006). At this pHu range, indeed, the activity of the most important enymes involved in meat tenderezitation, calpains and catepsines, is minimal. Moreover, another patway has been proposed: at intermediate pHu the affinity between small heat shock proteins and muscle protein and calpains is maximized and this limit both enzyme activity and muscle proteins susceptibility to the cleave activity of the protease (Lomiwes, 2014). The relationship between stress and meat toughness can also be unrelated with the pHu. The epinephrine released following a stress response is able to improve calpastatin activity as reported by Gruber et al. (2010), which found tougher meat in cattle that exhibithed an agitate behavior during lairage and a higher stress response (demonstrated by the higher blood lactate).

Carcass chilling is another critical step for meat quality, since a too fast or a too slow chilling rate can impair tenderness, water holding capacity and color. A too fast chilling is known to impair meat tenderness, due the phenomena called "cold shortening". Birefly, a too low muscle temperature determines membrane alteration and Ca^{2+} releasing from endoplasmic reticulum (ER), with consequent persistent contraction. It determines a sarcomere shortening that is not fully degraded during postmorted proteolysis, even slowered by the low temperature (Lawrie and Ledward, 2006). Moreover, during cold shortening conditions a higher calpastatin activity has been reported (Zamora et al., 1998). The opposite condition, namely "heat shortening", is determined by the combination of high temperature and low pH. It happens more frequentely in the deeper regions (leg and shoulder) and/or when excessive electrical stimulation is applied. In order to avoid these two quality defects, Thompson (2002) propose that, at pH 6.0, temperature should be comprising between 35° and 12°C. The combination of high temperature and low pH impairs calpains activity (Hwang and Thompson, 2001; Thompson et al., 2008; Pomponio e Ertbjerg, 2012); soft aspect and higher water loss due to protein denaturation (Kim et al., 2012; Warner et al., 2014), with higher drip loss determined also by the lower citoscheletal degradation due to the lower protease activity (Huff-Lonergan and Lonergan, 2005); pale color due to the higher light refraction, the higher drip loss and the pigment denaturation, which impairs also colour stability and promote lipid oxidation (Kim, 2014; Hopkins et al., 2014). Moreover, in conditions of slower chilling rate odour alteration, likely attributable to oxidation of phenylalanine, histidine, ornithine, tryptophane and lysine have been

reported (Stella et al., 2002). Individual susceptibility has to be considered, as animals with similar caracteristics (weight, fat cover, breed…) and processed the same day at the same condition may present or not the reported alterations. Indeed, the speed of glycolysis process is affected by genetic variability and different proportion of muscle fibres (Ouali et al., 2013). However, risk factors are represented by high carcass weigh and/or fatness, too high carcass density in the chilling room, too slow slaughter chain/delayed chilling of heavy carcasses (Sgoifo Rossi et al., 2009). Therefore, a proper chilling rate has to be set and pH decline has to be routinely monitored to avoid meat quality alterations.
Several strategies have been investigared to improve meat quality during post-mortem processing, however the most applied are represented by electrical stimulation and pelvic suspension of the carcasses.

Electrical stimulation

It is represented by the electrical stimulation of the carcassa t the end of slaughter chain, commonly applied on neck and leg, with the aim to boos the post-mortmen contraction and glycolysis to determine a fast pH drop to avoid cold shortening (Adeyemi and Sazili 2014). This technology is mainly applied for lambs, while for beef, especially if other electrical imput are given (e.g. to stop leg motility after stunning and to help skin removal), it increases the risk of heat shortening (Hopkins et al., 2014; Jacob and Hopkins, 2014).
As reviewed by Adeyemi and Sazili (2014), electrical stimulation (ES) is applied at different instensity and frequency: extra low-voltage electrical stimulation (ELVES), low voltage electrical stimulation (LVES) and high-voltage electrical stimulation (HVES). The ELVES is carried out at voltage of 110 V. Low voltage electrical stimulation is carried out between 100-110 V and is often regarded as medium-voltage electrical stimulation (MVES) (Adeyemi and Sazili, 2014). However, effects are controversial in beef, as positive, no or negative effects (due to heat shortening) has been reported (Adeyemi and Sazili, 2014). Basically, the most important tenderizing effect is determined by the prevention of cold shortening, even intensification of glycolysis and, consequently, proteolysis. Positive effects were reported even for meat color, where ES improved brightness, determined by the reduction of oxygen consumption, which increase oxymyoglobin formation and the lower pHu and higher protein denaturation that improve the interaction between proteins, thus increasing light reflaction.

Aichbone hanging

Known also as tenderstretch, hanging carcasses trough the haichbone instead of Achilles tendon is aimed to stretch back and high values leg muscles as rump and eye of round, thus increasing sarcomere length and reducing postmortem shortening and, consequent, toughening. Stretching carcass through aictchbone anging have to be done before rigor and it was demonstrated effective to improve meat tenderness (Park et al., 2008; Ahnström et al., 2012), and altought this technique should theoretically be more effective in counteracting cold shortening effects, it was reported to improve tenderness even with heat shortening conditions (Kim et al., 2014). The main limit to the high diffusion of this technique is represented by the higher space required for carcass storage and the expensiveness of the replacement of the carcass rail lines, more than the little impact on forequarter cuts.

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CHAPTER 2

Objectives

2. Objectives

Given the molteplicity of factors affecting quality perception of meat from intensively reared ruminants by the consumers, the undertaken studies aimed to evaluate the effectiveness of specific nutritional supplementation and on farm and post farm strategies to improve animal health, strictly related with sustainability, welfare, antimicrobial utilization and meat quality.

CHAPTER 3

A randomised trial to evaluate the impact of bovine respiratory disease and multivalent vaccination on veal calves' health, performance and carcass value

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3. A randomised trial to evaluate the impact of bovine respiratory disease and multivalent vaccination on veal calves' health, performance and carcass value

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3.1. Abstract

Bovine respiratory disease (BRD) represents a concern for veal industry, as it causes economic losses, reduces animal welfare and increases antibiotics utilization. A field study to evaluate the effects of multivalent vaccination on veal calves' health and performance was carried out. 944 healthy calves were randomly allotted into two separate barns. One group was vaccinated 7 days after arrival with quadrivalent vaccine against IBR, PI3, BRSV and BVDV Type 1 viruses plus booster administration 21 days apart (VAC; 675 calves), while the second group was not treated (CON; 269 calves). Vaccination did not affect overall mortality (P=0.75) and mortality due to BRD (P=0.28), while it reduced BRD morbidity (P=0.009). VAC group showed better average daily gain (ADG; P=0.03) and carcass weight (P<0.001). Vaccination delayed the peak of BRD by nearly two weeks (P<0.001) and represented a protective factor against BRD (OR 0.55; P=0.001). BRD increased mortality (P=0.01), lowered ADG (P<0.001) and carcass weight (P<0.001), increased the amount of discounted carcasses (P<0.001). Lung lesions and pleuritis and/or consolidations increased with BRD (P=<0.001) but were unaffected by vaccination (P=0.40 and P=0.43 respectively). In conclusion, BRD impaired veal calves' health, growth and carcass value and vaccination reduced BRD morbidity, thus improving carcass value.

3.2. Introduction

In European Union veal production is based on indoor fattening of young calves (nearly one month old) sourced from multiple herds (mainly dairy herds) by local tradesmen and delivered to fattening units in which they are kept until maximum 8 months (Regulation EC 566/2008). Bovine respiratory disease (BRD) is the most impacting disease in terms of morbidity, mortality and discounted carcasses (Pardon and others, 2012b and 2013). Inadequate passive immunitazion, immature immune system, transport and mixing in an indoor environment at high density represent the main predisposing factors for BRD. Etiological agents includes bacteria Pasteurella multocida and Mannheimia spp., mycoplasma such as Mycoplasma bovis, and viruses as BVDV (Bovine viral diarrhoea), PI3V (Parainfluenza type-3 virus), BRSV (bovine respiratory syncytial virus), BHV-1 and BAV-3 (bovine adenovirus 3) (Frankena and others, 1994; Salt and others, 2007; Arcangioli and others, 2008; Pardon and others, 2011; Rérat and others, 2013). BRD is mainly controlled through an all-in/all-out sanitary approach and with oral antimicrobial administration (Pardon and others, 2012). Authors reported that treatment for BRD account for more than 50% of total drugs administration. The extensive utilization of antimicrobials in intensive farming systems is a growing concern due to the transfer of bacterial resistance to human medicine. Veal calves is a critical farming system in this regard, since higher multiple bacterial resistance have been found compared to conventional cattle (Pardon and others, 2014) and higher livestock associated methicillin-resistant Staphylococcus aureus (LA-MRSA) prevalence has been reported in veal calves producers compared to poultry, beef, dairy cattle and pig farmers (Vandendriessche and others, 2013). Moreover, resistances against commonly employed antimicrobials have been reported for Pasteurellaceae (Vogel and others, 2001; Wettsein and Frey, 2004; Rérat and others, 2012), leading to an ineffectiveness of oral antimicrobial mass treatment. BRD has also been identified by EFSA (2006) as the major issue for veal welfare. Given the unkown level of passive immunization and that immunization against BRD viruses is a protective factor (Pardon and others, 2015), vaccination may represent an effective preventive strategy to reduce BRD occurrence. However, contrasting results about the effectiveness of vaccination against BRD for beef cattle in field trials are reported. Inconsistency due to improper timing of vaccination, incomplete protection against the different etiological agents and stress, that suppress immune function (Taylor and others, 2010). Within vaccines, modified live virus and multivalent vaccines were more effective than killed and monovalent vaccines (Faber and others, 2000; Schunicht and others, 2003). Vaccination is not routinely applied in veal industry (Pardon and others, 2011 and 2013) and few studies have been recently performed to evaluate the effectiveness of vaccination against viruses involved in BRD on veal calves' health and performance. This field study aimed to evaluate the effectiveness of multivalent vaccination against BRD viruses on health and performance of veal calves at high risk for BRD.

3.3. Materials and methods

3.3.1. Study population, experimental design, farm management and environment

The study was carried out in a commercial farm in the North-West of Italy (44°30'40"N 7°41'19"E) from October 2013 to April 2014. 944 healthy veal calves (98.8% entire male; 91.7% Holstein Friesian) were enrolled and randomly allotted in two separate barns from the arrival. One group was vaccinated 7 days after arrival with a quadrivalent vaccine with altered strains of IBR and PI3 viruses and modified live BRSV plus a liquid adjuvant preparation of inactivated cytopathic and noncytopathic BVDV (CattleMaster 4®, Zoetis Italy, Rome, Italy) plus booster administration 21 days apart of the same vaccine (VAC; 675 calves), while the second group was not treated (CON; 269 calves). Animals were similar (area of origin, age at arrival etc.) and this approach was aimed to replicate two different approaches currently applied in veal industry, vaccination or not. The different numerical distribution between groups was due to the different dimension of the two barns. Animals were fed twice a day with the same diet and housed in naturally ventilated barn with wooden slatted flor. Animals were individually penned for 50 days and then cages were removed, to create group pens with 6 calves each with a space availability of 1.8 m2/head. In order to limit interference factors, calves treated by farm personnel for diarrhoea before being affected by BRD were excluded from the study, as calves affected by diarrhoea are more at risk to develop BRD (Pardon and others, 2013) (Table 1). Calves were undergone to the same sanitary protocol, routinely applied in the farm.

Table 1. Study population (average±SD)

Group	n	Age at arrival (days)	Age at slaughter (days)	Excluded animals due to diarrhoea	Study population
Control	269	21±8	211±9	39 (14.50%)	230
Vaccinated	675	22±9	216±10	79 (11.70%)	596

3.3.2. In vivo and postmortem examinations

Animals were daily inspected by a bovine practitioner not informed about the treatment and overall mortality, mortality due to BRD, BRD prevalence and days on feed (DOF) at the first BRD treatment were recorded. Calves were considered affected by BRD if showed depression, anorexia dyspnoea, cough, nasal discharge and hyperthermia (>39.5°C). BRD diagnosed calves were treated with Florfenicol (20 mg/kg LW) + Spiramycin adipate (600

IU/kg LW) + Metamizole sodium (40 mg/kg LW) for three consecutive DOF. The amount of drug administered to each calf treated for BRD has been determined based on the estimated live weight by the bovine practitioner and cost was then calculated. Arrival age, arrival weight, DOF and cold carcass weight (CCW) were recorded. Final weight was estimated as follow (Story and others, 2000) to calculate average daily gain (ADG): Final weight= CCW/dressing percentage (0.535 for Holstein calves; 0.57 for Simmental calves and 0.60 for Holstein x Belgian Blue calves). ADG= [(CCW/dressing percentage)-arrival weight]/DOF. The amount of discounted carcasses (<115.0 kg CCW) was calculated. Carcass colour was measured 45 minutes post-mortem on rectus abdominis by abattoir operator using a Chromameter (Minolta CR400, illuminant D65, view angle 10°). Carcass colour score (1=white, 2=light pinkish, 3=pinkish, 4=red) was determined following the equation developed by Vandoni and Sgoifo Rossi (2009): Colour score = 10.50106 - 0.38185 (L*) - 0.02906 (b*) + 0.00316 (L^{*2}) - 0.00678 (b^{*2}) - 0.00602 (L*a*) + 0.47206 (Chr).

Blood haemoglobin (Hb) was determined on 100% of the animals at 30 and 90 DOF and on 10% of the animals at 125 and 160 DOF by a commercial lab. At post-mortem inspection, the severity of lung lesions was assessed by a trained veterinary following the scheme proposed by Leruste and others (2012): score 0 - no pneumonia (healthy lung with a normal pale orange colour); score 1 - minimal pneumonia (one spot of grey-red discoloration); score 2 - mild/moderate pneumonia (one larger or several small spots of grey-red discoloration with a total surface of less than 1 lobe); score 3 - severe pneumonia (grey-red discoloration area of at least one full lobe and/or presence of abscesses). The presence of pleuritis and/or consolidations was also recorded (binary outcome).

3.3.3. Statistical analysis

Data distribution was evaluated with Shapiro-Wilk test. ANOVA on ADG, CCW and Hb was performed using a mixed model that accounted for the fixed effects of vaccination and BRD status (BRD affected or not) and the random effect of the animal within the experimental treatment and BRD status (GENLIN function, SPSS 21.0, IBM). Arrival weight was used as covariate. Bonferroni post hoc test was carried out. The frequency of BRD, overall mortality, mortality due to BRD, discounted carcasses, pleuritis and/or consolidation and the frequency of the different colour and lung lesion scores were compared using a GEE (generalized estimating equations) in which the dependent variables shown a Poisson distribution and a log link function was used. Animal was considered as random effect. Odds ratio to be diagnosed as BRD affected was estimated by a logistic regression and in order to visualize the relationship to first BRD event at the different DOF Kaplan-Meier curve was created. The difference of the average DOF at the first BRD episode was evaluated using the non-parametric U test of Mann-Whitney. Values of P≤0.05 were discussed as significant, while for P≤0.10 were discussed as tendency.

3.4. Results

3.4.1. Morbidity and mortality

Vaccination did not reduce overall mortality and mortality due to BRD and BRD accounted for nearly 44% and 27% of mortality for CON and VAC respectively. Vaccination delayed BRD insurgence (P<0.0001; Fig 1). Indeed, BRD morbidity peaked during the third week in CON and at the fifth week in VAC. As evident form Kaplan-Meier curves (Fig 2), vaccination reduced also the relative risk to be affected by BRD (P=0.001) (Table 2).

