CHAPTER 1: INTRODUCTION

1.1 PROJECT THEME

Meat Science focusing on meat tenderness.

1.2 PROJECT TITLE

Effects of dietary beta-agonist treatment, Vitamin D_3 supplementation and electrical stimulation of carcasses on meat quality of feedlot steers.

1.3 AIMS

The aims of this project were to:

1) To determine the effect of different levels and the duration of Vitamin D_3 supplementation on the tenderness, colour, drip loss and water holding capacity of beef from feedlot cattle treated with a beta-adrenergic agonist (zilpaterol) and slaughtered under different abattoir practices.
2) To determine the effect of different levels and the duration of Vitamin D_3 supplementation on the calpain proteolytic system of beta-adrenergic agonist (zilpaterol) treated and control feedlot animals.
3) To determine the interaction between the effects of supplemented Vitamin D_3, electrical stimulation and aging on the tenderness, colour, drip loss and water holding capacity of zilpaterol treated and control feedlot animals.

1.4 MOTIVATION

Consumer choice regarding meat quality is dependent on a number of factors, the most crucial being meat tenderness. The 1995 US National Beef Quality Audit indicated that the top two quality concerns in the industry were the low overall uniformity and consistency of beef products and inadequate tenderness (Vargas, Down, Webb, Han, Morgan & Dolezal,

1999). It has therefore become a top priority to solve the problem of inconsistent meat tenderness (Koohmaraie, 1996) in order to satisfy the consumer (Dransfield, 1994). Meat tenderness is a combined function of production, harvesting, post harvest processing, value adding and cooking method used to prepare the meat for consumption by the consumer. Failure of one or more links in the chain increases the risk of a poor eating experience for the consumer (Thompson, 2002).

Many physiological factors affect meat tenderness. These can occur both pre- and post slaughter and need to be taken into account when studying meat tenderness. Most South African feedlots (75% of meat produced in South Africa) supplement with a beta-adrenergic agonist, usually zilpaterol hydrochloride, during the final weeks of finishing. Beta-adrenergic agonists improve feed efficiency and increase carcass meat yield efficiency (Dikeman, 2007). Beta-adrenergic agonists however have a negative effect on meat tenderness by increasing the activity of calpastatin, an inhibitor to the calpains. The degree of these changes depends on the species, type of muscle, the particular beta-adrenergic agonist as well as the time and duration of supplementation (Dransfield, 1994).

Some post-slaughter factors include chilling rate and electrical stimulation and their effect on *post mortem* pH and temperature ratios. The optimum scenario is a pH/temperature relationship of greater than a pH of 6 for muscle temperatures greater than 35°C, and a pH of less than 6 for muscle temperatures less than 12°C. If this optimum relationship is not adhered to then heat and cold shortening can occur, as well as increased autolysis of the calpains and a decrease in meat tenderness (Thompson, 2002). Strydom, Osler, Leeuw & Nel (1999) found that electrical stimulation could reduce aging time to reach a certain level of tenderness. This was due to electrical stimulation causing a lower pH to occur at a relatively high temperature, thereby resulting in an earlier initiation of the aging process (Dransfield, 1994).

Various attempts have been made to overcome meat tenderness problems. In recent years, supplementation of very high levels of vitamin D_3 during the final days of finishing have been investigated (Montgomery et al., 2002 & 2004), the theory being that vitamin D_3 is needed for calcium absorption in the small intestine. Higher levels of vitamin D_3 could therefore eventually lead to higher concentrations of calcium in plasma resulting in more calcium available for the calcium dependant proteinase system. Results however have been inconsistent and no local trials (using beta-adrenergic agonist supplemented animals and electrical stimulation) have been done.

Another critical link in the consumer satisfaction process is the physical appearance of meat cuts during display, with the two most important factors being the colour of red meat as well as the amount of drip loss in packaging. It is well-known that processes like electrical stimulation and *post mortem* aging may affect colour (Ledward, 1985; Ledward, Dickinson, Powell & Shorthose, 1968; Renerre, 1990) and water holding capacity (Kristensen & Purslow, 2001; Den Hertog-Meischke, Smulders, Van Logtestijn & van Knapen, 1997; Devine, 2009) and that these procedures combined with beta-agonists may have additive effects on these parameters (Geesink, Smulders, Van Laack, Van der Kolk, Wensing & Breukink, 1993).

In this study we look at the possibility of high levels of vitamin D_3 supplementation being able to counteract the negative effects of beta-adrenergic agonist supplementation on meat tenderness, as well as the efficacy of this when compared to other cheaper, non-invasive methods such as electrical stimulation. We also investigate the effect that the combination of these factors has on other meat quality traits such as colour and water holding capacity.

1.5 HYPOTHESIS

Ho: Vitamin D_3 supplementation does not significantly influence meat quality of zilpaterol supplemented feedlot steers.

Ha: Vitamin D_3 supplementation does significantly influence meat quality of zilpaterol supplemented feedlot steers.

Meat tenderness focus/ characteristics:

- Warner Bratzler shear force
- Myofibril filament length
- Sarcomere length
- Enzyme activity (µ-calpain, m-calpain, calpastatin)

Colour focus/ characteristics:

- a* (green)
- b* (yellow)
- L* (lightness)
- Chroma
- Hue

Variables in Vitamin D_3 supplementation:

- Treatment (various levels and durations)
- Electrical stimulation
- Aging

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CHAPTER 2: LITERATURE REVIEW

2.1 MEAT TENDERNESS

The concept of meat tenderness is very complex since it is dependent on many physiological factors such as connective tissue characteristics (total collagen and collagen solubility)(Morton, Bickerstaffe, Kent, Dransfield & Keely, 1999; Monin, 1998), the energy status of the muscle, which influences the extent of muscle contraction (studied by measuring pH-temperature decline, glycolysis and sarcomere length) and meat tenderisation by means of the proteolytic degradation of cyto-skeletal proteins (studied by measuring ultimate pH, myofibril fragmentation, proteolytic calpain system levels etc.). These physiological factors are influenced by genetic factors (species and breed), pre-slaughter factors such as age, gender and feeding practices and factors related to processing conditions (electrical stimulation, chilling rate and cooking).

2.1.1 Baseline tenderness

Baseline meat tenderness is that which cannot be changed by any external practices or processes. It is determined by the amount and solubility of connective tissue (Koohmaraie & Geesink, 2006) which consists primarily of perimycium, endomycium and epimycium (Harper, 1999). These connective tissues consist of collagens and these have the ability to form cross links. As an animal matures these cross links become heat-stable. The more heat-stable cross links present in a muscle the tougher the meat will be (Bailey, 1989). In the case of feedlot production, the more common form of production in South Africa, this is not really a factor as animals are slaughtered young before the cross links of collagen can become an issue (for the more tender cuts such as the *M. gluteus medius* and *M. longissimus lumborum*).

