AUSTRALIAN MEAT PROCESSOR CORPORATION

FINAL REPORT – Causes and contributing factors to dark cutting: Current trends and future directions

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Table of Contents

1.0 Executive Summary

Colour, flavour/aroma, texture/tenderness, juiciness and nutrient content all can influence the sale of meat. Among these, meat colour is the most important attribute that influences customer's decisions when purchasing fresh meat at the point of sale because consumers use meat colour as an indication of freshness and wholesomeness. The colour of normal meat is bright red or cherry red or pinkish to bright red and most of the population prefer this. Consumers tend to reject "dark cutting" meat due to the perception that this meat is from old animals, underfed animals, stressed animals, poorly handled animals, sick animals or spoiled and results in meat with an undesirable flavour, that is less tender (when pH is 5.8-6.1) and has a poor shelf life.

There is no universal system to define "dark cutting" across countries. Some classify meat based on colour whereas others use the pH of meat as a criterion for evaluation of dark cutting. It is risky to classify carcases as dark cutters on the basis of objective measures of ultimate pH using one muscle (example *longissimus*) to represent the whole carcass because not all muscles with a high pH will produce visually dark cutting meat and vice versa. As such this can lead to a loss of income across the supply chain, with product mis-described. The objective of this review was to identify the factors predisposing animals to "dark cutting" by examining available domestic and global literature and provide recommendations and directions for specific future research.

In Australia, the occurrence of dark cutting is estimated at about 10% in beef, which equates to a potential loss to the cattle industry of around $36 million AUS (MLA 2014) due to discounts up to $0.45/kg hot carcass weight. Some figures are even higher for sheep and lamb, with an estimate of 15% of sheep and lamb carcasses being categorised as dark cutters due to high pH meat (MLA 2014). However the 15% dark cutting in sheep and lamb in Australia might be an over estimation due to an inconsistency in measuring the ultimate pH between the time of kill and time of pH measurement and figures are based on a criterion that might not universally hold (e.g. high pH is not always visually dark).

Dark cutting in beef and lamb has been the subject of extensive research with numerous associations established with various production practices, yet "dark cutting" still occurs and causes significant financial losses globally in the fresh meat market. The review has found that "dark cutting" can be caused by a wide range of factors. These include nutrition level, age of animal, duration of transport to the abattoir, climate, hormone implant strategies, lairage times before slaughter, processing systems at slaughter plants, animal temperament and aggressiveness, stock person attitude, and to some extent subclinical diseases that cause glycogen depletion in muscle. Dark cutting meat is often defined in terms of muscle pH and is therefore a reflection of glycogen levels and as such, meat with a high pH is often referred to as, dark firm and dry (DFD). Such meat has several unique characteristics such as high water holding capacity, firm texture and the surface feels dry because the water is tightly held within the muscle. However, it should be noted that DFD meat is optimal for the production of manufacturing meat, but this results in a significant reduction in profitability.

The review has shown that there is no single main cause of "dark cutting" meat. Rather there is a range of factors or combination of factors and interactions that can lead to the production of "dark cutting" meat. The phenomena is complex, and needs the resolution of a number of issues so as to provide industry throughout the value chain with clear guidelines to minimise the incidence of "dark cutting" meat and improve the profitability of all sectors in the chain.

The review has identified the following issues that need resolution to reduce the incidence of dark cutting meat in sheep and cattle:

1. A clear definition of a pH point based on a standard measure at a particular post mortem time point needs to be identified and adopted so that it can be applied to enable the incidence of "dark cutting" to be consistently compared across systems. For example a pH of 5.7 is used (as per MSA), and this overestimates the incidence of the dark cutting (based on visual assessment) as opposed to higher pH thresholds. Further a low pH will overinflate the magnitude of the problem.
2. An objective common colour threshold (using instrument or colour chip grading system) for "dark cutting" is recommended for a universal grading system based on visual tolerance levels by actual consumers. (This approach has been developed for lamb, but not in beef).
3. The review has shown that there is no single main cause of "dark cutting" meat. Rather there is a range of factors or combination of factors that can lead to the production of the condition. How these factors contribute separately and in various combinations is not well understood. The implementation of strategies to avoid "dark cutting" meat will necessarily involve an integrated approach from different members along the value chain. This will isolate the magnitude of each contributing factor and allow development of a model for predicting the extent of "dark cutting" in the Australian meat industry. This approach would also provide opportunities to develop guidelines that will minimise "dark cutting".
4. Stress factors associated with the temperament of animals can lead to biochemical changes such as increased adrenal flow and cortisol concentration and depletion of glycogen in the muscle causing "dark cutting" in cattle and sheep. Early identification of stressed animals in the slaughter chain through development of non-invasive technologies (hair or saliva) could be a useful strategy to lower the incidence of "dark cutting" (through modified slaughter and packaging procedures). The technology needs to be confirmed in experimental work and then high throughput technology can be tested for practical use at industrial scale.

2.0 Introduction

Meat is a valuable commodity for humans because it provides nutrition, energy, satiety, satisfaction, taste and wellness when eaten in moderation. However, for beef and lamb to be attractive to consumers, it is important to ensure that all sectors including the production, transport and processing sectors are well managed with proper standard procedures from farm to fork. Any defect in the supply chain not only can cause dissatisfaction to consumers, but also lead to loss in revenue due to downgrading of carcasses and/or meat at both processor and retail sectors. Meat consumers demand high quality and safe meat products. The level of quality and safety are required to be attractive to a wide range of consumers as the main factors used for quality evaluation will vary among consumers. Colour, flavour/aroma, texture, juiciness and nutrient content can all influence the sale of meat. Among these factors, colour is the most important attribute influencing customer's decisions when purchasing meat because consumers use meat colour as an indication of freshness and wholesomeness. Meat colour is related to the concentration of pigments (mainly myoglobin and its chemical state), the antioxidant potential of meat (mainly vitamins and carotenoids), the fibre structure and physical state of muscle proteins and the type and level of intramuscular fat (Bekhit & Faustman, 2005; Bodas et al., 2007; Faustman et al., 2010, Ponnampalam et al., 2012a). The colour of normal (fresh or packaged) meat is bright red or cherry red and most of the population prefers this as it indicates freshness. Any deviation from this can be categorised as meat with defects and consumers tend to reject such meat due to a perceived degradation in quality (Tarrant, 1989; Sawyer et al., 2009). Given this, meat that is "dark" causes a substantial economic loss to the meat industry globally.

Dark cutting in beef and sheep meat is described as meat that is dark, firm and dry on the surface when examined by sight within the visible spectrum. This condition can be caused by many factors such as nutrition, age of animal, duration of transport to the abattoir, climate, hormone implant strategies, lairage times before slaughter, level of glycogen depletion, processing systems at slaughter plants, animal temperament and aggressiveness, and to some extent subclinical diseases. Dark cutting meat is often defined in terms of muscle pH and is therefore a reflection of glycogen levels (Lister, 1989) and as such, meat with a high pH is often referred to as dark, firm and dry (DFD). Such meat has several unique characteristics such as high water holding capacity, firm texture and the surface feels dry because the water is tightly held within the muscle. Factors which lead to a decline in muscle glycogen levels pre-slaughter will lead to a higher pH in post mortem muscle and potentially darker meat.

Not only the amount of water, but the distribution of water in muscle tissue can also affect meat colour, firmness and drip loss (Offer & Knight, 1988). Water is normally held in the myofibrils in the space between the filaments and a small portion of this water is bound to protein by electrostatic attraction. It also may be held in the space between myofibrils, in the intracellular space and in the interfascicular space. Under post mortem conditions with the onset of rigor, water moves from the myofibrillar compartment into the inter-myofibrillar space and then in turn into the extracellular space (Penny, 1975; Honikel et al., 1986). The degree of myofibril shrinkage will have an impact on the reflection of light from the muscle surface and the ultimate pH will impact on this by affecting the amount of water loss.

At the outset it is important to acknowledge that meat can have a dark colour due to high levels of myoglobin. Such an effect is clearly seen as animal's age, where an increase in myoglobin results in dark meat colour (Ledward & Shorthose, 1971). This leads to a reduction in the lightness (L* values) and an increase in redness (a* values) (Hopkins et al., 2007). The redness of meat has been shown in other studies to increase with animal age (Dawson et al., 2002) and is partly the reason that sucker (unweaned) lambs have superior meat colour because the meat is also lighter (Hopkins et al., 2005; Hopkins et al., 2007). Thus, it has been concluded that in sheep between 14 and 22 months of age, the increased redness and decreased lightness results in the meat being visually unacceptable to consumers based on thresholds of acceptability (Khliji et al., 2010). Such an effect is unrelated to pH (Hopkins et al. 2007), and reflects the increased oxidative capacity of say the *longissimus* muscle as animals get older (Greenwood et al., 2007). These effects will not be further discussed in this review as they can be controlled by slaughter age, but it should be noted that when interpreting pH or colour data, animal age must be considered.

The depletion of glycogen from muscle stores during the slaughter process (including all the immediate pre-slaughter stages) and after death leads to an decrease in the muscle lactate content and thus an increase in muscle pH. For anaerobic muscle, the metabolism of glycogen is the only source of energy available and to achieve a pH of 5.5, muscle must have 57 µmoles of glycogen available/g of muscle (Tarrant, 1989). A direct effect of pH on colour development has been found, whereby there is a reduction in the thickness of the oxymyoglobin layer on the surface of cut meat as the pH increases (Renerre, 1990) and an increase in the translucency of the meat (Tarrant, 1989). Despite the mechanistic links between pH and colour development the relationship is not so tightly coupled such that all dark meat has a high pH and vice versa. This is demonstrated in the recent data presented by Hughes et al. (2014), where of carcases with a *longissimus* pH of 5.8 only 28% had meat colour scores greater than 3, based on the AUS-MEAT colour chips (AUS-MEAT, 2005) and therefore were classified as unacceptable to consumers. This percentage was increased to 74% at pH 6.0. This is very important and there is a range of pH based thresholds that have been used to designate dark cutting meat. Some researchers refer to meat with a pH above 5.9 (Ferguson et al., 2001) as DFD, whereas others (McGilchrist et al., 2012) applied a pH of 5.7 and above as DFD, yet others used a pH of 6.0 and above (Apple et al., 2006). The latter threshold is in some ways, the most appropriate for muscle structure as it shows more dramatic changes when the pH exceeds 6.0 with increased water holding capacity (Huff-Lonergan and Lonergan 2005), and the development of a firm muscle texture and off-flavours. However, from a quality control point of view, a higher incidence of less tender meat at the early post-mortem stage can be found at pH > 5.8 to 6.3 (Purchas et al., 1999) and therefore pH 5.8 as a threshold should be used in fresh unaged meat.

In making an assessment of meat colour it is critical to know the time post-mortem when measurements of pH are taken along with the assessment of colour (using visual appraisal or comparison with colour chips) and the blooming time applied. For example, there is evidence that the time of assessment can have a significant impact on absolute colour scores with earlier assessment leading to darker scores (Hughes et al. 2014). Such variation between studies can introduce a bias in the assessment of the extent of dark cutting.

It should be also noted that in terms of the stability of meat colour, the colour of meat may be good and attractive at the time of blooming, but can rapidly change to a darker colour due to complex changes which take place, influenced by muscle biochemical components. This could be due to the levels and interactions between vitamins, minerals and lipids in muscles (or meat) (Ponnampalam et al., 2014). These issues prompt the question about what is the correct method to assess dark cutting; the use of a pH threshold or of a colour threshold using human perception (vision) and colour chips?

This review will cover the production factors and the processing issues that impact on the incidence of dark cutting in beef and sheep meat. This review aims to focus on issues related to live animal management from farm to stunning point, and strategies to identify susceptible animals and minimise the incidence of dark cutting. From the review a number of areas for future R&D investment will be outlined.

3.0 Project Objectives

Identify the factors predisposing animals to dark cutting using domestic and global scan of available literature and provide recommendations and directions for future research.

4.0 Methodology

An international panel of scientists with expertise in on-farm, off-farm and meat processing sector research has been assembled to prepare a literature review and provide the Australian Meat Processing sector with recommendations on the “Causes and contributing factors to dark cutting: Current trends and future directions.” The panel members are:

(1) Dr. Eric Ponnampalam, DEDJTR, Victoria, Australia

(2) Dr. Aladin Bekhit, University of Otago, New Zealand

(3) Dr. David Hopkins, NSW DPI, Australia

(4) Dr. Heather Bruce, University of Alberta, Canada

(5) Dr. Duo Li, Zhejiang University, China

This review will attempt to put all the issues in context as well as provide an indication of research directions most likely to assist in reducing the incidence of dark cutting in beef and sheep in Australia.

5.0 Project Outcomes

A literature review detailing the contributing factors and causes of dark cutting for the cattle and sheep industry submitted to AMPC. This outcome was achieved by three deliverables as stated below.

1. A literature review detailing the contributing factors and causes of dark cutting for the cattle and sheep industry.
2. A seminar/ workshop presented with AMPC and industry representatives to share the findings, consider recommendations and future directions prior to the finalization of the review.
3. An industry update covering recommendations and directions for future research to reduce incidence of dark cutting for the cattle and sheep industry.

The panel has constructed the literature review using their scientific knowledge and globally available literature relating to dark cutting meat. The literature review, recommendations and directions for future research and development are presented below.

6.0 Definition of dark cutting

Several production systems, on farm management practices, post-farm gate handling, slaughter procedures, and environmental factors can cause the occurrence of dark cutting and the extent of dark cutting differs depending on the factors involved. These in turn lead to speculations, and perceptions about dark cutting meat among processors, retailers, and consumers. Here we raise the question 'Is there any common definition for dark cutting? Researchers and consumers from around the world use different tools to interpret the quality of meat in term of the formation of meat that is known as dark cutting (DFD). Dark cutting meat exhibits various abnormal characteristics that can vary in their importance depending on the individual evaluating the meat quality across the production chain and the type of change taking place (dark colour, dry and shiny surface, high water content, increased microbial growth, off-flavour, toughness). So how do we define dark cutting? Probably the best way to define dark meat is to examine the various methods used to determine whether the meat is dark cut or not. In many studies and industrially the pH of meat at 24-48 hour post-slaughter has been used as a bench mark for detecting DFD meat. In this context 24 hour post-slaughter pH of meat ranging above 5.7 to 6.0 have been used as a threshold for DFD meat as outlined previously. The pH value at which a carcass is most likely to be demarcated as 'dark' was found in a survey by Page et al. (2001) to be greater than or equal to pH 5.87 and this is consistent with the suggested threshold above.

Some researchers have used glycolytic potential or muscle glycogen concentration in post mortem muscles as a marker for defining dark cutting meat. Regardless of the situation in which it occurs, dark cutting meat results from severe depletion of muscle glycogen during the ante-mortem period such that normal acidification processes do not occur in the early post mortem period as muscle is transformed into meat. Dark cutting beef with a pH above 6.0 usually has less than 40 µmol glucose unit/g of muscle (Pethick et al., 1996; Immonen et al., 2000a) while others have used a muscle glycogen concentration of 57 µmoles/g of glycogen as the critical level (Tarrant, 1989). For example, Apaoblaza et al. (2015) reported that muscle glycogen levels in steers at 30 min post slaughter were 65.5 µmoles/g in carcasses with normal muscle pH (5.71) and 29.5 µmoles/g in carcasses with a high muscle pH (6.34) at 24 h post slaughter. However, this criteria cannot be correct all the time because studies have shown that some carcasses that qualify as dark cutters as assessed by the Canada B4 grade system based on a colour standard had pH values less than 6. The reason why the *longissimus thoracis* muscles from carcasses did not produce a bright red colour is not clear but the authors stated that the rate of post mortem glycolysis may be an influence (Holdstock et al., 2014). This was similar to that reported by Hughes et al. (2014) which shows that colour perception and pH are not tightly coupled. It indicates that there might be a link between muscle glycogen and pH but the high pH meat does not always mean darker meat.

Some researchers have used muscle lactic acid concentration post mortem as a criterion for defining high pH meat. Davey and Gilbert (1976) reported meat samples having 40 mmol lactic acid/kg as indicative of high pH carcasses. Apaoblaza et al. (2015) reported that carcasses from steers having a muscle pH < 5.8 at 24 h post slaughter had a concentration of lactic acid at the beginning of the process (30 min post slaughter) of 50.7 mmol/kg compared with carcasses that did not reach muscle pH < 5.8 that had 36.5 mmol/kg. On the other hand, many researchers have used objective colour measurements as criteria for defining dark cutting meat. Objective measurements of dark cutting can be carried out using colour chips or instrumental colour standards. For example, Hopkins (1996) reported an acceptability threshold for lamb m. *longissimus* fresh colour of 34-35 for *L**, which elaborates lightness of meat using the CIE colour space coordinates from a Minolta Chroma Meter. This finding was supported by the outcomes from Khliji et al. (2010) who found that when *a** values, indicative of redness of meat, are equal to or exceed 9.5, on average consumers will consider lamb meat colour acceptable and the corresponding value for *L** is 34. No similar work has been undertaken for beef meat. Some consumers even define beef or lamb as a dark cutter after consumption through experiencing the off flavor taste, and because the meat can be less juicy and tough due to the high pH (Gregory and Grandin, 1998). Dark cutting carcasses are also discriminated against because the high pH increases the likelihood of microbiological growth (Egan & Shay, 1998). Further dark cutting carcasses are discriminated against because they have an ultimate pH between 5.8 and 6.2 and thus tend to produce tough meat which cannot be differentiated visually from meat with pH values greater than 6.2, which are tender (Jeremiah et al., 1991). A possible reason for this biphasic relationship could be lower proteolytic activity between pH 5.8-6.2 because it lies outside the pH optima for two separate enzyme systems and the increase in tenderness of meat from pH 6 to 7 is attributed to an increase in calpain activity. Another possible reason for tough meat might be that sarcomere length increases as ultimate pH decreases below pH 6.2 (Miranda-de la et al., 2009).

The above mentioned criteria for the definition of dark cutting were all objective measures. However, in real terms the incidence of dark cutting beef and sheep meat is mainly identified as such based on visual assessment within the visible light spectrum and has a dry surface that is firm when felt by touch. Consumers prefer bright cherry colour meat or bright red meat as this meat is not only attractive and appealing, but also provides better sensory attributes (e.g., texture, juiciness and flavour when eating) and shelf life. Dark cutting meat will have an abnormal dark purplish red to black colour and the muscle is firm and dry due to a high water holding capacity that binds water tightly within the muscle. For these reasons, consumers tend to avoid purchasing dark meat and therefore the meat is sold at lower prices or used for mince. There is no unique definition to define dark cutting across countries. Some classify meat based on colour whereas others use the pH of meat and/or muscle glycogen concentration. As is apparent there is risk associated with classifying carcasses as dark cutters on the basis of objective measures of ultimate pH using one muscle (example *longissimus*) because not all muscles having high pH will produce dark cutting meat or vice versa. Thus there may be a loss of income to processors or producers by downgrading dark cutting carcasses and selling the meat at lower prices. Further to this if processors purchase carcases on a carcass weight basis and then by misidentification the meat is deemed DFD meat there is also a loss of income. It is important to clarify what is the correct methodology to grade dark cutting in meat, the basis of dark cutting (due to on farm and off farm factors), and develop strategies to minimise the prevalence of dark cutting.

7.0 Incidence of dark cutting meat in Australia, North America, other countries and its economic implications

Dark cutting meat in the retail display is one of the leading causes of consumer rejection because consumers use off-coloured meat as an indicator of poor quality or degraded meat quality (Vidal et al., 2009). Consumers' discriminate against meat with dark colour because it can lead to spoilage as a result of increased microbial growth and toughness due to inadequate glycogen and pH fall post mortem. Tougher meat cuts from high pH carcasses (pH 5.8-6.2) in the US prompted a call for grading penalties applied to dark cutting beef to be increased because of the associated reduction in beef quality (Wulf et al., 2002). In Australia the occurrence of dark cutting is almost 10% in beef, which equates to a potential loss to the cattle industry around 36 million (MLA 2014) due to discounts up to $0.45/kg hot carcass weight. The figures are even higher for sheep meat with an estimated 15% of sheep carcasses are categorised as dark cutters due to high pH carcasses (MLA, 2014). An Australian sheep CRC study and a national audit of retail loin quality study (Safari et al., 2002) based on colour measurement showed 10% dark cutting incidence in Australia based on a pH measure. However the 15% dark cutting in sheep and lamb in Australia might be an over estimation perhaps due to an inconsistency in measuring the ultimate pH of meat between the time of kill and exact time of pH measurement carried out at the abattoir. Any measurement taken before 24 hour from the kill time will inflate the number of carcasses with a high pH meat. It also depends on which threshold is used to define dark cutting because when a pH of 5.7 is used (as per MSA), this overestimates the incidence of the dark cutting problem (based on visual assessment) as opposed to pH thresholds of 5.9 or 6.2. These are very important points and the threshold applied needs to be carefully considered and it is recommended that Australia adopts a threshold that is matched to visual tolerance levels by actual consumers.

The USDA Agricultural Marketing service reported the discount for dark cutters to be $30 per 100 lbs carcass weight, which equates about US$165 million loss to the beef industry or $5.43 per fed steer and heifer harvested in the year 2000 (Miller 2007, based on colour assessment). In Canada, the proportion of beef carcasses that are graded dark cutting (Canada Grade B4 carcass: 5.7-6.0 and > 6.0), has increased from an average of 0.8% of total carcasses processed in 1998/99 to 1.3% in 2010/11, which represents about $1.4 million in lost value each year and it is significant concern to the Canadian beef industry (Holdstock et al., 2014). The incidence reported from Canada is lower compared with other countries. Table 1 shows the research reported in the last 20 years on the prevalence of dark cutting meat in cattle and sheep. High pH beef has been an important problem in Chile (Gallo, 2004) and other countries with extensive pasture finishing systems like Mexico (Leyva-Garcia et al., 2012). Although the dark cutting defect in beef has been the subject of extensive research with numerous associations established with various production practices, dark cutting still occurs. Studies conducted in South Chile showed an extremely high incidence of high pH meat between 17-40% on a yearly basis (Gallo 2004) and this causes significant losses to the meat industry (Vidal et al., 2009). On this basis there is value in minimising the incidence of dark cutting carcasses and developing the ability to identify animals/carcasses that can be prone to dark cutters.