Figure 1. Relative distribution of morbidity due to BRD in the different weeks on feed

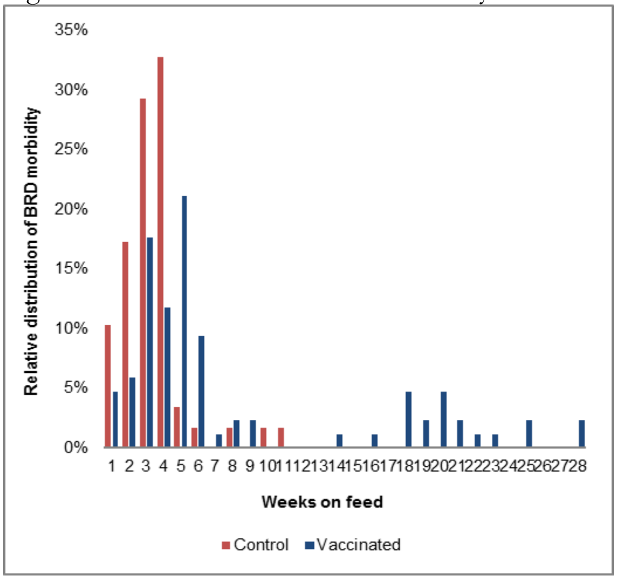

Figure 2. Kaplan-Meier curves of the cumulative probability to be diagnosed as affected by BRD at the different days on feed

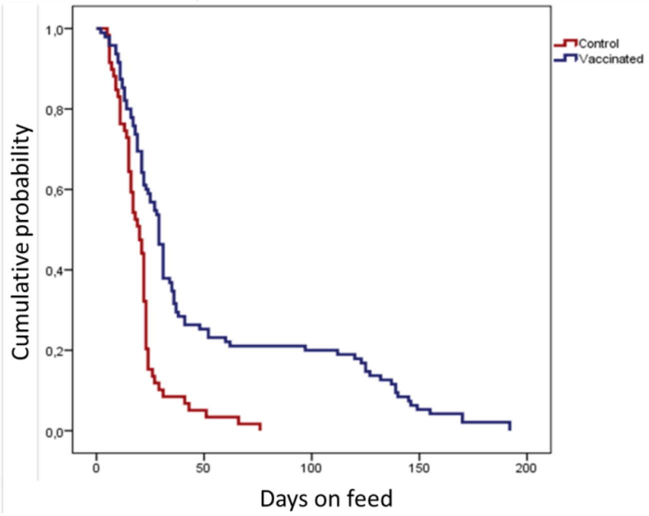

Table 2. Odds ratio estimates to be diagnosed as affected by BRD (CON as reference)

Effect	OR estimates	95% C.I.		P
		Min	Max	
Vaccination	0.55	0.38	0.79	0.001

The ratio between BRD morbidity and BRD mortality revealed that fatality occurred for 10.54% and 11.85% of the diagnosis of BRD in VAC and CON respectively. As expected, mortality of BRD diagnosed calves was higher than those not diagnosed for BRD (Table 3).

Table 3. Effect of vaccination and BRD diagnosis on BRD prevalence, overall **mortality and** mortality due to BRD

Group	BRD prevalence (%)	Overall mortality (%)	Mortality due to BRD (%)
Control	25.65	6.96	3.04
Vaccinated	15.94	6.21	1.68
P	0.009	0.74	0.28
BRD	Not analyzed	11.04	Not analyzed
No BRD		5.36	
P	-	0.01	-

3.4.2. Haemoglobin, growth performance and carcass traits

Hb at 90 DOF tended to be reduced (P=0.07) by BRD and was improved by vaccination (P=0.02), while it was not affected neither by BRD nor by vaccination at 30, 125 and 160 DOF (Table 4).

Table 4. Blood haemoglobin (Hb) at different days on feed (DOF)

Group	Blood Hb (mmol/L)							
	30 DOF	sem	90 DOF	sem	125 DOF	sem	160 DOF	sem
Control	5.42	0.06	5.57	0.05	5.61	0.16	5.64	0.17
Vaccinated	5.49	0.04	5.70	0.03	5.70	0.10	5.70	0.11
P	0.32		0.03		0.64		0.76	
BRD	5.47	0.03	5.65	0.03	5.64	0.09	5.68	0.09
No BRD	5.47	0.07	5.78	0.07	5.63	0.27	5.38	0.36
P	0.96		0.07		0.95		0.60	

Regarding growth performance (Table 5) VAC showed better ADG (P=0.03) and CCW (P<0.001), while the proportion of discounted carcasses was only numerically lowered (P=0.11) by vaccination. As expected, BRD reduced both ADG (P<0.001) and CCW (P<0.001) and increased the proportion of discounted carcasses (P<0.0001).

Table 5. Effect of vaccination and BRD diagnosis on growth performance, cold carcass weight (CCW) and frequency of discounted carcasses

Group	ADG (g/d)	sem	CCW (kg)	sem	Discounted carcasses (% <115kg CCW)
Control	1016	13.72	130	1.31	16.82
Vaccinated	1053	8.96	136	0.87	11.81
P	0.03		<0.001		0.11
BRD	989	16.62	129	1.71	25.55
No BRD	1058	7.18	136	0.74	10.53
P	<0.001		<0.001		< 0.001

The distribution between the different colour scores (Table 6) was affected by vaccination (P=0.004) and VAC group showed more carcasses falling in classes 1 and 2 (lighter, more desirable), while the distribution was unaffected by BRD diagnosis (P=0.29).

Table 6. Effect of vaccination and BRD diagnosis on veal carcass colour

Group	Carcass color score (% of carcasses)			
	1	2	3	4
Control	19.63	24.77	33.64	21.96
Vaccinated	30.77	30.59	25.69	13.96
P	0.004			
BRD	23.36	30.66	28.47	17.52
No BRD	28.62	28.62	26.89	15.88
P	0.29			

3.4.3. Post-mortem inspection

As expected, the distribution into the different lung lesion scores was affected by BRD diagnosis (P<0.0001), with larger proportion of scores 2 and 3 (moderate and severe lesions). BRD diagnosis increased the prevalence of pleuritis and/or consolidation as well (P<0.0001). On the contrary, neither the distribution between lung lesion scores nor the prevalence of pleuritis and/or consolidations was affected by vaccination (P=0.40 and P=0.43 respectively) (Table 7).

Table 7. Effect of vaccination and BRD on lung lesion score and prevalence of pleuritis and/or consolidations

Group	Lung lesion score (% of lungs)				Pleuritis and/or consolidations (% of lungs)
	0	1	2	3	
Control	17.29	21.50	43.93	17.29	58.88
Vaccinated	18.25	29.52	33.63	18.60	54.03
P	0.40				0.43
BRD	10.22	16.06	40.88	32.85	73.72
No BRD	19.65	29.72	35.53	15.09	51.42
P	<0.0001				<0.0001

3.4.4. Economic impact

For the economic evaluation (Table 8), the costs of vaccination and individual treatment for BRD were based on the price tags. Average market values for young stock and carcasses were obtained from the abattoir's technicians. The economic calculation does not consider feed and labour costs, the cost related to other pharmacological treatments, the fixed cost and the carcass disposal. From an economic perspective, VAC gained on average 40.97 €/head more than CON, thanks to the higher carcass weight, the lower proportion of discounted or unacceptable dark carcasses. BRD affected animals gained on average 97.24 €/head less than unaffected ones.

Table 8. Economic impact of vaccination and BRD at the present experimental conditions

	Control	Vaccinated	BRD	No BRD
n	230	596	154	672
Average LW at the entrance (kg)	48.02	48.72	48.45	48.55
Average purchasing price (€/kg)		1.68		
Overall cost of the young stock (€)	18555	48782	12535	54811
Average cost of the vaccination (€/head)*	0.00	6.35	n.a.	n.a.
Cost of the vaccination (€)	0.00	3785	n.a.	n.a.
Calves diagnosed and treated for BRD (n)	59	95	-	-
Average cost of BRD treatment (€/head)	7.03	9.48	8.54	0.00
Cost of the treatments for BRD (€)	415	901	1315	0
Standard carcasses** (n)	142	426	84	484
Average CCW standard carcasses (kg)	135	141	137	140
Market price for standard carcasses (€/kg)		5.00		
Income from standard carcasses (€)	96113	299925	57620	338752
Discounted carcasses (n)	36	66	35	67
Average CCW discounted carcasses (kg)	104	102	104	102
Market price for discounted carcasses (€/kg)		3.50		
Income from standard carcasses (€)	13136	23513	12771	23804
Dark carcasses (n)***	36	67	18	85
Average CCW dark carcasses (kg)	134	143	138	140
Market price for dark carcasses (€)		4.00		
Income from dark carcasses (€)	19236	38233	9908	47559
Overall income (€)	128484	361672	80298	410115
Income per head (€/head)	558.62	606.83	521.42	610.29
Gross profit (€)****	109514	308204	66448	355304
Gross profit per head (€/head)	476.15	517.12	431.48	528.73
Difference (€)	40.97		97.24	

* Labour cost not included

** CCW > 115 kg, colour score 1, 2 or 3

*** CCW > 115 kg, colour score 4

***** Overall income - Overall cost of the youngstock - Overall cost of the vaccination (not for BRD vs no BRD) - Overall cost of the treatments for BRD

n. a. = not analyzed

3.5. Discussion

The enrolled animals can be considered at high risk for BRD due to the light weight at arrival and the season. Calves were born from mother exposed to heat stress in the last third of pregnancy, that reduce colostrum quality (Tao and Dhal, 2013) and autumn is characterized by a significant thermal excursion between day and night. Overall mortality was slightly higher than the finding of Pardon and others (2012b and 2013), studies performed along the year, considering therefore even season with lower risk for BRD. Based on the different ratio between overall mortality and mortality due to BRD, is possible to state that vaccination reduced the severity of BRD in the present study. Pardon and others (20012b and 2013) reported that BRD accounted for 27.7% and 27.1% of overall mortality in unvaccinated veal calves' herds, similar to our findings in VAC. As previously reported, these studies cover all the seasons, therefore the higher ratio found in our unvaccinated calves is likely due to the high-risk season. An increasing of mortality in BRD affected calves agrees with Pardon and others (2013), while a lack of effects of vaccination against virus involved in BRD complex on mortality is consistent with Vahl and others (2014a, b) and Windeyer and others, (2012). Contrarily, Stilwell and others (2008) reported a reduction of mortality in beef calves vaccinated at weaning with quadrivalent vaccine. Frankena and others (1994) and Windeyer and others (2012) did not reported any significant effect of vaccination on BRD morbidity in veal and dairy calves treated with a trivalent (IBRV; BRSV and BVDV) or a quadrivalent vaccine (similar to the one administered in the present study) respectively. On the contrary, a reduction of the overall antibiotic administration during the most critical period for BRD (14 to 84 DOF) after intramuscular vaccination against BRSV, PI3V and M. haemolytica (Vahl and others 2014a) and intranasal vaccination against BRSV and PI3 (Vahl and others 2014b). As vaccination reduces pathogens shedding and transmission (Thurmond and others, 2001; Salt and others, 2007; Vangeel and others 2007), the inconsistencies between the reported studies could be related to the different experimental design. In the first two studies vaccinated and unvaccinated animals were raised in the same barn, while in the present study and in the two works of Vahl and others (2014a, b) vaccinated and unvaccinated calves were kept in two different barns, thus avoiding pathogens transmissions between groups and the confounding effect of different shedding. The lower shedding due to the vaccination may also explain the different timing of the onset of BRD. The delayed onset can be considered a positive effect, since older animals have a more mature immune system. This, couple to the lower pathogens burden, may explain the lower mortality due to BRD in respect of the overall mortality found in VAC. The timing of BRD is consistent with Pardon and others (2011 and 2012 a, b) and Leruste and others (2012). The lack of differences in lung lesions and pleuritis and/or consolidations, although the different morbidity, could be related by timing of BRD, as it peaked in the first two months after arrival and VAC showed higher proportion of late-onset. Indeed, Leruste and others (2012) found a weak correlation between lung lesion and BRD signs on farm at 3 weeks of fattening, while it was moderate with signs recorded at 13 weeks and high with sign recorded 2 weeks before slaughter. Authors stated that early respiratory disorders not always result in persistent lung lesion. In agreement with Leruste and others (2012), also in the present study the frequency of lungs with moderate and severe lesion was higher that the morbidity based on on-farm clinical observations. An impairment of growth performance due to BRD agrees with Frankena and others (1994) and Pardon and others (2013), which reported a reduction in HCW of 8 kg in calves that experienced one BRD episode, similar to our findings. Reduction in weight gain and carcass quality is attributable to anorexia coupled with higher energy and protein demand to support the immune reaction. Infection increases amino acid requirements for acute phase protein synthesis and given the low feed intake, protein requirements are filled increasing mobilization from muscle (Krehbiel and others, 2012). The reduction of feed intake may also explain the lower Hb after the peak of BRD, as nowadays veal calves are fed with a significant amount of solid feed even at 90 DOF. Difference in carcass colour has been reported only for chronically affected calves, while the proportion of unacceptable red carcasses did not differ in the study of Pardon and others (2013) comparing carcasses from animals that showed none or one episode of BRD. The improvement of ADG and, consequently, CCW of VAC animals can be attributed to the reduction of morbidity, which improved feed intake and feed efficiency. The higher feed intake can explain also the higher Hb at 90 DOF as discussed early. Colour variation in veal is primarily explained by lightness (Denoyelle and Berny 1999; Hulsegge and others 2001; Lagoda and others 2002; Vandoni and Sgoifo Rossi 2009), negatively correlated with meat pH (Klont and others, 2000). Although is difficult to elucidate the mechanism at the basis of the lighter colour found in VAC, vaccination may have promoted a better overall health status, reducing disease prevalence and severity, with a consequent higher feed intake and lower stress, that could have improved muscle glycogen and post-mortem pH decline. The reduction of discounted carcassesand the lower proportion of undesirable red carcasses led to an improvement of gross profit. The impact of BRD on veal carcass value was higher than that reported by Pardon and others (2013) for Holstein Friesian veal. The difference is likely due to the fact that in the reported study the economic impact was based only on carcass value and differentiated for the number of relapses, while in the present study also mortality was taken into account and relapses were not differentiated.

Results from the present study confirm the detrimental effect of BRD on veal calves' health, growth performance and carcass quality. In this scenario vaccination was effective to reduce BRD prevalence, thus improving animal health and welfare, growth performance and economic result. In conclusion, vaccination against viruses involved in bovine respiratory disease complex represents a useful strategy to reduce BRD prevalence and negative effects in veal industry.

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CHAPTER 4

The effect of different selenium sources during the finishing phase on beef quality

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4. The effect of different selenium sources during the finishing phase on beef quality

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4.1. Abstract

Selenium (Se) is involved in several biological functions and its supplementation is necessary for farm animals. Selenium can be provided in organic or inorganic forms, which are metabolized differently. The aim of this study was to compare the effects of switching the selenium source in the last 60 days of fattening on beef quality and Se content. Charolaise beef heifers supplemented since the beginning of the fattening period with sodium selenite (162 heads; 517±61 days of age) were divided into two groups, fed the same diet, in which Se (0.2 mg Se/kg DM of feed) was provided as sodium selenite (SS; 82 heads, 450.36±33.69 kg BW) or selenium-enriched yeast (Se-Y; 80 heads, 454.60±41.70 kg BW). The Se source did not affect growth performance, meat centesimal composition, thawing loss, cooking or drip losses, and pH during 8 days of aerobic storage. Se-Y supplementation improved the meat Se content ($P<0.001$) and tended to reduce shear force ($P=0.076$) at 48 h post-mortem. Lightness ($P<0.01$) and yellowness ($P<0.01$) decreased with the duration of storage and were higher in the Se-Y group compared with the SS group during 8 days of storage. Meat from group Se-Y also showed a better visual score for colour ($P<0.01$), odour ($P<0.05$), surface wetness ($P<0.05$), and overall appearance ($P<0.01$). Under the given experimental conditions, switching beef heifers from inorganic to organic selenium in the last two months of fattening improved meat tenderness, shelf life, colour stability, and muscle Se content.