2.1.2 Conversion of muscle to meat

When an animal is slaughtered, exsanguination takes place causing a drop in blood pressure. In an attempt to maintain blood pressure, the heart starts to pump faster and peripheral vasoconstriction takes place. This causes a stoppage of nutrient supply and removal of waste products to and from the muscle, stoppage of oxygen supply to the muscle and an increase in temperature of the carcass due to failure of the temperature control mechanism. Stored oxygen is depleted as myoglobin only stores small amounts of oxygen and ATP cannot be formed. This results in the onset of anaerobic glycolysis. There are two main sources of ATP produced from anaerobic glycolysis. The first is stored glycogen which is degraded to create energy for contraction of muscles and results in the production of lactic acid with the release of hydrogen ions, which accumulate in the muscle. The second is the transfer of phosphate from creatine phosphate to ADP to yield creatine and ATP. With no blood flow to remove the lactic acid, it is stored in the muscle resulting in a drop in pH (Pösö & Puolanne, 2005). The ultimate pH of the muscle depends on the amount of glycogen and creatine phosphate reserves in the muscle at the time of exsanguination, the buffering capacity of the muscle as well as extrinsic factors such as environmental temperature and the administration of drugs pre-slaughter (Pösö & Puolanne, 2005; Lawrie, 1985). Once no more ATP can be formed, the actin-myosin complex remains locked in permanent contraction called rigor mortis at a pH of 5.4 – 5.8 (Lawrie, 1985).

2.1.3 The pH temperature relationship

There are a number of factors which can influence the process of the conversion of muscle to meat and can therefore influence tenderness. The pH temperature relationship is one such factor. Normal pH drop should be from 7.0 to 5.6- 5.7 in 6 to 8 hours *post mortem* with an ultimate pH range of 5.3- 5.7 after 24 h. The rate of pH drop *post mortem* is inversely

related to meat tenderness, with a slower fall in pH yielding more tender meat (Hwang & Thompson, 2001b). This is all however also dependent on rate of decline of temperature. Locker and Hagyard (1963) showed that muscle shortening occurs when pre-rigor muscle is held at either low or high temperatures. At low temperatures cold shortening occurs which leads to increased toughness of the meat. In order for cold shortening to occur the muscle pH has to be greater than 6.0 at a temperature below 10ºC and still have ATP available for muscle contraction (Pearson & Young, 1989). Rigor or heat shortening is caused by a combination of a high temperature with a low pH. The low pH is usually due to a rapid pH drop causing early exhaustion of proteolytic activity (Dransfield, 1994; Simmons, Cairney & Daly, 1997). Both cold and heat shortening leads to decreased tenderness and increased drip loss (Thompson, 2002). A good relationship between pH and temperature seems to be a pH of more than 6.0 at temperatures above 35ºC and a pH below 6.0 for temperatures below 12ºC (Thompson,2002).

Electrical stimulation can have an effect on the pH/temperature relationship. Electrical stimulation can prevent cold shortening by causing a faster drop in pH in cases where carcasses are chilled rapidly or hot–deboning occurs (Hwang & Thompson, 2001a). Over stimulation however can lead to heat shortening and increased autolysis of calpains with the consequence of reduced aging potential. In addition increased drip loss could occur due to protein denaturation.

2.1.4 Electrical stimulation

Electrical stimulation is used as a means of accelerating the post- slaughter fall of pH and the onset of rigor. Electrical stimulation involves passing an electric current through the carcass after slaughtering. This stimulates the muscle to contract and utilize glycogen and ATP, thereby accelerating rigor mortis and causing a rapid decline in pH within the muscle (Taylor, 1981; Taylor, Perry & Warkup, 1995; O'Neill, Troy & Mullen, 2004; Strydom, Frylinck

& Smith, 2005). When the electrical current is interrupted, there is still sufficient glycogen and ATP in the muscle to enable the carcass to relax. Due to this low energy reserve, rigor mortis begins earlier while the muscle temperature is still high (Taylor, 1981). As a result, tenderization will start earlier at the prevailing temperature (Dransfield, 1994). When rigor mortis occurs in a relaxed muscle, the sarcomere lengths are not affected, allowing the meat to retain its inherent tenderness (Potter & Hotchkiss, 1995; Kerth, Cain, Jackson, Ramsey & Miller, 1999; Monson, Sanudo, Bianchi, Alberti, Herrera & Arino, 2007). Overstimulation however, can lead to a low pH at a high temperature resulting in heat shortening and therefore a decrease in tenderness. It has been shown that a good pH-temperature relationship seems to be a pH above 6.0 at high temperatures and a pH of below 6.0 at temperatures below 12ºC (Thompson, 2002). This means that under conditions where immediate chilling of the carcass occurs, it would be beneficial to implement electrical stimulation in order to cause a rapid drop in pH, thereby avoiding the potential of cold shortening. Electrical stimulation has also been shown to provide tender meat in half the aging time of non-stimulated meat but only under conditions of slow cooling (Dransfield, Etherington & Taylor, 1992). This corresponds with an experiment conducted by Strydom, Frylinck and Smith (2005), where *M. longissimus lumborum* muscles from electrically stimulated sides were more tender than the non-stimulated muscles at 2 days aging. At 14 days however, there was no significant difference between non-stimulated and stimulated muscles. This result coincided with decreased available µ-calpain activity at 24 h *post mortem*, meaning that initial tenderness was due to increased enzyme activity which was then exhausted.

Strydom, Osler, Leeuw and Nel (1999) found that electrical stimulation could reduce the aging time needed to reach a specific level of tenderness for meat of beta-adrenergic agonist supplemented animals and although it could not improve the tenderness to the same level as the control group, electrical stimulation could significantly reduce the difference between the two after prolonged aging. This could be explained by the ability of electrical

stimulation to advance the onset of rigor releasing calcium ions which activate µ-calpain causing muscle proteolysis and therefore tenderization (Ducasting, Valin, Schollmeyer & Cross, 1985). Likewise it can be attributed to electrical stimulation reducing the level of calpastatin activity leading to a lower inhibitory effect on µ-calpain (Ducasting, Valin, Schollmeyer & Cross, 1985). In agreement, Ferguson, Jiang, Hearnshaw, Rymill and Thompson (2000) obtained results showing that electrical stimulation increased µ-calpain and m-calpain activity as well as decreasing calpastatin activity, all leading to an improvement in tenderness in *Bos indicus* breeds of cattle. This situation could be regarded as being similar to beta-adrenergic agonist supplementation as an increase in *Bos indicus* content in a breed coincides with an increase in calpastatin activity and therefore a decrease in tenderness. Uytterhaegen, Claeys & Demeyer (1992) found no difference in µ-calpain and calpastatin activity at 1 h *post mortem* but did find a significant reduction in activity for stimulated samples of both compounds at 24 h which could confer accelerated aging. Hwang & Thompson (2001b) showed that rapid pH decline alone had no effect on enzyme activity when chilling was rapid, but that when chilling was slow, it caused a decrease in µ-calpain and calpastatin activity due to autolysis.