In support of this contention, in Canada, beef audits within the last 20 years have indicated that the incidence of dark cutting in beef has increased from about 1 to 1.3% (Donkersgoed, et al., 2001; Beef Cattle Research Council, 2013). Similarly, audits in the US have shown that the frequency of dark cutting beef has fluctuated from 2.3% in 2002 (McKenna et al., 2002) to 1.9% in 2005 (Garcia et al., 2008) only to increase to 3.2% in 2012 (Moore et al., 2012). In contrast to the frequencies observed in North American countries, the incidence of dark cutting beef in Australia is markedly higher than that in Canada and the US at 10% (Meat Standards Australia, 2010). The inconsistency in the definition of dark cutting and the grading procedure used for the assessment is a likely reason for variation between countries. Cumulatively, these trends also indicate that the incidence of dark cutting in beef carcasses is not declining in the major beef producing countries, suggesting that the causes of this meat quality defect within bovines are not completely understood.

Although the current value of beef is the highest it has been for over ten years, dark cutting beef is at best harvested from low connective tissue muscles with the remainder of the carcass sold as trim beef for use in manufactured products such as hamburgers. Scanga et al. (1998) estimated that the financial loss associated with dark cutting was "US$6.08 per animal harvested in the United States" which is the equivalent of US$172 million per annum (Underwood et al. 2007). Although this may not be seen as a significant sum in the multi-billion dollar meat industry, dark cutting often occurs in clusters within producer consignments, so the financial penalties are not spread across the industry and can be devastating to those producers affected.

Table 1: **Prevalence of dark cutting in beef and lamb carcasses across different countries**

Country	%Dark cutting	References	Finishing system
South Africa	11.8% (n=22178; pH>5.8)	Vilijoen (2000)	Beef, Feedlot
Canada	1.28% (n=16711; VE)	National Beef QualityAudit (2010-2011)	Beef, Feedlot
Canada	1.3% (pH> 5.8)	Holdstock et al. (2014)	Beef
USA	3.2% (n=9802; VE)	Moore et al. (2012)	Beef, Feedlot
USA	2.3% (n=697130; pH>5.9)	National Beef Quality Audit (2000)	Beef
Australia	4.8% (n=2436148; VE)	MLA (2013)	Beef, Feedlot/Pasture
Australia	~9-11% (pH> 5.7)	Warner at al.(2014)	Beef
Australia	8-32% (pH> 5.7)	Jacob et al. (2005)	Lamb, semimembranosus
Australia	7.3% (n=1614, pH> 6.0)	McPhail et al. (2014)	Lamb, spring and autumn
Australia	10% (n=909, pH> 6.0)	Safari et al. (2002)	Lamb
New Zealand	4.3% (n=1759; pH≥5.8)	Wiklund et al. (2009)	Beef, Pasture
New Zealand	7.3% (n=1536; pH≥6.0)	Devine and Chrystall (1989)	Lamb
Spain	13.9% (n=5494; pH≥5.8)	Mach et al. (2008)	Beef, Feedlot
France	3.4% (n=891; pH>6.0)	Mounier et al. (2006)	Beef, Feedlot
Great Britain	8.8% (n=717; VE)	Warriss and Brown. (2008)	Beef, Feedlot/pasture

[1]Reported data refer to *longissimus* muscle, unless mentioned.

[2]VE = visual evaluation (assessment method). n = number of surveyed carcasses.

8.0 Contributing factors to dark cutting

8.1 On farm factors

Dark cutting is a well-known meat quality defect that has been associated primarily with on farm and off-farm activities. On farm factors that can be considered as stressors to cause dark cutting are nutritional background or feeding systems, animal management, climate variability, gender, hormone implantation, genetics and individual animal temperament. In cattle, heifers are more likely to produce dark-cutting (DC) carcasses than male castrates (steers) because of increased mounting and low feed intake during estrus (Jones & Tong, 1989). Some cattle have temperaments that lead them to being more prone to stress than others.

8.1.1 Nutrition/feeding systems

Basically, cattle and sheep around the world are grown under two main feeding systems: 1) extensive grazing on traditional pasture finishing; or 2) intensive production systems maintained on concentrate diets. However, feeding systems can be combined depending on availability of resources, quality of feeds and the purpose of production. Sheep and cattle reared under extensive grazing systems receive diets with high protein (e.g. green pasture) and grow slower while animals reared under intensive finishing systems receive higher energy diets and grow faster. These diets can in turn have an impact on carcass weight, fat thickness, muscle glycogen content, depletion rate of glycogen during transport and slaughter, and post slaughter muscle cooling rate.

The prevalence of DC in cattle finished on concentrate diets normally ranges from 1.5% to 14% (Table 1). It has been reported that pasture finished animals are more prone to develop dark cutting carcasses, often with higher pHu, compared to feedlot cattle (Muir et al., 1998; Hughes et al., 2014). Animal age will be one of the contributing factors influencing the incidence of dark cutting in animals reared on pasture because such animals are slaughtered at older ages due to the low energy density of the diet and consequently take longer to reach market weight (Vestgaard et al., 2000). Pasture finished animals had higher muscular iron (pigment) concentration (Ponnampalam et al., 2012) due to a higher iron intake (Muir et al., 1998; Vestgaard et al., 2000). However, a lack of significant differences in myoglobin (Mb) content in pasture- or grain-fed cattle has been recognized as well (Priolo et al., 2001), reflecting differences in farming systems in different countries. In support of the importance of diet, lambs slaughtered in the autumn season had higher pH at 24 h post-mortem and greater occurrence of dark cutting than those slaughtered in the spring season in Australia (McPhail et al., 2014) and New Zealand (Devine & Chrystall, 1989).

Moreover, the lower dietary energy density of pasture diets compared to grain based diets, coupled with higher maintenance requirements involved with grazing and the higher morbidity lead to the production of leaner carcasses with high pH meat. For example a higher pHu and darker colour has been reported in beef carcasses with fat thickness lower than 0.76 cm (Page et al., 2001). Grain based diets can increase carcass fat thickness and intramuscular concentration (Yang et al., 2002). The increase in intramuscular fat can lead to lower myoglobin and sarcoplasmic protein concentrations in the meat, which may lead to lighter colour meat (Bodas et al., 2007) and also increases the lightness due to the colour of the fat . Studies have reported a consistent darkening effect of increased grazing time attributed to higher ultimate pH due to greater susceptibility to ante-mortem glycogen depletion. Feeding high energy diets or energy supplements such as grains and by-products during the finishing period of cattle and sheep may be useful to reduce the occurrence of dark cutting meat. A survey by Jacob et al. (2005a) found feedlot lambs were characterized by a higher muscular glycogen concentration at slaughter than pasture-fed lambs.

Immonen et al. (2000a) showed that the incidence of dark-cutting could be reduced by supplying increased high energy feed to cattle prior to pre-slaughter transport. Knee et al. (2007) also proposed that supplementing cattle that had been on low energy forage with a grain-based ration for 3 weeks prior to slaughter would reduce the incidence of dark cutting through an increase in muscle glycogen, and it was substantiated that an increase in muscle glycogen was possible with this strategy. McGilchrist et al. (2012) also recommended improved nutrition for cattle to increase subcutaneous rib fat, which was related to a lower incidence of dark-cutting. Page et al. (2001) found in their survey of carcasses across the USDA grades, including those to which a dark cutting demerit had been applied, that subcutaneous fat thickness threshold below which dark cutting likelihood increased was 0.76 cm.

That dark cutting persists within the North American system, which is based on finishing cattle on diets consisting of very high proportions (about 90%) of grain, suggests that even cattle receiving an energy-intense diet are susceptible to dark cutting and that other factors may be contributing besides nutritional level and type. For example, a high grain diet may lead to subclinical incidence of acidosis, which may reduce cattle feed intake relative to expected intake. Ferguson et al. (2008) showed that sheep fed concentrates prior to strenuous exercise recovered faster than those fed roughage, and these authors suggested that this may be due to the relationship between glycogen synthase and initial muscle glycogen concentration. The rate of glycogen synthesis is determined by the activity of glycogen synthase, the activity of which is governed by the levels of glucose-6-phosphate (Bräu et al., 1997). The levels of glucose-6-phosphate in muscle depend upon the supply of glucose to the muscle cell, and in cattle recovering from transport or other pre-slaughter stress without access to food, the only reliable source of glucose will be that from the liver stores. The results of Ferguson et al. (2008) therefore imply that ruminants fed concentrate diets prior to slaughter may have increased liver glycogen stores, which may be a key to supplying glucose to the muscle during recovery. Cattle with chronic acidosis will then be at risk of dark cutting as liver stores in these cattle will most likely be depleted by reduced feed intake associated with low rumen pH.

An adequate muscle glycogen concentration prior to slaughter is essential to ensure a normal muscle pH in the carcasses at 24 h post mortem. According to Warriss (1990) pH fall appears to be limited approximately below the glycogen concentration of 45 µmol/g. Lower muscle glycogen stores at the time of slaughter can lead to lower lactic acid formation in post mortem muscle and higher meat pH at 24 h post mortem that may lead to dark cutting. In order to produce meat with a desirable final pH, at least 50-60 µmoles/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, energy density of a diet is a fundamental factor affecting muscle glycogen content. Apaoblaza et al. (2014) reported carcasses with pH >5.8 at 24 h post mortem contained 14.9 mmol/kg glycogen while the same muscle in carcasses with pH <5.8 had 35.5 mmol/kg.

Concerning beef, Immonen et al. (2000a) reported an increase in both muscular glycogen content and glycogen repletion after an adrenalin challenge, coupled with a reduction of glycogen loss in the cold season in animals fed 90% corn + 10% hay vs animals fed 100% hay, although no differences were found in pHu. More interesting, these authors noted that switching from a high energy diet minimally decreased muscular glycogen, while feeding a high energy diet for 37 days compared 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 seasons (Immonen et al., 2000b). Accordingly, Gardner et al. (2001a) reported a higher glycogen repletion rate in *semimbranosus* muscle (prevalent oxidative) after exercise in cattle fed cereal-based vs silage- or hay-based diet while Hopkins et al. (2005) reported a lower pHu and a lighter colour in lambs fed on a high vs low nutritional plane. The reported data suggest that it is necessary to provide ruminants a high energy diet, at least during the finishing phase, to reduce the occurrence of dark cutting. McGilchrist et al. (2012) reported that a higher growth rate, achievable by administering a high energy diet, can reduce dark cutting prevalence due to a higher glycogen content.

Other than providing high energy diets or supplements, specific dietary strategies have been tested to reduce the incidence of dark cutting due to transport stress. Magnesium oxide supplementation reduced glycogenolysis after exercise, promoted higher glycogen repletion, improving both glycogen at slaughter and muscle pHu in sheep (Gardner et al., 2001b), while it was ineffective in cattle up to 0.75% of the diet in the two weeks pre-slaughter (Bass et al., 2010). The effectiveness of providing electrolytes, high sugar supplements or a mixture of electrolytes, sugars and amino acids during pre-slaughter and/or lairage has been reviewed by Schaefer et al. (1997). Schaefer et al. (1997) studied the role of oral electrolyte therapy in attenuating transport and handling stress in cattle and reported improvements in both live and carcass weight as well as a reduction in the occurrence of dark cutting. Pethick et al. (1999), based on the results of several experiments 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 during lairage, rather than through feed on farm or in lairage. This however relies on the animals drinking in lairage and this often does not occur (Toohey et al., 2006). The effectiveness of reducing dark cutting using other micronutrient supplementation such as tryptophan and tyrosine that is able to alleviate the stress response and glycogenolysis has been demonstrated (Schaefer et al., 2001).

8.1.2 Animal genetics and breed effect

A genetic and breed effect on the incidence of dark cutting in ruminants is evident through changes in cooling rate, pHu, pigment content, glycogen depletion and subcutaneous fat thickness. The genetic correlation of muscle glycogen with beef colour standards was 0.69 and glycogen heritability was estimated to be 0.34 in Japanese black cattle (Tomohiko et al., 2014). In lambs many studies have been carried out to evaluate the effect of genotype on meat colour and pHu. Most of the reported evidence suggests that, among different breeds (Merino, Border Leicester, Poll Dorset, Texel and their first and second crosses), Merino and its crosses exhibit higher ultimate pH and/or darker colour meat (Young et al., 1993; Hopkins and Fogarty, 1998; Gardner et al. 1999; Warner et al., 2006; Hopkins et al., 2007). Gardner et al. (1999) demonstrated that, in common market conditions (2 h transport 24 h lairage), Merino lambs exhibited higher glycogen depletion, pHu and redness index (a*) than its first (Poll Dorset x Merino) and second (Poll Dorset x Border Leicester x Merino) cross counterparts. This is supported by the findings of Jacob et al. (2005b), that showed a reduction in *semimembranosus* muscle glycogen from farm to slaughterhouse in 4 of 13 lambs consignments; of which 3 of the 4 were Merino lambs. Considering that the Merino is characterized by a lower muscular myoglobin content (Warner et al., 2006) and isocitrate-dehydrogenase (ICDH) activity (Mortimer et al., 2014) compared to other lamb breeds, a higher stress-sensitivity can be reasonably considered as a possible explanation of the higher prevalence of high pH in the Merino breed, but behaviour and physiological changes to animal response of stress work has not confirmed this. Indeed, myoglobin is positively correlated with a* and negatively related with lightness (*L**; higher lightness means paler meat), while ICDH is an aerobic metabolic enzyme and exhibits a negative genetic correlation with meat final pH (pHu). A genetic and diet effect on basal and hormone stimulated changes in blood glucose, lactate and adrenalin between Merino and second cross lambs has been reported (Ponnampalam et al., 2012b). Warner et al. (2006) concluded that, based on the available evidence, the Merino is not characterized by a lower glycogen level compared to other breeds, but deplete more glycogen between the farm and the abattoir. It is interesting to note that this breed seems to have a calm temperament, indeed, even if they exhibit a higher carcass pHu, Merinos and second-cross lambs had the lowest agitation score and Poll Dorset × Merino had the highest agitation score both at 8 and 14 months of age using the isolation-box test (behavioral observation during 1 minute of isolation) (Warner et al., 2006). These contrasting results between Merino and cross bred lambs demonstrate that future work is warranted to understand the mechanisms behind this phenomena.

Hopkins and Mortimer (2014) reviewed the evidence about the Merino breed having higher pHu meat and noted some inconsistencies are present in the literature. Hopkins et al. (2005a) found a higher pHu in 3 different muscles for Merino lambs sourced and slaughtered in New South Wales, Australia, but no similar results were obtained in Merino lambs slaughtered from Victoria, Australia. This was attributed to the greater weight gain of the latter flock, that could have led to a higher muscular glycogen, coupled with a lower pre-slaughter stress. Also an intra-species difference is present in the Merino breed. Due to lower activities of aerobic enzymes based on the data reported by Hopkins et al. (2005b), Warner et al. (2006) reported that superfine wool strains produce carcasses with higher pHu compared to medium and coarse wool,. Results indicate that it is necessary to pay attention to the handling of Merino lambs before stunning to minimize stress and apply strategies to maintain adequate glycogen levels in the muscle before slaughter so that the incidence of dark cutting can be minimized. Regarding other breeds, Hopkins and Mortimer (2014) reported that evidence of a higher pHu in Scottish Blackface and Romney vs Texel lambs exist. It is important to note that the meat may have a higher pH, but it does not translate into a dark colour (see Hopkins et al., 1998 and 2007). In this context, if meat that is of acceptable colour is rejected on the basis of pH this can also impact on returns.

The most recent investigations into the incidence of dark-cutting in beef have indicated that predisposition toward dark cutting may be indicated by animal phenotype. Based on using pH of meat above 5.7 and AUS-MEAT colour reference standards having a 1-7 scale, McGilchrist et al. (2012) found that dark cutting carcasses had reduced eye muscle area, carcass weight and subcutaneous rib fat depth. The results of this research were supported by those of Holdstock et al. (2014), which showed that dark-cutting carcasses had decreased carcass and muscle weights relative to normal carcasses based on using pH of meat 5.8-6.0 (atypical) and above 6.0 (classic) and subjective colour measurement done by an experienced agency grader having 1-8 scales. That physiological attributes may be indicators of the likelihood of dark cutting suggests that cattle that produce a dark cutting carcass may not be experiencing additional stress prior to slaughter through transport and handling, but that some cattle have less resistance to or recover more slowly from physical stress than others (McGilchrist et al., 2012; Holdstock et al., 2014). Preliminary research into the incidence of dark cutting in heifers and steers in Canada (Mahmood et al., 2015) substantiated that heifers most likely to cut dark had reduced weaning and slaughter weights, and that cattle regardless of gender most likely to cut dark had decreased carcass weights and rib eye areas.

Based on the m-ATPase activity, muscle fibres are broadly classified as Type I (slow ATPase activity, oxidative metabolism, rich in myoglobin) and Type II (fast ATPase activity, glycolytic metabolism mainly, rich in glycogen content). The latter is further classified as Type IIA, IIB, IIX and IIC based on their metabolic function (Peinado et al., 2004). Several authors concluded that the muscle fibre type can influence the muscle susceptibility to dark cutting (Young & Foote, 1984; Zerouala & Strickland, 1991). LaCourt and Tarrant (1985) suggested that Type II muscle fibres are affected by the type of physical stress that leads to exhaustion of glycogen, whereas psychological stress releases adrenaline that affects Type I fibres. Cattle that have predominantly β (Type I) - and α (Type IIA) -red muscle fibers tend not to be heavily muscled because β- and α-red muscle fibers are smaller than α-white (Type IIB), glycolytic fibers (Schiaffino & Reggiani 2010); therefore cattle that are small and lightly muscled may be at heightened risk of dark cutting because their muscle fibers are more easily depleted of glycogen with the same level of activity or stress than an animal with α-white muscle fibers (McGilchrist et al. 2011). Also, Tarrant (1989) indicated that muscles most likely to be dark in a beef carcass due to physical activity stress were those that had the largest proportion of fast glycolytic muscle fibers such as the *longissimus thoracis et lumborum* (LTL) and *semitendinosus* (ST), while those that responded to adrenaline administration and hence most likely to be depleted by anxiety were the slow twitch β-red muscle fibers. Although dark cutting muscle in carcasses sampled in Canada have shown no relationship to a specific type of muscle fiber (Holdstock et al. 2014), cattle that produce highly marbled beef may have an increased proportion of muscle fibers that are slow twitch with low levels of glycogen because the predominant energy metabolism for α-red fibers tends to be oxidative rather than glycolytic (Calkins et al. 1981). Underwood et al. (2007) found that cattle with increased intramuscular fat had lower concentrations of glycogen than cattle that had decreased intramuscular fat, and found that the differences were not related to muscle fiber type, supporting the conclusion that intramuscular fat simply displaces glycogen and makes less glycogen available to the animal. If future research establishes muscle fiber type as an important aspect affecting dark cutting, then recovery time at the abattoir may need to be varied based upon fiber type because slow oxidative muscle fibers recover pre-stress glycogen levels more slowly than fast glycolytic muscle fibers (Tarrant 1989; Bräu et al., 1997; Fournier et al., 2004).

Based on the findings of Martin et al. (2004) and Gardner et al. (2007), Warner et al. (2007) reported that the shift from fast oxidative-glycolytic fibers to fast glycolytic fibers impacted on glycogen content and is higher in animals with greater estimated breeding values (EBV's) for muscling. It is important to underline that this difference can emerge only if animals are properly fed, as muscling lambs need adequate energy to exhibit an higher repletion rate, otherwise, if inadequate energy is provided, their rate is slower than those with low EBV's for muscling (Warner et al., 2007).

McGilchrist et al. (2012) found that the occurrence of dark cutting in beef carcasses was decreased when carcass weight, rib eye muscle area and rib fat depth increased.

The possibility to improve pHu and color through genetic selection is related to the heritability (h^2) of these traits. Estimated h^2 of these traits in lambs was reviewed by Hopkins et al. (2011). A low to moderate h^2 was evident for pHu (0.09-0.27) and *a** (0.02-0.45), while a moderate h^2 was found for *L** (0.14-0.45). The most recent study on this topic by Mortimer et al. (2014), reported a lower h^2 for pHu (0.08), while values for *a** (0.08) and *L**(0.18) fell within the previously reported ranges. The study of Mortimer et al. (2014), was based on 8968 lambs from 372 sires and 5309 dams of the most common employed crosses. A low h^2 was reported for iron content both by Hopkins et al. (2014) and Mortimer et al. (2014), 0.12 and 0.04 respectively. Based on findings of Mortimer et al. (2014) hot carcass weight (HCW) is negatively correlated with pHu (-0.32) and positively correlated with fresh *L** (0.25), *a** (0.23) and *b** (0.19). The authors stated that increasing HCW through selection would reduce the ultimate pH and lead to a small increase in fresh meat redness and yellowness, concluding that reducing pH genetically is expected to lead to only marginal changes in *a** and *b** values. Regarding iron, increasing HCW would lead also to a small reduction in the iron content of lamb meat, reflecting less oxidative muscle, given the negative genetic correlation among these two traits (-0.28). It's interesting to note also that the genetic relationships of IMF and shear force with the fresh meat color traits were positive and negative respectively. Based on these findings, the authors concluded that a selection to increase IMF or reduce shear force values would increase the fresh meat measures of *b** value (meat more yellow in color) and *L** value (lighter or paler meat), as well as *a** value (redder meat) to a lesser extent.

