Improving meat tenderness with vitamin D3 and electrical stimulation

By

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Abstract

Meat tenderness is regarded as the single most important characteristic of meat quality. Fifty Bonsmara feedlot steers were fed a commercial feedlot ration (10,5 MJ ME/Kg DM, 12% CP), supplemented with 0,15mg Zilmax/kg live weight in the feed and with different levels of vitamin D_3 (1 to 5 X 10^6 IU Vit D_3 /day) for five days prior to slaughter. The steers were randomly allocated to the vitamin D_3 treatments and a control group that received no vitamin D_3 supplementation. The steers were fed from ca. 248 ± 3 kg live weight, while Zilmax was fed for the last 35 days to a target weight of ca 400kg. All steers were slaughtered at a commercial abattoir after a Zilmax withdrawal period of 7 days. Samples from *m. longissimuss lumborum* were collected 24h post-mortem for sheer force testing on an Instron apparatus equipped with a Warner Bratzler shear blade. Cooking loss was determined by measuring the amount of fluid loss after cooking. Feedlot performance, carcass characteristics and drip loss of meat samples did not differ significantly between the different vitamin D_3 treatments. The inclusion of 5 X 10^6 IU of vitamin. D_3 resulted in significantly lower shear force (SF) values compared to the steers in the control group. The results suggest that dietary supplementation of 5 X 10^6 IU of vitamin. D_3 may significantly improve the tenderness of meet from steers fed 0,15 mg Zilmax ®/kg live weight for the last 35 days in the feedlot.

The aim of the second study was to explore the effectiveness of the use of electrical stimulation on tenderness of mutton. In this experiment 22 wethers of class AB weighing between 45 and 50kg were used. The carcasses were assigned to two treatment groups, of which group one was electrically stimulated (ES) and the other group was not electrically stimulated (NES). The results revealed that electrical stimulation did not significantly affect of the fatty acid content of meat and crude fat content. Treatment however, significantly ($P< 0,038$) influenced the moisture content of the samples. There was a variation in SF values between the two treatment groups; SF of samples from the ES group were lower compared to that of the NES group. This suggests that ES can be successfully applied to reduce the variation in tenderness within the class- AB mutton.

Declaration: I declare that this thesis for the degree MSc (Agric) (Meat Science) at the University of Pretoria has not been submitted by me for a degree at any other University.

Signature:.....

Improving Meat Tenderness with Vitamin D_3 and Electrical Stimulation

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SUMMARY

Tenderness remains a significant characteristic in most meat products and is considered the primary attribute of eating quality in red meats. In the first study the effects of Zilpaterol hydrochloride (Zilmax®) in combination with high doses of dietary vitamin D_3 supplementation on the tenderness of meat from β-agonist treated steers was examined. The inclusion of 5 X 10^6 IU of vit. D_3 resulted in significantly lower shear force values compared to the control group, suggesting that supplementation with 5 X 10^6 IU of vit. D_3 may significantly improve the tenderness of meat.

In the second study, the effect of electrical stimulation did not have a significant effect on fatty acid composition and crude fat content of mutton. Treatment however, significantly affected ($P>0.038$) the moisture content of the samples. There was a variation in shear force values (SF) between two treatment groups: the variation in SF of samples from the ES group was less compared to those of the NES group. The results suggest that ES can be applied to reduce the variation in tenderness within the class- AB mutton.

Keywords: Tenderness, Electrical stimulation, beef, mutton, Vitamin D_3, β-agonists

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Chapter 1

INTRODUCTION AND MOTIVATION

1.1 PROJECT THEME

Meat tenderness

1.2 PROJECT TITLE

Factors that affect the tenderness of South African mutton and beef

1.3 AIM

- To investigate methods of improving meat tenderness in mutton and beef
- To find methods of reducing variation in tenderness of class AB-sheep carcasses

1.4 MOTIVATION

The thesis is divided in two parts namely beef and mutton. Part I on beef was done as part of a preliminary study to understand the mechanisms involved in meat tenderness. Part II on mutton was done specifically to evaluate the effect of electrical stimulation of the recently introduced class- AB on the tenderness of meat.