4.2. Introduction

Selenium (Se), in form of selenocysteine, is present at the active site of glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinase, seleno-phosphate synthetase 2, selenoprotein P, and different kinds of selenoproteins, and is, therefore, involved in the reduction of oxidized antioxidants, scavenging reactive oxygen species, synthesis of thyroid hormones, protection of DNA and proteins from oxidation, redox signals, and immune responses (Lu & Holmgren, 2009). Thanks to these biological roles, Se supplementation improves ruminants' immune response, neutrophil and lymphocyte activity and disease resistance (Finch and Turner, 1996; Spears, 2000; Chauhan et al., 2014). These results, coupled with the soil deficiency of Se in large areas of the world (Gissel-Nielsen, 1987; Cantor, 1997; McDowell, 1997; Oldfield, 2002), underline the need to administer supplementary selenium to farm animals. Dietary Se can be supplemented through inorganic or organic forms. The first are mainly represented by mineral salts such as selenite or selenate of sodium or cobalt, while, the latter, by selenium-enriched yeast (Se-Y), in which the main selenocompund is represented by selenomethionine (SeMet), or SeMet itself (mainly produced from selenium enriched yeasts; Korhola et al., 1986). Due to being differently metabolised, inorganic forms are characterized by a lower bioavailability than organic forms (Weiss, 2005). In terms of beef and veal quality and oxidative stability, although organic supplementation generally increased the meat selenium content compared with inorganic supplements at the same dosage (Juniper et al., 2008; Cozzi et al., 2011; Richards et al., 2011), inconsistencies in the effects on meat quality have been reported. Despite long-term organic Se supplementation significantly increasing dietary costs, only Cozzi et al. (2011) have studied short-term supplementation in bullocks. Thanks to the higher bioavailability of organic Se, we hypothesize that short-term supplementation may be able to improve meat quality and Se content, with a minimal impact on production cost. In the present study, a short-term supplementation strategy has been tested on beef heifers, chosen because they nowadays already represent a premium product at the retail level, and are, therefore, more suitable for high-Se beef production compared with bullocks.

4.3. Materials and methods

4.3.1. Animals and animal care

The study was performed in a commercial intensive beef fattening farm located in the northeast of Italy. A total of 162 Charolaise heifers, of average age of 517 ± 61 days, were enrolled and randomly assigned to the two dietary treatments: sodium selenite (SS) or selenium-enriched yeast (Se-Y). Animals were housed in two large open-air yards, one per treatment, with a full concrete floor covered with corn stoves. The trial was conducted during the last 60 days prior to slaughter. The SS group included 82 heads with an average BW of 450.36 ± 33.69 kg, while the Se-Y group included 80 heads with average BW of 454.60 ± 41.70 kg. Animal care and treatment were in accordance with the European Community 1986 guidelines n.609.

4.3.2. Experimental diets and feeding routine

The heifers were fed the same basal diet (43.3% corn silage, 14.4% ryegrass silage, 19.3% high moisture corn, 7.2% wheat bran, 4.8% corn gluten feed dry, and 11.0% of protein-fibrous mix containing as fed 26.2% CP, 4.5% EE, 10.5% CF, 13.8% ash, 40 000 UI/kg vitamin A, 4 000 mg/kg vitamin D3, 120.0 mg/kg vitamin E, 2.4 mg/kg vitamin B1, 1.6 mg/kg vitamin H, 400.0 mg/kg niacin, 0.8 mg/kg Co, 120.0 mg/kg Mn, 3.2 mg/kg Se, 200.0 mg/kg Zn, 85.5 mg/kg Fe, 4.0 mg/kg I, 24.5 g/kg urea) formulated to meet or exceed NRC nutritional requirements (NRC, 2000), differing only in selenium source: SS or Se-Y (Selsaf EC No. 3b8.12, produced by Saccharomyces cerevisiae strain CNCM-I3399, Lesaffre Feed Additives Italia, Italy), in which inorganic Se accounted for less than < 1 % of the total Se. Organic selenocompounds were mainly represented by selenomethionine (62.7%) and selenocysteine (2% to 4% of total Se), while the remaining organic Se compounds were not specified (EFSA, 2009). Se was supplemented through the mineral and vitamin mix, and its inclusion was targeted to provide 0.2 mg Se/kg DM of feed. Experimental diets were administered ad libitum and delivered in TMR form once a day in the morning by a feed mixer wagon, provided with a balance to weigh the inclusion of each ingredient. Water was available ad libitum.

4.3.3. Growth performance and health status

Individual weight was recorded prior to the morning feeding on enrolment (day 0) and the day before slaughter (day 60) and average daily gain (ADG) was subsequently calculated.
Health status was monitored daily by the veterinarian staff and no adverse clinical symptom was recorded.

4.3.4. Meat samples and analysis

At the end of the finishing period, the animals were slaughtered at the same slaughterhouse and carcass characteristics were recorded. After slaughtering, conformation (SEUROP) and fattening score (1–5) were assessed by an expert judge following EU legislation (Council Regulation (EEC) No 1026/91). Cold carcass weight was obtained after 48 hours of chilling at a temperature of 0°C to 4°C and, at the same time, samples of

the longissimus thoracis muscle between the 5th and the 7th rib were taken from 30 homogenous carcasses for the experimental group.
Each sample was divided into three subsamples, two 2.50 cm steaks, used fresh, the first one to evaluate meat colour, pH, WHC, and the second for shelf-life assessment, daily for 8 consecutive days. The third subsample was weighed, vacuum-packaged, and kept frozen at –20 °C until chemical and physical analysis.
The shelf-life of each steak, kept in a plastic box, overwrapped with polyethylene film and kept at 0 °C to 4 °C in a dark room, was visually assessed daily by a three-member expert food inspector panel, which evaluated lean colour (8 = bright cherry-red, 1 = extremely dark brown or green/grey), overall appearance (8 = extremely desirable, 1 = extremely undesirable), surface wetness (7 = humid and bright; 4 = dry), and odour (7 = “fresh beef” odour; 5 = no odour; 3 = slight odour development but still acceptable; 2 = definite off-odour indicative of spoiled beef; 1 = very strong off-odour associated with spoiled beef); the average of the three observations was considered for statistical analysis. On the other sample, kept under the same conditions, pH and instrumental colour were recorded daily, while drip-loss was assessed at the beginning and at the end of storage. Measurements of pH were made by a portable pH-meter (HI 98150, HANNA Instruments Inc., Woonsocket, RI, USA) equipped with a glass electrode (3 mm Ø conic tip) suitable for meat penetration; values for each sample came from the average of three measurements. Colour determination was performed using a CR310 Chromameter, set on D65 illuminance, calibrated on the CIE LAB colour space system using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras) and lightness (L*), redness (a*), and yellowness (b*), were calculated according to the CIELab system. The colorimeter had an 8-mm measuring area and the average of 10 repetitions was recorded as the value for each sample. For drip loss determination, samples were dried from superficial wetness and weighed at the start and at the end of storage time. Chemical composition (dry matter, ether extract, crude protein, and ash) was determined on samples trimmed from external fat and connective tissue and homogenized for 30 seconds according to AOAC (1990). Thawing loss was assessed by weighing, freezing, and weighing after 24 hours of thawing at 4 °C. Cooking loss was determined, as described by Honikel (1988), as the weight lost after cooking in a water bath until the core temperature reached 75 °C (monitored with a temperature meter Hanna Instruments HI98840) and 24 hours of storage at 4 °C. Before being weighed, the samples were blotted dry. The difference between pre- and post-cooking weights was used to calculate the percentage loss during cooking. After cooking loss determination, six cylindrical cores, 1.27 cm in diameter, parallel to fibre orientation, were obtained and used for shear force evaluation, using a

Warner-Bratzler shear force texture analyser (model 4466; Instron Corp., Canton, MA). The peak force (kg/cm2) was then recorded.

4.3.5. Meat selenium content

The selenium content in fresh meat was assessed by a commercial laboratory using ICP-MS methods (Agilent 7500cx). Analytical procedures were performed following UNI CEI EN ISO/IEC 17025:2005 standards.

4.3.6. Statistical analysis

Body weight, average daily gain (ADG), meat selenium content, shear force, and drip loss were analysed by one-way ANOVA (SAS Inst. Inc., Cary, NC) considering the main effect of treatment. Colour parameters, pH, and visual evaluation score were analysed by a two-way ANOVA using a general linear model for repeated measures, considering the effects of treatment, storage time and their interaction (SAS Inst. Inc., Cary, NC). The significance level was set and discussed for $P \leq 0.05$, while $P \leq 0.10$ was considered a tendency.

4.4. Results

Final body weight, average daily gain and carcass characteristics did not differ between groups (Table 1).

Table 1. Least square means for the effect of selenium source during finishing phase on rearing performance

Item	SS	Se-Y	sem	P
Average initial BW, kg	450.39	454.60	4.28	0.48
Average final BW, kg	529.46	535.33	4.94	0.40
ADG, kg/d	1.39	1.42	0.03	0.53

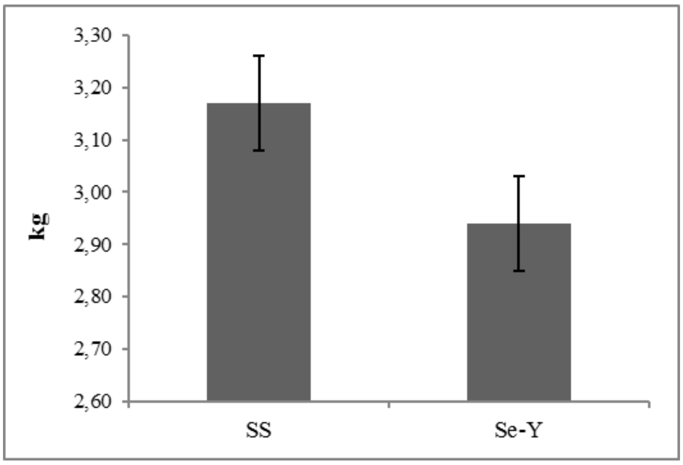
Meat centesimal composition in the selected carcasses was not affected by treatment, while Se-Y supplementation markedly increased the meat selenium content ($P < 0.001$) (Table 2).

Table 2. Least square means for the effect of selenium source during finishing phase on meat chemical composition, selenium content thawing, drip and cooking loss of 30 carcasses/group.

Item	SS	Se-Y	sem	P
Cold carcass weight, kg	318.06	319.27	2.85	0.77
Dressing percentage, %	55.08	54.95	8.03	0.42
Humidity, %	72.87	72.70	5.23	0.24
Crude Proteins, %	22.73	22.96	0.09	0.10
Ether extract,%	3.39	3.33	0.06	0.44
Ash, %	1.01	1.01	0.11	0.80
Se, mg/kg DM	0.425	0.791	0.07	<0.001
Thawing loss, %	0.70	0.76	0.033	0.22
Drip loss, % after 8 days of storage	2.06	2.05	0.09	0.98
Cooking loss, %	29.31	29.17	0.76	0.79

The replacement of SS with Se-Y tended to reduce shear force on cooked samples (P = 0.076) (Fig. 1). The selenium source did not affect thawing, cooking, or drip losses after 8 days of storage (Table 2).

Figure 1 Least square means (±SEM) for the effect of selenium source during finishing phase on shear force at 48 hours post mortem (P=0.076).



During storage, the pH increased (P < 0.001), but was not affected by treatment (Table 3).

Table 3. Least square means for the effect of selenium source during finishing phase on meat pH during 8 days of storage.

Days of storage	pH		
	SS	Se-Y	sem
Day 1	5.70	5.69	0.023
Day 2	5.70	5.77	0.033
Day 3	5.73	5.77	0.015
Day 4	5.79	5.77	0.023
Day 5	5.73	5.74	0.028
Day 6	5.75	5.76	0.021
Day 7	5.91	5.88	0.032
Day 8	5.92	5.99	0.040
P(s)[1]		0.60	
P(t)[1]		<0.001	
P(s*t)[1]		0.06	

[1] s: selenium source; t: storage time; s*t: selenium source*storage time

Regarding colour parameters, lightness (L*) was affected by treatment (P < 0.01), storage time (P < 0.01), and their interaction (P < 0.05), and the treated group was characterized by significantly higher L* values. In the same way, redness (a*) decreased with increasing storage time (P < 0.01), but was not affected by selenium source or their interaction. The treated group also showed a higher yellowness (b*) (P<0.01), which decreased in both groups during storage (P < 0.01), but was not affected by the interaction between treatment and storage time (Fig. 2). Considering also the decreasing trend of lightness and redness, samples from animals fed organic selenium showed higher L* and a* stability during the first days of storage (Fig. 2).

Table 4. Least square means for the effect of selenium source during finishing phase on color, odor, surface wetness and overall appearance score during 8 days of storage.

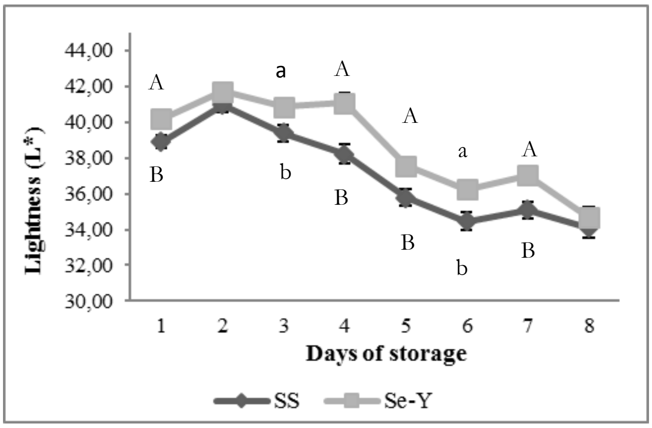
Days of storage	Color[1]			Odor[1]		
	SS	Se-Y	sem	SS	Se-Y	sem
Day 1	7.43	7.71	0.19	7.02	7.11	0.20
Day 2	6.86	7.29	0.23	6.42	6.71	0.33
Day 3	6.09	6.57	0.30	5.57	6.14	0.39
Day 4	5.03[a]	5.86[b]	0.24	4.14	4.71	0.35
Day 5	4.43[a]	5.11[b]	0.21	2.71	2.86	0.17
Day 6	3.57[A]	4.43[B]	0.20	1.43	1.71	0.19
Day 7	2.75[A]	3.95[B]	0.23	1.00[a]	1.45[b]	0.14
Day 8	2.14[A]	3.29[B]	0.27	1.00[a]	1.29[b]	0.13
P(s)[1]		<0.01			<0.05	
P(t)[1]		<0.001			<0.001	
P(s*t)[1]		0.68			0.84	
Days of storage	Surface wetness[1]			Overall Appearance[1]		
	SS	Se-Y	sem	SS	Se-Y	sem
Day 1	6.71	6.83	0.18	7.61	7.80	0.18
Day 2	5.81	6.14	0.27	6.85	7.14	0.26
Day 3	5.28	5.69	0.29	5.58	6.28	0.25
Day 4	4.29	4.57	0.19	4.46[a]	5.15[b]	0.24
Day 5	4.02[a]	4.31[b]	0.14	3.71[a]	4.28[b]	0.29
Day 6	3.15[a]	3.65[b]	0.14	2.42[a]	3.21[b]	0.19
Day 7	2.12[A]	2.97[B]	0.15	1.57[A]	2.42[B]	0.20
Day 8	1.56[A]	1.98[B]	0.18	1.14[A]	1.86[B]	0.14
P(s)[1]		<0.05			<0.01	
P(t)[1]		<0.001			<0.001	
P(s*t)[1]		0.76			0.49	

1 s: selenium source; t: storage time; s*t: selenium source*storage time
Within a row, means without a common superscript differ (A,B P≤0.01; a,b P≤0.05).