In several studies examining beef carcass colour characteristics, dark cutting prevalence and pH were compared between 2 or more breeds. Given the huge number of beef breeds reared all over the World, this review focuses on species (*Bos taurus* or *Bos indicus*) and breed type (dairy, beef, double muscle) rather than reporting the comparisons between the single breeds available in literature. Muscle pigment content differs between breed types, with dairy breeds (e.g. Holstein and Brown Swiss) characterized by higher levels of myoglobin and thus iron in their muscle (Dunne et al., 2004). A redder meat from dairy breeds compared to those from beef and double muscle (DM) breed or dairy x beef/DM crosses was reported by Dunne et al. (2004) due to higher haem content in high genetic merit New Zealand and Irish Friesian bulls compared to Holstein-Friesian x Belgian Blue bulls. The higher muscular pigment concentration is attributable to a more oxidative metabolism due to selection for milk production. For beef and dual purpose breeds, King et al. (2010) found in 464 steers with 0 to 50% inheritance of Angus, Charolais, Gelbvieh, Hereford, Limousine, Red Angus, and Simmental, no difference in pHu, while Angus showed the highest *L** values at the beginning of display life test and Limousine and Charolais a lower myoglobin concentration.

Differences in muscle fibres characteristics are at the base of the different meat colours and dark cutting prevalence that characterize DM breeds compared to other beef and, especially, dairy breeds. Double muscled cattle (e.g. Belgian Blue, Blonde d'Aquitaine, Piedmontese, Asturiana de los Valles and some Gasconne and Romagnola strains) are characterized by a myostatin gene mutation that results in a lack of myostatin activity (Fiems, 2012). Myostatin is involved in the regulation of the conversion from second generation myoblasts into muscle fibres (mainly fast glycolytic, as type I slow oxidative fibres originate mainly from first generation myoblasts) and its inactivity results in a higher number of muscle fibres in general and, specifically, fast glycolytic ones (type IIb). Myostatin promotes, thus, a shift from an oxidative to a more glycolytic metabolism, making the muscle more prone to glycogen depletion, especially during stressful conditions. Several factors other than a more glycolytic metabolism concur to promote a higher stress susceptibility in DM cattle. Double muscled cattle have lower capillary density, myoglobin content and lung weight compared to non DM cattle. All of these traits predispose muscle to a fast glycogen depletion under stress conditions. Given these findings, DM cattle seem to be more prone to dark cutting development, as evidenced in the study of Shackelford et al. (1994) in which Piedmontese crosses did have the second-highest frequency of unacceptable dark lean colour scores.

Contrasting data are available regarding the effect of bovine species on dark cutting prevalence, lean carcass colour and pH. In a survey on 4 slaughter plants located in different USA cities involving 724,639 cattle, a higher dark cutting prevalence based on colour assessment was reported in slaughter plants that processed *Bos taurus* and *Bos indicus* x *Bos taurus* crosses compared to plants that processed only *Bos Taurus* (Kreikemeier et al. 1998). On the contrary, Shackelford et al. (1994) and Page at al. (2001) did not report a significant species effect on both lean carcass colour and pH. The low h^2 of pHu (0.08 from Shackelford et al., 1998; 0.06 from King et al., 2010) and the low to moderate h^2 for L^* (0.24 from King et al., 2010 and 0.09 from Pratt et al., 2013) may represent a limit for genetic selection against dark cutting, although a higher h^2 was reported for fresh visual colour evaluation: 0.12 by Shackelford et al. (1998), and 0.34 reported by Pratt et al. (2013). Even if carcass colour and pH seems to be characterized by a low heritability, as stated by Pethick et al. (2005a) genetic selection could improve dark cutting incidence when other traits are considered such as glycogen depletion, because a sire effect on glycogen depletion was found in Simmental bulls. Steers had a higher incidence of dark cutting beef than did heifers (Jones and Tong, 1989).

8.1.3 Farm management conditions

Application of growth promotants or hormone implants in meat producing animals

Animal physiological status can be modified through management practices such as the administration of metabolic modifiers widely used in the beef (meat) producing countries except the European Union. 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 decade and most of the available data for ruminants are relevant to beef cattle.

Scanga et al. (1998) and Schneider et al. (2007) found that dark-cutting frequency increased with sustained use of steroid growth promotion implants. These researchers also found a significant interaction between cattle gender and implant type, with steers having decreased incidence of dark cutting when implanted with estrogenic implants and heifers having reduced incidence of dark cutting when implanted with androgens. Scanga et al. (1998) also found that feeding cattle beyond 100 days post-implantation decreased the likelihood of dark cutting regardless of cattle gender, suggesting that the re-partitioning effects of the implant had ceased by that time and that the cattle were able to build sufficient muscle glycogen for normal post mortem muscle pH decline. Sending animals for slaughter 100 or more days post implantation of growth promotants reduced the incidence of dark cutting on an average of 35% in heifers and 69% among steers. Beta-adrenergic agonists (β-AA) are also powerful tissue repartitioning agents that have the potential to decrease muscle glycogen and increase the incidence of dark cutting (Tarrant 1989). Ractopamine and zilpaterol hydrochloride are the only β-AA approved for commercial use in North America, and research to date has shown no association between the use of ractopamine according to manufacturer label directions and increased dark cutting (Vogel et al. 2009; López-Campos et al. 2012), although other β-AA that are more aggressive than ractopamine such as cimaterol and clenbuterol, both prohibited from use in livestock, have been shown to increase post mortem muscle pH and reduce muscle glycogen stores in lambs (Tarrant 1989).

The most recent reviews of Dikeman (2007) and Lean et al. (2014) reported 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 are available in literature. In 3 studies in sheep (using cimaterol and L-644 969®) reviewed by Dunshea et al. (2005) an increase in pHu was evident, while in a more recent work, according to the findings in beef cattle, no or only slight effects on meat colour were reported with the use of Zilpaterol in goats (López-Carlos et al., 2014) or unspecified β-agonists in lambs (Brand et al., 2013). Based on the available data it seems that β-agonists do not affect the prevalence of dark cutting in meat from ruminants, although limited studies are available on sheep and goats.

Meat production from female ruminants (heifers, cows, ewes, goats) accounts for a significant amount across the different countries and its quality can be affected by oestrous status. Kenny and Tarrant (1987) demonstrated that mounting behaviour during oestrus is negatively related with muscular glycogen content and, consequently, promotes a higher incidence of high pH meat. This data is confirmed by Scanga et al. (1998), who found a higher incidence of dark cutting in entire heifers compared with spayed ones. 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, 2008, MLA 2011).

Effect of sex

Contrasting evidence for a sex effect on meat pHu and dark cutting in sheep is present in the literature. Most of the studies reported a lack of difference in pHu when comparing female, entire male and wethers across different breeds, slaughter ages and dietary backgrounds (Horcada et al. 1998; Díaz et al. 2003; Texeira et al. 2005; Young et al. 2006; Okeudo & Moss, 2008; Tejeda et al. 2008). On the contrary, Craigie et al. (2012) reported a higher pHu in the *longissimus lumborum* in ewes compared to entire males, while no differences were found in the *semimembranosus*, whereas Johnson et al. (2005) and Bain et al. (2009) found a higher pHu in rams than in ewes.

In terms of meat colour, when comparing females and entire males no sex effect was reported (Vergara et al., 1999;, Díaz et al., 2003; Tejeda et al., 2008). By contrast Texeira et al. (2005) found a darker meat colour in females than in entire male, opposite to the findings of Craigie et al. (2012), which reported a lower lightness in *semimembranosus* from ewes compared to rams. Comparing wethers and ewes, a lighter meat colour in the first has been reported by Hopkins et al. (2007). In terms of castration, Okeudo and Moss (2008) found a lower redness in vasectomized rams compared to wethers and ewes, although a lack of differences in muscle lightness, while in the meta-analysis of Sales (2014) there was no effect of castration on meat colour, even if only 3 studies were considered in the comparison of wethers and rams.

From a metabolic point of view, Warner et al. (2007) reported that, compared to wethers, female sheep had a slightly higher relative area of fast-twitch glycolytic fibres, a lower relative area of oxidative–glycolytic fibres and a lower activity of the oxidative enzyme ICDH, based on the findings of Greenwood et al. (2007) and Gardner et al. (2007). No effect of castration on muscle glycogen content at slaughter was reported by Young et al. (2006). Taken together the reported data, seems to indicate that sex may exert a marginal effect on meat colour and pHu in sheep and, therefore, in dark cutting frequency.

More surveys had been conducted in beef cattle aimed to identify a sex effect on dark cutting incidence, meat colour and pHu. Studies, across different breeds and management on farm and from farm gate to slaughter, agree that there is a higher incidence of dark cutting in bulls compared to steers and/or heifers (Jeremiah et al., 1991; Mohan Raj et al., 1992; Dunne et al., 2004; Mach et al., 2008; Page et al., 2001; Miguel et al., 2014). The higher dark cutting incidence in bulls and vasectomized cattle is attributable to the flightier temperament, especially under stressful conditions. It has been shown that young bulls most often exhibit dark cutting meat due to their aggressive behavior. Bulls tend to produce more dark cutting if they are mixed prior to slaughter because they engage in fighting and mounting to re-establish social hierarchy that consequently depletes muscle glycogen through exhaustion (Warriss et al., 1984). No differences have been reported comparing surgical and immune castration (Miguel et al., 2014).

Intact heifers have been reported to be more susceptible to dark cutting and/or high pHu compared to steers in several surveys (Jeremiah et al., 1991; Scanga et al., 1997; Voisinet et al., 1997; Wulf et al., 1997). The flightier temperament, due to oestrogen secretion, is suggested as the basis of this finding. Indeed Wulf et al. (1997) and Gruber et al. (2006) reported a more excitable temperament in heifers compared to steers. No differences were reported in meat color and/or pHu and/or dark cutting incidence between steers and heifers (Kreikemeier et al., 1998; Page et al., 2001; Gruber et al., 2006). In contrast Bass et al. (2010) found higher pHu and dark cutting incidence in steers vs heifers. The authors attributed the findings to the different stress response. Indeed heifers, although having a higher temperament score when stressed 1 day before slaughter compared to steers, exhibited a higher glycolytic potential. Given this Bass et al. (2010) concluded that heifers had a short-term physiological response to stress, not enough to cause significant glycogen depletion, while steers reacted to stress with a sustained rather than an acute response. This effect, coupled with the greater physical activity during mixing, resulted in a higher glycogen depletion and, thus, higher pHu and dark cutting incidence.

The reported data suggest that bulls are more prone to develop dark cutting than heifers and steers, with the latter group the less susceptible sex type. This difference can be attributed to different hormonal status that affects stress susceptibility and dominance behaviour during pre-slaughter. Therefore, extremely careful handling and all the necessary strategies to reduce stress and promote a higher glycogen concentration have to be applied to minimize dark cutting, especially in bull and heifers.

Effect of age at killing

In considering the effect of age at slaughter on carcass quality, is necessary to consider that the 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 conditions. In this situation the different age at slaughter is often determined by factors such as breed characteristics, growth rate, diet and rearing system.

It is well established that as cattle age increases at slaughter the darker the meat (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). Similar effect occurs in lambs (Vergara et al., 1999; Diaz et al., 2003; Martínez-Cerezo et al., 2005; Pethick et al., 2005b; Wiese et al., 2005; Hopkins et al., 2007). This effect is due to an increase 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). The increase in myoglobin content is explained by a shift to a more oxidative metabolism with age. Indeed, Greenwood et al. (2007) reported a rise in the number of type 2A myofibers (fast oxidative) relative area from 13 to 20% in lamb *longissimus* muscle form 4 to 22 months and, a decrease in the relative area from 72 to 67% of type 2X fibers (fast glycolytic), with the major change between 8 and 14 months of age. This shift, however, was not reported for *semitendinosus* muscle, underlining a muscle specificity. Mlynek et al. (2006) and Girard et al. (2011) reported an increase in slow oxidative fibers with increasing age at slaughter (range 12-24 months), in *Longissimus* and *gluteus medius* respectively, while Wegner et al. (2000) reported that an increase of type IIB fast glycolytic fibers and a decrease frequency of type IIA fibers in *semitendinosus* muscle was evident from 0 to 2 months, while type I fiber frequency remained constant. From 2 to 6 months the rate of change from type IIA fibers into type IIB fibers strongly decreased and linear changes rarely occurred from 6 to 24 months. Different breeds and muscles were examined in the reported studies, underlining a possible breed and/or muscle specificity on muscle fiber development in beef.

Hopkins et al. (2007) reported a lower pHu in *semitendinosus* muscle in 4 months old lambs compared to those slaughtered at 8, 14 or 22 months, all of them fed the same diet. Differences were also found when comparing suckling lambs from European breeds at 3 to 4 weeks of age, with a higher pHu in the older animals (Diaz et al., 2003). Kelava et al. (2008) reported a slight increase in pHu when comparing 10-14 month old vs 15-18 and 19-24 months old Simmental cattle slaughtered in Croatia, while from the report of Sanudo et al. (2007) it is possible to see a numerical increase (trend not statistically analyzed) of pH as the slaughter age increases in 10 Spanish and French local breeds under local production systems. Comparing veal, young cattle and cows Hughes et al. (2014) reported an increase in unacceptable dark carcasses increasing slaughter age. As suggested by Hopkins et al. (2007), an increase in pHu with age could be attributable to a rise in muscle adrenalin sensitivity, such that this boosts glycogen depletion in older animals. This hypothesis is supported by a lack of change in fiber types with age in *semitendinosus* muscle as previously reported by Greenwood et al. (2007) and by Gardner et al. (2005), who reported an increase in adrenalin sensitivity in both Angus and Piedmontese cattle at 36 months of age vs those 15 months of age. By contrast a number of studies have not reported an effect of slaughter age on pHu in both sheep (Wiese et al., 2005; Martínez-Cerezo et al., 2005) and beef (Troy et al. 2002; Warren et al., 2008; Girard et al., 2012; Mpakama et al. 2014). It can be stated that older animal have darker meat due to a higher muscle pigment content and/or less glycogen concentration in the muscle and therefore are more prone to dark cutting.

8.1.4 Climate condition or seasonal variation

Dark cutting is conceivably a by-product of animal management practices and entirely preventable if the predisposing conditions are understood and recognized. Of the factors known to contribute to dark cutting, the environment may be the most difficult to control. Prolonged exposure of slaughter cattle to wet, cold weather that is conducive to shivering will deplete muscle glycogen and this was substantiated anecdotally during southern Australian winters when certain Asian markets required cattle to be washed prior to slaughter, with this practice producing daily abattoir dark cutting percentages as high as 40% (Bruce, personal communication). The incidence of dark cutting in Canada increases in both the Northern Hemisphere spring (March to April) and autumn (September to November) when daily temperature fluctuations are large, and is thought to be related to the cattle using additional muscle and liver glycogen to adapt to the cool nights after warm days. This phenomenon has been noted in other Northern Hemisphere countries such as Ireland (Tarrant & Sherington 1980). Scanga et al. (1998) found that daily temperature fluctuations greater than 5.6 °C produced an increased incidence of dark cutting. Ambient temperatures above 20°C (95 F) 24-48 h prior to shipment for slaughter increased the occurrence of dark cutting beef in both steers and heifers, while ambient temperatures below 0°C (32 F) 24-48 h prior to slaughter increased the incidence only in heifers, but not in steers. Temperature fluctuation more than 10 F between the low and high ambient daily temperature 24-72 h prior to transport for slaughter increased incidence of dark cutting in both heifers and steers. Barns or shelters provided by trees, fences or sheds can be used to minimize the effect of temperature on cattle in North America. High daily temperatures may also increase the frequency of dark cutting, as temperatures above 35°C were found by Scanga et al. (1998) to increase dark cutting in heifers. This presumably would be due to the heifers not eating during the hot weather and accessing muscle and liver glycogen to sustain homeostasis.

Seasonal variability in dark cutting incidence is clearly evident. reported. Interestingly most of the studies report a higher dark cutting incidence, and/or higher pHu and/or darker colour in warm rather than in cold seasons in both beef (Kreikemeier et al., 1998; Immonen et al., 2000b; Kadim et al., 2004; Mach et al., 2008; Wiklund et al., 2009; Węglarz, 2010;) and sheep (Kadim et al., 2008; McPhail et al., 2014). Two reasons may explain these findings: poor pasture quality during the summer and the beginning of autumn and heat stress. Under poor pasture quality conditions glycogen synthesis will be reduced. Knee et al. (2004) reported, a higher glycogen level in lambs during spring, concomitant to the higher quality pasture. Increased body temperature at slaughter was, shown to be associated with a higher dark cutting prevalence in beef (Jacobson et al., 1997), while a negative correlation with glycolytic potential and a positive relationship with pHu has been reported in sheep (Pighin et al., 2014). It should be noted that DM animals are more sensitive to hot condition due to reduced capillary density, lower body surface per unit of weight and lung weight (Fiems, 2012). Although most focus is on hot conditions with cold weather, especially when combined with rain can increase body heat loss in exposed animals (Scanga et al., 1998), and will represent a stressful effect , especially during transport. In fact a higher dark cutting prevalence has been reported by Kim et al. (2003) in Korean cattle and by Miranda-de la Lama et al. (2011) in lambs transported and slaughtered in winter.

8.1.5 Temperament and human-animal interaction on farm

Variability in incidence of dark cutting between animals of the same breed maintained under the same diet and transportation to the same slaughterhouse can be essentially explained by two different factors: 1) individual variability in temperament and stress susceptibility; 2) quality of handling practices.

Operators play a crucial role in the incidence of dark cutting in ruminants. The effect of farmer behaviour and gentle handling is evident in the study of Lensink et al. (2001) in which calves (exposed 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). Lensink et al. (2001) concluded that this result was attributable to a lower fear reaction to handling before slaughter. and these workers showed that gentle handled calves were less agitated and showed a higher muscular glycolytic potential.

There is a need to minimize stress during handling i.e., with gentle animal handling by the operators and with a proper design and maintenance of handling facilities (see http://www.grandin.com/ website for more details). Individual variability in temperament and the relationship between on-farm and pre-slaughter behaviour and prevalence of dark cutting has been widely investigated, especially in cattle. 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 relationship between agitated temperament and higher pHu and/or incidence of dark cutting has been reported in several studies (Voisinet et al., 1997; Wulf et al., 1997; Cafe et al., 2011; Hall et al., 2011). This underlines that animals with more excitable temperament seem to be more susceptible to stress. From a general point of view, agitated animals seem to be more stress sensitive and therefore careful handling practices have to be applied in order to minimize the risk of dark cutting. Results such as these serve to strengthen the hypothesis that dark cutting in bovine carcasses is due to some cattle having a temperament which predisposes them to stress.

Phenotypic characteristics as whorl head position and cannon bone dimension have been associated with temperament. Animals without hair whorl or with a hair whorl located above or between eyes exhibited a more excitable behaviour than animals with a hair whorl below the line of the eyes (Grandin et al., 1995; Randle, 1998; Lanier et al., 2001;), although Olmos and Turner (2008) reported that hair whorl position was only associated with some of the behavioural assessment criteria cited above. A larger and wider cannon bone has been associated with calmer temperament in *Bos taurus* reported by Lanier and Grandin (2002). Within breeds, a sire effect on cattle temperament has been shown (Grandin & Deesing, 1998). In light of the overall moderate to high h^2 of temperament traits such as chute behaviour (0.08-0.67), flight speed and flight time (0.08-0.54) and flight distance and docility (0-0.70) as reviewed by Haskell et al. (2014), temperament could be considered in genetic selection programs for meat producing ruminants. Dark cutting can be minimized by reducing animal stress through appropriate management decisions. Animals must be monitored through proper feeding, disease prevention, weaning, penning, handling and mixing.

8.2 Off farm factors

The pre-slaughter phase from farm gate to stunning can lead to several stress events due to many activities taking place individually or in parallel. These include human handling, transport, unfamiliar environments creating fear, feed and water restriction, mixing with unfamiliar animals, fighting of animals for dominancy and changes in climate conditions (especially in case of long transport and/or prolonged lairage). These factors can lead to physical, physiological and emotional changes in the animal body, which can affect the ultimate product quality and profitability of meat industry. The intensity of these factors is essentially affected by management or voluntary fasting on farm, marketing conditions (direct consignment or saleyard), transport length, lairage time and stock person attitudes. Dark cutting is a well-known meat quality defect that has been associated primarily with pre-slaughter stress (Lacourt & Tarrant, 1985; Mach et al., 2008) such as transport of animals to the abattoir (Immonen et al., 2000 a,b) or with an excitable temperament changes (Voisinet et al., 1997). Bulls have been shown to have an increased likelihood of producing dark-cutting carcasses because of activity associated with re-establishing social hierarchy during transport to slaughter (Warriss et al., 1994).

8.2.1 Livestock management conditions associated with marketing and transport pre-slaughter

Marketing conditions

Lambs and cattle can be direct consigned from farm to slaughterhouse or be sold through saleyard/auction markets. A higher dark cutting incidence in cattle sourced from saleyard compared to those directly consigned from farm to slaughterhouse has been reported by Shorthose et al. (1998) and Warren et al. (2010), while Warner et al (1998) found a higher pHu in saleyard cattle, despite their being no differences in dark cutting prevalence. By contrast, Ferguson et al. (2007) did not report any differences in meat color between directly consigned or saleyard cattle and McPhail et al. (2014) did not find any marketing method effect (direct consignment or saleyard) on the pHu in lambs.

Although the contrasting results are likely due to confounding factors such as genotype, dietary background, transport length and pre-slaughter management as underlined by Ferguson et al. (2001), sourcing animals from auction markets can be considered a risk factor for dark cutting due to the fact that the animals are exposed to more handling, mixing and feed restriction compared to those sourced directly from the farm (Ferguson et al., 2001; Ferguson & Warner, 2008). Direct consignment is considered as preferred option by the Meat Standard Australia (MSA) grading system (Pethick, 2005).