Hwang, Devine & Hopkins (2003) postulated that there were a number of possible explanations why stimulation would increase the activity of enzymes like the calpains. One is that the calpain/calpastatin ratios are affected by some intrinsic effect associated with the rapid pH decline that results in a low pH at increased temperatures, and a second could be due to a significant increase in the levels of ‘free’ calcium, leading to activation of the calpains, particularly µ-calpain. Dransfield (1994) predicted that calpain activity would be increased by a factor of six in rapidly glycolysing muscle compared to muscle with more normal rates of glycolysis. µ-Calpain however is likely to undergo autolysis under these conditions making the interplay with temperature and the levels of free calcium important (Hwang, Devine & Hopkins, 2003). Hwang, Devine and Hopkins (2003) also speculated that electrical stimulation may also protect those muscle fibres that enter rigor soon after

stimulation and therefore avoid prolonged pre-rigor exposure to high temperatures at a low pH, maintaining optimum calpain levels. Electrical stimulation also accelerates pH decline which is mirrored by an increase in 'free' calcium, suggesting that at the same temperature, stimulated muscle will be exposed to higher levels of 'free' calcium and this could lead to increased proteolysis (Hwang, Devine & Hopkins, 2003).

In general electrical stimulation has its advantages, in that it can counteract cold shortening where carcasses are chilled quickly. It can also result in tender meat at an early stage without the prolonged aging (Strydom, Frylinck & Smith, 2005). Electrical stimulation does not however improve inherently tender meat beyond baseline tenderness (Hwang, Devine & Hopkins, 2003).

2.1.5 Calcium dependant proteinases

Tenderisation during the storage of meat occurs by proteolysis of myofibrillar and cytoskeletal proteins (Dransfield, Etherington & Taylor, 1992). The calpains (calcium-activated neutral proteinases) degrade myofibrillar and cytoskeletal proteins while lysosomal acidic proteinases (cathepsins B, D, and L) also hydrolyse myofibrils and isolated proteins (Ouali, Garrel, Obled, Deval, Valin & Penny, 1987). Calpains appear to have primary involvement at a pH of more than 6 while the activity of cathepsins seems more important at pH's lower than this. In our study we focus on the calpains and their inhibitor calpastatin.

There are two isoforms of the large subunit of calpain, namely µ–calpain and m-calpain. Both are calcium dependant. The two subunits differ in the concentration of calcium required to induce their activity, with m-calpain requiring calcium concentrations in the millimolar range, and µ–calpain in the micromolar range (Geesink & Koohmaraie, 1999).

Calpain is a protease that is abundant in the cytoplasm of the cell, and can cleave many structural proteins. Calpain is tightly regulated by many mechanisms including calcium requirements and calpastatin. Calpastatin is a polypeptide that is specific for inhibiting the proteolytic activity of the calpains and does not inhibit any other proteolytic enzyme (Goll, Thompson, Taylor, Edmunds & Cong, 1995a).

The optimum pH for calpain activity is between 7.0 and 7.5 (Ouali, 1992), but is 20-25% active at the normal end-pH-value of *post mortem* muscle, around pH 5.5 (Geesink, Smulders, Van Laack, Van der Kolk, Wensing & Breukink, 1993). Calpains do not cause bulk degradation of the sarcoplasmic proteins, but they do however specifically degrade those structures and proteins that are responsible for maintaining the assembled myofibrillar proteins in the myofibril structure. The calpains can remove Z-disks (necessary to keep adjacent sarcomeres together) and degrade titin, nebulin (probably function as a scaffold that strengthens the myofibrillar structure) tropomysin, troponin and c-protein (Zeece, Robson, Lusby & Parrish, 1986a). Specific degradation of these structures would result in the release of thin and thick filaments from the surface of the myofibril.

Calpain activity is regulated by calcium concentrations as well as calpastatins (in the living animal there is not enough calcium in cells to activate the calpain system). It appears however, that active calpains generate only a limited amount of cleavages, but this limited proteolysis may nevertheless initiate myofibrillar protein breakdown (Béchet, 1995).

As well as being regulated by calcium concentration and calpastatins, the calpain system is also dependant on pH and temperature. At higher temperatures calpains are inactive, but activity increases with a drop in temperature, but below 10ºC inactivity increases with a drop in pH (Dransfield, 1994).The activity of µ–calpain decreases very quickly *post mortem*, while the activity of the m-calpain decreases very slowly during the aging period (Ducastaing, Valin, Schollmeyer & Cross, 1985; Koohmaraie, Seideman,

Schollmeyer, Dutson & Crouse, 1987). Active m-calpain can also degrade calpastatin, resulting in a decrease of calpastatin activity (Melloni, Salamino & Sparatore, 1992). The higher the activity of calpains, the more autolysis occurs and the more tender the meat becomes (Steen, Claeys, Uytterhaegen, De Smet & Demeyer, 1997).

2.1.5.1 Prolonged aging

Aging refers to the improvement in palatability that occurs as meat is held *post mortem* beyond the normal time taken for setting and cooling to enhance tenderness (Moran & Smith, 1929). Aging can therefore be seen as the later part of tenderization and can be measured. The extent of aging is largely related to the level of calpains at 24 h *post mortem* and varies according the initial levels and their inactivation during the development of rigor (Dransfield, 1992).

Temperature also plays an important role in governing aging, as once rigor is complete, time and temperature are the only variables which can be controlled (Dransfield, 1992). Freezing stops calpain activity but does not destroy the enzymes. This means that while the meat is frozen, enzyme activity remains halted, but is regained after thawing (Dransfield, 1992). Freezing doubles the rate of aging after thawing, when compared to aging of fresh samples, and this increased rate is probably due to cellular damage.

Aging has been proven to significantly reduce shear force values in *M. longissimus lumborum* muscles (Wulf, Tatum, Green, Morgan, Golden & Smith, 1996). Geesink, Koolmees, Smulders and Van Laack (1995) also found that shear force was reduced after 14 days of aging and Mitchell, Giles, Rogers, Tan, Naidoo and Ferguson (1991) found that aging significantly increased sensory tenderness up to 10 days, but with no further improvement at 21 days aging.

Rathmann et al. (2009), Hilton et al. (2009) and Kellermeier et al. (2009) all found that prolonged aging did improve WBSF in zilpaterol supplemented animals. In all three experiments however, the control groups were still more tender than the zilpaterol supplemented groups even after 21 days of aging. This is mainly attributed to the increase in calpastatin activity caused by beta-adrenergic agonists which retards *post mortem* aging (Geesink, Smulders, Van Laack, Van der Kolk, Wensing & Breukink, 1993).