Beef

Interest in the methods to improve the efficiency of animal production has focused among others on β- andrenergic agonists (Moloney et al., 1994). Several compounds from this class of substance, particularly clenbutarol and cimaterol have been shown to reduce intramuscular collagen, induce muscle hypertrophy and reduce body fat content in several species of animals (Ricks. et al., 1984; Moloney et al., 1991). In this study, the effect of a combined β -agonist and vit D_3 supplementation in the feed was explored. Vit D_3 has been used in dairy cattle to treat hypocalcaemia; in this case it was used to manipulate the chemical reactions that are responsible for tenderisation in the muscles, particularly those involving calcium Ca

Mutton

Meat tenderness is generally regarded as the single most important component of meat quality for the consumer. According to Koohmaraie (1996), reducing the variation in tenderness of aged beef by understanding the mechanisms involved and understanding the causes of variation would enable the meat industry to manipulate the process of meat production and processing to ensure consistent quality. Environmental factors, such as postmortem cooling, electrical stimulation and ageing, proved to have major influences on meat quality, especially tenderness (Koohmaraie, 1996; Olsson et al., 1994; Ouali, 1990).

In this study the effect of ES on the tenderness of mutton carcasses from class AB sheep was evaluated. The current South African red meat classification system gives consumers the perception that class- A carcasses produce best quality meat and can therefore have a higher price. The effect of electrical stimulation of class- A is only important from a chilling point of view, while the effect on tenderness per se is negligible. The recent introduction of the class-AB in the Lamb and Mutton carcass classification system was mainly done to the unique qualities of the class- AB sheep carcass. The effect of electrical stimulation on meat tenderness of older sheep (notably class- AB carcasses) is uncertain and this prompted the study.

Chapter 2

PART I: EFFECT OF DIETARY β-AGONIST AND VITAMIN D_3 SUPPLEMENTATION ON BEEF TENDERNES

2. Literature overview: Factors that affect beef tenderness in feedlot cattle

Seasonal changes in feed and cattle costs usually dictate the length of time that cattle are fed (Van Koevering et al., 1995). It is stated that longer feeding period for cattle of a given starting weight typically increase final weight, hot carcass weight, *m. longissimus* cross sectional area, subcutaneous fat thickness and yield grade and quality grade (Zinn et al., 1970a; Hicks et al., 1987; Dolezal et al., 1982). While some of these changes increase the value of cattle, other changes decrease the value. Increases in subcutaneous fat thickness and yield grade are often not appealing to consumers. Other qualities of significance include cholesterol content and tenderness of ribeye steaks.

2.1 Effect of length of time on feed

Tenderness in feedlot cattle is believed to increase with time on feed up to some point (139d, Epley et al., 1968; 150-180d, Zinn et al., 1970b), after which animal age may have a greater influence resulting in reduced tenderness.

Thompson, (2002) in his study managing meat tenderness adds that meat tenderness is a function of production, processing, and value adding and cooking method used to prepare the meat for consumption by the consumer. Therefore failure on one or more links in the beef supply chain increases the risk of poor eating experience for the consumer. Thompson further states that a guarantee for eating quality can only be given if the links that most affect tenderness are controlled along the meat production chain. In their study of the effect of time on feed on performance of feedlot steers, carcass characteristics, tenderness and composition of *m. longissimus* muscles, Van Koevering et al., (1995) noted the following; that the effect of time on feed on carcass traits presented as hot carcass weights, increased linearly with time on feed ($P<0.01$). This was congruent with previous reports (Zinn et al., 1970a; Hicks et al., 1987; May et al., 1992). Tenderness of the *m. longissimus* muscle in their study was measured as the inverse of the Warner Bratzler shear force and tended to increase ($P<0.07$) with time fed. Their findings contrasted those of Matthews and Bennett,(1962), who reported that tenderness, as measured by taste panel or Warner Bratzler shear force, did not change as time fed increased. May et al., (1992) on the other hand fed steers between 0 and 196 days and reported that the lowest shear force value was at 112d. According to Van Koevering, (1995), tenderness of rib steaks tends to increase with time on feed. With increased consumer demand for lean but high quality beef, feeding cattle for more than 119d may not be advantageous. The above statement conforms to the report by Dolezal et al., (1982).