Other than the marketing system, stressful practices in the days before slaughter can predispose animals to dark cutting development due to glycogen depletion. Cattle should not be mixed with other cattle unfamiliar to them so that fighting and dominance activities do no occur, which can deplete muscle glycogen from fast glycolytic muscles (Tarrant 1989). Mounier et al. (2006) reported that cattle mixed in the latest stages of finishing phase had an increased dark cutting incidence due to the stress caused by the fighting to establish a new dominance hierarchy. In case of stressful events in the days pre-slaughter, a recovering period, necessary to restore glycogen reserves, has to be guaranteed in order to minimize dark cutting incidence. Warris et al. (1984), slaughtered bulls (fed a high energy diet) 0, 1, 2, 4, 7, 9, 10 days after been mixed overnight with unfamiliar bulls, and found that pHu reached nearly normal levels in animals allowed to recover from mixing stress for at least 2 days. As such the authors suggested that 4 to 7 days appeared necessary for the bulls to fully recover and prevent a negative effect on meat color. Devine et al. (2006) reported that Merino weathers allowed to recover on pasture for 8 or 10 days after stressful handling due to weighing and shearing had a lower dark cutting prevalence than animals that were allowed to recover only 1 or 3 days. It can be concluded that stressful operations should be avoided in the immediate days before slaughter and, if stressful events happen, a proper recovery period has to be guaranteed in order to reduce the prevalence of dark cutting.

Transport conditions

Transport is one of the most stressful steps in beef and lamb meat production chain due to pre-transport management requirements, 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 & 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. Tarrant (1990) stated that distress may be avoided by observing statutory rest periods on long journeys, applying good animal handling, considerate driving technique, and using correctly designed pens, loading ramps and stock crates. Several studies have been carried out to compare the effects of transport length on meat dark cutting prevalence and contrasting results are found in the literature. 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 seem to confirm this assertion, as no effects on dark cutting prevalence have been reported after short transport periods 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 increase in dark cutting incidence in cattle transported for 16 hours compared to those transported for 3 hours. In contrast to the conclusion of Ferguson et al. (2001), Jones and Tong (1989) reported an increase in dark cutting frequency as transport distance increased from < 100 km to more than 300 km and Kadim et al. (2012) found an increase in pHu in sheep transported for 75 min compared to those not transported. Moreover, Mounier et al. (2006) found that as transport duration increased, unloading became more difficult. With reference to sheep Dalmau et al. (2014) did not report any differences between lambs transported for 1 or 24 hours, while the season played an important role, as short transport increased 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.

The frequency of dark cutting beef increased as transportation distance from the farm to the slaughter plant changed from less than 60 miles to more than 180 miles (Jones & Tong 1989). Based on the reported data it is assumed that, generally, short transport does not increase dark cutting prevalence, while longer transport distance is more likely to increase the probability of dark cutting, especially for beef, but this is affected by the season. The 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 journeys shorter than 8 hours. Concerning sheep, the 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, and as reported by Schwartzkopf-Genswein et al. (2012) in Canada the maximum transport time is 52 h, higher than that allowed in the EU (30 hours) and USA (28 hours). In case of long journeys, however, Tarrant and Grandin (2000) suggested to avoid rest stops and unloading animals, as they only prolong the overall journey time, unless adequate resting facilities are available and animals are carefully handled.

In Australia, during transport of sheep, care is taken in planning a journey and the facilities provided are of a high standard. Sheep aged over 12 months can be transported without spelling for up to 24 hours without any problems. However animals should be inspected regularly during transit. For weaned sheep less than 12 months, the relevant time period is 12 hours. This time period includes loading and unloading and stops when the animals are not unloaded. The 24 hour transport period for mature sheep may be extended to 30 hours if, and only if, the entire journey can be completed within this time. For young sheep, the 12 hours period may be extended to 18 hours. During every specified rest period, sheep of all ages must be unloaded, have access to food and clean water (except during the last 8 hours before reloading). Water and feed must be provided at least once in every 24 hours to animals older than 12 months, the only exception is animals travelling on a journey which will be entirely completed in 30 hours (Department of Local Government and Regional Development Western Australia, 2003).

Mounier et al. (2006) reported that loading was easier on 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 they both can induce stress. Indeed, Warren et al. (2010) reported that agitated cattle at unloading had a higher dark cutting incidence than calm cattle. 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 (mixing) animals from different farms and/or of different sexes should be avoided, as this has been shown to lead to a higher frequency of dark cutting (Jones and Tong, 1989; Mach et al., 2008; Warren et al., 2010). Warren et al. (2010) reported, a higher incidence of dark cutting when mixed-sex groups were not-separated on trucks. Mixed load of cattle had a higher occurrence of dark cutting beef than unmixed loads.

Space allowance requirements are calculated by allometric equations (see Broom 2003, 2008, and Petherick and Phillips, 2009) and, taken together the equation developed, recommends a space allowance for polled/dehorned cattle of 1.20-1.60 m^2/head for 550 kg animals or higher than 1.4-1.6 m^2/head for >700 kg animals, while for sheep an allowance of 0.2-0.3 m^2/head has been suggested 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 m^2/head more space for sheep. Eldridge and Winfield (1988) however did not find any difference in pHu when comparing a space allowance of 0.89, 1.16 or 1.39 m^2/cattle during a 320 km journey. In the same manner, no difference in dark cutting frequency has been reported when comparing animals transported with a density higher or lower than 0.86 cattle/m^2 (average 0.83±0.23, compliant with EU regulations) in the survey of Mach et al. (2008).

Regarding further factors that significantly affect dark cutting prevalence, Warren et al. (2010) reported a higher prevalence in consignments for which the truck driver didn't undertake specific training for transporting livestock, and noted that drivers with more experience delivered cattle with reduced dark cutting prevalence. In the same study it was found that increasing the ventilation during the cold season increased the frequency of dark cutting. Also road type may affect dark cutting prevalence, as Miranda-de la Lama (2012) reported a higher pHu and a lower *L** in lambs transported on unpaved rural secondary roads compared to those transported on paved roads for 3 hours.

Conditions during transport that are conducive to increased dark cutting will include any situation that prevents the animal from eating and requires the animal to use its muscles to maintain balance. Not removing feed or water from cattle prior to transport to abattoir is now recommended in Canada to ensure good carcass quality, as ruminants may be shipped for slaughter for up to 48 hours without having to be fed or watered (González et al. 2012); therefore, cattle should be fed and watered within the 5 hours preceding transport if the regulations of the country allow this. Under Australian condition, All sheep must be given access to water as soon as they are unloaded. Feed should be provided to sheep which have just travelled for more than 16 hours (and thus not been fed for 24 hours) or sheep which will be yarded for more than 24 hours. The only exception is that feed should not be provided to animals which will be slaughtered within the next 8 hours (Department of Local Government and Regional Development Western Australia, 2003). Conditions most conducive to dark cutting are those which cattle experience at auctions or sale yards, where the animal may not receive feed for 24 hours and may be loaded and unloaded at least twice. The results of Warner et al. (1998) supported this, as these authors found that blood corticosteroids were increased and muscle glycogen reduced under simulated sale yard handling, with muscle glycogen particularly depleted in cattle that received only pasture prior to treatment. The simulated sale yard treatment of cattle by Warner et al. (1998) also substantiated that the sale yard treated cattle produced beef that was darker, that had a higher mean pH and that was tougher than beef from cattle shipped directly to the abattoir.

8.2.2 Livestock management conditions associated with lairage pre-slaughter

Scanga et al. (1998) reported that dark cutting beef can result from pre-slaughter stress, which depletes muscle glycogen stores and thus reduces the glycogen needed to produce the lactic acid that reduces the pH of post mortem muscles. This study indicated that the occurrence of dark cutting in beef was affected by different management philosophies, lairage construction facilities and handling practises and this was supported by other studies by Grandin (1993) and Smith et al. (1993). Miller (2007) reported that the most common stressors are mounting behaviour and the mixing of unfamiliar animals.

As previously highlighted for transport, contrasting data are present in literature concerning the link between pre-slaughter lairage duration and dark cutting prevalence in ruminants. An increase in dark cutting frequency with increasing lairage time has been widely reported in both surveys and controlled studies, across different countries. Llewelyn et al. (2002) reported an increase in dark cutting frequency when comparing 12 to 24 hours versus 25 to 36 hours lairage in Australian cattle. In the survey of Mach et al. (2008) cattle which underwent more than 15.8 hours in lairage exhibited a higher dark cutting frequency in a Spanish abattoir, while in a US survey Kreikemeier et al. (1998) found that cattle held at slaughter plant over weekend or holiday (36-84 h) showed a higher dark cutting frequency than those processed less than 12 h after consignment. Similarly, Warren et al. (2010) found that Canadian cattle held overnight at the plant tended to cut dark 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 resulted in a 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 and; longer periods in lairage were also associated with an increase in dark cutting 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 consignments, a marked increase in the frequency of carcasses with pHu >5.7 was 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 deprivation l and a longer exposure to stress, especially if lairage is not properly conducted. Mounier et al. (2006) in a survey conducted in France reported a reduction of pHu in short transported cattle as the lairage time increased (from 20 min to 48 hours). Similarly del Campo et al. (2010) reported a lower pHu in cattle which underwent 15 hours of lairage overnight compared to those slaughtered after 3 hours lairage . These authors suggested that, in this case the effect of 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 Jacobs et al. (2005a), 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. (2007) and between lambs slaughtered at arrival or after 12 hours of lairage by Liste et al. (2011).

During lairage, mixing unfamiliar animals has to be avoided, as it increase 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 behavior, especially in agitate animals; Mach et al. (2008) reported indeed a higher DC frequency increasing stocking density over 0.26 bulls/m^2. 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 behavior, can be effective to promote glycogen restore, hydration and calm behavior. 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, Pethick 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 improved 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 glycogenolysis, 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).

Fasting is aimed to reduce digestive tract filling, reducing thus the risk of carcass contamination during evisceration and wastes at the slaughterhouse. Often fasting is not voluntarily applied, but is due to the market conditions. As pointed out by Ferguson and Warner (2008), for cattle and sheep directly consigned from farm to slaughterhouse, feed and water restriction is likely to be lower than 24 h, while it can reach 48 h in some situations as marketing from saleyard/auction market. Even in that case contrasting data are present, as Jones et al. (1986) reported that increasing fasting from 4 to 24 hours increase steers and bulls pHu, while in another study Jones et al. (1988) reported a lack of effect of 24, 48 or 72 hours of fasting on pHu in steers and heifers, even if a darker meat from animals fasted more than 24 hours was evident. The same research group, however, found that increasing feed and water withdrawal from 0 to 48 h led to a reduction of L* and an increase of pHu in cattle (Jones et al., 1990). The effect of water deprivation has been investigated by Vogel et al. (2011) in cull cows and no differences in pHu have been reported after 0, 18 or 36 hours of water deprivation (average pH were border line for DC), while in the survey of Llewelyn et al. (2002) emerged that cattle undergone to a water restriction lower than 2 h exhibited lower DC frequency than those experienced water restriction for 4 hours. Based on the reported data, is possible to conclude that prolonged fasting may act as predisposing factor for DC prevalence and it should be as short as possible, as it may impair glycogen synthesis and promote a nervous behavior.

Cattle should not be mixed with other unfamiliar cattle so that fighting and strenuous physical activity does not occur (Tarrant 1989). Cattle should be sheltered from heat or inclement weather, particularly if they have been transported a long distance, as prolonged fasting may have reduced their energy reserves making them less resistant to environmental stress. Ruminants are notoriously slow at recovering muscle glycogen, most likely because there is limited circulating glucose because the major source of energy is volatile fatty acids from the rumen. Ruminants may have even slower muscle glycogen repletion if they are fasted, again most likely because glycogen must be mobilized first from the liver to circulating blood, carried to muscle as glucose and then transformed to glycogen again; therefore, muscle glycogen repletion can only be accelerated through feeding (McVeigh and Tarrant 1982). In North America, no feed is given to slaughter cattle unless they are held overnight or over a weekend; therefore, limiting additional stress during recovery from transport to the abattoir and providing an environment conducive to rest and recumbence will facilitate glycogen repletion in muscle in the absence of feed.

Cattle should not be allowed to become wet during cold weather, but should be housed under shelter during lairage prior to slaughter (Scanga et al. 1998). Also, resting of cattle at the abattoir, particularly after long haul transport, may be beneficial to decreasing post mortem muscle pH (Tarrant 1989). Shorthose et al. (1972) and Wythes et al. (1980) found that resting steers 4 or 2 days, respectively, after long haul transport reduced post mortem muscle pH relative to 2 and 1 days, respectively. A minimum lairage time of 2 hours is practiced in Canada, as extended lairage times have been associated with increased incidence of dark cutting (Warren et al. 2010). This may be due to extended lairage being applied to cattle that have been unloaded at the abattoir but not slaughtered due to abattoir malfunction or mis-scheduling, thus requiring the cattle to be loaded onto a truck again, transported to a nearby feedlot, and fed an unfamiliar feed until return transport to the abattoir for processing. This practice, although necessary as Canadian abattoirs do not have extensive facilities for feeding and holding cattle, exposes a load of cattle to unloading and loading a total of five times with 72 hours of slaughter. Loads of cattle that experience this type of handling can produce 30 to 40% dark cutters (Bruce, personal communication). There have been inconsistencies on defining dark cutting and methodologies used for grading dark cutters between countries and between researchers within a country (see recent publications Apaoblaza et al., 2015; Holdstock et al., 2014; Hughes et al., 2014; McGilchrist et al., 2012; McPhail et al., 2014; Teke et al., 2014). These are very important points and the threshold applied needs to be carefully considered and it is recommended that Australia adopts a threshold that is matched to visual tolerance levels by actual consumers.

9. Techniques to assess and manage (minimize) dark cutting animals

9.1 Techniques to assess dark cutting animals on farm and off farm

Operations equipped with ultrasound and weigh scale capabilities may wish to monitor the live weight and back fat thickness of cattle being finished for slaughter. Cattle that are at increased risk of producing a dark cutting carcass have been shown to have rib subcutaneous fat levels of 0.76 cm or less (Page et al. 2001), decreased carcass weights (McGilchrist et al. 2011; Holdstock et al. 2014). A deep knowledge of animals' background and management at slaughterhouse, coupled with a strict observation of their behavior, are necessary to identify those susceptible to develop DC. Other than the evaluation of animal background, health status and temperament, given the reported association between increased body temperature at slaughter and DC prevalence in beef (Jacobson et al., 1997) and higher pHu in sheep (Pighin et al., 2014), a screening with thermographic camera may help to objectively identify DC susceptible animals, as proposed by Schaefer et al. (2001). However, studies conducted in that field did not assured high accuracy of DC prediction using infrared thermographic camera, as reviewed by Gazzola and Gibson (2001).

- Animals affected by clinical or subclinical disease. Animals affected by clinical or subclinical diseases often weak and susceptible to environmental changes. Live weight gain and muscle energy storage may not be ideal for producing better carcasses that yielding good quality meat. The identification of susceptible animals on farm plays a key role in reducing DC incidence, as can allow producers to apply specific nutritional intervention or even delay the slaughtering by few days to overcome this situation.
- Aggressiveness of females on heat. Proper management needs to be applied during transporting those animals that are aggressive due to estrous behavior, perhaps separation of these animals from normal animals when marketing.
- Animals that are implanted with growth promotants especially during the latest 2-3 months prior to slaughter are more agitated and more aggressive. Animals given combination of

growth promotants are more aggressive and the occurrence of dark cutting epidemics is higher in these animals compared with animals given single growth promotants.

- More susceptible breeds. Merinos have different temperament than animals selecting for high muscularity. Some animals are fearful to dogs and mixings flocks on farm prior to transport can cause physical and physiological changes.
- Older animals specially dairy cows tend to be weak and often get injuries or bruises when mixed with younger animals
- Entire animals in a mixed flock show dominancy and cause trouble to others during eating and relaxing. On the other hand if cattle are excited and anxious at being handled or being separated and these animals will show signs of stress, which can deplete muscle glycogen before slaughter.
- Pasture-fed animals or animals fed with low energy diet prior to slaughter
- Extreme weather conditions or weather variability in the days prior to slaughter
- Agitated animals or animals undergone to stressful practices in the latest days before slaughter. Animals that exhibit agitated behavior at unloading and during lairage should be separated from normal stocks. Animals that are difficult to loading and unloading may prone to dark cutting.
- Animals showing signs of fear by jumping or positioning head down or running away from stock persons often show elevated levels of blood cortisol and adrenaline which can lead to more dark cutting due to depletion of energy sources from the muscle before death.

9.2 Techniques to manage (minimise) dark cutting events on farm and off farm

Most of the published research on dark cutting meat has focused on the pre-slaughter factors that play a key role in excessive antemortem muscle glycogenolysis and the development of dark cutting meat (Jones et al., 1986; Kenny & Tarrant, 1987; Voisinet et al., 1997; Kreikemeier et al., 1998; Scanga et al., 1998). Although most of these studies were conducted to prevent antemortem stress in meat animals; stress cannot be totally removed before slaughter as many of the stress conditions can be related to environmental, transportation and unavoidable mixed lots of animals. Strategies to minimize stress during on-farm loading, transportation, unloading and at housing at the abattoir are very important and need to be considered as the first line to reduce the incidence of DFD meat. Several methods have been suggested to control problems with aggressive behaviour of animals during regrouping, such as mounting, mock fighting and butting (pre-slaughter strategies). Also few strategies have been examined to improve the quality of DFD meat (post-slaughter interventions).

9.2.1 On-farm techniques to manage (minimize) dark cutting events

- Strategies for habituate cattle handling (positive handling and contact with human). Early interaction with the animal and gentle touching while the animal still young can reduce the level of stress in beef cattle (Probst et al., 2012).

- Castration and spaying can reduce DC prevalence leading to a calmer temperament associated with a lack of heat.
- Animals in heat tend to produce higher levels of dark cutting. Heifers in oestrous yielded a higher incidence of dark cutting carcasses than steers due to the expression of breeding behaviour. By applying specific hormone implants suitable to heifers and steers, the incidence of dark cutting can be reduced (eg, testosterone implant can increase the DFD carcasses). Also separating heifers showing signs of heat may lower the incidence of dark cutting.
- Concerning breed, particular attention has to be paid when dealing with susceptible breeds. It would be good to select animals or stock on the basis of temperament and performance.
- From a nutritional point of view, providing a high energy diet, especially during finishing phase and prior to marketing, increase glycogen storage and, improving growth rate, allow to reduce the age at slaughter.
- High energy diet has to be properly balanced and prepared to avoid acidosis, as acidosis impairs feed intake (Schwartzkopf-Genswein et al., 2003) and promote agitation and aggressive behaviour (Commun et al., 2011).
- Moreover, specific dietary supplements, as magnesium oxide (example, 1% of feed four days prior to slaughter) or hyperglycaemic substances as sugars, glycerol and propylene glycol, can improve glycogen level and reduce the negative effect of pre-slaughter stress on dark cutting.
- Heat stress often promotes DC frequency, which can be reduced by providing shade. For animals kept in close barns improved ventilation through proper barn design and provision of fans can minimise the prevalence.
- Genetic selection for moderate heritability of temperament traits.
- Stressful practices should be avoided in the days prior to slaughter, in case of unavoidable stressful practices a proper recovery period, longer if animals are fed with low energy diet, has to be guaranteed. Familiarise animals with handling process is always good to minimise unnecessary stress. Direct transport of animals to abattoirs than sending them via saleyards can dramatically reduce dark cutting.
- Sending animals for Slaughter 100 or more days post implantation of growth promotants can reduce the incidence of dark cutting in substantial rate.
- Use of Zeranol implants to reduce the developments of sex characteristics (Staigmiller et al., 1985; Greathouse et al., 1983) that increases the carcass fat content and decreased the incidence of DFD meat. However, these improvements have not general found in bulls with Zeranol implants (Jones et al., 1986) and castration was more effective in reducing DFD meat.
- Ensure that cattle that have been fed on forage-based diets, particularly pasture, receive a high energy diet such as that provided by grain until subcutaneous back fat at the 11-12th rib is greater than 0.76 cm (Page et al., 2001). Cattle should receive feed up to the time of transport, particularly if they are scheduled for long-haul (more than 10 hours) transport. Feeding of high energy diets two weeks prior to slaughter to animals that are susceptible to stress may reduce the development of dark cutting

- Caffeine administration in pigs inhibited the effect of isoproterenol, non-selective beta-adrenergic agonist, and produced normal pH and lactate in the muscle even when nicotinic acid, an inhibitor of lipolysis, was co-administrated with isoproterenol (Spencer et al., 1983). The authors suggested the stimulation of lipolysis in the animals prior to slaughtering to reduce the incidence of DFD meat.
- The use of mentholated ointment applied on the nostrils of horses was effective in reducing the concentrations of plasma epinephrine and norepinephrine (Micera et al., 2012). Mentholated ointment appears to affect the olfactory cognition and may be useful with other animals, but this yet to be established.
- The feeding of supplemental chromium may help in increasing glycogen deposition which in turn reduces the depletion rate of muscle glycogen before slaughter (Hanson et al., 2000).
- Other than reported strategies above, it is necessary to underline that all the efforts have to be enforced to reduce stress during animal handling. This goal is achievable through proper handling strategies, design and maintenance of handling facilities (see Grandin 2008 for proper handling and handling facilities design)

9.2.2 Off farm techniques to manage (minimize) dark cutting events

In order to reduce the prevalence of dark cutting carcasses, minimum stress conditions have to be applied from farm gate to slaughter point (stunning point). Human population are increasingly demanding that meat producing animals should be reared, handled, transported and slaughtered using humane practices (Appleby & Hughes, 1997). By maintaining a high welfare standard, the events that causes dark cutting can be minimised. Loading, transporting, unloading, holding in lairage, moving, stunning and exsanguination can cause fear, pain, agitation that can cause physical, emotional and physiological changes in the animals – all these can lead to increased incidence of dark cutting. Schaefer et al. (1997) reported that there is a growing public concern regarding the welfare of animals during transportation and handling that causes stress to animals.