2.2 BETA-ADRENERGIC AGONISTS

2.2.1 Mode of action of beta-adrenergic agonists

Growth rate and feed efficiency are both important traits in livestock production, and because consumers demanded leaner meat, more emphasis has been placed on carcass composition with less fat and more muscle (Monson, Sanudo, Bianchi, Alberti, Herrera & Arino, 2007). The introduction of beta-adrenergic agonists (hereafter referred to as beta-agonists) represents the latest use of pharmacologically active compounds which have opened up new prospects for improving efficiency and quality of meat products (Dransfield, 1992). The beta-agonist is added to feed for the purpose of promoting protein synthesis in muscle tissues and lipolysis in adipose tissue, resulting in a reduction of carcass fat and an increase of muscle mass of the carcass (Baker, Dalrymple, Ingle & Ricks, 1984). Beta-agonists achieve this by binding to certain beta-receptors on fat and muscle cell surfaces, thereby modifying the biochemical processes of tissue growth by increasing lipolysis, decreasing lypogenesis (Dunshea, 1993; Liu & Mills, 1989; Mersmann, 1998), decreasing protein degradation (Koohmaraie & Shakelford, 1991; Wheeler & Koohmaraie, 1992) and increasing protein synthesis (Eisemann, Huntington & Ferrell, 1988; Strydom, Frylinck, Montgomery & Smith, 2009). Beta-agonists significantly influence growth by improving lean content, reducing carcass fat and overall by having a positive effect on growth rate without there being a change in feed intake (Casey, 1998a).

Beta-agonists have the properties of a neurotransmitter and of a hormone. As a neurotransmitter, beta-agonists are closely related to norepinephrine. Norepinephrine is a naturally occurring catecholamine produced by tyrosine, and together with epinephrine, are the two neurotransmitters of the sympathetic nervous system. Beta-agonists are also related in their physiological effects to epinephrine and norepinephrine (and are accordingly analogues of these hormones) in that they stimulate glycogenolysis and lipoloysis (Casey, 1998b). Beta-agonists achieve this by binding to beta-adrenergic receptors (beta-AR). These receptors are similar to those that are responsive to epinephrine and norepinephrine. There are three subtypes of the receptors namely, $beta_1$-AR, $beta_2$-AR and $beta_3$-AR. All three receptors are present on most cells, but the distribution of subtypes and proportion of each varies between tissues in a given species. The beta-AR subtype distribution also varies within a given tissue between species. The pharmacological and physiological responses of an individual cell results from the particular mixture of the three beta-AR subtypes present on that cell. Amino acid sequence also causes modification of a given beta-AR subtype. The beta-AR subtype population may change with the stage of differentiation of a cell, but there tends to be more of a particular kind of beta-AR subtype in a particular kind of cell (Mersmann, 1998). In cattle, competitive ligand binding studies suggest that there are predominantly $beta_2$-AR on skeletal muscles cells and adipocytes (Sillence & Matthews, 1994). These factors together with the use of several different agonists make the mechanisms to produce the pharmacological effects observed with oral administration of a beta-agonist complex (Mersmann, 1998).

Beta-agonists have an affinity for either $beta_1$-AR or $beta_2$-AR receptors. Their efficacies are determined by their chemical structure, the number of receptors which need to be stimulated for an effect to occur as well as on the physiological effect of stimulating the respective beta-AR. A beta-agonist with a high efficacy would achieve a high response from a relatively small number of receptors, the situation however, can also be complicated if both types of receptors are present on an organ and both could be mediating a pharmacological

effect. Desensitising of beta-AR can also occur and the rate of this is determined by the intrinsic activity of the particular type of beta-agonist (Casey, 1998b).

The mechanism of action of beta-agonists is that they bind to the receptors in such a way that the agonist receptor complex activates the G_s protein (some compounds are antagonists and therefore bind to the receptor but do not activate the G_s protein and thus block the receptor function). The α-subunit of the G_s protein then activates adenylyl cyclase which is the enzyme that produces cyclic adenosine monophosphate (cAMP). cAMP is one of the major intracellular signalling molecules. cAMP then binds to the regulatory subunit of protein kinase A to release the catalytic subunit that then phosphorylates a number of intracellular proteins. Phosphorylation activates these proteins, some of which are enzymes such as hormone sensitive lipase (the rate-limiting enzyme for adipocyte triacylglycerol degradation). The cAMP response element binding protein (CREB) is phosphorylated by protein kinase A. The CREB binds to a cAMP response element in the regulatory part of a gene and then stimulates the transcription of that gene. Phosphorylation increases the transcriptional activity of the CREB, providing the mechanism for beta-agonist mediated transcription of a number of genes in the cell (Mersmann, 1998). Phosphorylation inactivates other enzymes such as acetyl-CoA carboxylase which is the rate-limiting enzyme for long-chain fatty acid biosynthesis (Mersmann, 1989a; Liggett & Raymond, 1993).

With beta-agonists mimicking the hormones of the sympathetic nervous system it is obvious that they would have an effect on the systems major activities namely, cardiac function, blood vessel tone, gut and bronchiole muscles as well as the metabolic systems already discussed (lipolysis and glycogenolysis). All organs have both $beta_1$-AR and $beta_2$-AR but $beta_1$-AR are more predominant in the heart and $beta_2$-AR more predominant in the other organs. Beta-agonists have the following effects on the cardiac system, namely, positive inotropy (increased contractility), positive chronotropy (increased heart rate) and positive dromotropy (increased conduction velocity). Beta-agonists also cause relaxation of

smooth muscles (vasodilation) and bronchial muscles (broncholdilation) as well as release of rennin from the kidneys, insulin from the pancreas and hepatic glycogenolysis (Morris, 1997).

The zilpaterol hydrochloride molecule is a physiologically highly active beta-adrenoreceptor agonist which acts on beta$_2$-AR on skeletal muscle, smooth muscle and adipose tissue and is intended for use in beef cattle as a repartitioning agent. The molecular structure of zilpaterol hydrochloride is (±)-trans-4,5,6,7-tetrahydro-7hydroxy-6-(isopropylamino) imidazo[4,5,1-jk]-[one] benzazepin-2(1H)-one hydrochloride and its formula is $C_{14}H_{19}N_3O_2$.HCL. Tests for interactions with various pharmacological agents indicate zilpaterol hydrochloride to be non-interactive, with the possibility of even complimenting selected pharmacological agents (Casey, 1998a). Casey, Montgomery and Scheltens (1997) showed that treatment with zilpaterol hydrochloride (0.2 mg/kg) in combination with an anabolic implant (24mg oestradiol + 120mg trenbolone acetate) proved to be agonistic, improving the biological efficiency of production but without the fat reducing properties of zilpaterol hydrochloride being affected. A unique characteristic of zilpaterol is that unlike other beta-agonists which are lipophillic, zilpaterol hydrochloride is not (Casey, 1998b).