2.2 Effect of breed on tenderness

According to Sherbeck et al., (1995) there are concerns in the American beef industry regarding the use of Brahman cross bred cattle in the supply of beef products in a new branded program- certified Hereford beef. Previous research has consistently shown that beef tenderness decreases almost linearly, as the proportion of Brahman breeding increases (Damon et al., 1960; Palmer, 1963; Peacock et al., 1982; Crouse et al., 1989; Johnson et al., 1990). Sherbeck further adds that as a result, cattle with any discernible evidence of Brahman ancestry are excluded completely from other branded beef programs. In their study of feedlot performance, carcass traits, and palatability traits of Hereford* Brahman steers (Sherbeck et al., 1995) had the objective to compare feedlot performance, carcass traits and beef palatability characteristics of straight bred Hereford (100H) 75% Hereford*25% Brahman (75H*25B), and 50%Hereford*50% Brahman (50H*50B) steers, and to determine whether steers with 25% or 50% Brahman breeding could be included in the Certified Hereford Beef program.

It was noted that the use of certified Hereford and Brahman carcass specifications seemed to improve tenderness only slightly within each breed grouping. Brahmans are excluded from the Hereford Certified Beef Program due to concerns about product tenderness.

The results of the study by Sherbeck et al., (1995) suggest that, including Hereford crossbred steers with 50% Brahman breeding would adversely affect the tenderness of Certified Hereford Beef. However, Hereford cross breeds with 25% Brahman breeding could be allowed in the certified Hereford program without significantly increasing the risk of decreased product tenderness.

The study of De Bruyn, (1991) has indicated that Brahman and Afrikaner, although similar in carcass weight, conformation and fatness, differed significantly in tenderness. O'Conner et al., (1997) and Pringle et al., (1997) ascribed the lower tenderness of indicus breeds and crosses to a slower rate of post-mortem ageing due to higher muscle calpastatin activity. Cattle indigenous to Africa were often in the past misnamed as *Bos indicus* based on their phenotype (Strydom et al., 2000), while Mayer (1984) showed that most of these breeds are more related to *Bos taurus* than *Bos indicus*. The palatability of meat from continental and British breeds compare favourably with that of Tuli (Shackelford et al., 1995) and Africaner cattle (Naudé and Boccard, 1973).

2.3 The effect of ageing on tenderness

Following death of the animal, muscles undergo physical and biochemical changes which are responsible for their conversion to meat (Bendall, 1978).

After slaughter there is a change in osmotic pressure (Bonnet et al., 1992) and a decrease in temperature and pH (Marsh et al., 1988) followed by an increase in

expressible juice (Offer & Knight, 1989). A common sequel is the weakening of the myofibrillar structure and an improvement in tenderness. The post-mortem proteolysis responsible for tenderisation has been demonstrated to be Ca^{2+} dependent. Under normal circumstances the high intracellular Ca^{2+} concentrations needed to trigger the ageing process do not occur until rigor mortis, when ATP levels become exhausted (Jeacocke, 1993). Ageing of meat is however, according to (Ouali, 1991), a complex phenomenon which depends on physiochemical parameters, extent and rate of acidification, changes in osmotic pressure and on glycolitic and proteolytic enzyme systems. Devine et al., (1993) suggests that ageing begins when the first muscle exhausts its ATP, but only advances to include all muscles when pHu is achieved. Other workers (Olson et al., 1976; Olson and Parish, 1977; Gann & Merkel, 1978), indicate that ageing is accompanied by deep changes in chemical composition and structure of the muscle tissues.