Transport and handling stressors from farm to slaughter affect the welfare of meat producing animals (Grandin, 2007 & 2010) as well as the quality of meat at the processor and retailer sectors (Knowles, 1998). Stress at lairage can be due to environment, management, handling facilities and the practices of livestock personnel. There are increasing community concerns about the treatment of farm animals post-farm gate, particularly handling pre-slaughter (Grandin, 2007). Recent research has shown considerable variation between abattoirs and within cohorts within abattoir in the pre-slaughter behaviour and stress of sheep and cattle (Hemsworth et al., 2011). Furthermore pre-slaughter handling has been shown to be significantly associated with behaviour and stress of sheep and cattle (Hemsworth et al., 2011) and stockperson attitudes were significantly associated with pre-slaughter handling in pigs as well as in sheep and cattle (Coleman et al., 2003, Coleman et al., 2012). Research also shows that handling of livestock, by affecting the animal's fear of humans, can markedly affect the stress physiology and productivity of livestock (Hemsworth & Coleman, 2011; Rushen et al., 1999; Waiblinger et al., 2002).

Lairage should be integrated with other on-farm and processing practices in the meat production system to achieve the best results for the producer, the processor, the consumer and for the animals (Liste et al., 2011). A period of rest in lairage is generally recommended to allow the lambs to recover from transport and associated handling (Cockram, et al., 1997). The effects of different lairage times on both animal welfare and meat quality have been reported by many researchers (Díaz et al., 2014; Ferreira et al., 2006; Jacob et al., 2006; Teke et al., 2014). There are however discrepancies among researchers about the effects of different lairage and transport times on meat quality. Little information is available on the relationship between animal behaviour characteristics, stock person attitudes towards handling, animal behavioural responses pre-slaughter and dark cutting or degraded meat quality. The effects of lairage on meat quality can be explained by temperament, breed and handling procedures from post farm to slaughter and conditions at the abattoir (del Campo et al., 2010; Grandin, 2000).

Feeding strategies to reduce the stress response considered vitamin supplements, electrolyte therapy and high energy diet after transport (Cole et al., 1979; Hutcheson & Cole, 1986; McVeigh & Tarrant, 1982; D'Souza et al., 1998; Gardner et al., 2001a; Dunshea et al., 2005). Electrolytes supply the required nutrient for healthy performance of body functions and maintain the neutral pH of the body (acid-base balance). Stress caused by environmental factors (heat/cold), transportation of the animals, animal behaviour and improper diet can cause physiological imbalance of body compounds. Electrolytes have been used for treatment of heat stroke and perspiration loss (Wagner, 1987; Armantano & Solorzano, 1987). The commercial mixtures available can vary in composition but can contain cobalt, copper, manganese, zinc, magnesium, selenium, potassium, sodium and sulphur (http://www.beachportliquidminerals.com.au/__files/f/2163/Green_Cap.pdf) and several commercial brands are available in Australia (e.g. DEB9®; Selectolyte®; Glucotrans®; Topstock Electrolytes; Solulyte Concentrate®; Green Cap® and Selectrolyte®). Schaefer and co-workers filed a patent in 1995 that described the use of mixture of minerals, amino acids and energy source to alleviate the stress in animals that can be administrated prior to transportation or before slaughter (Schaefer et al., 1995). The inventors suggested 6-24 h post administration time for best effect. However, recent work from the same research group on cull dairy cows did not show significant effects on LM or SM quality for electrolytes treatment pre- or post-transportation compared to control (Arp, et al., 2011) similar to earlier observations by Apple et al. (1993) who reported no effect for electrolyte (Biolyte®) drenching on stressed sheep blood parameters. Magnesium supplementation, mostly from work carried out on pigs and sheep, was shown to decrease the loss of glycogen loss of the muscle and reduce the production of catecholamine (D'Souza et al., 1998; Lahucky, et al., 2004; Gardner et al., 2001a), but this was not found in some studies (Apple et al., 2000a; Lowe et al., 2002; Apple et al., 2005; Bass et al., 2010) or negative effects were reported (Apple et al., 2000b). It is worth noting that magnesium oxide at 1% of ration exhibited various effects depending on the mode of supplement (in feed or in water). Electrolytes therapy need to be carefully implemented as negative effects have been reported (e.g. diarrhoea and potassium depletion) upon improper use (Schaefer et al., 1997). The high energy diet is useful to replenish the glycogen stores, but the process is slow and may require up to 14 days to return to normal glycogen level (McVeigh & Tarrant, 1982; Immonen et al., 2000a). The use of sugar rich feed (sugar beet pulp, molasses and barley; monopropylene glycol, lactoserum) during rest times is useful to replenish the muscle glycogen during overnight lairage. A patent describing the use of sorbitol in solid form or in solution before slaughter is available (Bignon, 1992). Recovery of glycogen in different muscles varies. Warriss et al. (1984) found that the *Longissimus dorsi* (LD) of stressed young bulls was recovered after 4 days of resting whereas the *M. psoas* was recovered after 2 days only. High energy feed for 3 weeks increased the muscle glycogen content linearly (Knee et al., 2007) and thus avoiding hypoglycaemia by high energy feeding prior to transportation of animals may be a successful strategy.

Transport

- Application of electrolyte therapy has been reported to reduce physical stress of cattle during transport and handling. Supplement cattle and sheep with commercial electrolytes at least 24 h prior to transport.
- The use of beta-adrenergic blockade agent and inhibitor of epinephrine, such as propranolol (0.5 mg/kg live weight) can be used to prevent the antemortem exhaustion of glycogen and thus prevention of DFD meat incidence caused by subcutaneous adrenaline injection (Ashmore et al., 1973). However, it appears that beta-adrenergic blockade compounds effectiveness in preventing glycogen loss is dependent on the mode of stress. Propranolol was partially successful in blocking the breakdown of glycogen in lambs during transport (Monin & Gire, 1980) but was not successful in inhibiting glycogen breakdown in cattle stressed mixing (McVeigh & Tarrant, 1983).
- Animals have to be carefully handled during loading and unloading. Use of dogs may aggravate the animal behaviour through form of fear, emotional stress, jumping, changes in blood adrenalin, cortisol and body temperature or defence mechanism that all can increase the occurrence of dark cutting.
- Farms and slaughter plants should have properly designed loading and unloading ramps, as it easy the operations. Improper facilities can cause dark cutters through bruises and injuries. Loading ramps have to be properly designed in order to facilitate animal's movement and avoid injuries (Grandin, 2008).
- Loading animals of different sex and\or from different farms or mixing of unfamiliar animals on the same truck should be avoided. In case of unavoidable mixing, animals have to be separated with proper partitions. Fighting during the 24-48 h prior to slaughter can increase the incidence of dark cutting (Livestock Conservation Institute, 1999). Try to separate bulls and rams from other flock or animals during transport and lairage to reduce mounting and aggressive fighting activities.
- Transport of animals to abattoirs within a short distance is preferred. With long transport distance, paved roads have to be used and it is always preferred truck drivers undertake a specific training course for livestock transport and welfare of animals. Make sure animals are given adequate water before transport, especially during long drive and hot seasons. A study of market cows/bulls at three plants in Texas revealed that truck driver differences (stop, start, speeding, rough cornering) accounted for most of the variability in bruise incidence and severity (Livestock Conservation Institute, 1999; Mies 1999).
- With long distance, hotter or colder days should be avoided as it can cause extreme stress due to transport exhaustion and withholding of water. Avoiding transportation of animals for

slaughter during very hot days or cold days is important, as they utilise more energy to acclimatise to the exposure to harsh weather conditions.

- Avoid transporting animals for slaughter when the weather fluctuates 10 F between high and low ambient daily temperature during 24-72 h prior to slaughter. It is also important to take care of shorn sheep during transport of very hot and cold days.

Lairage

- 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.
- Cattle should not be allowed to become wet during cold weather, but should be housed under shelter during lairage prior to slaughter.
- Lairage time should be as short as possible for well managed with loading and unloading animals, as it may increase pre-slaughter stress.
- A minimum lairage time of 2 hours is practiced in Canada, as extended lairage times have been associated with increased incidence of dark cutting. As Canadian abattoirs do not have extensive facilities for feeding and holding cattle, exposes a load of cattle to unloading and loading and transported to a nearby feedlot and rescheduling for slaughter can produce 30 to 40% dark cutters (Heather Bruce, personal communication).
- Animals undergone prolonged fasting due to long lairage can increase dark cutting as it may impair muscular glycogen content and therefore feed and water have to be provided to restore muscle energy sources. Recovery from stress by allowing access to feed for at least 48 h (Warriss et al., 1984). Lairage times less than that appear to have negative impact and increased ultimate pH and DFD incidence (Gallo et al., 2003).
- Provision 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 the incidence of dark cutting.
- Allow additional time to recover from fear and fatigue after long transports. Lairage longer than 24 hours seems to exert a detrimental effect on dark cutting frequency. Resting of cattle at the abattoir, particularly after long haul transport, may be beneficial to decreasing post mortem muscle pH. It was found that resting steers 4 or 2 days, respectively, after long haul transport reduced post mortem muscle pH relative to 2 and 1 days, respectively.
- Mixing of unfamiliar animals has to be avoided during lairage, as it promotes fear, fighting and competitive behaviour.
- High stocking density should be avoided as it can cause injury, bruise, restlessness, tiring due to inadequate space for relaxing. Adequate spacing should be allowed if animals are kept longer time in lairage.
- Mixing animals of different sex, specially bulls. The occurrence of dark cutting can arise due to stress associated with mounting and aggressiveness. The installation of electrify overhead wire

grid can effectively reduce mounting behaviour, with positive effect on muscle glycogen and frequency of dark cutting. Use of overhead electric fence/grid above the animals to prevent mounting was effective in reducing the incidence of DFD in bulls by about 57% (Kenny & Tarrant, 1987; Bartos et al., 1988). The authors also investigated the effect of darkness and combination of electric fence and darkness. There was no effect for darkness alone, but the combination of darkness and electric fence was 100% effective in eliminating DFD incidences.

10.0 Stunning and slaughter protocols impacting on dark cutting

One of the most critical events in meat production is pre-slaughter handling of livestock (Cockram & Corley, 1991). There is a likely correlation between effective slaughterhouse design and technology for increased animal welfare and occupational safety, because calm animals are easier and safer to work with than stressed, anxious animals. For instance, loud slaughterhouses cause animals to easily become nervous, creating a dangerous workplace environment, especially for the handlers of large animals such as cattle. Currently, different technologies (e.g. ventilation equipment in lairage facilities; indirect lighting; noise reducers; blinders; limited use of electric goads; and wide passageways for animals to walk side-by-side for as long as possible) are used in slaughterhouses to reduce the stress in animals in order to prevent dark cutting (Grandin, 1980; Smulders & Algers, 2009). Plants and equipment for restraining, stunning or slaughtering animals also play an important role in inducing stress and dark cutting, therefore they need to be designed and constructed to achieve rapid and effective stunning or slaughtering. Brown et al. (1990) studied the effect of plant size (small = number of slaughtered animals< 50 per day vs large = number of slaughtered animals > 100 per day) on the incidence of dark cutting in eight slaughter plants on the incidence of dark cutting beef in the United Kingdom. The authors found a significant effect of plant size with a higher incidence of dark cutting in large plants (4.6%) compared to small plants (2.0%), which was attributed to the pre-slaughter treatment (short rest period, long transportation and mixing in holding pens) to which the animals were subjected to achieve high output in large plants.

Stunning is employed prior to sticking to fulfil the animal welfare requirement of humane slaughtering where animals become unconscious so that they can be stuck and bled without causing pain or distress (Gregory, 1998). Improving animal welfare at slaughterhouses and controlling the stunning and slaughter protocols can reduce the incidence of dark cutting meat. High animal welfare standards reduce physical injuries to animals and prevent the release of stress hormones in the animal, which can have a damaging effect on meat quality (Anil & Lambooij, 2009).

Stunning is a legal requirement in many of the meat producing countries, but some countries do not use pre-slaughter stunning due to religious reasons. Mechanical (captive bolt pistol, percussion stunner or free bullet), electrical or CO_2 gas stunning methods are used in animal slaughtering (Farouk, 2013). For stunning of cattle, mechanical stunning techniques with free-bullet firearms, electrical and percussive captive bolt stunning have been used effectively and recognized legally acceptable in most countries (Van Logtestijn & Romme, 1981; Lambooy & Spanjaard, 1982; Önenç & Kaya, 2004). Captive bolt pistols are noisy and can frighten the animals. Also there is a high cost associated with the cost of the blanks and the damage caused by brain haemorrhages making the brains unacceptable for consumption. These undesirable aspects of captive bolt stunning led to the rapid development of electrical stunning as an alternative stunning method. Electrical stunning is the most common stunning method for sheep (Henckel et al., 1998). Much attention has been devoted to the physiological (Kallweit et al., 1989), behavioural reactions (Ewbank et al., 1992), brain function (Daly et al., 1988; Lambooy & Spanjaard, 1981) and blood flow (Anil et al., 1995) of sheep and cattle with stunning, but few comparative studies have examined the impact of stunning methods on meat quality (Velarde et al., 2003; Önenç & Kaya, 2004) of poultry and pigs. Electrical and percussive captive bolt stunning of Friesian young bulls resulted in higher muscle glycogen concentrations than for muscle from non-stunned animals (Önenç & Kaya, 2004), and therefore may be less likely to result in dark cutting meat. It is worth mentioning that consumers in that study scored the acceptability of meat from stunned groups higher than that of non-stunned animals.

High epinephrine concentrations in plasma of heifers as a result of short-term stress was found to increase the shear force without an increase in muscle pH or change in lean meat colour (Warner et al., 2007a,b; Gruber, 2009). The significance of this release is not well established in beef and sheep meat, but it has been shown to have considerable impact on the quality of pork and poultry (Sensky et al., 1996; Ertbjerg et al., 1999; de Fremery, 1966; Sayre, 1970). This may be due to the rapid clearing of these compounds from the animal body with fast sticking, which is practiced in the meat processing plants. While an increased pH as a result of epinephrine release is expected to improve the activity of proteases and increase tenderization rates, some reports indicate slight toughening of meat from lambs injected with epinephrine five minutes before slaughter (Pearson et al., 1973) or stressed immediately before slaughter. The bioavailability of calcium ions in muscles is compromised significantly with epinephrine injection because calcium ions are translocated from plasma to adipose tissue (Moseley & Axford, 1973), which consequently may reduce calpain activity and concomitant proteolysis. More recently, it has been reported that injection of lambs with epinephrine five minutes prior to slaughter did not affect the shear force of the *semimembranosus* (Bond et al., 2004), but injection at 24h prior to slaughter reduced the shear force of *Longissimus* and *semimembranosus* (Warner et al., 2006). Moderate stress through shackling and hoisting of calves or sheep reduced the tenderness of meat (Westervelt et al., 1976). Therefore, the toughening effect of epinephrine appears to be dependent on release time and possibly the level of stress, given the known curvilinear relationship between pH and toughness. Additional research is needed to confirm the conditions that give rise to this increase in shear force for different species, with an emphasis on age-related effects. It is worth mentioning that the glycolytic potential of beef LL muscle (2 x [glycogen + glucose + glucose-6-phosphate] + lactate) also had a curvilinear relationship with ultimate pH (Wulf et al., 2002).

Electrical stimulation is a standard practice used in the meat industry to avoid muscle cold shortening that has several positive effects on meat quality (Strydom & Frylinck, 2014; Wiklund et al., 2001). Electrical stimulation has no effect on muscle colour, ultimate pH, shear force or any of the sensory characteristics of muscle fibre tenderness, overall tenderness, connective tissue amount, juiciness, flavour desirability or overall palatability of dark cutting meat (Dutson et al., 1982). However Fjelkner-Modig & Ruderus (1983) found that dark cutting meat tested by sensory evaluation at 1 day after slaughter, whether electrically stimulated or not, was significantly more tender than meat with an ultimate pH at or below 5.8. This difference disappeared for electrically stimulated samples after 5 days of storage and for non-stimulated samples after 14 days of storage. The authors reported a tendency for electrically stimulated dark cutting meat to be less tough than non-stimulated dark cutting meat as early as the day after slaughter, but the difference was only significant after 14 days of ageing. Recently, McPhail et al. (2014) investigated the ultimate pH of 3 muscles (*rectus femoris*, *longissimus* and *infraspinatus*) from a total of 1614 carcasses. The authors found that electrically stimulated carcasses had an increased incidence of dark cutting. No relationship has been noted between post-slaughter processing plant practices and the occurrence of dark cutting, and so whether post slaughter practices contribute to the incidence of dark cutting is unknown.

10.1 Post-slaughter interventions

10.1.1 Use of chemical compounds

Use of lactic acid

Because the obvious difference between normal pH meat and DFD meat is the lack of lactate in the muscle and the increased pH of the dark cutting meat, several studies have investigated the effects of organic acids, especially lactic acid, to reduce meat pH. Lactic acid (LA) enhancement through multi-needle injection followed by vacuum tumbling was found to alleviate some of the negative aspects of DFD meat, but the efficacy of the treatment is dependent on the concentration of LA and the level of enhancement. Apple et al. (2011) examined the level of enhancement (105% or 112%) and the LA concentration (0.15, 0.35 and 0.5%) on 7-d aged DFD beef LL muscle (pH = 6.70- 6.78). Only enhancement with LA at 0.5% concentration at a 112% enhancement level was successful in producing meat with similar tenderness and colour (fresh and cooked) characteristics similar to normal LL samples. Sensory evaluation of the enhanced samples indicated that while the colour and texture of LA enhanced DFD meat were similar to normal meat, the enhanced DFD samples had significantly increased sour, bitter, and soapy flavour notes and decreased roasted and cooked beef flavour compared to normal meat samples. With LA enhancement of DFD meat, it appears that "more is less", with a 0.5% LA concentration added at 10% of the meat weight improving the colour of cooked DFD beef better than higher concentrations of LA (Sawyer et al., 2007). The best effect of enhancement was found with 0.5% LA with 0.5% NaCl, which was similar in colour to normal meat. The authors reported that 0.25% LA was the best concentration within the range 0.25, 0.50, 0.75 and 1.00% LA enhancement of high pH (= 6.56) meat (Sawyer et al., 2009). The authors reported significantly higher lipid oxidation in DFD samples treated with LA at a concentration ≥ 0.5% compared with untreated DFD and normal meats. While LA has been shown to improve the colour stability of normal fresh meat (Kim et al., 2006), high levels of LA (> 0.75%) solutions imparted a darkening effect on the colour of DFD meat (Sawyer et al., 2007; 2009). Ramanathan et al. (2013) reported an increase in mitochondrial OCR upon stimulation with lactate, which consequently would deplete oxygen and cause dominance of the purple dark colour deoxymyoglobin. Kim et al. (2006) suggested that NADH , the limiting cofactor for NADH metmyoglobin reductase, was regenerated upon the addition of lactate to meat. Indeed recent studies indicated increased metmyoglobin reducing activity as a result of lactate addition in model system at pH of 5.6 (Mancini & Ramanathan, 2014).

Use of other organic acids

Lowering meat pH can confer several other benefits to meat and has been a subject of interest for long time due to the safety benefits achieved by low pH environment. Several organic acids (e.g. acetic, citric, formic, and lactic) demonstrated effective control of microbial growth and increased the stability and shelf life of meat (Bell et al., 1986; Gill & Newton, 1981 & 1982). Several studies have shown that enhancement with organic acids swells the meat fibres and activates cathepsins, leading to increased protein degradation (Berge et al., 2001), and consequently increased meat tenderness. Lactic acid is effective in tenderizing meat cuts with high levels of connective tissues (Wendham & Locker, 1976; Medyński et al., 2000) and pre-rigor injection with LA activates lysosomal enzymes in beef and increases the solubilisation of collagen (Ertbjerg et al., 1999; Berge et al., 2001; Aktaş et al., 2003). These biochemical changes may be useful for intermediate pH meat that usually has tougher texture.