2.2.2 Effects of beta-adrenergic agonists on skeletal muscle and adipose tissue

Treatment of mammals with a beta-agonist causes an increase in the amount of RNA transcript for many skeletal muscle proteins. The result being that the mRNA for myosin light chain (Smith, Garcia & Anderson, 1989), α-actin (Koohmaraie, Shackelford, Muggli-Cockett & Stone, 1991) and calpastatin (Killefer & Koohmaraie, 1994) are increased after beta-agonist treatment with the most obvious effect being an increase in muscle mass. The other obvious effect of dietary beta-agonist supplementation is a decrease in carcass fat due to the beta-agonist stimulating adipocyte triacylglycerol degradation and inhibiting fatty acid and triacylglycerol synthesis (Mersmann, 1998).

Maritz (1996) found that zilpaterol hydrochloride had a significant effect ($P < 0.05$) on growth performance with there being an increase in both average daily gain and feed efficiency. The most prominent improvement occurred during the first few weeks of treatment. Zilpaterol hydrochloride also significantly ($P < 0.05$) reduced the proportion of carcass fat (subcutaneous, intramuscular and total dissectible fat), with this shift in carcass composition giving rise to a corresponding increase in the muscle-to-bone and muscle-to-fat ratio. This was in agreement with Morris (1997) whose steers receiving zilpaterol hydrochloride during the growth phase had significantly increased ($P < 0.01$) mean daily body weight gain and were therefore significantly heavier ($P < 0.01$) and had better feed conversions ratios ($P < 0.001$). Steers receiving an additional low dose of zilpaterol hydrochloride, during a phase where other treatment groups were in a withdrawal period, showed higher protein and lower fat content in rib analysis samples compared to the groups that were no longer being supplemented. O'Neill (2001) also found zilpaterol hydrochloride improved both average daily gain and feed efficiency although the improvement was not significant. This trial however showed no changes for percentage lean or subcutaneous fat thickness in carcasses.

Results obtained by Webb and Casey (1994) suggest that a beta-agonist may influence the proportions of fatty acids synthesised in both the subcutaneous adipose tissue and *M. longissimus lumborum* of feedlot steers, with the beta-agonist resulting in a shift towards the deposition of saturated fatty acids in the *M. longissimus lumborum*. This shift is presumed to be related to the increased rate of lipolysis resulting in a subsequent release of free fatty acids in the subcutaneous fat and muscle. In the same trial, Webb (1994) found that the lipolytic effects of zilpaterol hydrochloride may elicit insulin secretion but also blunt insulin sensitivity up to 12 h post treatment with these changes ultimately influencing the synthesis or deposition of fatty acids in ruminants.

Beta-agonists however, tend to have a negative effect on tenderness and animals supplemented with beta-agonists seem to produce tougher meat. It has been found that a potential cause for the decrease in tenderness of beta-agonist supplemented meat is due to the effect it has on the activity levels of calpains and their inhibitor, calpastatin. Kretchmar, Hathaway, Epley and Dayton (1990) reported that in lambs there was a 15% decrease in µ-calpain activity in animals fed beta-agonists compared to the control group. Not only do beta-agonists decrease µ- calpain activity, they can also increase the level of calpastatin by up to 150% (Koohmaraie & Shakelford, 1991) as well as increasing the level of m- calpains which is the less active calpain out of the two (Dransfield, 1992). This is in agreement with Strydom, Frylinck, Montgomery and Smith (2009) who found that calpastatin activity was 2.4 and 3.2 units lower on *M. longissimus* muscles from the control group compared to the two beta-agonist groups (zilpaterol and ractopamine) and with Geesink, Smulders, Van Laack, Van der Kolk, Wensing and Breukink (1993) who also found a significant increase in calpastatin in clenbuterol supplemented animals. In both cases however, there was no difference between any of the groups for µ- and m-calpain activity.

Strydom, Frylinck, Montgomery and Smith (2009) found that beta-agonists increased the WBSF values of both the *M. longissimus* and semitendinosus muscles compared to a control group at 2, 7 and 14 days aging. Schroeder, Polser, Laudert and Vogel (2003a), reported a significant negative effect on shear force tenderness for ractopamine supplemented animals, while more recent studies by Rathmann et al. (2009), Hilton et al. (2009) and Kellermeier et al. (2009) found that zilpaterol increased WBSF at 7, 14 and 21 days aging. Monson, Sanudo, Bianchi, Alberti, Herrera and Arino (2007) found that beta-agonists only caused a small increase in shear force values. These animals were however also supplemented with dexamethasone which could have caused an increase in soluble collagen. O'Neill, Casey and Webb (2010) concluded however that with the implementation of electrical stimulation coupled with a 10 day aging period, that zilpaterol hydrochloride could be supplemented for 35 days without any detrimental effect on meat quality.

2.3 VITAMIN D_3

2.3.1 Function of Vitamin D_3

Vitamin D_3 is a fat soluble vitamin usually stored in the liver. Dietary vitamin D_3 is absorbed through the small intestine and transported in the blood to the liver, where it is converted into 25-hydroxycholecalciferol. 25-hydroxycholecalciferol is then transported to the kidney where it is converted into 1.25-dihydroxycholecalciferol, which is the most biologically active form of the vitamin (McDonald, Edwards, Greenhalgh & Morgan, 1995). From there the compound is transported in the blood to the various target tissues of the body. One of the most important functions of the compound 1.25-dihydroxycholecalciferol is the absorption of calcium from the intestinal lumen (McDonald, Edwards, Greenhalgh & Morgan, 1995).

The need for supplementing the diets of cattle with vitamin D_3 is generally not large, as adult ruminants can receive adequate amounts of the vitamin from irradiation (McDonald, Edwards, Greenhalgh & Morgan, 1995). The act of supplementing vitamin D_3 is therefore an attempt to increase the levels of calcium absorbed from the intestine and thereby increase calcium levels in the blood and possibly the muscle at slaughter.

2.3.2 Homeostasis of Vitamin D_3

The amount of 1.25-dihydroxycholecalciferol that the kidney produces is controlled by the parathyroid hormone. When the level of calcium in the blood is low, the parathyroid gland is stimulated to secrete more parathyroid hormone. Parathyroid hormone induces the kidney to produce more 1.25-dihydroxycholecalciferol which in turn enhances the intestinal absorption of calcium and phosphorus (since calcium is combined with phosphorus in the bone), as well as enhancing calcium and phosphorus resorption from the kidney and the bone (McDonald, Edwards, Greenhalgh & Morgan, 1995). However, when blood calcium

content increases, the hormone calcitonin is released from the thyroid gland. Once released into the blood, calcitonin has the opposite effect to that of parathyroid hormone and inhibits the resorption of bone and decreases the release of calcium from bone to the blood. High levels of calcium, as well as high levels of 1.25-dihydroxycholecalciferol, in the blood also inhibit the production of parathyroid hormone. Therefore the control system that keeps the blood's calcium supply at a stable level consists of two feedback loops. These two loops are parathyroid hormone operating to sustain the supply of calcium, and calcitonin operating to prevent calcium from rising above the desired level in the blood (Frandson & Spurgeon, 1992).