It is worth noting that many post-mortem changes in muscle tissue vary strongly from individual to individual (Valin et al., 1975; Ouali & Valin, 1984; Ouali, 1990). According to Bruas & Brun- Bellut, (1996), species, breed, sex, age and type of muscle all affect the ageing process. The above statement conforms to that of Campo et al., (1999), who indicated that breed type had an important effect on the sensorial perceived tenderisation. The tenderisation process which occurs post-mortem is the result of several enzyme systems degrading the myofibrillar structure (Roncalès,

1995). Nashimura et al., (1998), stated that, although connective tissue is the main limiting element of meat tenderness, it is also partially degraded through ageing.

In their study on the assessment of breed type and ageing time effects of beef, using two different texture devices, Campo et al., (2000) concluded that the Warner Bratzler test was not as good in assessing meat texture evolution through ageing when compared to the modified compression device using raw meat. The device avoids transversal elongation of the sample. They further concluded that aging has a more important effect on the myofibrillar tenderisation than breed type and that some related genetic factors, such as connective tissue composition, can significantly affect meat texture

2.4 Effect of production system on tenderness

In many developed countries, concentrate by-products and cereal grains are the main feed stuffs used in intensive beef production. In developing countries, livestock production is based on low quality feeds such as pasture, stubble and some forages (Araba & Byers, 2002). Feed costs present a major proportion of total variable costs in beef systems and grazed grass is often the cheapest feedstuff in temperate climates (French et al., 2000).

Despite the above factors, the value of beef from grass finished cattle is often discounted compared with concentrate finished beef because of perceived differences

in meat quality. There are factors that influence consumer perception of meat quality, these include tenderness (Chrystall, 1994), colour (Baardseth, Skrede et al., 1998), juiciness (Hutchings & Illford, 1988) and flavour (Melton, 1990).

Browling et al (1975), stated that many workers have previously made comparisons of forage finished and grain finished beef (Wanderstock and Miller, 1948; Carpenter et al., 1969; Bull et al., 1952; Craig et al., 1959; Schupp et al., 1976). Bowling et al., (1978) indicated that the problem with such comparisons is that of establishing the appropriate slaughter end point. Their argument stemmed from the fact that, if cattle from two management systems are slaughtered on a time constant basis, differences in fatness obscure real differences in meat palatability, they also added that if cattle from grain vs. forage feeding programmes are slaughtered at the same degree of fatness, differences in maturing would confound the analysis.

According to French et al., (2000), concentrate-fed animals have heavier and fatter carcasses than forage - fed animals when grown for a constant time period or may be younger when grown to a specific body weight or back fat thickness. Meat quality characteristics, especially tenderness and flavour, are influenced by carcass weight, back fat thickness, age at slaughter and pre-slaughter growth rate (Spanier et al., 1990).

French et al., (2000) conducted a study on meat quality of steers finished on autumn grass, grass silage or concentrate-based diets. Their objective was to evaluate the effect of diet on the quality of meat from cattle while maintaining similar mean carcass growth rates between diets. For marbling, their findings contrasted those of Binder et al., (1981) and (Purchas & Davis, 1974) who observed lower intra-muscular and subcutaneous fat concentrations on grass fed animals relative to concentrate fed animals when grown to similar carcass weight.

They measured the Warner Bratzler shear force according to the procedure of Shackelford, Koohmaraie, Cundrif, Gregory, Rother & Savell (1991). Steaks (25cm) were cooked in retortable vacuum pack bags to an internal temperature of 70°C, by immersing in a water bath (Model Y38, Grant instruments Ltd) at 80°C. Five cores (1.25cm diameter) were cut from the steaks parallel to the direction of the muscle fibres and sheared using an Instron Universal testing machine equipped with Warner Bratzler shearing device. French et al., (2000) noted and interaction between diet and aging time post mortem for longissimus dorsi pH. The animals offered grain and concentrates (GC) had lower ($P<0.05$) mean pH values at 2,3,4 and 5 hours post mortem than all other treatments.