Use of ionic solutions and other chemicals

- The use of polyphosphates (found in acidic and alkaline forms) can be useful in improving the pH of DFD meat without affecting its desirable functionality (Young et al., 2005).
- The persistent pinking of DFD meat is a problem for its marketability. The use of food-grade oxidant and browning agents to enhance the browning of cooked DFD meat (pH = 6.6) was investigated by Moiseev & Cornforth (1999) to reduce the undesirable pink colour in beef patties. Lactic acid effectively reduced the dark colour of the raw meat and persistent pink appearance in cooked patties made from DFD beef. However, the authors reported an increase in off-flavour in the treated samples. The use of calcium peroxide (0.3%) promoted myoglobin denaturation and accelerated the browning of cooked beef patties, but green discolouration and off flavour formation were noted. The use of caramel colour improved the colour of cooked beef patties and increased the oxidation of myoglobin, but led to very dark colour formation.
- The effects of chemical compounds on the quality of meat have been reviewed by Bekhit et al. (2013) and the following section summarizes some of the relevant information. Several formulations containing various ratios of salt, polyphosphate salts, sugars and carbohydrates have been used at experimental and commercial levels to enhance meat eating qualities. Several sodium salts (e. g., sodium chloride, sodium pyruvate, sodium lactate, sodium acetate, sodium ascorbate, sodium citrate and sodium bicarbonate) have been investigated for meat enhancement. Potassium lactate, potassium chloride and ammonium hydroxide seem to offer the advantage of improving the tenderness without adding sodium or affecting the sensory attributes. Lactate salts (sodium and potassium) have been used in beef tenderizing solutions at a level of 2.5–3%. The use of polyphosphates is regulated and the maximum level is 0.5%. Trends toward less sodium intake and the saltiness desired (depending on personal and cultural factors) will limit the level of inclusion and the use of different salts (e.g. potassium). Several functions have been assigned to salts in enhanced meats, such as flavour development, improving the water-holding capacity, increasing ionic strength, and solubilisation of myofibrillar proteins. All these factors will promote protein modification including effects on the endogenous enzymes.
- Sodium chloride has been used at various concentrations (0.5–6% solution). Generally, the addition of salt increases the negative charge on proteins above their isoelectric point, which will increase the electrostatic repulsion forces between myofibrillar proteins. This in turn allows more side groups to be available for interactions with water, removing some of the structural constraints to retain water (lattice swelling). This will lead to a better ability to bind water and increase the water-holding capacity. Salt injection/ marination (0.6 M) has a tenderizing effect which matches that obtained with $CaCl_2$ after ageing. Salt produced about

20% of the tenderization achieved with $CaCl_2$, and a higher tenderization rate early post-mortem has been reported. There is general agreement regarding the tenderizing effects of phosphates and sodium chloride used with other ingredients in several meat cuts. The phosphate type does not play an important role, but the level of pumping seems to play an important role in the sensory assessment. Sensory panellists normally find injected meat to have increased myofibrillar tenderness and less connective tissue present. This effect can be a result of the ability of salt to solubilize myofibril proteins. However, calpains may also be activated leading to higher proteolysis. The maximum water holding capacity can be obtained with 0.8–1.0 M (4.6–5.8%) salt, although best functionality is achieved at 0.4–0.6 M. A synergistic effect between salts and phosphates exist and the salt requirements to improve the water-holding capacity can be halved by the addition of 10 mM tetrasodium pyrophosphate. Sodium chloride and polyphosphates accelerate the degradation rates of titin and troponin-T as well as the appearance of 95 kDa and 30 kDa degradation products, which leads to higher tenderization rates. The effects are thought to be due to an increased pH owing to the high buffering capacity of polyphosphates. Sodium chloride, unlike calcium chloride, does not cause a bitter taste in meat, but there are some conflicting results on the impact of enhancements with sodium chloride and polyphosphates on meat flavour. An increase in beef flavour was found but the literature also reported a decrease or no change in beef flavour. Off-flavours have also been reported in meat injected with high salt concentrations. Salt and polyphosphates decreased redness in meat and the effect paralleled an increase in concentration. The impact of sodium chloride and phosphate enhancement is different among different muscles and this might explain, in part, some of the reported contradictory outcomes.

- The literature describes several techniques (marination, tumbling, injection and infusion) for the introduction of the compounds in the meat, but in practice injection and tumbling have been adopted (possibly because of the familiarity with these methods, which are commonly used for processed meat products and small goods, and the capability of handling a large volume output). The purpose of the injection step is to accelerate the penetration rate and generate a uniform distribution of the infused compounds. The injection sometimes causes localized effects and the addition of tumbling ensures a more uniform distribution of the compounds within the meat cut. The compounds used in meat enhancement do not have the same mechanism of action or the same impact on meat quality. It would therefore be appropriate to discuss the effects separately for each compound. Several benefits can be gained from meat enhancement with the most obvious being the ability to modify the textural attributes (tenderness and juiciness). It has also been suggested that meat enhancement might have a protective effect by maintaining the desirable palatability even when meat is overcooked. The use of Rinse & Chill™ technique is common in several countries, including Australia. The technique is based on infusing carcasses after bleeding with a solution containing 2% salt and sugar to a level of 10% of the live animal weight. A lighter colour and no change in pH have been reported (Anonymous, 2003). While a decrease in myoglobin concentration is expected as a result of the infusion process, the impact of the process on metmyoglobin reducing activity, the colour stability and the suitability for DFD meat remain to be evaluated.

10.1.2 Packaging systems

Packaging systems used for the sale of meat play an important role in dictating the oxygenation rate of meat and consequently the colour of meat. The use of modified atmosphere packaging (1 atm pressure consist of 20% CO_2:80% O_2) and vacuum skin packaging have been shown to improve the blooming and stability of the colour of DFD meat. López-Campos et al. (2013) reported very similar blooming of ribeye and striploins from cattle with pH range of 5.65-6.0 and 6.0-6.4 to corresponding normal meat samples with pH range of 5.4-5.65 under modified atmosphere packaging and vacuum skin packaging. The colour stability of DFD was better under modified atmosphere packaging compared to normal meat , with discolouration in normal meat occurring after 11 days whereas it occurred after 13 and 21 days in 5.65-6.0 and 6.0-6.4 meat groups, respectively. The study of López-Campos et al. (2014a) provided evidence for potential improvement in colour stability in DFD meat using modified atmosphere packaging, but the impact of the longer colour shelf life on the microbial status in the meat was not reported and it will be crucial for practical use of the technology.

Slaughter plants or stunning point

- Forceful handling in slaughter plant may play a key role in increasing dark cutting prevalence, therefore it must be minimised or avoided if possible.
- Poor or improper design of slaughter facilities. Shadows, fences, floor type or slick floor can cause welfare problems.
- Application of stunning and handling equipment. Use of electrical prods or sticks may cause unnecessary stress to animals and agitation and jumping on other animals and internal blood flow. Workers have to be remain calm, avoid sudden jerky motions and noise, and minimize electric prods usage.
- Distraction which affects smooth animal movement. For example, cattle will move easily through a curved race compared to a straight one. The most frequent distractions impede animal movement found in Canadian slaughterhouses surveyed were light problems (animals tend to move from a darker to an illuminate place, flash lights), air blowing towards animals, objects and noises along movement area.
- Lack of employee training and supervision. Aggressive action of stockperson and improper stunning and bleeding can cause bruising, pain and elevated cortisol levels that can increase stress and lead to a depletion of muscle glycogen. The most common mistake made by slaughterhouse employer is that they try to move too many animals at the same time and it was suggested that forcing pens should not be filled more than three-quarters full.

11. Quality aspects of dark cutting beef/sheep (including manufacturing beef)

When the glycogen reserve is reduced prior to slaughter, little or no lactate can be produced resulting in a high ultimate pH. Variations in pH affect protein hydration and water holding capacity of the meat and several biochemical processes, which could affect the sensory properties (tenderness, colour, flavour and juiciness), keeping quality of the meat as well as the technical aspects associated with handling and functional use. Dark-cutting results in reduced carcass value because of reduced quality grade (USDA-AMS-MNS, 1989; 1997). The handling and preparation of dark cut meat at retail level is more difficult compared with normal meat due to its sticky characteristics (Sornay et al., 1981). The abnormal characteristics of dark cutting meat (dry firm and dark, DFD meat) compared to normal meat makes it more difficult to keep and to use as table meat cuts. The dark colour of DFD meat is not appealing to consumers as they associate DFD meat with meat from older cattle, and of poor flavour and keeping quality. As mentioned earlier, the quality attributes of meat (pH, colour, texture, flavour and water holding capacity) and the microbiological stability of the meat are all affected by the occurrence of dark cutting (Tarrant, 1989).

11.1 Tenderness

Tenderness is one of the most important meat quality attributes and is the limiting factor in the eating quality of meat (Bekhit, 2013; Bekhit et al., 2014). Modern consumers place great emphasis on meat flavour (Behrends et al., 2005a, 2005b; Bekhit, 2013), but this quality attribute can be easily manipulated by the use of additives during preparation and various cooking styles whereas meat toughness is difficult to alter by consumers (Bekhit et al., 2014). The relation between ultimate pH and tenderness varies depending on the pH. However, there is some confusion regarding the impact of pH on meat tenderness due to the various pH limits for considering a muscle or carcass to be dark cutting. In New Zealand, Australia, South Korea and many places a pH > 5.7 is used as the cut off for dark cut, but not in North America.

Dark cutting meat with a high ultimate pH (> 6.2) is usually similar or more tender than normal meat with an ultimate pH of about 5.5 (Bouton et al., 1973; Jeremiah & Gibson, 1991; Watanabe et al., 1996; Lahucky et al., 1998). In the pH range of 5.8-6.2 less tender meat is generated (Dransfield, 1981; Purchas, 1990; Jeremiah et al., 1991; Watanabe et al., 1996; Wulf et al., 1996), which was attributed to low titin and nebulin degradation rates (Watanabe, & Devine, 1996). These results have been recently confirmed in dark cutting meat from young bulls, where meat with intermediate pH was tougher than meat with > 6.1 or normal meat (Holdstock et al., 2014). Fjelkner-Modig & Rudėrus (1983) reported that electrical stimulation can improve the tenderness of DFD compared to non-stimulated DFD at 1 day post-mortem, but differences were not significant after aging, although Dutson et al. (1982) did not find any such effect of electrical stimulation in DFD muscle at 48 hrs post-mortem. Ultrastructural changes, i.e. severe contractions and complete disorganization of the tissue, were observed in electrically stimulated DFD meat (Fabiansson et al., 1985), but these changes were not reflected in the shear force measurements. The main mechanism involved in meat tenderness is assigned to proteases, with calpains having been generally accepted as the major proteases involved in post-mortem meat tenderness (Kemp et al., 2010), and their activities are regulated by the early post mortem pH and temperature of the meat. Yu and Lee (1986) reported increased calpain activity as the pH increased to neutral pH. Pulford et al. (2009) suggested the involvement of heat shock proteins in regulating the proteolytic activity at various pH levels. The authors hypothesised that at pH >6.3, where little protein denaturation can take place, the muscle proteases (e.g. calpains and caspases) have better access to desmin and troponin T because heat shock proteins are not needed to protect these structural proteins and consequently the tenderization of the meat can occur quickly. At intermediate ultimate pH (5.7 < pHu < 6.3) in response to protein denaturation, heat shock proteins shield myofibrillar proteins to protect them and in doing so, the enzymatic cleavage by proteases is hindered. At pH <5.7, high amounts of protein denaturation take place to the point that myofibrillar will be exposed and susceptible to enzymatic degradation. The same group reported that the degradation of titin, filamin and desmin was lower in bull *M. longissimus thoracis* with intermediate ultimate pH (5.8-6.19) compared to high (pH ≥ 6.2) and normal ((pH ≤ 5.79) pH samples (Lomiwes et al., 2013). Katsaras & Peetz (1990) studied the structural changes of dark cut meat heated at a temperature range from 55° to 95° C after 7 days of storage at 2°C using a scanning electron microscope. At temperatures ≥ 65°C, dark cut meat showed definite transverse breaks in the L-bands and Z-line connections and signs of granulation or breakdown of connective tissues. The fragmentation of myofibrils was greater in dark cutting meat than in normal meat and increased as heating temperature increased. It is worth noting that the toughness related to pH can vary among different muscles. Minimum tenderness in mutton was found in *M. biceps femoris, M. semitendinosus* and *M. longissimus thoracis* at pH 5.6, 5.9 and 6.1 respectively (Bouton & Shorthose, 1969). In bull *M. semitendinosus*, a linear increase in tenderness with the increase in pH range 5.5-6.8 was observed (Dransfield, 1981). Consumer perception of tenderness of DFD and normal meat was marginally in the favour of DFD under various cooking conditions, i.e. grilling, frying, roasting and casserole (Dransfield, 1981). However, other studies did not find any difference in the tenderness between DFD and normal beef (Viljoen et al., 2002). More insights on the tenderness-pH relationship were provided by the excellent work reported by Grayson et al. (2014). The authors examined four levels of DFD *Longissimus lumborum* at pH 6.1, 6.4, 6.6 and 6.9 compared to normal beef (< 5.7). LL samples with pH 6.1 and 6.4 had higher shear force (251 and 225 N, respectively) than LL samples with pH 6.6, 6.9 and normal meat (mean shear force were 190, 165 and 174 N, respectively). The rating of meat tenderness by a trained sensory panel was different from the instrumental shear force (6.5, 6.1, 5.2, 4.9 and 4.7, for meat with pH 6.9, 6.6, 6.4, normal meat and 6.1, respectively). The normal meat samples had longer sarcomere length and 10% more desmin proteolysis compared to the DFD samples. Purchase (1990) suggested that sarcomere length of myofibrils may be a contributing factor for the tenderness found in normal pH meat since the sarcomere length increases with the decrease in pH below 6.2. However, the sarcomere length of LL at pH 6.9 and 6.6 is shorter than LL with pH 6.1 (Grayson et al., 2014), which cannot explain the opposite relationship between tenderness and pH. These results suggest that many of the normal meat tenderization mechanisms are unable to explain the tenderizing effects in severe DFD meats. This distinction of pH within DFD meat seems to explain some of mixed outcome found in literature in relation to the tenderness of DFD meat.

A 15 min exercise period before sheep were slaughtered increased the ultimate pH of LL, SM and *Semipinalis capitis* to > 5.9 and low voltage ES (unipolar rectangular waveform of 146.5 mA, frequency 13.5 Hz and pulse width of 8 ms) did not affect the ultimate pH (Warner et al., 2005). Sensory evaluation of LL indicated better scores for tenderness and juiciness in samples from stressed animals whereas applying ES significantly decreased the tenderness and likening of Gluteus medius (GM). The shear force of LL was decreased as a result of the acute stress, but not that of the SM muscle. These results indicate a positive effect for acute stress on the texture of the LL, but not of other muscles. This is in contradiction to other reports on lamb, which reported a toughening effect of exercise stress (Geesink et al., 2001). Warner et al. (2005) suggested that the selective negative impact of ES on GM was due to heat shortening as a result of heat generated in GM that increased the temperature to near the critical 25°C threshold (Thompson et al., 2005; Warner et al., 2005).

11.2 Juiciness

Roasted DFD was found to be either significantly less juicy or juicier than roasted normal meat depending on the meat source (Dransfield 1981). For example, roasted steer *M. longissimus thoracis* was scored higher for juiciness as the pH increased, but not bull *M. longissimus thoracis.* Differences in the water holding capacity of bulls and steers can vary depending on farm and slaughter management (e.g. use of implants and level of stress) (Jeremiah et al., 1988). The majority of the available studies however found no differences in the consumers' acceptability of juiciness of meat from high pH and normal meat (MacDougall et al., 1979; Wulf et al., 2002; Viljoen et al., 2002). Electrical stimulation of dark cutting carcasses reduced the perception of juiciness (Jeremiah et al., 1997). Juiciness ratings for DFD meat decreased with the reduction in pH (5.9, 5.7, 5.4, and 5.2 for pH 6.9, 6.6, 6.4 and 6.1, respectively) and juiciness of LL of pH of 6.1 was similar to that found in normal meat (Grayson et al., 2014).

11.3 Flavour

Dransfield (1981) reported that consumers found DFD beef to have less intense beef flavour compared to normal meat and consumers disliked the meat flavour as the pH increased (an average of 3 points on 14 point scale for pH range 5.5 to 7.0). On the other hand, Viljoen et al. (2002) found that female consumers only preferred the flavour of fried normal meat over DFD meat. A trained sensory panel found DFD LL beef to be less sour compared to normal LL beef, but meat with a pH of 6.9 or 6.6 was ranked higher for musty flavour (Grayson et al., 2014). The fat flavour scores were increased by the increase in pH (1.4, 1.6, 1.9, 2.0 and 2.2 for normal, 6.1, 6.4, 6.6 and 6.9 pH meat samples, respectively) (Grayson et al., 2014). This is in contrast to the findings of Jeremiah et al. (1991) of lower fatty aroma in roasted DFD meat compared to normal pH roasts.

Yancey et al. (2005) studied the effect of pH on the flavour profile of several beef muscles and found DFD meat to have less beef flavour intensity and less brown-roasted flavour than normal pH meat cuts. The beef flavour in DFD meat was decreased with the increase in aging time, but not in normal meat (Yancey et al., 2005). This is due to low carbohydrates in high pH meat and a lack of reactants required for the Maillard reaction. Vacuum aging of DFD sirloin steaks for 21 or 35 d increased metallic, rancid and sour flavours. Generally, DFD meat was frequently reported to have off-flavours compared to normal meat (Dransfield, 1981; Katsaras & Peetz, 1990; Viljoen et al. 2002). These off-flavours or abnormal tastes in DFD meat were likely to be as a result of very high ATP and CP metabolism early post-mortem (Fabiansson et al., 1985) and the production of inosine monophosphate degradation products (i.e, inosine and hypoxanthine) in higher concentrations compared to normal meat (Potthast & Hamm, 1976). The off flavour might be the result of higher lipid oxidation as well. Yancey et al. (2005) reported significantly higher rancid flavour in high pH meat compared with normal meat samples. Other significant flavours that have been found in high pH meat are; serumy/bloody, sour, metallic that appear to increase with the increase in aging time (Jeremiah et al., 1988; Warren & Kastber, 1992; Yancey et al., 2005) and peanutty and bitterness (Wulf et al., 2002). Bulls' meat generates high levels of off flavours compared to steers meat (Jeremiah et al., 1988). Jeremiah et al. (1988) showed that dusty and metallic flavours can be eliminated by reducing pre-slaughter stress and the use of Zeranol-6 and Zeranol-3. The study of Jeremiah et al. (1988) also indicated that minimum stress can increase bloody flavour and reduce sweet flavour in steers. The difference in flavour desirability for normal and DFD meats was 0.64 and 0.54 on an 8 points sensory scale carried out by expert panel.

11.4 Colour

The importance of fresh normal meat colour stems from consumers' perception at the point of sale. Consumers prefer a cherry red colour as they associate that colour with meat freshness. In DFD meat, the colour can vary from dark purple colour (Bekhit, 2004) to almost black (Vilijoen et al., 2002), which is unacceptable to consumers. Another problem with DFD meat is its persistent pink colour (Mendenhall, 1989; Trout, 1989; Moiseev & Cornforth, 1999; Sawyer et al., 2008; Apple et al., 2011), which is problematic in cooked DFD meat. High pH protects myoglobin from denaturation during cooking (Trout, 1989), leading to the pink colour in cooked DFD meat.

High pH meat can permit the growth of *Alteromonas putrefaciens* that produce hydrogen sulphide and cause the green pigment sulfmyoglobin formation and green discolouration of meat (Nichol et al. 1970). The formation is a maximum when the pH is above 6.0 and the oxygen tension is 1%.

High pH_u increases the water-holding capacity and oxygen consumption rate (OCR) (Ledward, 1985). High pH meat also appears dark due to the maintained swelling of the fibres, which results in closer spacing of the fibrils causing decreased light scattering and, as a consequence, the meat is more translucent. Under such conditions, the light penetrates deeper into the meat and hence, the Mb absorbs the light more strongly, causing the dark colour of the meat. The increased water holding capacity and a more closed myofibril structure resist deep O_2 diffusion which, in addition to the high OCR and lower oxidation rate, makes deoxymyoglobin the dominating pigment (Bekhit & Faustman, 2005). Hence, this meat has a dark purple colour. Inactivation of mitochondria and decreasing the mitochondrial respiration capacity of DFD meat is important to regulating the colour of the meat as otherwise myoglobin will be deoxygenated and the muscle will remain dark. Cornforth et al. (1985) showed that mitochondrial respiratory inhibitor, rotenone, or high oxygen atmosphere can improve the colour of DFD meat.

A significant correlation between beef colour and pH_u has been reported (Cridge et al., 1994; Purchas et al., 1999). The ultimate pH was found to correlate to a* and b* components of Commission International de l`Eclairage (CIE)-L*a*b* system, but not with L* in beef (Warner, 1989). The rate of discolouration of beef *M. longissimus thoracis*, in terms of L*a*b*, decreased with the increase of pH_u (Purchas et al., 1999). The rate of OxyMb formation at pH 8 can be 3-4 times that at pH 5-7 (Schwimmer, 1981). Lowering the pH from 7.4 to 5.7 during the muscle to meat conversion process tends to denature some of the active reducing enzymes and cause a decrease in OCR (Schwimmer, 1981). In support of this view, Asghar et al.(1990) found that the presence of mitochondria and microsomes in sarcoplasmic extracts influenced the proportion of OxyMb and MetMb at high pH (OxyMb was increased and MetMb was decreased), but at a low pH their presence had little effect. They suggested that the observation was due to the effect of pH on the enzymes available in these organelles.

Warner et al. (2005) studied the effect of acute exercise on the lamb colour and reported that the exercise increased the pH_u of the *Longissimus thoracis* (LL) and *Semimembranosus* (SM) muscles, which was associated with reductions in *L**, *a** and *b** values, particularly in the SM. Similar observation were found by other researchers (Murray, 1989; Park et al., 2007). The level of stress has been reported to play an important role in inducing abnormal meat colours in beef, with low levels of stress leading to pale colour development while an extensive stress level can lead to DFD meat (Hedrick, 1981).

The colour variation in muscles from dark cut classified carcasses is much higher than normal meat classified carcasses (NCBA, 2000; Bass et al., 2008) and the colour of the LL muscle was recommended not to be used as an indication for the colour of the remaining muscles of the carcass (Bass et al., 2008).

Recently, Hughes et al. (2014) examined the factors which could contribute to beef meat colour at grading in Australian beef processing plants. Beef carcasses at seven Australian processing plants were assessed at grading for the colour of the *M. longissimus thoracis*. Statistical modelling was used to determine the animal, carcass and processing factors contributing to the meat colour score at carcass grading. As the time from slaughter to grading increased, the meat was found to have a light meat colour. Muscles graded at 14 h post slaughter had a dark-cutting incidence (MSA colour score >3) of 8%, whereas those graded at 31 h post slaughter had an incidence of 3%. The authors postulated that this was due to structural shrinkage of the muscle fibres and myofibrils, which increases the light scattering properties of the muscle. Also, a high temperature at pH 6 was associated with a light colour at grading and an optimal temperature around 25°C was recommended to minimise meat colour issues such as dark-cutting and heat-induced toughening. Less than 30% of carcasses with non-compliant pH displayed a dark noncompliant meat colour score >3, indicative of an opportunity to determine the mechanism behind this pH-induced colour development and thus to reduce the incidence of noncompliance. These authors recommended that the time from slaughter to grading could be lengthened to minimise economic penalties to that particular carcass.