2.3.3 Hypervitaminosis D_3

The pathophysiology of vitamin D_3 toxicity is due partially to the severe hypercalcemia that is the result of the exaggerated response the body has to the vitamin. The symptoms of hypercalcemia are renal calculi (calcium phosphorus salts which form in the renal tubules eventually leading to kidney failure), joint and skeletal pain, weakness, decrease in feed intake (leading to anorexia), vomiting and polyuria (increased urine output). Toxicity can also lead to salt depositions in other soft tissues such as various organs as well as the inner walls of large blood vessels. In acute cases of vitamin D_3 toxicity death of bone cells can occur. Vitamin D_3 toxicity is a very serious disease and is difficult to treat as many of the pathological changes it causes are either difficult or impossible to reverse. Initial treatment is to alleviate the hypercalcemia to relieve clinical signs (Dukes, 1993).

2.3.4 Vitamin D_3 supplementation of feedlot cattle

A survey in Australia found that 77% of consumers would buy more beef if they knew it was always going to be more tender than previously purchased beef (Lawrence et al., 2006), whilst it was found in the USA that the top three quality concerns of consumers

included low overall consistency of beef products, inadequate tenderness and overall palatability (Vargas, Down, Webb, Han, Morgan & Dolezal, 1999). Post-mortem aging of carcasses at 0-2ºC for 7-21 days has been proven to increase tenderness in beef with proteolysis of key myofibrillar proteins by the calpains (especially µ–calpain) being implicated as the major cause of this process (Veiseth, Shackelford, Wheeler & Koohmaraie, 2001). Research has focused on increasing intracellular stores of calcium, thereby activating both µ–calpain and m-calpain to increase *post mortem* rates of proteolytic activity (Lawrence et al., 2006). Although the results vary as to the efficiency of dietary vitamin D_3 and beef tenderness, vitamin D_3 is in general a nutritional means of elevating muscle calcium concentration, with the ability to enhance the calcium dependant myofibrillar protein degradation *post mortem* to improve tenderness (Koohmaraie, 1996; Koohmaraie & Shakelford, 1991).So a potential means of improving tenderness in beef is to add supplemental vitamin D_3 to the diet shortly before cattle are slaughtered.

In a trial conducted by Karges et al. (2001), beef steers received supplemental vitamin D_3 of 6 x 10^6 IU for four or six days. Steaks were aged at 2 ºC for 7, 14 or 21 days. Feeding vitamin D_3 to feedlot steers for six days decreased (P = 0.04) WBSF values of *M. longissimus lumborum* steaks compared to control steers or steers fed vitamin D_3 for four days. Blood plasma calcium concentrations were significantly greater (P < 0.03) for all animals supplemented with vitamin D_3, and even more so for those supplemented for a longer period of time, compared to non-supplemented animals. Swanek et al. (1999) supplemented 7.5 x 10^6 IU vitamin D_3 for 10 days resulting in a significant (P < 0.05) increase in both plasma and muscle calcium concentrations. There was also a significant improvement in WBSF at 7 (P = 0.02) and 14 (P = 0.07) days aging. This is in agreement with previous findings of Karges, Morgan, Owens and Gill (1999) and Montgomery, Parrish, Beitz, Horst, Huff-Lonergan and Trenkle (2000). Tipton, King, Paschal, Hale and Savall (2007) supplemented 3 x 10^6 IU vitamin D_3 for 5 days immediately before slaughter and then a second group of 3 x 10^6 IU vitamin D_3 for 5 days followed by a 7 day withdrawal period

before slaughter. Serum calcium levels increased after supplement removal but not immediately following supplementation. There was no improvement in tenderness for the first group but tenderness did improve at day 7 of the withdrawal period. It was concluded that a withdrawal period made vitamin D_3 supplementation more effective as well as safer, as increased levels of vitamin D_3 that occurred during supplementation were back to normal levels at day 7 of the withdrawal period.

Montgomery, Parrish, Beitz, Horst, Huff-Lonergan and Trenkle (2000) found that all steaks from steers orally administered vitamin D_3 (5×10^6 IU or 7.5×10^6 IU for 9 days and slaughtered 1d later) had numerically lower WBSF values than control steaks at 3, 7 and 21 days aging. Oral supplementation of vitamin D_3 did however cause a significant difference ($P < 0.05$) in shear force in steaks aged for 14 days with shear force values being lower by about 0.5kg for steaks from supplemented steers compared to control steers. It was also found that the treatment groups had increased levels of vitamin D_3 in the muscle by approximately twenty four fold, and the levels were even higher in the liver and kidneys. Vargas, Down, Webb, Han, Morgan and Dolezal (1999) found similar results with steaks from control animals being tougher ($P < 0.05$) than steaks from treated groups (6×10^6 IU for 6.5 days prior to slaughter) up to 7 days *post mortem* storage. Shear force did not differ for steaks aged for more extended time periods, however, the steaks from supplemented animals did require fewer aging days to become more tender, which indicates that vitamin D_3 supplementation can be used to accelerate the aging process and improve the tenderness of beef products. Montgomery et al. (2002) achieved similar results when supplementing beef steers with various levels of vitamin D_3. It was found that plasma calcium increased linearly with vitamin D_3 treatment ($P < 0.01$) with there being a significant increase in muscle calcium ($P < 0.05$) as well. Calpastatin and calpain activity were however not influenced by treatment ($P < 0.05$) but there were differences in tenderness. Vitamin D_3 treatments of 0.5, 1.5 and 7.5×10^6 IU/d reduced strip loin steak WBSF values at 7 days aging but WBSF values did not decrease at any other time *post mortem*. Montgomery et al. (2004) also found that giving

beef steers vitamin D_3 supplementation increased total cytosolic calcium, phosphorus and magnesium concentrations in meat. Free cytosolic calcium could stimulate calcium-activated calpains and could be responsible for muscle structural alterations. It however remains unclear whether the activation of the calpain system and increased proteolysis are a result of increased cytosolic calcium or from *post mortem* changes in cytosolic calcium (Montgomery et al., 2004)

It must also be mentioned that in many of these experiments (Vargas, Down, Webb, Han, Morgan and Dolezal, 1999; Montgomery et al., 2004; Karges et al., 2001; Lawrence et al., 2006) it has been shown that steers receiving vitamin D_3 supplementation show a decrease in feed intake which can lead to a decrease in average daily gain. Factors such as this, as well as increased levels of vitamin D_3 in the muscle, liver and kidneys have to be taken into consideration regarding vitamin D_3 toxicity, although vitamin D_3 levels tend to drop in meat during the cooking process.