However, all dietary treatments had similar longisimuss pH at 24h-post slaughter. Muir et al., (1998), indicated that grass-fed steers had higher ultimate pH values than grain fed steers and suggested that grass -fed steers were more susceptible to pre-slaughter stress than grain- fed steers, his argument was based on the fact that the latter would be more accustomed to penning and would be less likely to suffer glycogen depletion in the abattoir pre-slaughter. According to French et al., (2000), all animals used in their study were accustomed handling. Other workers, Wanderstock & Miller (1948).

Binder et al., (1981 , 1986) and Morris et al., (1997) have also found no differences in ultimate muscle pH between grass and grain finished cattle. French et al., (2000) reported an interaction ($P<0.05$) between diet and ageing time post mortem of LD tenderness as measured by WBSF and sensory analysis. Animals offered GC had lower WSBF values, higher tenderness scores and higher overall acceptability scores for the 2-day aged steak than animals from all other treatments. The observation above supported the conclusion by Marsh et al. (1987). French et al., (2000) also noted that in the 7 and 14 day aged steaks, there was no effect of dietary treatment on tenderness. Some studies have revealed forage finishing of cattle to have negative consequences on tenderness.

2.5 Effect of transportation on tenderness

Animal welfare, meat quality and yield are the important issues in moving cattle from farm to the abattoir (Tarrant, 1992). Stocking density, group size and method of loading and unloading are the main considerations on short journeys. Live weight losses are high, even on short journeys due to loss of gutfill (Shorthose, 1965). According to Grandin (1993), mixing animals has been shown to cause mobilisation of glycogen and should be avoided at all costs. They came to this conclusion after analysing data for bulls that had been mixed, they noted the high prevalence of dark cutters. Thompson (2002) stated that stress in a number of forms would deplete glycogen reserves of the animal.

Ferguson et al., (2001) supported the above statement in his conclusion, he described the emotional state of the animal as probably more critical in mobilising glycogen reserves than was activity that was not physically demanding (e.g. during transportation). Ante- mortem stress entails significant modifications in post mortem biochemistry of muscle and meat quality (Beltran et al., 1997). The major effect being exerted through its influence on mobilisation of muscle glycogen stores; if these are reduced or depleted at slaughter, the resultant is the extent of post-mortem acidification, which is consequently reduced (Warris, 1990; Sauz et al., 1996).

Ultimate pH in post- mortem bovine muscle is believed to have a direct relationship with stress intensity. (Pethick et al., 1999) stated that cattle which are well fed up until dispatch for slaughter will have muscle glycogen concentrations in the range from 60 to 120 µmol/g. 57 µmol/g of glycogen is required in the muscle to achieve an ultimate pH $_{ca}$ 5.5 in the post slaughter muscle. The statement by (Pethick et al., 1999) is supported by Beltrain et al., (1997), and they also noted that ultimate pH in post-mortem bovine muscle may be between 5.4 and 7.2, showing a direct relationship with stress intensity. High pH is said to result in dark colour, susceptibility to bacterial spoilage and reduces flavour (Tarrant, 1989).

High pH has also been associated with an increased tenderness of meat (Sánudo et al., 1993; Guignot et al., 1994) and even higher tenderisation throughout the ageing process (Watanabe et al., 1996). On the contrary, Shaefer et al., (1990) believed that ante- mortem stress increased the degree of toughness in meat. In their study of the effect of transport time on the sensorial aspects of beef meat quality (Villarroel; et al., 2003) stated that they found no differences in sensory quality with respect to transportation time in terms of tenderness, and overall liking, the tenderness of the beef tended to decrease with an increase in pH_{24} from 5.5 to 6.1, after which it increased. The reasons for the above behaviour according to Purchas & Augsupakorn, (1993) are obscure but, seemed to be directly affected by the change in sarcomere length which is intensified by cold shortening.