The quality of manufacturing meat (e.g., water holding capacity, protein solubility, oxidation of protein and lipids, cooked batter torsion stress and other mechanical properties) is negatively affected during frozen storage (Miller et al., 1980; Farouk & Swan, 1998a,b; Farouk & Wieliczko, 2003). Zhang et al. (2005) reported significantly higher sarcoplasmic protein solubility, water holding capacity, cooked batter torsion stress and strain, yield and emulsion stability in high pH meat (pH ranged 6.1-6.79) compared to normal pH meat (pH ranged 5.40- 5.79). These properties were degraded over storage time, but the deterioration rate was lower in high pH meat compared to normal pH meat. The hue angle of high pH batter was lower than normal pH, indicating better colour. High pH meat offers meat processors the ability to formulate meat products with reduced salt and phosphate due to the high water capacity of high pH meat. Also, cooking of roast beef could be performed at high cooking temperatures, allowing improved food safety measures without compromising desirable attributes such as rare/medium cooking. While the solubility of myofibrillar proteins in normal pH meat is higher than that of high pH meat, it is the sarcoplasmic protein fraction that is the key for emulsion quality (Young et al., 2005). Roasted bull inside rounds (semimembranosus and adductor muscles) from high and normal pH meat indicated less purge in high pH samples compared to normal pH meat in frozen, but not chilled samples (Swan, & Boles, 2002). Increased cooking yields were obtained from high pH meat samples and, therefore, they have better yield and economically hold an advantage over normal pH meat. High pH meat offers better emulsion capacity, strain to fracture and stress to fracture attributes that normal meat will not have even if the pH of the normal meat was increased (Young et al., 2005).

12. Microbiological concerns and eating quality

The spoilage brought about by microorganisms progresses in two steps depending on the availability of preferred nutrients. The first step is the utilization of low molecular weight compounds, such as glucose, and in fresh meat this does not lead to the formation of off-odours immediately. It takes about 14 days of normal pH meat to develop off-odour under aerobic storage conditions (Patterson & Bolton, 1981; Gill & Newton, 1981-2). Under anaerobic conditions, i.e. vacuum packaging, lactic acid bacteria can produce odours that are objectionable to consumers that are often described as dairy, cheesy or sour odours (Borch et al., 1996). The 2nd step is the use of high molecular weight compounds for energy which require secretion of enzymes and several signs of deterioration appear (changes in sensory attributes).

In addition to the poor sensory properties of dark cutting meat, it has a limited shelf life (Vanderzant et al., 1983). Due to its elevated pH, DFD meat is prone to microbial spoilage more so than normal pH meat (Gill & Newton, 1979; 1981; Ferguson et al., 2001). The increasing trend of marketing meat products in vacuum packed and chilled form is especially problematic to DFD meat as it promotes the development of off-flavour and potentially green colour formation (discussed below). The change of meat's pH from normal (5.5-5.7) to high (> 6.0) does not affect the generation time of *Pseudomonas*, *Enterobacteriaceae*, *Moraxella*, *Acinetobacter* and *Flavobacterium,* but it reduces the generation time of *Aeromonas* (Gill & Newton, 1981). At the pH of normal meat the growth of *Acinetobacter* and *Altermonas putrefacien* is inhibited, where they are able to grow at high pH. Both *Enterobacter liquefaciens* and *Yersinia enterocolitica* are able to grow aerobically or anaerobically. These bacteria grow faster on DFD meat under vacuum packaging condition than on normal pH meat (Gill & Newton, 1977; 1979; 1981-1982) and they produce off-odours.

Many spoilage microorganisms (e.g. *Pseudomonads* under aerobic storage and *lactobacilli* under anaerobic bacteria) preferentially use glucose as a source of energy rather than other nutrients (e.g. amino acids, fatty acids). Only when glucose is not available will the bacteria switch to utilization of amino acids and other nutrients for energy. In doing so off-odours are generated and some species can generate H_2S that can lead to green discolouration in vacuum packed meat (Gill & Newton, 1981). During glycolysis, some residual glycogen remains in normal meat due to the decline in pH and the loss of adenosine monophosphate that inhibits enzymes involved in glycolysis (Bendall, 1973; Hamm, 1977). Consequently, incomplete exhaustion of glycogen/glucose provides a substrate for the microorganisms and thus delays the use of other nutrients and the production of off-odours. In dark cutting meat where the glucose is absent, spoilage microorganisms will use amino acids as their source of energy immediately and the spoilage odours will begin to accumulate. Therefore, the onset of spoilage is faster in dark cutting meat compared to normal pH meat. The addition of small amounts of glucose can prevent the spoilage of dark cut meat, but glucose is not able to prevent the green colour formation caused by *Alteromonas putrefaciens* (Gill & Newton, 1981). The use of citric acid can prevent the green colour formation as the preference by microorganisms for citrate is higher than that for amino acids. Fat contribution to off-odour formation is similar to that found in proteins (Patterson & Bolton, 1981), but this is not a problem for carcases where the fat surface is normally too dry for bacterial growth. The generation of off-odours becomes a problem in packaged meat where the fat is bathed in meat drip, which supports the growth of spoilage bacteria (Gill & Newton., 1981-2).

13. Biochemical causes of dark cutting

Several psycho-physical factors can trigger the fight, fright and flight (3F) animal response, leading to animal stress and the dark cutting phenomena if the animal has not recovered from stress before slaughter. Mechanisms that can cause fear and/or physical exhaustion (e.g., dog chase, transportation, walk, run, extreme hot and cold weather, feed restriction, and mixing of unfamiliar animals) can lead to depletion of muscle glycogen (Warriss et al., 1990, McVeigh & Tarrant, 1983) and therefore, less glycogen will be available for post-mortem glycolysis. Glycolysis potential ((2x [glycogen + glucose + glucose-6-phosphate] + lactate) of less than 100 µmol/g meat was found to be the threshold for DFD in beef (Wulf et al., 2002). While several factors can cause the formation of dark cutting meat, the main reason is the low muscle glycogen content at slaughter, preventing the achievement of a normal meat acidification level (pH < 5.8). Interventions that prevent antemortem muscle glycogen depletion, such as propranolol, may be useful tools to control dark cutting meat.

The response and recovery from stress are regulated by the sympathetic and parasympathetic nervous systems. The sympathetic nervous system is activated when the animals is exposed to stress leading to the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla. These catecholamines stimulate the generation of energy required for the animal's response, i.e. the 3F, by stimulating glycogenolysis and lipolysis via the effects of epinephrine and norepinephrine, respectively. This eventually depletes the muscle and liver glycogen stores and cell fatty acids as they are used for ATP generation. It was suggested that the conditions leading to DFD meat can be species-dependent with glycogenolysis being the dominant reason for DFD in cattle whereas increased stress hormones are the main reason for DFD in sheep (McVeigh & Tarrant, 1983; Tarrant, 1989)

The onset of stress can affect several blood enzymes (e.g. glycogen phosphorylase, creatine phosphokinase, lactate dehydrogenase, aspartate aminotransferase, glutamate-oxaloacetate-transaminase) (Apple et al., 1995; Mohan Raj et al., 1992; Schaefer et al., 1997), but these serum enzymes were increased only in extreme DFD cases and there was wide variation among the samples due to animal variation (Mohan Raj et al., 1992). The injection of epinephrine into heifers' muscles produced dark cut meat (Ashmore et al., 1971). The authors found no significant change in mitochondrial function, mitochondrial protein yield, specific activity of succinic dehydrogenase, phosphorylase and glycerol phosphate dehydrogenase, but injection of epinephrine resulted in a higher percentage of active phosphorylase in the muscles compared with non-treated muscles. AMP-activated protein kinase (AMPK) is involved in the regulation of the uptake and the synthesis of glycogen (Liang et al., 2013) and thus these enzymes can be an important factor in understanding the events causing depletion of recovery of glycogen.

Factors that can affect heat transfer during chilling or the temperature of post-mortem meat can regulate these enzymes activities. Several studies reported trends that suggested the involvement of such factors in meat ultimate pH. For example, McGilchrist et al. (2012) examined data of 204,072 beef carcasses and found that low incidence of dark cutting meat in carcasses with large eye muscle area and increased subcutaneous fat depth. The authors rightly suggested that these phenotypic measurements are reflection of better nutrition and probably increased glycogen reserves in the muscles. However, these measurements also suggest that the cooling rate is expected to be lower and this will affect the activities of the above mentioned enzymes. Recently

McPhail et al. (2014) examined data from 1614 lamb carcasses and found low ultimate pH compliance in electrically stimulated carcasses and low incidence of dark cutting in heavy carcasses. Collectively, these results suggest the involvement of the rate of cooling post-mortem in regulation of enzymes involved in glycogenosis, but this has yet to be established.

14. Model systems available for studying dark cutting

Reliable animal models to study dark cutting are extremely important to understanding the reasons that lead to animal stress, to the investigation of the mechanisms involved, and to the evaluation of solutions to prevent the occurrence of dark-cutting meat (Apple et al., 2006). Given the wide range of environmental, nutritional and psychological reasons that can lead to stress in animals, several model systems have been used to study stress in animals and to predict the incidence of dark cutting. Emotional stressors (restraint, isolation, or mixing of unfamiliar animals) as well as physical stress (walking long distance, use of dogs to chase the animals, hot season transportation, or forcing sheep to swim) can induce stress and dark-cutting syndrome (Warriss et al., 1984; Bond et al., 2004; Petersen, 1983; Tarrant, 1989; Warriss, 1990). Thus these methods individually or in combination have been used in studies on dark-cutting meat. Some of the methods used to induce stress such as swimming, dog-chase or human chase were criticised for being relatively unnatural to animals and thus not considered helpful for distinguishing the mechanism of stress (i.e. physical vs emotional) (Apple et al., 2006).

Mixing of unfamiliar animals can upset the group's hierarchy and cause increased behavioural actions that can cause stress. Warriss et al. (1984) reported increased creatine phosphokinase activity and free fatty acids in plasma of unfamiliar young Friesian bulls mixed overnight. Also the treatment led to decreased glycogen in liver and muscle. Animals from mixed mobs slaughtered after mixing directly produced DFD in the *M. longissimus thoracis*.

Treadmill exercise was suggested as a controlled method to estimate precise physical stress (Apple et al., 1994; 1995; 2006). Treadmill exercise caused a significant increase in serum cortisol concentrations of sheep (Apple et al., 1995) and in steers (Apple et al., 2006). The level of serum cortisol increase in steers was dependent on the extent of exercise with a significant effect only evident after 6 minutes of exercise and the level of change was increased with an increase in the treadmill speed. This model was shown to cause changes in blood parameters (plasma glucose, cortisol and lactate), but the model was not effective in influencing the meat quality of sheep and steers compared with control animals (Apple et al., 1994; 2006).

Restraint and isolation of lambs for 6 hours increased plasma epinephrine and cortisol concentrations. The *longissimus* muscle from stressed lambs had lower glycogen and lactate concentrations and produced meat with ultimate pH > 6.0 (Apple et al., 1995). A similar effect was found with Holstein steer calves (Apple et al., 2005a). Restraint and isolation for 6, 4, and 2 hours resulted in 75%, 25% and 25% dark-cutting incidence in the calves, respectively.

Chemically induced dark firm dry meat

Beta adrenergic stimulant, such as isoproterenol, can reduce muscle glycogen. Lister & Spencer (1983) found that infusion of isoproterenol alone in sheep decreased muscle glycogen, but was not sufficient to cause dark-cutting. Co-administration with pyrazole carboxylic acid (an inhibitor of lipolysis) resulted in DFD in all treated animals, indicating that a complete exhaustion of muscle glycogen requires blocking the metabolism of other energy sources.

Subcutaneous injection of epinephrine mimics the conditions leading to DFD meat and sheep injection with epinephrine (8.8 mg/100 kg) stimulate muscle glycogen metabolism and produced DFD meat (Ashmore et al., 1973). Injection of adrenaline (LaCourt & Tarrant, 1985; McVeigh & Tarrant, 1982) caused increase in the pH of selected muscles.

15. Measurement techniques or methodologies available to identify potential dark cutters

The identification of animals and carcases with the potential to exhibit dark cutting meat has been considered by many researchers with the idea of establishing reliable measurement techniques. Several methods have been developed based on the assessment of the stress of live animals, including animal behaviour and blood parameters, and techniques applied to sampling of blood pre-slaughter, soon after stunning for plasma lactate, cortisol, adrenalin concentrations and meat post slaughtering for ultimate pH measurement, colour measurement, muscle glycogen or lactate concentrations.

15.1 Pre-slaughter techniques

15.1.1 Body temperature

Animal stress as the major cause of DFD was intensively investigated to determine the level of stress that causes DFD meat in various animal species and breeds, the environmental and management factors leading to dark-cutting, and to investigate the biochemical markers involved in the process. The ultimate goal of all these studies was to have the ability to predict the conditions leading to dark-cutting meat and to design corrective measures. The heat emission by the animal body or the temperature of the animal has been used as indicators of stress events. It has been well known that the animal temperature and the respiration rate of cattle are affected by heat stress (Shrode et al., 1960) and that rectal temperature of the animals is correlated to environment temperature. Warriss et al. (2006) stated that the stimulation of the sympatho-adrenal axis by stress can cause redistribution of the blood and alter the body temperature, which can be useful to assess the level of stress that the animals exposed to. Recently, Delić et al. (2014) reported an average 3°C increase (range in various experiments was 2.6-3.7°C) in young bulls due to transportation and pre-slaughter handling, but the range of increase within individual animals was much larger (0.9 – 4.7°C). The relationship between body temperature of sheep due to stress and post-mortem muscle glycogen was investigated by Pighin et al. (2014). The authors found a significant negative correlation between the changes in the core body temperature and *M. Longissimus* glycogen levels at slaughter. Unfortunately, in that study the researchers were not able to establish the relationship between the muscle's ultimate pH and the changes in the body temperature because the carcases had normal pH. Infrared thermography is a non-invasive technique that can be useful in evaluating the temperature of the animals without the need to be in contact with the animal. Schaefer et al. (1988) investigated the use of infrared thermography to evaluate the impact of transportation and fasting on cattle. The authors found that animals exposed to high stress treatment (transported long distances and fasted for long periods) had increased skin temperature and thus the technique may be useful tool in evaluating the stress level in animals pre-slaughter.

15.1.2 Temperament rating

Temperament rating can be based on flight speed (also known as exit velocity), pen scoring, and/or chute scoring. Temperament ratings based on a numerical scale (chute score) were assessed during weighing and handling of cattle at a feedlot (Voisinet et al., 1997). Temperament rating was effective in detecting dark-cutting carcases as well as those on the borderline and had a significant effect on the shear force of the meat at 2 weeks post mortem. Calm animals had lower shear force compared to agitated and violent animals. This result was confirmed in several other studies (Behrends et al., 2009; King et al., 2006). Recently, Coombes et al. (2014) evaluated the effect of animal temperament measured using flight speed (FS) on plasma lactate, muscle glycogen and lactate concentrations at slaughter plus ultimate pH in 648 lot finished cattle of mixed breed and sex but no relation between the flight speed and the incidence of dark cutting was found.

15.1.3 Biopsies Technique

This technique was streamlined for the use in investigation of stressed animals by Tarrant and co-workers (Tarrant, & McVeigh, 1979; Tarrant & LaCourt, 1984; Lacourt, & Tarrant, 1985). In this procedure, the animal is restrained in a chute and the muscle of interest is sampled (sample size is 100-200 mg) by a percutaneous needle biopsy technique as described by Bergstrom (1962) after applying local anaesthesia. The sampling site can be used for repeated sampling at various times to monitor the impact of treatments on muscle biochemistry. Through this method Tarrant and co-works reported vital information on glycogen depletion during stress and the recovery rates in different muscle fibres as well as examining the efficacy of different interventions to counter the development of dark-cutting (Kenny, & Tarrant, 1987; LaCourt & Tarrant, 1985; McVeigh & Tarrant, 1982; McVeigh, & Tarrant, 1983; Tarrant, 1989; Tarrant, & McVeigh, 1979; Tarrant & LaCourt, 1984).

15.2 Post-slaughter techniques

15.2.1 pH and glycogen measurements

Measurement of ultimate pH is the most common technique for establishing the incidence of DFD meat. The pH standards can vary considerably (Viljoen, 2000) and the time of ultimate pH measurement will depend on the species, which may be problematic for production and trade. Meat with pH ≤ 5.8 is considered normal everywhere. At pH values of ≥5.8 the keeping quality and the dark colour become important issues in discrimination against DFD meat (Tarrant, 1981). The use of pH meters can be problematic as it requires constant calibration and the readings may be become less sensitive as deposition of fat and proteins accumulate on the pH probe. Also, the contact of the probe with many carcases is hygienically not preferable. Modified methods for an early predication of ultimate pH that involve pH measurement by accelerating the glycolysis rate by freeze/thaw cycle (Davey & Graafhuis, 1981) and electrical stimulation of small sample (Braathen, 1984) have been reported.

Young et al. (2004) described a patented method (WO 00112844) that measures the glycolytic potential of pre-rigor meat as a mean of predicting the muscle's ultimate pH. The method is based on the enzymatic conversion of muscle glycogen, using an *Aspergillus amyloglucosidase*, to glucose, which then can be measured using a glucometer. The method requires homogenization of the sample and only 5 minutes of incubation after which the measurements can be done directly on the homogenate. This method offers the potential of early prediction of dark cutting meat early post-mortem, but the method is labour intensive and thus may be useful only for testing suspect animals.

15.2.2 Near Infrared Spectroscopy

Prieto et al. (2015) investigated the potential use of visible and near infrared spectroscopy (Vis–NIRS) to separate normal and dark cut beef. The authors reported 95% correct classification of both meat groups. Similarly Reis & Rosenvold (2014) used Vis-NIRS at 20 to 40 min post-mortem and reported >90% successful segregation between carcasses with ultimate pH of > 5.8 and <5.8. Therefore, this technology appears to be promising and have the potential to be integrated in an on-line system for earlier prediction of dark-cutting meat early in the post-mortem period.

15.2.3 Colour and optical properties measurements

Colour grading is commonly carried by the meat industry in the US also in Australia (MSA cattle and AUS-MEAT chiller assessed carcasses), but it is not common in New Zealand. A colour grading system assigns colour scores based on a colour chart, which is subjective and dependent on the grader experience and interpretation of the colour chart. Several colorimeters are commercially available (e.g. HunterLab, Minolta) for the measurement of the meat surface colour, but this is not easily done at grading. The use of a fibre optic probe to measure the scatter coefficient of raw meat was reported 3 decades ago (MacDougall & Jones, 1981) with effective identification of 80% of samples with a pH value of ≥6. A similar approach was reported by Swatland (1990) and Chrystall (1987) to measure the optical properties of meat using fibre optic probe in a system termed (Colormet) and Hennessy probe, respectively. Swatland (1990) reported a superior segregating power of the Colormet over both subjective and objective evaluations for dark-cutting. A Colormet L* cut-off point was very useful for the identification of dark-cutting meat since lightness was shown to be the most important parameter related to dark cutting and it was suggested that the Colormet could be used in commercial operations with a standardized post mortem grading time (Gariepy et al., 1994).

Methods of assessing the stress status of cattle at the time of slaughter could be of value in investigations into the specific causes of dark cutting beef. The measurement of concentrations of various plasma or serum constituents in blood samples collected during exsanguination has been used in several species to assist in investigations into the handling of animals prior to slaughter and its effect on meat quality.

15.3 Analyses applicable to pre- and post-slaughter

Blood glucose, plasma epinephrine, norepinephrine, and cortisol concentrations, and serum concentrations of insulin, glucose, lactate, and free fatty acids can be evaluated in blood samples drawn from live animals and upon slaughtering. Most of these analyses can be performed using commercial kits (Apple et al., 1995). It should be noted that changes in blood profile may not indicate a DFD case (Warriss et al., 1984; Rulofson et al., 1988). The concentrations of the muscle glycogen and lactate can be carried out on samples taken from live animals and after slaughter (Tarrant, & McVeigh, 1979; Tarrant & LaCourt, 1984; Lacourt, & Tarrant, 1985). New methods that have not been investigated in animals yet and show promise are the detection of glucocorticoid hormones, especially cortisol, in saliva (de Weerth et al., 2003; Trilck et al., 2005; Perogamvros et al., 2010) and hair (Raul et al., 2004; Stalder et al., 2013). These methods may hold some benefits as they are not invasive and can have minimum effect on the animal even agitated ones. An added benefit from the hair analysis of glucocorticoids analysis, is that it provides a history of stress events that can be useful indicator for the time of stress occurrence.

Other enzymatic systems found to increase with mixing stress include; creatine phosphokinase (CPK), lactate dehydrogenase and glutamate oxaloacetate transaminase (Warriss et al., 1984).

16. Chilling regimes, packaging type influence and retail shelf life consequences

Vacuum-packed normal fresh chilled meat can be stable at 0°C for about 12 weeks or more. High pH meat can grow *Janthinobacterium lividum* that causes the production of hydrogen sulphide. Apart from the off flavour that can be caused by H_2S, green pigment formation, sulfmyoglobin, can be high. Nichol et al. (1970) found that the microorganisms produced H_2S at pH≥6.0 and low oxygen tension only.

Some details of packaging systems on reducing dark cutting have been reported in section 7.1.2.

17. Nutritional value and purchasing power of Australian beef and lamb meat from a Chinese perspective

There are 56 Chinese ethnic groups and habitual dietary intake is very different among these groups. The Han Chinese ethnic group is the largest accounting for more than 80% of Chinese population. Han Chinese cuisine is based on Soy products and pork. However, the consumption of beef and lamb is increasing in China due to socioeconomic status changes (increased salaries and purchase power) and more exposure of Australian foods in Chinese markets. China's per capita consumption of beef is second after pork.