There have however also been many studies showing that supplementing vitamin D_3 has no effect on meat tenderness. In a study conducted by Foote, Horst, Huff-Lonergan, Trenkle, Parrish and Beitz (2004), results indicated that feeding supplemental 1.25 - $(OH)_2D_3$ and 25 – OHD_3 increased plasma calcium concentrations significantly ($P < 0.05$). All levels of treatment lead to an increase in plasma calcium concentration, with the highest concentrations of vitamin D_3 leading to the highest concentrations of calcium. However, even with elevated levels of plasma calcium concentration, total calcium concentration in the muscle was not affected ($P > 0.10$). Supplementation did however cause an increase in concentration of vitamin D_3 in the blood, liver, kidneys and muscles. There was also a trend for vitamin D_3 to decrease ($P < 0.01$) shear force values of *M. longissimus lumborum* steaks aged for 14 days, compared with those of controls aged for 14 days, but with further aging the control steaks became more tender. This interestingly showed that vitamin D_3 had the potential to improve tenderness at a faster aging rate but only until a point after which aging

alone is enough to produce the desired effect. These results were in agreement with an experiment conducted by Rider Sell, Mikel, Xiong and Behrends (2004) who found that vitamin D_3 supplementation did not statistically increase muscle calcium concentrations, but did show a tendency (P = 0.14) to increase numerically with increasing dietary vitamin D_3. As for WBSF values, supplementation had no effect on un-aged steaks, but did have lower values at 7 days of aging. However, at 14 days WBSF values were actually higher than for control steaks. Results from this study therefore indicate that vitamin D_3 supplementation provided little benefit to muscle tenderness (Rider Sell, Mikel, Xiong and Behrends, 2004). These animals were however cull cows and were therefore older and more likely to produce tough carcasses. Lawrence et al. (2006) showed that supplementation had no significant effect on pH, sarcomere length, muscle colour or cooking loss. There was also no increase in calcium and vitamin concentrations in the muscle or blood plasma. Supplementation also had no effect on WBSF values with there being no difference between treated and control groups after aging for 1, 7 and 14 days.

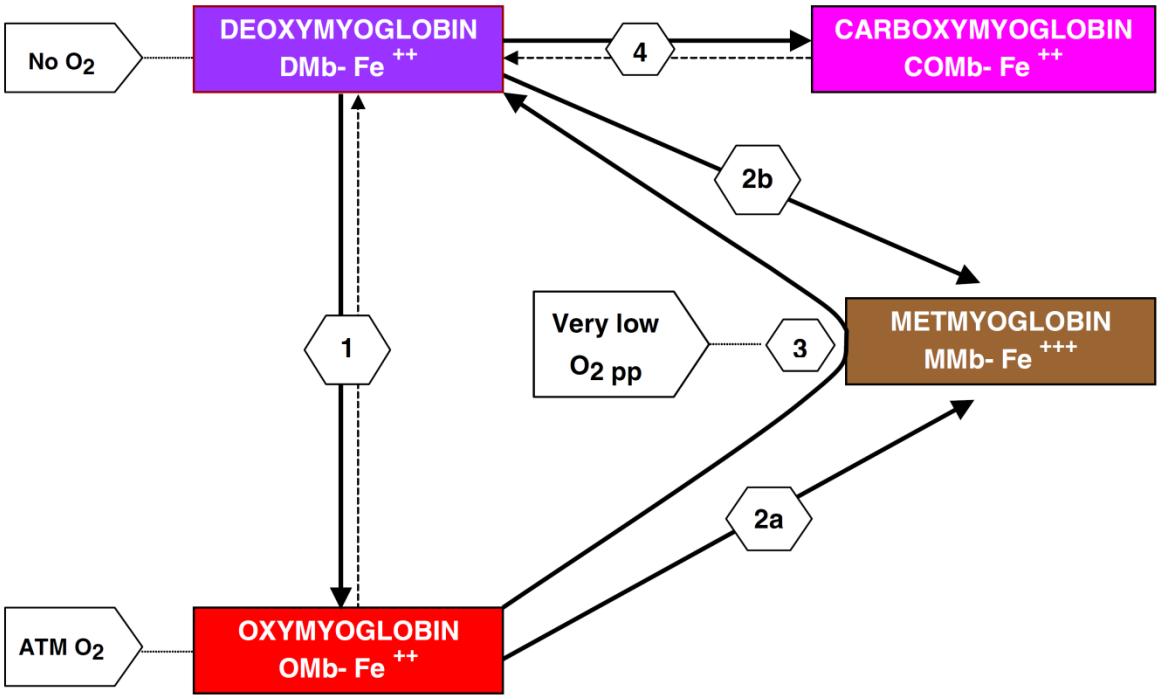
2.4 OTHER FACTORS AFFECTING MEAT QUALITY

There are many factors affecting meat quality. The most important quality attributes of beef include the tenderness, taste, juiciness (drip loss and water holding capacity), freshness, colour, lean content (and fatty acid composition), healthiness, nutrient content, safety and convenience (Webb, 2003).

2.4.1 Colour

The colour of meat is mainly determined by the amount of myoglobin in the muscle as well as the amount of oxygen available for it to react with. The amount of myoglobin in a muscle depends on many factors, namely species, breed, sex (more myoglobin in steers

and bulls than cows), age (more myoglobin in older muscles), and the type of muscle (more myoglobin in muscles that work more). The ligand present and the valence of iron present dictate muscle colour. There are three forms of myoglobin which can occur, namely, oymyoglobin, deoxymyoglobin and metmyoglobin formed by oxygenation, reduction and oxidation reactions respectively. Oxygenation occurs when myoglobin is exposed to oxygen forming oxymyoglobin. The formation of oxymyoglobin gives meat its bright cherry red colour. This is the colour that consumers associate with fresh meat. Oymyoglobin penetrates deeper into the meat's surface with increased exposure to oxygen (Mancini & Hunt, 2005). Oxygen consumption rate is associated with residual mitochondrial respiration in *post mortem* muscle and is related to the depth of oxygen penetration into the exposed surface of the muscle. Lower oxygen consumption rate allows for greater penetration of oxygen into the muscle and is associated with more colour stable muscles (McKenna, Mies, Baird, Pheiffer, Ellebracht & Saval, 2005) and oxygen diffusion into meat is also greater at lower temperatures (MacDougal, 1977). Deoxymyoglobin gives meat a purplish-red/grey colour. Very low oxygen tension is required to maintain myoglobin in a deoxygenated state, such as in vacuum packaged meat or meat just after cutting. When oxygen partial pressure is low, or there is oxygen consumption, metmyoglobin is formed giving the meat a brown colour. Discolouration results from oxidation of both ferrous myoglobin derivatives to ferric iron (Fe^{2+} → Fe^{3+}) and is defined as the amount of surface area covered by metmyoglobin (Fig. 1). Metmyoglobin beneath the surface of the meat, located between superficial oxymyoglobin and interior deoxymyoglobin, can gradually thicken and move towards the surface (Mancini & Hunt, 2005). Colour in meat can be measured as L* (lightness), a* (redness), b* (yellowness) and chroma (saturation index).