The increase in shear force values reported by Feins et al., (1990), was attributed to lower proteolytic enzyme activity in cimaterol treated bulls. In their study on the performance, muscle composition and meat texture in veal calves administered a B-agonist (Clenbutarol), Berge et al (1993) came to the following conclusions

That the negative effect of β-agonist on meat tenderness is mainly due to a reduction in post mortem degradation of myofibres that is not compensated by a reduction in the concentration of connective tissue in muscles

That the influence of clenbuterol in meat texture components and characteristics is muscle dependant and finally that the administration of a relatively low clenbuterol dose, followed by a two week withdrawal period, limits the toughening effect of clenbuterol without compromising its positive effect on animal performance and body fat disposition.

2.8 Effect of Vitamin D_3 supplementation in the feed on meat tenderness

Early studies using lactating dairy cows have showed that orally administered vitamin D_3 at $5*10^6$iμ daily for two weeks prepartum increased serum calcium (Hibbs et al., 1951; Hibbs and Pounden, 1955). Injections of 1α hydroxyvitaminD_3 also increased serum calcium concentrations of dairy cows (Bar et al., 1985, 1988; Sachs et al., 1987; Hoduett et al., 1992). The underlying thought is that calcium – activated tenderization system elevates muscle calcium levels and both μ- and m- calpains are activated during the post-mortem aging process.

In 1994, the US National Beef Quality Audit ranked inadequate tenderness as the second most important beef quality problem (Smith et al., 1995). These authors estimated that the annual economic loss associated with beef tenderness equalled $ 217 million for the US beef industry.

(Swanek et al., (1999) conducted experiments to evaluate the use of dietary vitamin D_3 supplementation as an alternative method of elevating muscle calcium concentration to activate the calpains, thereby improving beef longissimuss tenderness. They observed that steaks from orally administered vitamin D_3 Preceding slaughter had numerically lower Warner Bratzler shear values as indicated in the table below. Supplemental vitamin D_3 decreased Warner Bratzler shear force at all post-mortem aging times, but they noted maximal improvement for those steaks post-mortem aged 14 days (14d) ($P<.05$).

Table 6: Sensory evaluation of strip loin steaks post-mortem aged 14d (means ± SE)

Treatment Group	Tenderness	Juiciness	Flavour	Overall Palatability
Control	5.07± .25[b]	4.47± .33	4.96± .17	4.98± .29
$5*10^6$ iμ/d	5.31± .25	4.47± .28	4.90± .15	4.98± .28
$7.5*10^6$ iu/d	5.19± .19	4.56± .26	5.23± .15	4.99± 21

[a]Panel scores are based on an 8 point descriptive scale as follows: tenderness, 1= extremely tough, 8= extremely tender; juiciness, 1= extremely dry, 8= extremely juicy; flavour, 1= extremely bland, 8= extremely flavourful; palatability, 1= extremely unpalatable, 8= extremely palatable.

[b]All values within a column are similar (P<.05)

From: (Montgomery et al., (2000)

Montgomery et al., (2000) concluded that giving supplemental daily doses 5 or $7.5*10^6$ iμ of vitamin D_3 to feedlot cattle decreased Warner Bratzler shear force (P< .05) of beef strip loin and top round steaks that were aged for 14d. They further concluded that feeding vitamin D_3 may offer an effective way to improve tenderness (decrease Warner Bratzler shear force values) 14d of post-mortem aging of loin and round muscles.

It was noted that future research studies need to address the optimal storage time for maximal tenderization and to elaborate on why different responses seem to occur in top rounds and strip loins.

This increased tenderness is believed to occur because of increased intracellular calcium concentration, which is believed to augment proteolysis during post-mortem aging.