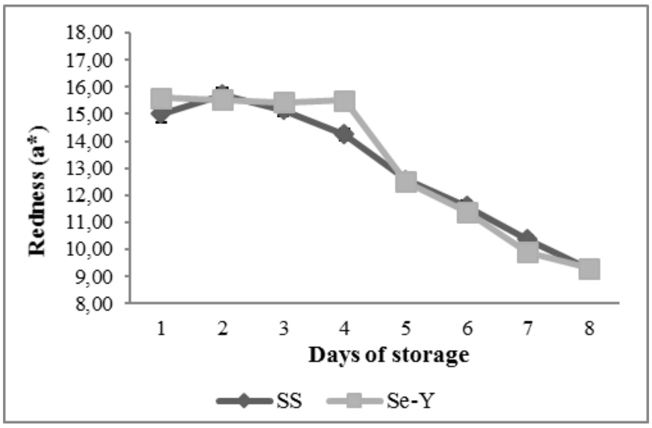
Meat shelf-life was also affected by treatment and storage time (Table 4). As expected, colour, odour, surface wetness, and overall appearance scores decreased during storage ($P < 0.001$) and the selenium source positively affected all of these visual parameters. The colour score was higher in the Se-Y group ($P < 0.01$) from the fourth day, the odour score was higher ($P < 0.05$) on the last two days, while surface wetness was higher ($P < 0.05$) starting from the fifth day of storage. The overall appearance benefited from these ameliorating effects and its score was increased by administration of organic selenium ($P < 0.01$) starting from the fourth day of storage.

Figure 2 Least square means (±SEM) for the effect of selenium source during finishing phase on Lightness (a), Redness (b) and Yellowness (c) during 8 days of storage (A,B P≤0.01; a,b P≤0.05).

a



b



4.5. Discussion

The absence of the effect of selenium source on growth performance and carcass traits is consistent with other studies in fattening cattle (Nicholson et al., 1991 and Cozzi et al., 2011), male goats (Shi et al., 2011), and lambs (Vignola et al., 2009). Similarly, absence of an effect of selenium source on meat centesimal composition was also reported by Taylor et al. (2008) and Cozzi et al. (2011) in beef, and by Vignola et al. (2009) in lambs. Given that Se is mainly implicated in improving immune response, as previously reported, this result was expected, as the final stage of the fattening cycle is not a critical stage for infectious diseases. Indeed, the disease most impacting beef cattle production is bovine respiratory disease (Radostits et al., 2007), which affects, especially, newly received beef cattle (Galyean et al., 1999). The rise in the muscular selenium concentration achieved by supplementing Se-Y in respect to SS is consistent with the findings of Juniper et al. (2008) and Cozzi et al. (2011) in beef, Juniper et al. (2009) and Vignola et al. (2009) in lambs, and Shi et al. (2011) in growing male goats. This confirms the greater bioavailability of the organic compared with the inorganic form. Indeed, at the rumen level, part of the dietary SeMet, the prevalent form in the provided Se-Y, is incorporated into bacterial protein, thus becoming part of metabolizable protein. The remaining amount is efficiently absorbed at the gut level through methionine carriers. In contrast, most of the selenate is reduced to selenite and subsequently converted into weakly absorbable compounds or used by microbes to synthesize seleno-amino acids. The escaped selenate is actively absorbed via a cotransport pathway along with sodium ions, while selenite is absorbed via passive transfer. After absorption, SeMet can be incorporated into proteins, in replacement of methionine, or catabolized and the Se utilized for SeCys synthesis. The absorbed selenite is reduced instead to selenide, utilized to synthesize SeCys, while the portion not immediately converted to SeCys is methylated and excreted (Weiss, 2005). Increasing the meat Se content is an important outcome, as meat is one of the major contributors to Se intake in the human diet and a low Se status is linked to a higher risk of mortality, poor immune function, and cognitive decline (Rayman, 2012). Moreover, in the majority of EU countries, Se intake is lower than the recommended value of 55 μg/day (Rayman, 2004; Thomson, 2004). Overall, from an economic perspective, given the short-term supplementation and, especially, the small amount provided, it is possible to conclude that the replacement of inorganic selenium with selenium-enriched yeast does not significantly affect beef production costs. Moreover, the higher bioavailability can allow producing selenium-enriched meat with a consequently higher added value. An increase in beef tenderness has also been reported by Cozzi et al. (2011). In our trial we found a reduction of shear force at 48 hours post mortem and, considering the positive relationship found between meat selenium content and glutathione peroxidase (GSH-Px) activity (Juniper et al., 2008), this may be explained by lower calpain oxidation due to the highest GSH-Px activity in the Se-Y group. Indeed, during post-mortem muscular proteins undergo marked oxidation that could impair meat tenderness by decreasing protease activity and inducing myofibrillar protein cross-linking (Huff Lonergan et al., 2010; Estevez, 2011; Lund et al., 2011). As reviewed by the same authors, both μ- and m-calpains contain histidine and cysteine thiol groups in their active site; these groups are highly susceptible to oxidation resulting in inactivation. Moreover, cross-linking of myofibrillar proteins reduces their susceptibility to degradation and improves the strength of the myofibrillar structure, increasing meat toughness. In this scenario, is possible to hypothesize that the increase of muscular antioxidant activity due to the higher Se content could have reduced the extent of enzymatic and myofibrillar protein oxidation. This hypothesis is supported by the findings of Rowe et al. (2004a, b), who reported that dietary antioxidant supplementation in beef (vitamin E) reduced protein oxidation, positively promoting post-mortem proteolysis and, consequently, beef tenderness. Nevertheless, further investigations are necessary to better clarify the role of Se in the meat tenderization process, protease activity, and protein oxidation.

The lack of effect of selenium source on meat drip loss is consistent with the findings of Cozzi et al. (2011) in beef and Vignola et al. (2009) in lambs. In contrast, some authors reported an increase in WHC in subjects supplemented with an organic selenium source in respect to those fed an inorganic one in studies on poultry (Downs et al., 2000 and Wang et al., 2011a,b) and pigs (Mateo et al., 2007 and Zhan et al., 2007).

Regarding meat pH, our findings are in agreement with those of Zhan et al. (2007) in pigs, Vignola et al (2009) in lambs, and Juniper et al. (2011) in turkeys. Cozzi et al. (2011), in contrast, reported a higher beef pH after 6 days of ageing in animals fed organic selenium compared with those fed sodium selenites or switched from it to selenium yeast in the last 70 days, but no differences were found by the same authors at 11 days post mortem or between animals fed for the entire fattening period with sodium selenite or switched to selenium yeast, as we did in this trial, at both ageing times. Furthermore, Wang et al. (2011b) found that the pH tended to be higher in the breast of broilers fed with selenomethionine in comparison with those receiving sodium selenite.

Improvement of lightness due to supplementation of organic selenium was also reported by Cozzi et al., (2011), who found an increased L* value after 6 and 11 days of vacuum packaged ageing. In contrast, Vignola et al. (2009) reported no effects of selenium source on lamb meat lightness, redness, and yellowness during 9 days of simulated display life. The same authors did not report differences in meat redness or yellowness. Regarding these two parameters, however, Taylor et al. (2008) found that meat from cattle fed a selenium-enriched diet had a higher Se content and tended to have a higher average a* and b* during 12 days of display, compared with animals fed an unenriched diet. Given that lightness is related to meat protein structure (MacDougall, 1982) and not with myoglobin status (McKenna et al., 2005), therefore, this excluded reduction of myoglobin oxidation as an explanation; the effect of organic selenium on this parameter still remains to be clarified, considering also the above reported inconsistency between studies.

4.6. Conclusions

Under the presented experimental conditions, switching selenium supplementation from sodium selenite to selenium-enriched yeast during the last two months of fattening did not affect heifer performance or meat water holding capacity, but improved meat tenderness and colour stability during storage, with a positive impact on meat shelf life. The present study also confirmed that short-term supplementation represents a valid strategy for increasing meat Se content, an important outcome from the market and human health perspectives. Further investigations are necessary to better clarify the magnitude of and mechanisms involved in the effects of organic Se supplementation on meat colour, as well as the potential impact on the post-mortem tenderization process and protein oxidation.

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CHAPTER 5

Comparison of a grain-based diet supplemented with vitamin E versus a lucerne-based diet on carcass traits, muscle vitamin E and fatty acid contents, lipid oxidation, and retail color of meat in lambs

5. Comparison of a grain-based diet supplemented with vitamin E versus a lucerne-based diet on carcass traits, muscle vitamin E and fatty acid contents, lipid oxidation, and retail color of meat in lambs

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5.1. Abstract

Inadequate concentration of vitamin E affect retail color and oxidative stability. Vitamin E (VitE) can be supplemented as synthetic all-rac α-tocopheryl acetate or through antioxidant-rich feed as lucerne. The study compared the effects of feeding lambs with a grain based diet at moderate (MOD 42 mg·kg^{-1} VitE E as all-rac α-tocopheryl acetate) or supranutritional (SUP 285 mg·kg^{-1} of vitamin E all-rac α-tocopheryl acetate) levels of vitamin E and organic selenium or a lucerne based diet (LUC; 37 mg·kg-1 VitE) for 8 weeks before slaughter. Forty-eight lambs blocked by sex (wethers or females) and feed intake in the last three days of adaptation were randomly assigned to one of the three dietary treatments (16 lambs/treatment). Treatment did not affect DMI (P=0.46) and ADG (P=0.76). LUC group showed lower n-6 and PUFA compared to both MOD (P<0.01) and SUP (P<0.01). Despite a similar VitE intake, muscle vitamin E was higher for LUC compared to MOD (P<0.05), while SUP fed animals showed the highest content (P<0.01). Although that, fresh muscle from LUC showed a better a* stability during 4 days display time compared

to MOD and SUP, while no differences were evident considering 6-weeks aged muscles. Oxidative stability measured with T-bars method, did not differ between groups at the beginning and at the end of display life for both fresh and aged muscles. Increasing dietary vitamin E in finishing lambs increased muscle vitamin E content. However, finishing lambs on Lucerne improved color stability of fresh lamb meat, compared to the artificial form.

5.2. Introduction

Nowadays consumers are well informed about nutritional characteristics of food and its effects on human health, thus increasing the focus or selection criteria on food that has a high nutritional value (Schmidt, 2000; European Commission, 2010). In this scenario an improvement of the nutritional value of lamb represents a market opportunity for the lamb industry, as nutritional characteristics are recognized as important traits by consumers (Lamb et al., 2010). Other than nutritional aspects, color is one of the major factors that can affect consumer perception of meat at the retail level (see e.g. Mancini and Hunt, 2005; Font-i-Furnols and Guerrero, 2014) because it indicates the freshness of meat. Vitamin E is known to improve meat color and oxidative stability by reducing susceptibility of muscle to lipid peroxidation (Liu et al., 1995; Faustman et al., 1998; Sales and Koukolová, 2011) and inadequate concentration of vitamin E was demonstrated to affect retail color and oxidative stability of lamb (Ponnampalam et al., 2012a, Ponnampalam et al., 2014a). Nutritional background is one of the main factors influencing muscle antioxidant statusand pasture fed ruminants are characterized by a higher level of muscle antioxidant (mainly α-tocopherol) when compared to those fed concentrate based diets (Daley et al., 2010; Zervas and Tsiplakou, 2011; Bekhit et al., 2013; Van Elswyk and MacNeill 2014). In the case of scarce pasture or low quality senescent pasture, vitamin E supplementation is required to improve growth performance of lambs as well as meat quality (Ponnampalam et al., 2012b). Therefore, the inclusion of supranational levels of antioxidant in supplemental feed, among which synthetic vitamin E (ester acetate all-rac α-tocopherol) is the most commonly employed (Vagni et al., 2011), represent a useful strategy to improve muscle antioxidant status, when sufficient availability of selenium is guaranteed. Alternatively, antioxidants can be provided by feeding high quality forages as lucerne, which contains vitamin E in the natural form (RRR α-tocopherol), carotenoids, flavonoid and phenolic compounds which exert antioxidant activity (Ferreira et al., 2015). Lucerne represents an important alternative to grass pasture especially in the summer season due to its tolerance to drought and the positive impact on animal productivity and carcass quality (Humphries, 2012; Ponnampalam et al., 2014b). This study aimed to compare the effects of feeding lambs with a grain based diet at moderate or supranutritional levels of vitamin E and organic selenium or a lucerne based diet during late summer and autumn. We hypothesized that the lucerne based forage diet would ensure carcass traits and muscle quality similar to the grain based diet.

5.3. Materials and methods

All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004). The study was approved by the Animal Ethics Committee (AEC #2014-19), Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR). The experiment was conducted from February to April 2015 at Rutherglen research center, DEDJTR, Victoria, Australia.

5.3.1. Animals background

Sixty Poll Dorset x [Border Leicester x Merino] wether and ewe lambs with similar live weight and age were selected at weaning (3 months) from the DEDJR Rutherglen Research Farm Flock and allowed to graze an improved perennial pasture without lucerne until the beginning of the study. Six month old lambs were then selected, weighed and allocated to individual pens (1.25 m^2) with meshed floor and walls and monitored during ten days of adaptation to the new diet and environment. The adaptation diet consisted of perennial hay plus pellets with a moderate amount of antioxidant (MOD), with the former gradually reduced and the latter gradually increased over the first 14 days. Ad libitum pellets were provided for the last 3 days of adaptation and individual feed intake during these days was recorded. Fresh water was provided daily ad libitum. Forty-eight animals were selected for the feeding experiment, based on health status and average pellet intake in the last 3 days of adaptation. Animals were blocked by sex (wethers or females), feed intake in the last three days of adaptation and randomly assigned to one of the three dietary treatments (16 lambs/treatment): 1. moderate level of antioxidant (MOD, 42 mg·kg-1 vitamin E as all-rac α-tocopheryl acetate and 0.1-0.15 mg·kg-1 mg of organic Se as Selenosource® (Diamond V Mills, Inc. USA)), 2. supranutritional level of antioxidant (SUP, 285 mg·kg-1 of vitamin E all-rac α-tocopheryl acetate and 0.5-0.6 mg·kg-1 of organic Se as Selenosource® (Diamond V Mills, Inc. USA)) or 3. lucerne based diet (LUC, 37 mg·kg-1 vitamin E). Diets were formulated by Farm Balance, Kerang, Victoria 3579, Australia. CON and SUP diets were pelleted to a 3 mm diameter in order to avoid feed selection, potentially impairing antioxidant intake, while LUC was provided as a total mixed ration. Vitamin E inclusion in the MOD pellet was designed to achieve a similar content of VitE as in the LUC diet, because vitamin E concentration in the lucerne was determined prior to the beginning of the study. One hundred g of feed samples were collected weekly and pooled to determine chemical composition (Table 1).