Rx 1 (Oxygenation): DMb + $O_2 \rightarrow$ OMb

Rx 2a (Oxidation): OMb + [oxygen consumption or low O_2 partial pressure] – $e^- \rightarrow$ MMb

Rx 2b (Oxidation): [DMb – hydroxyl ion – Hydrogen ion complex]+ $O_2 \rightarrow$ MMb + O_2^-

Rx 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activty $\rightarrow$ DMb

Rx 4 (CarboxyMb): DMb + carbon monoxide $\rightarrow$ COMb

Fig. 1. Visible myoglobin redox interconversions on the surface of meat (Mancini & Hunt, 2005).

Another factor which has a great impact on the colour of meat is the rate and extent that muscle pH declines *post mortem* and the temperature that this occurs at. An increasing paleness in meat is inversely proportional to pH meaning that a decrease in pH results in an increase in paleness. If the pH decline happens too rapidly, resulting in a very low pH at a high temperature, it will result in very pale meat. If the ultimate pH is high (where glycogen depletion occurs pre-slaughter resulting in little or no lactic acid production) the meat will be dark with a dry surface (DFD). DFD meat occurs when there is exercise or stress prior to slaughter resulting in the muscle being deficient in glycogen and therefore having a higher ultimate pH (5.7 and higher). DFD meat allows the growth of spoilage organisms which are inhibited at the usual ultimate pH of meat (Newton & Gill, 1981).

As electrical stimulation causes a more rapid decrease in pH it has to be taken into account when discussing colour. As mentioned previously, depth of oxygen penetration into meat depends on oxygen pressure, temperature and oxygen consumption rate by residual enzyme activity. The latter decreases with duration of aging after slaughter (MacDougall, 1977). Electrical stimulation will therefore speed up this process. Both Strydom, Frylinck & Smith (2005) and McKenna, Maddock & Savell (2003) found that electrical stimulation had no effect on L*, a* or b* values. Strydom, Frylinck & Smith (2005) however, concluded that chilling rates could make the effects of electrical stimulation negligible with regards to meat colour. Devine, Payne, Peachey, Lowe, Ingram and Cook (2002) found that the onset of rigor at a higher temperature usually results in a higher L* value which is a paler colour.

In this study we also have to consider the effects that zilpaterol and vitamin D_3 could have on colour. No differences in L*, a* or b* where recorded by Quin et al. (2008) in heifers fed a beta-agonist or by Avendaño-Reyes, Torres-Rodriques, Meraz-Murillo, Pérez-Linares, Figueroa-Saavedra and Robinson (2006), who observed no difference in meat colour during display from steers fed a beta-agonist. This does not agree with Geesink, Smulders, Van Laack, Van der Kolk, Wensing, & Breukink (1993) who found that beta-agonists significantly increased L* resulting in paler meat. This difference was attributed to L* being associated with water holding capacity in muscle. In this experiment electrical stimulation was applied resulting in a pH drop causing protein denaturation and therefore an increase in drip loss leading to increased L* values. In both Quin et al. (2008) and Avendaño-Reyes, Torres-Rodriques, Meraz-Murillo, Pérez-Linares, Figueroa-Saavedra and Robinson (2006), there were no differences in drip loss between beta-agonist supplemented and control groups. Hilton et al. (2009) obtained similar results regarding L* (no significant difference) but found that a*, b* and chroma were all significantly decreased by zilpaterol supplementation. Strydom, Buys & Strydom (2000) found that zilpaterol supplementation increased colour shelf-life by one day by improving colour stability by decreasing metmyoglobin development. Lawrence et al. (2006) found that vitamin D_3 supplementation had no effect on colour at all in

beef, while both Lahucky et al. (2007) and Wiegand et al. (2002) showed significantly higher a* values (and a significantly lower L* value in the case of Wiegand et al., 2002) in pork loin chops.

2.4.2 Water holding capacity/ drip loss

Water holding capacity is the ability of meat to bind its own water or, under the influence of external forces such as heat and pressure, to bind additional water. When meat loses water it is known as drip loss. There are three kinds of water found in muscle. The first is bound water. Bound water is found near non-aqueous constituents like proteins and does not easily move to other compartments. The second is immobilized water which is held either by steric effects or by attraction to the bound water. This water is held within the structure of the muscle but is not bound to the protein. This water does not flow freely from the tissue in early *post mortem* tissue. The third is free water whose flow from the tissue is unimpeded. This fraction of water is held to the meat by weak surface forces (Huff-Lonergan & Lonergan, 2005). Immobilized water is the most affected by the rigor process and the conversion of muscle to meat and can eventually escape as drip loss (Offer & Knight, 1988b).

pH has a large effect on water holding capacity. During the conversion of muscle to meat, water holding capacity will be reduced. The rate at which pH falls as well as the ultimate pH of the meat will have an effect on this. The higher the ultimate pH, the higher the water holding capacity will be. A fast rate in pH decline, as well as a fast rate of pH decline at high temperatures, will both result in a loss of water holding capacity. This can be attributed to the denaturation of muscle proteins, in particular myosin (Offer, 1991). The accelerated pH decline caused by electrical stimulation can contribute to reduced water holding capacity in beef. Strydom, Frylinck, & Smith (2005) found a small but significant increase in drip loss and attributed this to a rapid pH drop at a slightly slower chilling rate.

Both Strydom, Frylinck, Montgomery, & Smith (2009) and Kellermeier et al. (2009) found that beta-agonist supplementation led to a significant increase in drip loss. Kellermeier et al. (2009) suggested that this was due to zilpaterol supplementation causing an increase in carcass protein and moisture while Strydom, Frylinck, Montgomery, & Smith (2009) agreed with the increased moisture content as well as speculating that higher glycogen breakdown rates led to the increase in drip loss. Montgomery et al. (2002) found that supplementation with various levels of vitamin D_3 had no effect on the percent free, bound or immobilized water. This does not agree with Karges et al. (2001) who found that water holding capacity was increased with vitamin D_3 supplementation and increased with an increase in duration of supplementation.

2.5 CONCLUSION

Results regarding the effect of vitamin D_3 on meat tenderness are still varied. Vitamin D_3 has the potential to increase plasma calcium levels and therefore increase levels of calcium in the muscles, resulting in more calcium being available for the calcium dependant proteinases. This increase in calcium levels does not however always occur and could be due to the counteractive effect that the two feedback loops have, which are in place for calcium homeostasis. Even so, when calcium levels of the muscle are raised this does not seem to always result in increased calpain activity. Other negative effects of vitamin D_3 also have to be taken into account. These include vitamin D_3 toxicity in the animal or high levels of vitamin D_3 in the liver, kidneys and muscle leading to toxicity in humans after consumption. High levels of vitamin D_3 have also been shown to reduce feed intake and therefore average daily gain of supplemented animals. All these factors, as well as the high cost of vitamin D_3 and the possible positive effects on tenderness have to be weighed up. More research needs to be conducted on vitamin D_3 supplementation before it can be confirmed that it does indeed improve beef tenderness.

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