Ten years ago, China started importing beef and lamb from Australia and these meats are now recognized. Chinese domestic beef consumption has increased substantially since 1980, although beef consumption growth remains low compared to pork and poultry consumption growth over the same period. USDA domestic data indicate per capita beef consumption in China was 9 pounds in 2010. In total beef consumption, China ranks fourth globally behind the United States, the European Union, and Brazil (USDA - FAO, 2010). With a population of 1.3 billion, a relatively small change in per capita consumption in China has an enormous impact on aggregate beef consumption. A one percent increase in per capita beef consumption would increase total beef consumption in China by 63,339 tons (USDA - FAO, 2010).

Chinese recognize that Australian beef is high quality, low in fat, delicate, tender and juicy. Food safety is a concern and Chinese consumers believe that Australian beef is safe given the production systems and standard welfare procedures. Australian beef accounted for 56 percent of China's beef imports in 2014. The agribusiness research department of the Australia and New Zealand Banking Group Ltd has forecast that the total value of Australian beef shipped to China will reach 130 billion $AUS by 2030.

China is a significant importer of Australian lamb and mutton, however, to be competitive and attractive internationally, the quality of the exported lamb and mutton should be consistent and of good keeping quality. It should be stressed that the Asian population are familiar with eating meat from aged animals such as meat from hoggets and sheep, where the product is called mutton. Sectors of the population from different demographic areas prefer eating meat from animals from 12 to 24 months of age, since these older animals have high muscle fat (juicy) and iron (nutritious) contents and produce bigger carcasses and therefore larger cuts (more attractive).

With a growing population and the increase in socio-economic status of the Asian population (example, China and India), it is expected that there will be increasing demand for red meat as a source of animal protein. It is predicted that the consumption of red meat from cattle and sheep will increase approximately 200% by 2050 while pork will increase by 158% (Alexandratos and Bruinsma, 2012). In this context global animal production will face significant challenges over the coming decades in order to meet the increasing demand for protein specially from developing countries due to the rise in income of middle class population.

Sustainable production, efficient use of natural resources, and improvement of animal welfare all need to share the focus when attempting to meet this increased demand. Hence, animal nutrition and standard welfare procedures can be employed to play a major role in achieving these requirements. Consequential benefits related to improved productivity and carcass traits associated with welfare and animal nutrition includes improvements in muscle composition and meat quality mainly colour, juiciness and tenderness. Focusing along the supply chain at on farm and off farm activities to reduce the prevalence of dark cutting and other eating quality aspects of fresh and packaged meat will attract Australian beef and lamb in Asian markets. It should be noted that Chinese consumers perceive quality characteristics of beef quite differently than western consumers, but as incomes continue to increase, consumers tend to place more emphasis on food safety, product branding, packaging, and quality (Anderson et al., 2011) and thus issues like colour will be important. It would be interesting to survey the Chinese consumers to see what they really look for whether the colour or flavor/taste or texture or the nutritional value of lamb and beef from Australia.

18. Consumer thresholds

Consumers have indicated that colour is an important aspect of meat selection and purchase, with it being seen as an indicator of freshness and eating quality (Savell et al., 1989; Forbes et al., 1974). The interesting paradox is that despite the importance of colour this trait is not strongly related to eating quality and as stressed by Troy and Kerry (2010) consumer eating satisfaction will depend on traits like tenderness and juiciness. In spite of this however, as a consequence of the perception that dark meat is considered unacceptable, the question is at what point does the meat become unacceptable and how can this be measured? Further to this meat, specifically beef can also be deemed too light and pale under certain conditions (Hopkins et al., 2014), but this is not the focus of this review.

If pH which impacts on the development of meat colour (Renerre, 1990), could be used as the proxy for a consumer threshold of acceptability this would simplify grading schemes. However, the recent study by Hughes et al. (2014) reiterated that the relationship between meat colour and pH is not perfect. This study used data from 1512 beef carcases and found, when m. *longissimus* pH was 5.8 that only 28% of these received meat colour scores greater than 3, based on the AUS-MEAT colour chips (Anonymous, 2005), and were therefore classified as unacceptable to consumers. This means that large proportions of beef carcases produce visually acceptable meat despite having a high pH. Those with a colour score above 3 increased to 74% at pH 6.0 (Hughes et al., 2014). Similarly, Purchas (1989) described a curvilinear relationship between ultimate pH and muscle redness with a spread around the line of best fit. This implied variation was also evident in the relationship between colour scores from a panel and redness measured with a Minolta Chroma Meter.

During the development of the Australian meat grading system, 'Meat Standards Australia' (MSA), 86,000 consumers were used for the sensory testing of more than 603,000 beef samples and 90,000 sheep meat samples and data regarding consumer acceptability of a number of different meat quality traits was collected (Anonymous, 2014). Meat colour and pH are two of the traits used by the MSA beef model. The acceptable range for beef was defined as a visual colour score between 1B and 3 and the pH must be below 5.70 (Anonymous, 2012a). For sheep meat graded in the MSA scheme no pH or colour thresholds are applied Anonymous (2012b).

18.1 Colour thresholds

Objective methods for measuring meat colour are based on the measurement of the spectral profile of reflected light from the surface of the meat. While these approaches provide quantifiable numbers, it is conceded that these objective methods need to be calibrated against consumer acceptance levels (Hunt et al., 1991). A review of literature will show that there are only limited studies that have examined the relationship between objective and subjective assessments of meat colour in either lamb and beef meat.

In terms of fresh colour, Hopkins (1996) reported an acceptability threshold for lamb m. *longissimus* fresh colour (using a Minolta Chroma Meter with an open cone) of 34-35 for L^*, which is the CIE colour space coordinates which denotes lightness (Table 2). This was higher than the report by Hopkins (1995) which used retailers and wholesalers responses to assess lamb meat colour. But, in general these results support the outcomes from Khliji et al. (2010), which suggested that when L^* values are equal to or exceed 34, on average consumers will consider the lamb meat colour acceptable. However the absolute values for L^* differ depending on whether the Minolta Chroma Meter cone is closed or open – as L^* values are lower when measured using an open cone which can explain 71% of the variation observed in L^* measured using a closed cone (Kerr & Hopkins, 2010). Furthermore, it has been shown that calibration tile colour (Hopkins et al., 2005) and colour instrumentation aperture size selection (Holman et al., 2015) can also influence absolute CIE measurements. This means that any subjective acceptability thresholds must be defined by the parameters under which the matching objective measures were obtained.

In the literature there are few studies which consider the CIE a^* value when assessing consumer acceptability of lamb meat colour. This should be addressed as, Hopkins (1996) describes *a** values as being useful when assessing consumer colour acceptability. This observation is shared by Khliji et al. (2010) as they found that *a** values explained more of the variation in consumer scores than *L** values. This same study found that when *a** values are equal to or exceed 9.5, on average consumers will consider lamb meat colour acceptable. The corresponding value for *L** is 34. When the threshold values for *L** and *a** are calculated using a 95% confidence interval for when a randomly selected consumer would score a randomly selected lamb meat sample acceptable, they were found to be much higher – with these thresholds set at 14.5 (*a**) and 44 (*L**) respectively (Khliji et al. 2010). This illustrates the large variability between individuals perception of fresh meat colour and reaffirms the findings reported by Hopkins (1996) that a low correlation (r = 0.18) exists between *L** values and colour acceptability.

Table 2. Summary of findings from studies that have examined the relationship between objectively measured colour traits and consumer acceptance of colour

Study	Method	Country	Species	Number of consumers	Consumer threshold
Strange et al., 1974	Beckman D.U. model 2400 spectrophometer	USA	Beef	10 member panel	Below 35 (*L**) considered unacceptably dark
Shorthose et al., 1988	Minolta Chroma Meter CR-200	Aust.	Beef	192 households	Consumers found values below 30 (*L**) as equally unacceptable, with also no change in acceptability above 40 (*L**)
Hopkins, 1995	Minolta chromameter CR-300	Aust.	Lamb	5 retailers & 5 wholesalers	32 (*L**)
Hopkins, 1996	Minolta Chroma Meter CR-300	Aust.	Lamb	195 consumers	34-35 (*L**)
Khliji et at., 2010	Minolta Chroma Meter CR-400 & Hunter Minilab	Aust.	Lamb	541 consumers	*L** between 14.5-44 *a** between 3.3-14.8

Within a beef context, Shorthose et al. (1988) reported a non-linear relationship between *L** values and consumers ratings of colour appeal upon a seven point scale (1 = much too pale to 7 = much too dark). This showed little decrease in colour acceptability when the *L** values declined below 30, and if a score of 3.5 (the mean) was considered an ideal colour then increasing *L** values up to 40 proved optimal. The low correlation (r = 0.32) between *L** values and consumer scores does reflect, to some degree, the varying conditions (domestic settings) under which consumers evaluated the meat colour. The wide variation around the line of best fit reflects significant variation in the relationship. Strange et al. (1974) found comparable results when investigating beef, showing that an *L** value of 35 as critical to consumer acceptability. The application of this latter study is, however, limited as it used an assessment panel of only 10 individuals.

Although earlier studies, such as those aforementioned (Strange et al. 1974; Shorthose et al. 1988), reported low correlations between the objectively measured beef colour and consumer acceptance scores, the more recent study by Khliji et al. (2010) on sheep meat was able determine confidence intervals of consumer acceptance. Further work needs to be carried out in this area, looking at the reflectance correlations when measured with other colorimeters, such as the HunterLab Miniscan, and consumer acceptance. In this context, replicating the experiment described by Khliji et al. (2010) to study beef is an apparent need given the paucity of data for beef. Determining dark cutting consumer thresholds is not straight forward because consumers frequently have widely differing perceptions of acceptable meat colour which is often arduous to quantify and is prone to subjective errors (Khliji et al., 2010). But, as demonstrated, there is value in understanding this relationship between objective and subject colour evaluation.

Visual scoring of meat colour is frequently used in research and in grading schemes, such as AUS-MEAT. This is usually used when testing consumer preferences and is valuable to identify large differences in meat colour. Results of studies carried out in this manner are summarised in Table 3.

Table 3 Summary of findings from studies that have examined the relationship between visually measured colour traits and consumer acceptance of colour

Study	Method	Country	Species	Number of consumers	Results
Forbes at al., 1974	- Visual colour assessment by meat physiologist - Graded A or B according to Canadian grading system. B was seen to be darker, but consumers were also asked to describe colour.	Canada	Beef	170	Respondents preferred “red” or “bright red” lean to a “dark” or “deep red” colour. (No values given in paper)
Viljoen at al., 2002	-Visual colour assessment by trained assessor (1 to 8 colour score with 7 & 8 considered dark cutting) -preference test	South Africa	Beef	64	67% preferred ‘normal’ meat colour to 33% who preferred the dark cutting steaks.
Killinger et al., 2004	-Visual colour assessment by trained assessors -Dark red or bright cherry red steaks -preference test	USA	Beef	220	Study 1: 67.6% preferred bright cherry red to 32.4% preferred dark red. Study 2: 76.5% preferred bright cherry red to 23.5% preferred dark red.

Apparent in these studies is that a portion of consumers (~one third) preferred darker meat, linking this characteristic to ageing and product tenderness (Forbes at al., 1974; Killinger et al., 2004). This perception is somewhat accurate as red meat does darken and dry while on commercial display, and will generally become more tender. Further there is new evidence which demonstrates that beef eating quality can be independent of colour at the time of grading (Hughes et al. 2014). Clearly there is a need to derive the relationship between objectively measured beef colour and consumer colour acceptability as this will enable studies where objective measurement data is obtained to be given consumer context. Related to this there is also a paucity of information which links colour chips as used by AUS-MEAT to the objective measure of red meat colour. Furthermore, the loose coupling between pH and colour calls for improved ways of ranking meat given discounting for one trait based on the other suggests a loss of potential revenue.

19.0 Conclusions

- The colour of normal meat is bright red or cherry red or pinkish to bright red and most of the population prefer this. Dark cutting can be described as a dark colour on the surface of meat when examined by sight within the visible spectrum. Consumers tend to reject "dark cutting" meat due to the perception that this meat is from old animals, underfed animals, stressed animals, poorly handled animals, sick animals or spoiled and results in meat with an undesirable flavour, that is less tender and has a poor shelf life.
- Dark cutting meat is often defined in terms of muscle pH and is therefore a reflection of glycogen levels and as such, meat with a high pH is often referred to as dark firm and dry. Such meat has several unique characteristics such as high water holding capacity, firm texture and the surface feels dry because the water is tightly held within the muscle.
- Dark cutting condition can be caused by incidences associated with many factors such as nutrition level, age of animal, duration of transport to the abattoir, climate, hormone implant strategies, lairage times before slaughter, processing systems at slaughter plants, animal temperament and aggressiveness, stock person attitude, and to some extent subclinical diseases that cause glycogen depletion in muscle.
- There is no universal system to define dark cutting across countries. Some classify meat based on colour whereas others use the pH of meat. However it should be noted that DFD meat is optimal for the production of manufacturing meat, but this results in a significant reduction in profitability.
- Several strategies that can be implemented during the handling of sheep and cattle on the production property, as they leave farms, feedlots and saleyards, as they are transported to processing plants, and as they are held at lairage, managed at lairage (mixing and stock person handling) and slaughtered at the stunning point to reduce the incidence of dark cutting.
- Several physical (exhaustion, weight loss, bruise), emotional (anxiety, fear, pain), physiological (rise in temperature, heartbeat, blood flow) factors individually or in combination lead to biochemical changes (increase in cortisol, adrenaline, glycogen depletion, reduced lactic acid formation) that cause the occurrence of "dark cutting". Dark cutting is conceivably a by-product of animal management practices and entirely preventable if the predisposing conditions are well understood and steps are taken to reduce the events that enhance the incidence.
- Implantation of aggressive growth promotants or application of combinations of implants, especially in cattle, leads to an increase in the prevalence of "dark cutting" as well as leading to tougher meat. The application of growth promotants increases protein deposition while reducing the fat and glycogen content in the muscle, which might be one of the reasons why animals have less energy reserves that can be used during stress events. Dark cutting meat from carcasses showing pH ranges 5.8-6.2 at 24 h post slaughter is reported to produce

tougher meat than that from normal carcasses showing pH ranges of 5.4-5.7 at 24 h post slaughter.

- Dark cutting may also be linked to the feed used on-farm as well as off-farm. It has been reported that pasture finished cattle are more prone to develop "dark cutting" carcasses, often with a higher ultimate pH, compared to feedlot cattle. Poor pasture quality during summer and the beginning of autumn and heat stress may explain this variability because poor pasture quality does not allow sufficient glycogen synthesis and deposition, which is important for the maintenance of animals to cope with events that deplete muscle glycogen levels. Feeding high energy diets or energy supplements such as grains and by-products during the finishing period of cattle and sheep may be useful to reduce the occurrence of "dark cutting" meat.

- Apart from diets, the effectiveness of providing electrolytes, high sugar supplements or a mixture of electrolytes, sugars and amino acids during pre-slaughter and/or lairage has been recommended by many researchers to reduce the incidence of "dark cutting".

- Of the many factors known to contribute to dark cutting, environment may be the most difficult to control. Daily temperature fluctuations greater than 5.6 °C increase the incidence of "dark cutting". High daily temperatures may also increase the frequency of "dark cutting", as temperatures above 35 °C were found to increase "dark cutting" in heifers. Seasonal variability in incidence of "dark cutting" has been widely reported. Most of the studies report a higher pH 24 h post mortem and/or darker colour meat in warm seasons rather than in cold seasons in both beef and sheep.

- Stress factors prior to slaughter are thought to be main factors for the production of "dark cutting", which depletes the muscle glycogen concentration. Transport distance and lairage time are some stressors causing physical, physiological and emotional stress affecting the welfare of animals and meat quality. Not all researchers have reported that longer lairage time reduces the incidence of "dark cutting". Some studies recommend at least 24 h lairage time whereas others recommend more than 48 h to recover from long journeys in order to prevent the adverse effect of transportation stress on muscle glycogen depletion in live animals, lactic acid production in post mortem muscle and pH of meat. This lack of clarity makes recommendations difficult.

- Animals also differ in their stress levels depending on previous experiences and familiarity with people. Stock person attitude and handling of animals is an important area to reduce the incidence of "dark cutting" or low quality meat. Stockperson attitude can change the behaviour of animals and the response of animals to stress events vary with individual animal temperament.

- Dark cutting carcasses is classified using different methodologies such as standard colour chips, instrumental colour, using pH as a threshold, or by identifying the levels of glycogen, lactate or glycolytic potential of muscle. Among all, use of colour at 24 h post mortem or pH of carcases at 24 h post-mortem are the most commonly used. Using pH as a guide for dark cutting meat is more problematical as animals from different nutritional backgrounds can have different carcass fatness levels, which in turn can influence the temperature decline and indirectly ultimate pH of meat (fat insulation and more glycogen). Currently the "dark cutting" grading system is not applicable across countries because the definition varies from country to country.

20.0 Recommendations

1. A clear definition of a pH point that predicts the incidence of dark cutting needs to be identified. It is important to ensure that this common pH threshold can be applicable to beef and sheep carcasses around the world at a particular post mortem time point.

2. Development of a colour threshold using common equipment or colour chip grading system for dark cutting is recommended for a universal grading system. This needs to be related to visually assessed consumer grades so that there could be one instrumentally objective measurement system developed for grading of “dark cutting” around the world. It is recommended that Australia adopts a threshold that is matched to visual tolerance levels by actual consumers. This approach has been applied to lamb, but not in beef.

3. Any measurement taken before 24 hour from the kill time will inflate the number of carcasses with high pH meat. It also depends on which threshold is used to define dark cutting because when a pH of 5.7 is used (as per MSA) , this overestimates the incidence of the dark cutting problem (based on visual assessment) as opposed to pH thresholds of 5.9 or 6.2. So results must be given context and the impact of grading carcases prior to 24 h post-mortem is fully elucidated so guidelines for assessment can be established.

4. The literature clearly indicates that on farm nutrition, transport, lairage and animal response to stockperson attitude can all contribute to dark cutting individually or in combination. The impact of the combination of two or more contributing factors on dark cutting meat has not been identified from research conducted in the past. Therefore a multi factorial level experiment covering major contributing factors needs to be designed. This will isolate the magnitude of each contributing factor and allow development of a model for predicting the extent of dark cutting in the Australian meat industry.

5. Stress associated with poor temperament animals can cause a depletion of glycogen in the muscle and biochemical changes such as increased adrenal flow and cortisol concentration causing dark cutting in cattle and sheep. Early identification of stressed animals through development of non-invasive technologies (hair or saliva) could be a useful strategy to identify stressed animals before slaughter so as to lower the incidence of dark cutting. The technology needs to be confirmed in experimental work and then high throughput technology can be tested for practical use at industrial scale.

21.0 Directions for specific future research and development

Study area 1

Stress associated with poor temperament animals or body pro- & anti-oxidant defence systems can cause change in blood energy metabolites (biochemical changes such as increased adrenal flow, cortisol concentration, glucose metabolism) and depletion of glycogen in the muscle causing dark cutting in cattle and sheep. Early identification of stressed animals or animals that can produce dark cutters, for example at lairage stage, through development of non-invasive technologies (hair or saliva) could be a useful strategy to handle them separately before slaughter so as to lower the incidence of dark cutting. The technology needs to be confirmed in experimental work and then high throughput technology can be tested for practical use at industrial scale.

Study area 2

The efficacy of muscle glycogen repletion is a crucial factor in recovery from stress and reducing dark cutting meat. Investigation of various high polysaccharide diets for recovery from stress could potentially provide insights on interventions for elimination of DFD.

Study area 3

The review shows that there were few studies in sheep investigating the sensory properties of meat from carcasses graded as dark cutters. However there are no studies conducted from cattle identifying the visual sensory properties of beef from carcasses categorised as dark cutters and relating this to objective measurement of meat colour. This area needs further investigation so as to relate the consumer panel findings of eating quality to objective colour measurement of dark cutting beef.

Study area 4

Setup an experiment to measure pH of lamb and beef carcasses at critical time points that resembles the pH of meat when processors record at the plant and relating the pH to meat colour using an instrumental and colour chip measurements taken at a particular time points. This will allow the colour variation over the post-mortem period to be relating to the pH of meat and establish what is the critical time for the evaluation of colour post mortem. Currently there is no universal grading system to identify dark cutting threshold. This study will allow development of a threshold to interpret dark cutting in beef and lamb and it is important to use this finding as a threshold around the world.

Study area 5

The impact of dark cutting from the interactions of nutrition, transport length and lairage time is not very well investigated in relation to 24 h pH and colour of meat. In this context, a multifactorial experimental study is essential to understand the synergistic effect and/or the additive effect of each component on the prevalence of dark cutting carcasses. From the literature we can predict that the contribution of one component (e.g., nutrition) in conjunction with another component (eg, lairage time) to the formation of dark cutting can be substantial. Further research is needed to understand the combination of several contributing factors affecting dark cutting and to isolate which component is causing or impacting more to dark cutting.

Study area 6

With a growing population and the increase in socio-economic status of the Asian population (example, China and India), it is expected that there will be increasing demand for red meat as a source of animal protein. It is predicted that the consumption of red meat from cattle and sheep will increase approximately 200% by 2050 while pork will increase by 158%. In this context global animal production will face significant challenges over the coming decades in order to meet the increasing demand for protein especially from developing countries due to the rise in income of middle class population. It is recommended to survey the Chinese consumers to see what they really look for, whether they choose meat on the basis of colour or flavour/taste or texture or the nutritional value of lamb and beef from Australia. The impact of darkening may not be of significant importance giving the culinary style of Chinese cuisine. This is yet to be confirmed through designed research plans.

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