Table 1: Chemical composition of the grain based diets with moderate (MOD) or supranutritional (SUP) levels of antioxidant or lucerne based diet (LUC) fed to lambs.

Components, $g \cdot kg^{-1}$	MOD	SUP	LUC
Oat hay	300	300	-
Lucerne hay	-	-	600
Barley	530	530	390
Canola expeller	150	150	-
Limestone	10	10	-
Acid Buf® (Celtic Sea Minerals)[1]	10	10	10
Chemical composition			
DM	91.6	91.8	89.2
CP, $g \cdot kg\ DM^{-1}$	156	138	150
NDF, $g \cdot kg\ DM^{-1}$	230	246	222
ADF, $g \cdot kg\ DM^{-1}$	140	145	462
Estimated IVDOMD[2], %	76.7	76.6	67.3
Ash, $g \cdot kg\ DM^{-1}$	87	81	36
Estimated ME, $MJ \cdot kg\ DM^{-1}$	13.1	13.4	10.6
Vitamin E, $mg \cdot kg\ AF^{-1}$	41.92	284.79	37.19

[1] DM 95% ¸Ca 30%, Mg 5.5%, K 0.7%, P 500 $mg \cdot kg^{-1}$, Bo 10 $mg \cdot kg^{-1}$ Fe 800 $mg \cdot kg^{-1}$, Co 0.1 $mg \cdot kg^{-1}$, Cu 10 $mg \cdot kg^{-1}$, Zn 10 $mg \cdot kg^{-1}$, Mn 50 $mg \cdot kg^{-1}$, Mo 0.2 $mg \cdot kg^{-1}$ Se 1.8 $mg \cdot kg^{-1}$, I 30 $mg \cdot kg^{-1}$

[2] In vitro digestibility of organic matter.

Temperature (T°) and relative humidity (RH) were recorded every 20 min for the entire experimental period using data loggers (Tynitag®, Gemini Data Loggers, Chichester, UK) placed in 5 different locations of the feeding shed. THI was calculated according to Marai et al., (2007): THI = db °C -[(0.31 – 0.31RH) (db °C – 14.4)] where db°C is dry bulb temperature in C and RH = relative humidity percentage/100.5.

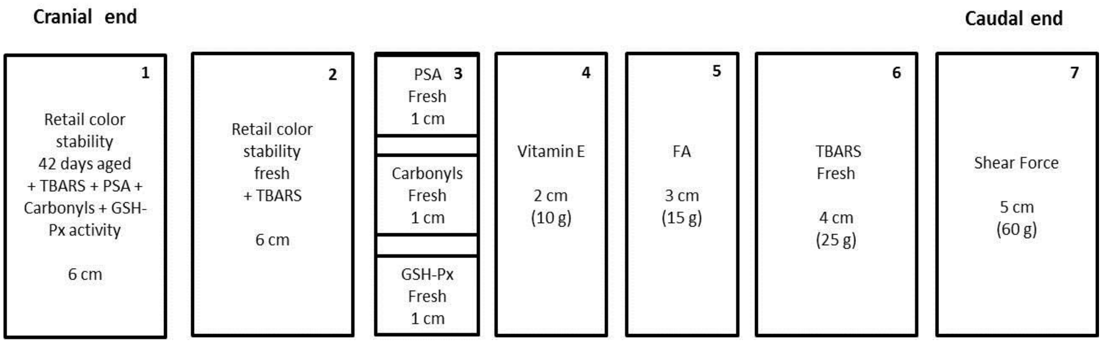
The average THI was 25.14 in the first 4 weeks (summer) and 19.20 in the latter 4 weeks (autumn) of the experimental period.

Animals were fed twice a day and the amount of feed refused was recorded prior to the morning feed in order to calculate daily feed intake. Lambs were weighed weekly prior to morning feeding with a portable scale (SO 33 Manual Weigh Crate, ProWay-Prattley Pty Ltd, Wagga Wagga, NSW, Australia) with an accuracy of 0.1 kg. Seven animals were excluded from the experiment due to poor body condition score or impaired health status and 1 carcass was condemned at the abattoir. Therefore, carcass data and muscle samples for the determination of meat quality characteristics from these animals were not available.

5.3.2. Slaughtering and meat quality analysis

At the end of the feeding period the animals were transported (300 km) to a commercial abattoir where they were kept in lairage (in a separate pen at the abattoir) for seventeen hours overnight. The lambs were slaughtered after head only stunning. All carcasses were electrically stimulated approximately 30 minutes after stunning before being chilled. Carcasses were chilled over a 24 h period. Hot carcass weight (HCW) was recorded and the tissue depth (muscle and fat) at 11th/12th rib intersection, 110-mm from the midline was measured using a GR knife (GR). At 24 h post-slaughter, the muscle longissimus lumborum (LL) was removed from the left side of the carcass (from the 9th and 10th lumbar vertebrae to the caudal end), trimmed of external fat and connective tissue and sampled as reported in Figure 1 for meat quality analysis.

Figure 1. Sampling diagram of the left loin for meat quality analysis (samples 1, 2, 4, 5 and 6 have been processed to obtain the data reported in the present paper, while data obtained from samples 3, part of the sample 1 and 7 will be presented elsewhere).



Samples for ageing were vacuumed packed, stored for 42 days at 2°C and then used for the evaluation of retail color. For retail color stability, the designated portion of muscle LL was sliced to create a fresh surface (2 slices of 2 cm thickness each), placed on a black foam tray and over wrapped with a PVC food film (15 μm thickness) as retail packs. For both fresh and aged meat retail colour assessment, the LL muscle was sliced at 1 and 42 days post slaughter, respectively. Trays were maintained at 3–4 °C under fluorescent light (1000 lux) for 72 h. Lightness (L*-value), redness (a*-value) and yellowness (b*-value) of meat at 0 (allowed to bloom 30 minutes), 24, 48 and 72 h of retail display were measured in duplicate on each sample using a Hunter Laboratory Mini Scan XE Plus meter with a 25-mm aperture, light source set to illuminant D-65 with a 10 degree standard observer (model 45/0-S, Hunter Associates Laboratory Inc., Reston VA, USA) as described by Ponnampalam et al. (2012b). An estimate of the oxy/met myoglobin ratio was calculated by dividing the percentage of light reflectance at wavelength 630 nm by the percentage of light reflectance at wavelength 580 nm ($RF_{630/580}$), which was used as an indicatior of brownness formation on the meat surface. Lipid oxidation was assessed by measuring the concentration of malondialdehyde (MDA, expressed in mg/kg of muscle) using the thiobarbituric acid reactive substances (TBARS) procedure (Witte et al., 1970) on samples collected at 0 h and 72 h of retail display for both fresh and aged meat. A homogeneous sample of freeze dried ground material (approximately 0.5 g) was used for the determination of fatty acid composition with a method described by Ponnampalam et al. (2014a). Fatty acid levels in the experimental diets (Table 2) and in the muscle were described as mg/100 g of fresh sample to be consistent with the nutrition information used to label foods according to Food Standards Australia & New Zealand (FSANZ). The vitamin E content of both experimental diets and muscle tissue was determined as described by Ball (1988).

Table 2 Fatty acid composition of the experimental diets (mg FAME·100g-1 AF)

FAME	MOD	SUP	LUC
C12:0	7.9	1.3	12.3
C13:0	2.6	2.6	2.6
C14:0	22.1	29.4	20.8
C14:1	1.3	2.6	2.6
C15:0	2.6	2.6	7.5
C15:1	2.6	2.6	2.6
C16:0	848.6	1117.3	848.0
C16:1	20.4	27.4	15.0
C17:0	5.8	7.6	9.2
C17:1	2.6	2.6	2.6
C18:0	198.2	167.0	261.4
C18:1n9c	4659.0	3208.9	6785.6
C18:2n6c	1714.5	2011.9	1733.0
C18:3n3 (ALA)	241.9	248.6	334.0
n3	254.7	263.7	356.55
n6	1717.1	2169.1	1735.6
n6/n3	4.6	5.3	3.7
Saturated Fatty Acids (SFA)	1165.2	1391.0	1307.1
Mono Unsaturated Fatty Acids (MUFA)	4688.5	3246.7	6810.9
PoliUnsaturated Fatty Acids (PUFA)	1974.4	2435.5	2094.7
PUFA/SFA	1.7	1.8	1.6
Total fatty acids	7856.8	7100.1	10226.6

5.3.3. Statistical analysis

Statistical analysis was carried out using SAS 9.3 (SAS Inc, Cary, NC, USA). Data distribution was tested using the Sahpiro-Wilk test and data were Log_{10} transformed, as necessary. For intakes of feed and vitamin E, live weight gain, carcass traits (HCW and GR), muscle FAMES and vitamin E, a REML procedure (PROC MIXED) was carried out with a mixed model that accounted for the fixed effect of dietary treatment and the random effect of animal within dietary treatment.
For TBARS, color coordinates (L*, a* and b*) and brownness formation (R580/630) of meat, a REML procedure (PROC MIXED) was carried out with a mixed model that accounted for the fixed effects of dietary treatment and display time and the random effect of the animal within dietary treatment. F-tests were used to determine the overall significant difference among the predicted means, whereas the difference between two predicted means was judged to be significant if it was at least two times the average standard error of difference (SED).

5.4. Results and discussion

5.4.1. Growth performance and carcass quality

Dietary treatment did not affect growth performance and carcass quality (Table 3). Although having lower dietary energy content, LUC fed animals produced carcasses similar to those from lambs fed MOD and SUP pellet diets. A lack of differences in growth rate and carcass quality in feeding lambs a grain based diet or lucerne has been previously reported (Ponnampalam et al., 2014b). Further several authors reported that increasing dietary vitamin E supplementation, ranging from 0 to 1020 mg/kg of synthetic vitamin E, did not affect lamb performance (Macit et al., 2003a; Berthelot et al., 2014).

Table 3 Growth performance, feed intake and carcass characteristics

	MOD	SUP	LUC	SED	P
Number of animals	13	13	15		
Initial weight, kg	38.04	38.22	37.30	0.75	0.40
Final weight, kg	44.13	44.59	45.35	2.46	0.87
Average daily gain, $g \cdot d^{-1}$	102	106	129	41.56	0.76
Average daily feed intake (ADFI), $kg \cdot d^{-1}$	0.90	1.03	1.05	0.13	0.46
Estimated ME intake, $MJ \cdot d^{-1}$	11.83	13.85	11.08	1.58	0.19
CP intake, $g \cdot d^{-1}$	141	143	157	18.36	0.61
Number of carcasses	13	13	14		
Hot Carcass weight (HCW), kg	21.30	22.15	22.16	1.12	0.68
GR, mm	13.08	13.46	15.50	1.69	0.30

5.4.2. Fatty acid composition

The fatty acid (FA) composition of the LL muscle is presented in Table 5. LUC fed animals showed a higher C18:0 and CLA content and a lower C18:2n-6 and C20:4n-6 content. These differences led to a lower total n-6 and PUFA content for the LUC group, reducing therefore n-6/n-3 and PUFA/SFA ratios. The higher C18:0 can be explained by the higher C18:0 content of LUC diet compared with the other two dietary groups. For C18:2n-6, it is possible that the LUC diet might have been retained in the rumen for longer due to its fibrous nature, leading to a higher biohydrogenation of the unsaturated FA C18:2 and therefore its content in the meat was significantly reduced despite the small difference in feed concentration among all groups. Moreover, the biohydrogenation of linoleic acid can lead to elevated C18:0 and CLA content in the meat compared to grain based diets. Indeed, 70 to 95% of the dietary linoleic acid is biohydrogenated in the rumen (Lock and Baumann, 2004) and FA biohydrogenation increases following an increase in feed particle size (Gerson et al., 1988). Higher CLA after feeding lucerne compared to a grain based diet has been previously reported by Gonzaléz-Calvo et al. (2015) supporting our current findings. Regarding n-3 fatty acids, our results are comparable to the value obtained in lambs fed a roughage-based diet (Ponnampalam et al., 2015), therefore meat from these lambs can deliver more than 30 mg/portion (135 g), a threshold to allow a claim of lamb as a source of n-3 (Williams, 2007).

Table 4 Loin fatty acid composition (mg FAME·100g-1 of fresh meat)

	MOD	SUP	LUC	SED	P
C12:0	6.41	7.34	7.33	1.20	0.67
C13:0	0.69	0.70	0.68	0.01	0.49
C14:0	104.53	112.6	114.10	14.94	0.79
C14:1	2.76	2.75	2.72	0.62	1.00
C15:0	14.26	14.65	13.97	1.66	0.92
C15:1	2.44	2.45	2.21	0.18	0.27
C16:0	804.46	845.59	853.36	75.81	0.78
C16:1	41.71	42.13	40.74	5.15	0.96
C17:0	39.10	40.95	38.81	3.71	0.82
C17:1	6.81 AB	7.79 A	5.99 B	0.67	0.03
C18:0	452.69 B	520.44 b	609.84 aA	42.37	0.0023
C18:1n-9c	1083.21	1257.24	1308.10	112.31	0.12
C18:2n-6c	198.42 A	179.12 A	124.33 B	11.13	<0.0001
C20:0	2.60 A	3.24 A,B	3.50 B	0.33	0.03
C18:3n-6	0.68	0.70	0.68	0.01	0.49
C18:3n-3 (ALA)	31.25	33.24	30.69	3.02	0.67
C18:2 9c,11t (CLA)	7.17 a	7.70 ab	10.48 b	1.63	0.10
C21:0	1.27	1.43	1.73	0.34	0.37
C20:2n-6	1.27 A	1.00 AB	0.68 B	0.20	0.02
C22:0	0.68	0.70	0.68	0.01	0.49
C20:3n-6	4.67 A	4.83 A	3.90 B	0.21	0.0001
C20:3n-3	0.70	0.70	0.68	0.01	0.49
C20:4n-6	49.08 A	49.68 A	40.33 B	2.67	0.001
C23:0	1.36	1.89	1.51	0.35	0.30
C22:2n-6	0.69	0.70	0.68	0.01	0.49
C20:5n-3 (EPA)	14.26	15.54	13.77	0.86	0.11
C24:0	0.69	0.70	0.68	0.01	0.49
C24:1	0.69	0.70	0.68	0.01	0.49
C22:5n-3 (DPA)	24.29	24.64	23.40	1.07	0.48
C22:6n-3 (DHA)	6.78	6.97	7.58	0.63	0.40
EPA+DHA	21.04	22.51	21.36	1.26	0.48
EPA+DPA+DHA	45.33	47.15	44.76	2.10	0.49
n-3	77.27	81.09	76.14	4.37	0.49
n-6	254.81 A	236.03 A	170.61 B	11.92	<0.0001
n-6/n-3	3.30 a	2.91 bB	2.47 C	0.19	<0.0001
SFA	1428.74	1550.24	1646.21	132.41	0.26
MUFA	1137.62	1313.08	1360.44	117.11	0.14
PUFA	339.24 A	324.83 A	257.23 B	14.75	<0.0001
PUFA/SFA	0.24 A	0.22 A	0.16 B	0.02	<0.0001
TOTAL FAT	2905.61	3188.16	3263.90	250.67	0.32

a,b,c P≤0.05; A,B P≤0.01

5.4.3. Vitamin E intake and muscle content

Lambs in treatment SUP had a higher vitamin E intake compared to the other two experimental groups ($P<0.01$), while no differences were observed between MOD and LUC groups (Table 5). There was an effect of dietary treatment on muscle vitamin E content such that, the higher dietary intake of the SUP fed lambs resulted in a higher ($P<0.01$) muscle vitamin E concentration compared to both MOD and LUC groups (Table 5). Despite a similar vitE intake between MOD and LUC, muscle vitamin E content was higher ($P<0.05$) in LUC compared to MOD group. Indeed, the concentration of muscle vitamin E per unit of vitamin E consumed was 1.37 times greater for LUC compared to the MOD group, underlining a difference in bioavailability between natural and synthetic forms, similar to the conversion factor for international unit (IU) calculation from all-rac α-tocopheryl acetate to RRR α-tocopherol of 0.73 proposed by EFSA FEEDAP PANEL (EFSA, 2010). Higher efficiency of absorption rate of natural vitamin E compared to all-rac α-tocopheryl acetate has been previously reported in lamb muscle (Kasapidou et al., 2009), in sheep (Gallardo et al., 2015) and cow milk (Weiss et al., 2009; Vagni et al., 2011). LUC fed lambs showed muscle vitE levels slightly lower to those reported by Hopkins et al. (2013) in lambs fed lucerne pasture + supplement (3.3 vs 3.8 mg/kg LL muscle). This difference could be due to the fact that in the current study lambs were fed lucerne hay (LUC) whereas Hopkins et al. (2013) fed fresh lucerne pasture. On the contrary lambs fed the MOD diet showed higher muscle vitE compared to the findings of Ponnampalam et al. (2012a) in animals fed a barley and capeweed based diet (2.4 vs 1.7 mg/kg meat).

Table 5 Average vitamin E intake and muscle concentration

	MOD	SUP	LUC	SED	P
Average daily vitamin E intake, $mg \cdot d^{-1}$	41.14 A	323.33 B	41.89 A	29.35	<0.001
Muscle vitamin E, $mg \cdot 100\ g^{-1}$ of fresh muscle	2.37 c	4.80 A	3.30 Bb	0.06	<0.001

a,b,c $P \leq 0.05$; A,B $P \leq 0.01$

5.4.4. Color stability

Dietary treatment affected overall color traits of both fresh and aged meat during display life (Table 6 and 7). Although the interaction between treatment*time only tended to be significant for redness ($P=0.08$) and surface brownness formation (metmyoglobin) ($P=0.05$), a different pattern of a* and $RF_{630/580}$ evolution during retail display was evident (Table 5).

Table 6 Retail color stability of fresh loin

	MOD	SUP	LUC	SED	P diet	P time	P diet*time
L*				0.77	0.07	<0.0001	0.87
Day 1	31.76 X	31.55 X	33.36 X				
Day 2	35.02 Y	34.97 Y	36.70 Y				
Day 3	34.10 Y	34.00 YZ	35.41 YZ				
Day 4	33.66 y	32.76 XZ	34.61 XZ				
a*				0.53	0.18	<0.0001	0.08
Day 1	17.74 Xx	17.48 x	17.46				
Day 2	16.52 xy	16.30 xy	17.10				
Day 3	16.11 y	16.43 xy	17.79				
Day 4	15.57 Y	15.70 y	16.94				
b*				0.30	0.05	<0.0001	0.61
Day 1	14.98 X	14.62 xX	14.90 X				
Day 2	16.67 Y	15.86 xy	16.50 Y				
Day 3	16.67 Y	16.44 Y	17.28 Y				
Day 4	16.78 Y	16.04 y	17.12 Y				
$RF_{630/580}$				0.26	0.66	<0.0001	0.05
Day 1	6.25 X	6.02 X	5.80 X				
Day 2	3.99 Yy	4.07 Y	4.25 Y				
Day 3	3.36 yz	3.66 Y	3.98 Y				
Day 4	3.12 z	3.57 Y	3.59 Y				

Different superscript indicate significance difference on the same column x,y,z $P \leq 0.05$: X,Y,Z $P \leq 0.01$.

Meat from LUC fed lambs was characterized by higher redness retention for fresh meat as shown by less decline in the a*-value during the 72 h retail display period than the other two groups. Furthermore, formation of brownness on the meat surface as assessed by metmyoglobin formation was stable from day 2 to day 4 in the LUC and SUP group, while a progressive decline was evident until day 4 (72 h) of retail display for the MOD group.

Table 7 Retail color stability of 6 weeks-aged loin

	MOD	SUP	LUC	SED	P diet	P time	P diet*time
L*				1.15	0.76	<0.0001	0.55
Day 1	37.82 X	38.28 X	37.64 X				
Day 2	35.23 Y	35.31 Y	34.85 Y				
Day 3	34.74 Y	35.22 Y	34.19 Y				
Day 4	34.62 Y	35.07 Y	33.82 Y				
a*				0.70	0.04	<0.0001	0.07
Day 1	20.08 X	19.06 X	19.40 X				
Day 2	17.83 Y	15.55 Y	17.16 Y				
Day 3	15.08 Z	12.90 Z	14.76 Z				
Day 4	13.16 W	11.35 W	12.86 W				
b*				0.54	0.24	<0.0001	0.53
Day 1	17.43 X	16.98 X	17.02 X				
Day 2	17.92 X	16.89 X	17.37 X				
Day 3	16.60 Y	15.53 Y	15.82 Y				
Day 4	15.49 Z	14.36 Z	14.77 Z				
$RF_{630/580}$				0.29	0.13	<0.0001	0.02
Day 1	5.59 X	5.27 X	5.36 X				
Day 2	4.39 Y	3.63 y	4.38 Y				
Day 3	3.25 Z	2.63 Z	3.37 X				
Day 4	2.53 W	2.16 Z	2.66 W				

Different superscript indicate significance difference on the same column x,y,z,w $P \leq 0.05$: X,Y,Z,W $P \leq 0.01$.

For aged meat a diet effect was evident for the a* value (redness) and the SUP group showed a lower overall a* value compared to the MOD group (14.71 vs 16.54, P=0.04) but the values did not differ from the LUC group. A diet*time interaction was evident for brownness (metmyoglobin) formation (P=0.02) such that MOD and LUC groups had lower brownness formation (higher $RF_{630/580}$) than the SUP group. As expected a decline of L*, a*, b* and $RF_{630/580}$ (P<0.001) values was evident during simulated retail display in all of dietary treatments. A lack of effect of increasing dietary synthetic vitamin E up to 500 mg/kg of feed on lamb meat color stability during the first 4 days of display has been reported by Macit et al. (2003a,b), Kasapidou et al., 2012 (under modified atmosphere with 75% CO2 and 25% of O2). In contrast, meat from lambs that received supplemental vitamin E up to 1000 mg/kg of feed showed a reduction of metmyoglobin formation in the studies of Guidera et al. (1997); De la Fuente et al., (2007) and Jose et al., (2008) under natural atmosphere and Lauzurica et al. (2005), under modified atmosphere (70% O_2 and 30% CO_2). The greater color stability in LUC group despite a lower muscle vitamin E concentration compared to lambs fed SUP diet could be attributed to the additive effect of phytonutrients other than vitamin E exerting antioxidant activity, as carotenoids, flavonoid and phytonutrients (Ferreira et al., 2015). There is further evidence from recent findings by the same group (Vahedi et al., 2014c) that mRNA gene expression and enzyme activities for superoxide dismutase (SOD) and glutathione peroxidase (GPX) in LL muscle from lambs fed lucerne pasture were superior to those fed feedlots or ryegrass diets indicating that muscle (or animal) from these lambs had lower oxidative stress. The later might have been a reason for the improved retail colour stability with LUC feeding in the current study compared with SUP lambs even though SUP lambs had higher levels of muscle vitamin E.

5.4.5. Oxidative stability

Oxidative stability of both fresh and aged meat as assessed by lipid oxidation (TBARS) did not significantly differ between dietary treatments (Table 8).

Table 8: Lipid oxidative substances (TBARS, expressed in mg MDA/kg meat) in meat displayed for 1 day and 4 days at simulated retail condition in loin stored at 2-3oC for 1 (fresh) or 42 days (aged).

	MOD	SUP	LUC	SED	P diet	P time	P diet*time
				Fresh			
Day 1	0.09	0.07	0.06	0.07	0.22	<0.0001	0.93
Day 4	0.18	0.15	0.14				
				Aged			
Day 1	0.32	0.26	0.31	0.08	0.34	<0.0001	0.70
Day 4	1.93	1.38	1.48				

By contrast, in a previous study conducted by the same group at the same research location, but with dietary treatments compared under pasture (lucerne or ryegrass), feedlot or the combination of pasture and feedlot significant differences in lipid oxidation were found for fresh and aged meat stored for 5 and 60 days, respectively at 2°C and then displayed for 96 h. The latter findings could be due to a wider variation with muscles having lower vitamin E concentrations thus leading to higher TBARS formation (Ponnampalam et al., 2014b) than the present study where muscle samples were stored for 42 days. In the Ponnampalam et al. (2014b) study, lambs fed the feedlot diet for 6 weeks had a lower muscle vitamin E concentration and higher lipid oxidation for both fresh and aged meat compared to lambs fed either lucerne or ryegrass which had a higher muscle vitamin E concentration and lower lipid oxidation. Ripoll et al. (2013) reported no differences in oxidative stability of meat from lamb grazing lucerne pasture or those fed a grain diet supplemented with 500 mg of dl-α-tocopheryl acetate/kg of feed for 10 days before slaughter.
Other than the presence of other antioxidants in lucerne, it is also possible that some other factors may have contributed to the lack of effect of supranutritional level of dietary vitamin E plus organic selenium on color and oxidative stability when compared with LUC fed lambs. Warner et al. (2010) reported that copper and iron content in muscle can contribute to significant variation in retail color stability of lamb meat. Due to the fact that the LUC diet contained different dietary components compared to both MOD and SUP diets (lower amount of barley, no canola expeller), it is possible that the different dietary ingredients used in diet formulation might have influenced muscle iron and copper levels, and they demonstrated that muscle iron and copper concentrations are negatively correlated with oxy/met (RF630/580) ratio. However, iron and copper concentration in the muscle were not available for the present study.

5.5. Conclusions

The results of the present study have confirmed that increasing dietary vitamin E in lambs increases muscle vitamin E content. Therefore, it is suggested that higher incorporation of vitE to improve the nutritional value of lamb meat can be achieved in muscle through supranutrtional supplementation in the diet during a finishing period of 6-8 weeks. However, finishing lambs on Lucerne improved color stability of fresh lamb meat, possibly due to the higher bioavailability of vitamin E provided through natural sources compared to the artificial form and the presence of other micronutrients influencing antioxidant activity in the muscle. Therefore, to improve the retail color stability of lamb meat for key markets, lambs may be finished on lucerne-based diet.

5.6. References

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CHAPTER 6

Effect of ageing time in vacuum package on veal longissimus dorsi and biceps femoris physical and sensory traits

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6. Effect of ageing time in vacuum package on veal longissimus dorsi and biceps femoris physical and sensory traits

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6.1. Abstract

The study evaluated the effects of vacuum ageing (2, 4, 6, 8, 10, 12, 16 days) on veal loin (longissimus dorsi; LD) and silverside (biceps femoris; BF) physical and sensory characteristics. Ageing did not affect cooking loss, increased LD pH and L*, a* and b* in both muscles. shear force (SF) decreased until day 6 in LD and day 10 in BF. Aroma, flavor and taste were not affected, while texture traits were improved. SF was negative correlated with tenderness and juiciness and positive correlated with BF fibrousness and stringy sensation. Ageing improved texture properties without altering other sensory traits.

6.2. Introduction

Tenderness is one of the main factors affecting consumer preferences in terms of meat (Reicks et al., 2011). Since in EU veal calves are slaughtered at no more than 8 months of age (EU Regulation 1234/2007), consumers expect the meat to be tender. Ensuring a tender product is thus critical for veal producers and retailers because tenderness is closely related to consumer's satisfaction and they are also willing to pay more for tender meat (Dransfield et al., 1998; Feuz et al., 2004; Alfnes et al., 2005). Post mortem ageing improves tenderness due to the proteolysis of myofibrillar, structural and connective proteins starting from the onset of the post-mortem phase (Kemp et al., 2010; Nishimura, 2010; Ouali et al., 2013).
Nowadays meat cuts are extensively vacuum packaged, which does not significantly affect the veal aroma, color, appearance, flavor and texture traits compared to the traditional ageing of bone-in carcass (Ngapo and Gariépy, 2006). In the light of the different degrees of tenderness and tenderization rates between different muscles (Rhee et al., 2004), vacuum packaging can allow to maximize tenderization based on the characteristics of specific muscle or commercial cuts, though this depends on the duration of postmortem ageing.
Since veal is not commonly aged in commercial practices, it is necessary to evaluate the effects of long-term chilling storage not only on meat

tenderness, but also on the physical and sensory properties that can affect it. For example, color is important for veal quality, thus preserving the appearance of veal is essential. However, in some studies prolonged ageing has led to the development of an off-flavor in beef (Spanier, 1997).
The aim of this study was to evaluate the effect of postmortem ageing time in vacuum packaging at a refrigeration temperature on the physical and sensory parameters of veal loin, (m. longissimus dorsi; LD) and silverside (m. biceps femoris; BF). The meat was frozen after ageing and then thawed before quality evaluation, in order to simulate typical consumer habits (Jeremiah, 1996). The first cut was selected for to its economic significance, while the second cut is recognized as a less tender hindquarter beef cut when cooked with dry-heat cooking methods (Sullivan and Calkins, 2011), thus failing the consumer expectation for tenderness.

6.3. Materials and methods

Two days post mortem, eight right loins (LD muscle from the 6th rib to the 6th lumbar vertebrae) and silversides (BF muscle) whole primal cuts were collected from the carcasses of eight male milk-fed Holstein veal calves. Calves were similar in age (231±16 d) and sourced from the same farm; they had been fed the same diet and were slaughtered on the same day. Cold carcass weight (163.50±15 kg), conformation (SEUROP conformation score: R), fatness score (European fatness score 1-5: 2) and serum lactate (54.52±1.32 mg/dL) were similar. The serum lactate was determined using blood samples collected during exsanguination by the Central Laboratory of the Veterinary Hospital of the University of Milan using a commercial kit (Sentinel Diagnostics, Milan, Italy).
The aim was to assess the differences in individual animal stress levels, which can impair meat tenderness (Gruber et al., 2010). After collection, each muscle was divided into eight subsamples, each of which was then vacuum packaged. Subsamples were assigned to one of the seven different postmortem ageing treatments: 2, 4, 6, 8, 10, 12 and 16 days randomized, while the remaining subsample was used to determine the chemical composition. Subsamples between treatments were distributed, ensuring that each portion of the muscle was equally represented in each ageing time (Mandell et al., 2001). All subsamples were kept at 0°C until the end of the established ageing period before being frozen at -20°C, in accordance with Campo et al (2000) and Mandell et al. (2001). Prior to measurement, subsamples were thawed for 24 hours at 4°C, and a 1.50 cm steak was removed from each subsample for the sensory evaluation, while the remaining part was used for physical and chemical analysis.

6.3.1. Physical and chemical analysis

Chemical composition (dry matter, ether extract, crude proteins and ash) was determined, according to AOAC (2000), on designated samples trimmed from the external fat and connective tissue, and homogenized for 30 seconds. On each subsample subjected to a different aging time, a fresh cut surface was created by removing a slice perpendicular to the fiber axis. After blooming for 60 minutes in a dark room at 4°C, the color of the subsample was assessed by a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan) calibrated on the CIE L*a*b* (CIE, 1976) color space (Calibration Plate 21533131 Y 93.4 x 0.3456 y 0.3321, Minolta Cameras). The Chroma Meter had an 8-mm measuring area, was set in D-65 lighting, and an average of 10 repetitions was recorded as the value for each sample. pH was measured with a portable pH-meter (HI 98150, HANNA Instruments Inc., Woonsocket, RI, USA) on a homogenate prepared by grinding the slice removed to create a fresh cut surface and mixing it with deionized water. Cooking loss was determined, as described by Honikel (1988), as the weight lost after cooking in a water bath, until the core temperature reached 75°C (monitored with a temperature meter - Hanna Instruments HI98840) and 24 hours of storage at 4°C. Before being weighed after cooking, samples were blotted dry. The difference between pre- and post-cooking weights was used to calculate the percentage lost during cooking (cooking loss). After cooking loss determination, six-cylindrical core 2.5 cm thick cooked samples (1.27 cm in diameter), parallel to fiber orientation, were obtained and used for shear force evaluation with a Warner-Bratzler shear force texture analyzer (model 4466; Instron Corp., Canton, MA). Peak force (kg/cm2) was then recorded.

6.3.2. Sensory analysis

Steaks of BF and LD samples were cut to 1.5 cm thick, cooked for 60 seconds at the greatest power (200 °C) on double-plated grills, and then cut into 1.5 cm cubes. Core temperature was monitored with a thermocouple (Pentronic AB, 198 Gunnebobruk, Sweden) and was not allowed to exceed 68°C. Sensory evaluations was performed on each sample aged for 2, 4, 8, 10 and 16 days by 10 expert and trained judges (UNI EN ISO 13299:2010), confident with meat sensory evaluation. Three cubes per samples were presented on white plastic plates to each panellist, who during the training and sampling, had access to unlimited water and unsalted crackers, and each sample was identified by 3-digit codes. Judges were trained in two tasting sessions aimed at familiarizing them with the sensory descriptors relative to veal aroma, taste, flavor and texture. Each judge was asked to evaluate the intensity of each attribute by assigning a score between 1 (absence of sensation) and 9 (extremely intense sensation). Descriptors

(Table 1) include the main beef sensory parameters and some of the defects that could affect vacuum packaged aged meat.

Table 1. Descriptors, definitions and standards for sensory analysis

Attribute		Definition
Aroma	Veal	Aroma associated with cooked veal loin
	Metallic	Aroma associated with blood or rare meat
	Off flavor	Aroma associated with meat at the end of shelf life
Taste	Salty	Salty taste
	Sweet	Sweet taste
Flavor	Veal	Flavor associated with cooked veal loin
	Metallic	Flavor associated with blood or rare meat
	Off flavor	Flavor associated with meat at the end of shelf life
Texture	Tender	The force needed to masticate the meat ready for swallowing (chewing 5 times)
	Fibrous	Presence of fibers during swallowing
	Juicy	The degree of juice released while chewing the meat
	Stingy	Production of a large quantity of saliva for swallowing

6.3.3. Statistical analysis

Statistical analysis was performed using SAS® 9.3 (SAS Institute Inc., 2012 Cary NC) software. Data from the physical analysis were processed by one-way ANOVA, considering post mortem ageing time as the main effect. Data from the sensory profile were processed by three-way ANOVA considering the effects of judge, replications and ageing time and their interactions. Least square means were compared according to the F test, with the level of significance set at $P \leq 0.05$. Pearson correlation analysis was also performed to evaluate the relationship between SF and sensory texture characteristics.

6.4. Results and discussion

6.4.1. Physical and chemical characteristics

The average chemical composition of LD and BF muscles is summarized in Table 2. Results are consistent with data reported for lean veal meat in the Danish and US food composition databases (Denmark: National Food Institute, 2009; USA: United States Department of Agriculture, 2011).

Table 2. Average chemical composition of the muscles sampled for the trial (least square means±SD)

Trait	Longissimus dorsi	Biceps femoris
Moisture, g kg^{-1}	751.72±5.12	754.91±3.32
Ash, g kg^{-1}	12.34±1.04	11.72±0.65
Crude Protein, g kg^{-1}	211.50±3.22	212.24±3.96
Ether extract, g kg^{-1}	24.52±3.24	21.20±2.47

Although a significant effect (P=0.05) of ageing time on LD pH was found (Table 3), it increased only from 2 to 8 days of ageing, while no significant differences were found from day 4 of ageing. Regarding BF, its pH was not affected by the ageing time, which is consistent with other studies that found no differences in veal pH after 7 days (Revilla et al., 2006) or 14 days of ageing (Oliete et al., 2006), and the review by Ngapo and Garyépi (2006), which suggested that postmortem ageing did not increase veal pH. However, despite the slight differences in LD as pH values across post mortem times, pH values fell within the normal range (5.40-5.70) for both muscles. Cooking losses (Table 3) were not affected by ageing time in either muscle. Our findings are in agreement with previous work evaluating veal (Klont et al. 2000 and Mandell et al. 2001), however a comparison is difficult due to the different cooking methods and endpoint temperatures. However, for both muscles, our results are located an intermediate point between the cooking loss values reported by Klont et al. (2000) and Mandell et al. (2001) (19.1-38.2% respectively). Regarding color (Table 3), ageing increased lightness (L*), redness (a*) and yellowness (b*) in both muscles (P≤0.01). This concurs with Mandell et al. (2001), who suggested that color parameters tended to increase only during the first week of ageing, before becoming stable. Insausti et al. (1999) also found that L* increased during vacuum storage in longissimus dorsi of young cattle. Increased lightness can be attributed, as proposed by Klont et al. (2000), to the increase in light scatter properties in the meat due to post mortem protein denaturation and degradation. Oliete et al. (2006) found an increase in a* and b* measured 1 hour after blooming in vacuum packaged veal and young cattle longissimus dorsi. These authors attributed the increase in redness to the faster blooming of aged meat. In fact, the more meat is aged, the faster it blooms because of the reduced activities of enzymes that compete for oxygen with Mb. The increased yellowness was, instead, attributed to the increase in metmyoglobin formation during storage time. Increased lightness can exert a positive effect on the appearance of veal, while an improvement in redness can be negative.

Table 3. Effect of ageing time on veal LD and BF physical traits (least square means±SEM) a,b,c,d in the same row indicates significant differences between the different ageing times

	Ageing time							
	2 d	4 d	6 d	8 d	10 d	12 d	16 d	p
				pH				
LD	5.54±0.04 a	5.62±0.04 ab	5.63±0.04 ab	5.70±0.04 b	5.68±0.04 b	5.71±0.04 b	5.70±0.04 b	0.05
BF	5.60±0.04	5.59±0.04	5.63±0.04	5.63±0.04	5.68±0.04	5.68±0.04	5.67±0.04	NS
				cooking loss, %				
LD	25.41±0.53	26.16±0.53	25.72±0.53	25.96±0.53	25.98±0.53	25.79±0.53	25.66±0.53	NS
BF	28.81±0.11	28.55±0.11	29.87±0.11	28.81±0.11	29.85±0.11	27.62±0.11	28.77±0.11	NS
				L*				
LD	48.20±0.68 a	50.32±0.47 b	51.00±0.54 bc	52.91±0.93 cd	52.80±0.90 cd	52.49±0.86 cd	53.08±0.90 d	≤0.01
BF	48.51±0.53 a	50.46±0.43 b	50.71±0.52 b	52.74±0.75 c	53.04±0.55 c	53.12±0.56 c	53.88±0.55 c	≤0.01
				a*				
LD	9.71±0.47a	10.27±0.34 a	12.82±0.40b	12.49±0.68 b	12.52±0.66 b	12.57±0.63 b	12.53±0.66 b	≤0.01
BF	10.85±0.25 a	11.27±0.20 a	13.70±0.25b	14.86±0.34 b	14.87±0.27 b	14.11±0.25 b	14.03±0.26 b	≤0.01
				b*				
LD	9.86±0.25 a	10.52±0.17 b	11.97±0.20c	12.22±0.34 c	12.41±0.33 c	12.73±0.31 c	12.87±0.33 c	≤0.01
BF	10.58±0.25 a	11.23±0.20 b	12.46±0.25c	13.62±0.34 d	13.65±0.27 d	13.39±0.25 d	13.22±0.26 d	≤0.01
				SF, kg				
LD	2.89±0.15 a	2.59±0.11 ab	2.42±0.16 bc	2.21±0.15 c	2.12±0.18 c	2.09±0.21 c	2.05±0.17 c	≤0.01
BF	2.89±0.13 a	2.73±0.09 ab	2.65±0.13 ab	2.45±0.13 bc	2.22±0.11 cd	2.19±0.11 cd	1.96±0.11 d	≤0.01

In several studies on veal carcasses, a decrease in lightness and an increase in redness from the lightest to darkest veal was reported, while b* was not related to color score (Denoyelle and Berny, 1999; Hulsegge et al. 2001; Lagoda et al. 2002; Vandoni and Sgoifo Rossi, 2009). The magnitude of the increases in L*, a* and b* in our study were higher than those found in the study of Mandell et al. (2001), event that could be due to the combination of freezing and thawing and blooming time, in fact Mandell et al. (2001) did not bloom meat before color evaluation, practice that may have exacerbated the impact of ageing on the meat color stability. Freezing and thawing are known promote myoglobin denaturation, susceptibility to oxidation and reduce the activity of metmyoglobin reducing enzymes. These effects, coupled with the loss of NADH (a cofactor of these enzymes) in the exudate, reduce meat color and oxidative stability as reviewed by Leygonie et al. (2012). Post mortem ageing of LD reduced SF ($P \leq 0.01$), however there were no further improvements in tenderness after 8 days of ageing (Table 3). These findings are consistent with Mandel et al. (2001), who reported decreases in SF for LD and semimembranosus muscles comparing veal aged for 2 days with veal aged for at least 7 days, however the same study found no differences in SF for ageing periods beyond 7 days. Revilla et al. (2006) also found a reduction in SF loin within 7 days of ageing. Eight days of ageing was needed to significantly decrease SF values for 2-day aged BF; there was no further improvement in SF values until BF was aged for 16 days. This slower tenderization rate of BF compared to LD agrees with data on this muscle from USDA graded 'select' lean beef carcasses (Gruber et al., 2006).

6.4.2. Sensory analysis

The F values of ageing time for aroma, taste, flavor and texture parameters of LD and BF sensory profiles are reported in Tables 4 and 5, respectively. Results indicated that postmortem ageing time affected ($P < 0.01$) the sensory texture of both LD and BF. Postmortem ageing improved LD sensory tenderness ($P \leq 0.01$). Tenderness was higher at day 4 than at day 2, and at day 16 compared to day 4 (Tab. 4). Increased ageing from 2 to 4 days also improved juiciness ($P \leq 0.01$) and reduced stringiness ($P \leq 0.01$). Improvements in eating quality associated with ageing were also perceived for fibrousness, with a significant reduction ($P \leq 0.01$), starting from day 8 post mortem. These results are consistent with Mandell et al. (2001), who found an increase in perceived LD tenderness, comparing samples aged 2 days with the average values recorded for samples aged 7 and 14 days, while no significant difference was detected by increasing the ageing period from 7 to 14 days.

Table 4. Effect of ageing time on LD sensory profile (least square means)

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	p ageing time
				Aroma				
Veal	1.47	6.98	6.77	6.62	6.92	6.65	0.20	N.S.
Metallic	1.32	4.18	4.29	4.59	4.17	4.87	0.32	N.S.
Off flavor	3.02	2.33	2.15	2.52	2.37	2.87	0.22	N.S.
				Taste				
Sweet	1.14	4.67	5.27	5.07	5.20	5.38	0.32	N.S.
Salty	1.47	3.81	4.32	3.83	3.68	4.22	0.30	N.S.
				Flavor				
Veal	0.89	6.62	6.82	6.50	6.82	6.95	0.24	N.S.
Metallic	1.56	4.12	4.61	4.34	3.92	4.77	0.32	N.S.
Off flavor	1.64	2.97	2.21	2.96	2.88	3.08	0.31	N.S.
				Texture				
Tender	16.21	4.92 a	6.35 b	6.77 bc	6.88 bc	6.94 c	0.23	≤0.01
Juicy	10.82	4.75 a	5.79 b	5.95 b	6.34 b	6.33 b	0.26	≤0.01
Fibrous	4.94	5.05 a	3.95 b	3.87 b	3.70 b	3.57 b	0.30	≤0.01
Stringy	3.89	4.68 a	3.96 ab	3.62 b	3.32 b	3.34 b	0.29	≤0.01

a,b,c in the same row indicates significant differences between the different ageing times

The BF sensory analysis (Table 5) showed that perceived tenderness significantly increased with ageing (P≤0.01) from days 2 and 4 to day 8, and also from day 8 to day 16 post mortem. Juiciness was improved (P≤0.01) from 2 to 8 days of ageing, but no further. Fibrousness was also reduced (P≤0.01) from 2 to 8 days and from 8 to 16 days of ageing and stringiness decreased (P≤0.01) from 2 to 4 days and from 4 to 10 days of ageing, but no further. The improvement in perceived tenderness and juiciness, as well as reductions in fibrousness and stringiness rankings, are common when sensory panels evaluate the effects of postmortem ageing on beef palatability attributes (Jeremiah and Gibson, 2003; Miller et al. 1997 and Campo et al. 1999). Campo et al. (1999) used PCA to differentiate between aged and unaged meat. This indicated that aged meat was characterized by tenderness and juiciness, while unaged meat was characterized by fibrousness and residue (similar to stringiness). In our study post mortem ageing did not affect aroma, flavor and taste for either muscles. This disagrees with Mandell et al. (2001), where meat flavor was improved by ageing veal for more than 7 days. Our panel detected a low incidence of undesirable palatability attributes such as metallic aroma and flavor, and off-flavor. This concurs with Jeremiah and Gibson (2003), who found low levels of off-flavor and metallic aroma/flavor attributes in beef, regardless of post mortem ageing time. Furthermore, the same authors did not find differences in off or metallic aroma, and salt and metallic flavor, by prolonging ageing time until 28 days. The lack of effect of ageing time on negative sensory descriptors is an important outcome, as several previous studies have reported increases in an undesirable flavor and aroma defects for beef after prolonged ageing (Spanier et al. 1997 and Monsón et al. 2005).

Table 5. Effect of ageing time on BF sensory profile (least square means)

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	P ageing time
				Aroma				
Veal	1.00	6.69	6.60	6.80	7.04	6.93	0.18	N.S.
Metallic	1.50	4.15	4.43	4.31	3.81	3.71	0.29	N.S.
Off flavor	0.19	2.31	2.40	2.42	2.35	2.26	0.19	N.S.
				Taste				
Sweet	0.74	5.00	5.12	5.34	5.41	5.31	0.27	N.S.
Salty	2.27	3.73	3.84	4.33	3.88	4.33	0.28	N.S.
				Flavor				
Veal	1.47	6.38	6.48	6.67	6.94	6.52	0.20	N.S.
Metallic	2.47	4.04	4.78	4.44	4.25	4.36	0.29	N.S.
Off flavor	1.54	3.04	3.00	2.58	3.27	2.29	0.22	N.S.
				Texture				
Tender	15.43	3.80 a	4.52 a	5.65 b	6.38 bc	6.64 c	0.31	≤0.01
Juicy	5.83	4.27 a	4.57 ab	5.13 b	5.80 b	5.80 b	0.29	≤0.01
Fibrous	9.88	6.58 a	5.34 b	4.78 bc	4.15 cd	3.76 d	0.33	≤0.01
Stringy	21.37	6.64 a	5.81 ab	5.00 bc	4.41 cd	3.39 d	0.30	≤0.01

a,b,c,d in the same row indicates significant differences between the different ageing times

6.4.3. Correlation between SF and texture sensory traits

Based on Pearson correlation coefficients to examine the relationship of sensory texture characteristics and SF for both muscles, there was a negative relationship between SF and sensory tenderness ($r=-0.67$; $P\leq0.01$ and $r=-0.83$; $P\leq0.001$ for LD and BF respectively) and juiciness ($r=-0.53$; $P\leq0.05$ and $r=-0.72$; $P\leq0.01$ for LD and BF respectively). The negative correlation between SF and sensory tenderness is in agreement with Shackelford et al. (1999) for beef and Monteiro et al. (2013) for veal. We found positive correlations between SF and fibrousness ($r= 0.78$; $P\leq0.01$), as well as SF and stringiness ($r= 0.78$; $P\leq0.01$) for BF. These findings agree with the positive correlation between fibrousness and SF found by Caine et al. (2003) and the negative correlation between SF and juiciness found by Monteiro et al. (2013). The absence of a relationship between SF with stringiness and fibrousness rankings for the LD muscle compared to the BF muscle could be explained by the lower collagen content of LD relative to BF (Rhee et al. 2004). In fact, fibrousness and stringiness were lower in the LD muscle, which could have been the basis for the lack of significant interaction.

Our results indicate that postmortem ageing under vacuum conditions improved the instrumental and sensory tenderness rankings for veal m. longissimus dorsi and m. biceps femoris, without any negative effects on the main sensory traits such as aroma, flavor, taste and juiciness measured after frozen storage and thawing. Ageing, coupled with freezing and thawing, did, however, reduce the oxidative stability in both muscles, without affecting other technological properties such as cooking loss and pH. There were different postmortem tenderization trends for each muscle evaluated in the study. The improvements in LD tenderness and related sensory traits occurred mainly during the first week of postmortem ageing, while in BF, postmortem ageing effects were also evident until day 10. Under these experimental conditions, a minimum period of 4 days for LD muscle, and 8 days for BF muscle was necessary to obtain a clear tenderizing effect. Prolonged ageing, for at least one week for veal LD, and two weeks for veal BF, is suitable for frozen veal, mainly destined for the ho.re.ca market, in which product appearance is a secondary trait, while tenderness is the primary goal. Vacuum ageing could also be used for the fresh veal market, considering its potentially lower impact on oxidative stability than found in this study, as the veal is not frozen and thawed before being prepared for retail.

6.5. References

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CHAPTER 7

General Discussion

7. General discussion

The main findings can be summarized as follow:

- BRD reduced by 6.5% ADG and doubled the amount of discounted carcasses. Multivalent vaccination nearly halved BRD occurrence, thus ameliorating carcass quality and reducing drug consumption. On overall vaccination doubled gross margin.

- Thanks to its higher bioavailability, switching selenium supplementation from inorganic to organic form in beef heifers during the latest 60 days of fattening improved meat selenium content by 46%, tenderness, color stability and shelf-life.

- Feeding finishing lambs with lucerne-based diet improved color stability of fresh meat, compared to feeding a grain based diet either with moderate or supranutritional level of organic selenium and vitamin E. This is possibly due to the higher bioavailability of vitamin E provided through national level of synthetic vitamin E and organic selenium, likely due to the higher bioavailability of natural vs synthetic vitamin E and the presence of other micronutrients able to enhance muscle antioxidant activity.

- Postmortem ageing under vacuum conditions improved veal tenderness without affecting aroma, flavor, taste and juiciness. The improvements in tenderness and related sensory traits occurred mainly during the first week postmortem for l. dorsi muscle, while in b. femoris muscle tenderizing effects were evident until 10 days post moretm. Therfore, under the study conditions, a minimum of 4 days and 8 days of ageing have to be guarantee to obtain clear tenderization for l. dorsi and b. femoris muscle respectively.

From literature review is evident that, nowadays, consumers' perception of meat quality is affected not only by appearance and eating quality, which however remain fundamental, but even ethical and psychological factors play a key role. Consumers are also concerned about sustainability, animal welfare, antibiotics utilization and meat sensory and nutritional quality, which therefore represent the actual hot topics for beef, veal and lamb production chain. Industry need to address to these concerns both to buck the decreasing consumption of meat from ruminants in western countries and to improve production efficiency to satisfy the growing demand from developing countries.

If the worldwide trend toward an increasing of farms dimension is allowing to exploit the economies of scale and to improve producers' specialization, on the other hand represents a challenge from a sanitary point of view, due to the higher pathogen circulation within the single farm and the reduction of the personnel:animal ratio. Given this, preventive strategies are necessary in order to improve animal welfare and reduce antimicrobial administration, especially for intensive farming system, with a positive impact on farming sustainability and human health. In this regards, veal is one of the most critical production system for animal welfare and antibiotics utilization, as it is characterized by high morbidity, often counteracted with oral antibiotic group treatments. The undertaken study demonstrates the highly detrimental impact of bovine respiratory disease (BRD) on veal performance, health status and carcass quality. In this scenario, quadrivalent vaccination against the main viruses involved in BRD complex represented an effective preventive strategy able to reduce BRD prevalence, with a positive impact on health, growth performance, carcass quality and profitability. Taken together, these positive effects can lead to an improvement of animal welfare, thanks to the reduction of the pathologies, and sustainability and profitability thanks to higher efficiency and product quality, reducing therefore antibiotics utilization and improving meat safety, reducing antibiotic resistance in human medicine and improve consumers' satisfaction.

Aspect and tenderness, followed by taste, are the main historical driver of consumer satisfaction about meat. The declining trend in beef, veal and lamb consumption in Western countries can be ascribed to perceived unhealthiness of meat and to unsatisfactory eating experience. In these regards switching beef heifers form inorganic to organic selenium supplementation during finishing phase significantly improved meat tenderness, color stability and selenium content in beef heifers. Regarding feedlot lamb, the comparison of a lucerne-based diet, naturally rich in antioxidants, against grain-based diets supplemented with moderate or supranutritional levels of synthetic vitamin E, demonstrated a higher bioavailability of natural form of vitamin E from lucerne compared to the synthetic one, which led to an improvement of color stability of fresh meat in lucerne-fed lambs. These results highlight that supplementing organic instead of inorganic selenium during finishing phase is more effective to improve meat sensory and nutritional quality. Moreover, the administration of feed naturally rich in antioxidant components is more effective than the supplementation of artificial vitamin E to improve color stability and antioxidant concentration in meat from lamb.

Tenderness is another pillar of sensory quality and failing with this expectation will lead to consumers' dissatisfaction. Regarding veal, consumers expect it very tender, given it is slaughter not older than 8 months. Increasing ageing time can reduce inconsistency and improve veal tenderness, although that it is not currently applied at retail level. Testing the effect of different ageing time on veal longissimus dorsi and biceps femoris the undertaken study demonstrated that increasing ageing time improved veal tenderness and texture properties without affecting aroma, flavour and taste measured after frozen storage and thawing. However, ageing, coupled with freezing and thawing, reduced oxidative stability. From a practical perspective emerges that prolonged ageing, for at least one week for veal longissimus dorsi, and two weeks for veal biceps femoris, is suitable for frozen veal, mainly destined for the ho.re.ca market, in which product appearance is a secondary trait, while tenderness is the primary goal. Regarding fresh retail market, vacuum ageing of veal could represent a useful strategy as well, considering that a lower impact on oxidative stability compared to those emerged in the present study have to be expected, as it was exacerbated by freezing and thawing, not applied for fresh product.

In a future perspective, the integration of preventive strategies, such as vaccination and boosting of the immune system through nutrition, with risk assessment system based both on specific biomarkers assessment and the evaluation of risk factors related to animal, structural and management characteristics, can lead to a reduction and a more rational and sustainable utilization of antimicrobials in intensive farms. The achievement of this goal will be beneficial for farming profitability as well, and reduction of production cost and losses can represent the main founding body for future researches. On the other end, the improvement of appearance, eating and nutritional quality achievable feeding specific micronutrients or applying well known or new technologies have to be supported by proper marketing initiatives, in order to inform the consumers and improving the appeal of red meat. Indeed, the increasing of added value, coupled with the reduction of discounted or wasted product, will assure a proper return on investment.

CHAPTER 8

Summary

8. Summary

Nowadays meat quality perception is multifactorial and, especially for red meat, consumers are becoming more and more critics about sensory traits and concerned about psychological and ethical aspects, such as sustainability, animal welfare, antibiotics utilization, safety and healthiness. Address to these topics is fundamental for red meat industry, in order to counteract the decreasing consumption in western countries and to improve production efficiency to fulfill the growing demand from developing countries.

Improving all the aspects related to perceived quality will lead to meet consumer satisfaction, with positive impact on demand, and to increase production efficiency and profitability. The following trials aimed to evaluate specific dietary supplements and on farm and post farm strategies to improve animal health, strictly connected with sustainability, animal welfare and production efficiency, and meat appearance and eating quality.

8.1. Trial 1

Bovine respiratory disease (BRD) represents a concern for veal industry, as it causes economic losses, reduces animal welfare and increases antibiotics utilization. A field study to evaluate the effects of multivalent vaccination on veal calves' health and performance was carried out. 944 healthy calves were randomly allotted into two separate barns. One group was vaccinated 7 days after arrival with quadrivalent vaccine against IBR, PI3, BRSV and BVDV Type 1 viruses plus booster administration 21 days apart (VAC; 675 calves), while the second group was not treated (CON; 269 calves). Vaccination did not affect overall mortality ($P=0.75$) and mortality due to BRD ($P=0.28$), while it reduced BRD morbidity ($P=0.009$). VAC group showed better average daily gain (ADG; $P=0.03$) and carcass weight ($P<0.001$). Vaccination delayed the peak of BRD by nearly two weeks ($P<0.001$) and represented a protective factor against BRD (OR 0.55; $P=0.001$). BRD increased mortality ($P=0.01$), lowered ADG ($P<0.001$) and carcass weight ($P<0.001$), increased the amount of discounted carcasses ($P<0.001$). Lung lesions and pleuritis and/or consolidations increased with BRD ($P=<0.001$) but were unaffected by vaccination ($P=0.40$ and $P=0.43$ respectively). In conclusion, BRD impaired veal calves' health, growth and carcass value and vaccination reduced BRD morbidity, thus improving carcass value.

8.2. Trial 2

Selenium (Se) is involved in several biological functions and its supplementation is necessary for farm animals. Se can be provided in organic or inorganic forms, the former characterized by a higher bioavailability. Despite organic supplementation generally increased the meat Se, inconsistencies in the effects on meat quality have been reported and only one short–term supplementation study has been performed. The study aimed to compare the effects of switching the Se source in the last 60 days of fattening on growth performance, beef quality and Se content. Charolaise beef heifers supplemented since the beginning of the fattening period with sodium selenite (162 heads; 517±61 days of age) were divided into two groups, fed the same diet, in which Se (0.2 mg Se/kg DM of feed) was provided as sodium selenite (SS; 82 heads, 450.36±33.69 kg BW) or selenium-enriched yeast (Se-Y; 80 heads, 454.60±41.70 kg BW). Live weight was measured at the beginning and at the end of the trial and meat samples were collected from 30 homogeneous carcasses/group 48 hours post mortem to evaluate centesimal composition, Se content, shear force and cooking loss. Colour, pH, water holding capacity and appearance were evaluated daily for 8 consecutive days of aerobic storage. The Se source did not affect growth performance, meat centesimal composition, thawing loss, cooking and drip loss and pH during 8 days of aerobic storage. Se-Y supplementation improved the meat Se content ($P<0.001$) and tended to reduce shear force ($P=0.076$). Lightness ($P<0.01$) and yellowness ($P<0.01$) decreased with the duration of storage and were higher in the Se-Y group compared with the SS group. Meat from group Se-Y also showed a better visual score for color ($P<0.01$), odor ($P<0.05$), surface wetness ($P<0.05$), and overall appearance ($P<0.01$). Under the presented experimental conditions, switching selenium supplementation from sodium selenite to selenium-enriched yeast during the last two months of fattening did not affect heifer performance or meat water holding capacity, but improved meat tenderness and color stability during storage, with a positive impact on meat shelf life. The present study also confirmed that short-term supplementation represents a valid strategy for increasing meat Se content.

8.3. Trial 3

Inadequate concentration of vitamin E affect retail color and oxidative stability. Vitamin E (VitE) can be supplemented as synthetic all-rac α-tocopheryl acetate or through antioxidant-rich feed as lucerne. The study compared the effects of feeding lambs with a grain based diet at moderate (MOD 42 mg·kg^{-1} VitE E as all-rac α-tocopheryl acetate) or supranutritional (SUP 285 mg·kg^{-1} of vitamin E all-rac α-tocopheryl acetate) levels of vitamin E and organic selenium or a lucerne based diet (LUC; 37 mg·kg-1 VitE) for 8 weeks before slaughter. Forty-eight lambs blocked by sex (wethers or females) and feed intake in the last three days of adaptation were randomly assigned to one of the three dietary treatments (16 lambs/treatment). Treatment did not affect DMI (P=0.46) and ADG (P=0.76). LUC group showed lower n-6 and PUFA compared to both MOD (P<0.01) and SUP (P<0.01). Despite a similar VitE intake, muscle vitamin E was higher for LUC compared to MOD (P<0.05), while SUP fed animals showed the highest content (P<0.01). Although that, fresh muscle from LUC showed a better a* stability during 4 days display time compared to MOD and SUP, while no differences were evident considering 6-weeks aged muscles. Oxidative stability measured with T-bars method, did not differ between groups at the beginning and at the end of display life for both fresh and aged muscles. Increasing dietary vitamin E in finishing lambs increased muscle vitamin E content. However, finishing lambs on Lucerne improved color stability of fresh lamb meat, compared to the artificial form.

8.4. Trial 4

Tenderness is one of the main factors affecting consumer preferences and consumers expect veal to be tender. Post mortem ageing improves meat tenderness but, since veal is not commonly aged in commercial practices, it is necessary to evaluate the effects of long-term chilling storage not only on meat tenderness, but also on the physical and sensory properties. The study evaluated the effects of vacuum ageing (2, 4, 6, 8, 10, 12, 16 days) on veal loin (longissimus dorsi; LD) and silverside (biceps femoris; BF) physical and sensory characteristics. Entire cuts were collected from 8 homogeneous carcasses and each divided into eight subsamples, vacuum packed and assigned to one of the seven different ageing treatments and the remaining one was used for chemical composition. Distribution has been done ensuring that each portion of the muscle was equally represented in each ageing time. LD was selected due to its economic significance, while BF is recognized as a less tender hindquarter beef cut when dry-heat cooked. Ageing did not affect cooking loss, increased LD pH and L*, a* and b* in both muscles. shear force (SF) decreased until day 6 in LD and day 10 in

BF. Aroma, flavour and taste were not affected, while texture traits were improved. SF was negative correlated with tenderness and juiciness and positive correlated with BF fibrousness and stringy sensation. Postmortem ageing under vacuum conditions improved the instrumental and sensory tenderness without alteration of aroma, flavour, taste and juiciness measured after frozen storage and thawing. Ageing, coupled with freezing and thawing, did, however, reduce the oxidative stability in both muscles, without altering cooking loss and pH. The improvements in LD tenderness and related sensory traits occurred mainly during the first week of postmortem ageing, while in BF, effects were also evident until day 10. Under these experimental conditions, a minimum period of 4 days for LD muscle, and 8 days for BF muscle was necessary to obtain a clear tenderizing effect.

CHAPTER 9

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