Montgomery et al (2000) also suggested that feeding $5*10^6$ iμ of vitamin D_3 per day for 9 days before slaughter could be implemented in a commercial feedlot system to improve tenderness (based on decreased Warner Bratzler shear force values) of strip loin and top round steaks within 14d post-mortem. Ante mortem feeding of supplemental vitamin D_3 therefore may hold the potential of improving beef tenderness and increasing consumer acceptance of beef.

Swanek et.al. (1999), adds that studies have to be conducted to investigate the effect of the procedure on other important economic characteristics (e.g., live animal performance and tissue residue concentrations) before any recommendations about its commercial applications can be made. One such study was by Scanga et al., 2001 who indicated that because of the inconsistencies in supplementation, they embarked on their study to develop a recommendation for the optimum dose level and duration of administration of vitamin D_3 for the purpose of improving the tenderness of cooked beef. They also intended to evaluate the effects of various dose levels and duration of administration and their effect on feedlot performance and blood calcium levels.

They came to the following conclusions: that vitamin D_3 did not influence feedlot performance; vitamin D_3 was successfully administered via the oral bolus since serum calcium increased in the animals. These findings were consistent with those of Swanek et al., (1999); Hibbs and Pouden (1954), which stated that serum calcium levels increased when cattle were fed supranutritional levels of vitamin D_3

From an economic stand point, they found that supplementation with a lower dose of vitamin D_3 for fewer total days, while attaining an equal increase in blood calcium concentrations, would thus be less expensive to incorporate into a production system. They came to a conclusion that, supplementing cattle with vitamin D_3 does not influence (improve or impair) post-mortem beef tenderness, which is in disagreement with previous studies. It is therefore apparent that more work needs to be done to determine the impact of vitamin D_3 on beef tenderness.

Chapter 3

THE EFFECT OF A COMBINED DIETARY β- AGONIST AND VITAMIN D_3 SUPPLEMENTATION ON THE TENDERNESS OF LOIN SAMPLES FROM FEEDLOT STEERS

Abstract

In the present study, the effect of zilpaterol hydrochloride (Zilmax®) in combination with high doses of dietary vitamin D_3 supplementation on the tenderness of meat from β- agonist treated steers was examined. Fifty Bonsmara feedlot steers were fed a commercial feedlot ration (10,5 MJ ME/kg DM, 12% CP), supplemented with 0,15mg Zilmax®/kg live weight in the feed and with different levels of vitamin D_3 (1 to 5 X 10^6 IU Vit D_3 /day) for five days prior to slaughter. The steers were randomly allocated to the vitamin D_3 treatments and a control group that received no vitamin D_3. The steers were fed from ca. 220 kg live weight, while Zilmax was fed for the last 35 days to a target weight of ca 450kg. All steers were slaughtered at a commercial abattoir after a Zilmax® withdrawal period of 7 days. Samples from m. *longissimuss lumborum* (LD) were collected 24h post-mortem for sheer force (SF) testing on an instron apparatus equipped with a Warner Bratzler shear blade. Cooking loss was determined by the amount of fluid after cooking. Feedlot performance, carcass characteristics and drip loss of meat samples did not differ significantly between the

different vitamin D_3 treatments. The inclusion of 5 X 10^6 IU of vit. D_3 in the feed resulted in significantly lower SF values compared to the steers in the control group. The results suggest that dietary supplementation of 5 X 10^6 IU of vit. D_3 may significantly improve the tenderness of meet from steers fed 0,15 mg Zilmax ®/kg live weight for the last 35 days in the feedlot.

3.1 Introduction

Interest in methods to improve the efficiency of animal production has focused on β-andrenergic agonists (Moloney et al., 1994). Improvements in growth rate, feed conversion efficiency, carcass fat and muscle content as well as the composition of fatty acids in the fat has been well documented (Webb et al, 1997; Webb & Casey, 1995).

Several compounds from this class of substance, particularly clenbuterol and cimaterol have been shown to reduce intramuscular fat, induce muscle hypertrophy and to reduce body fat content in several animal species (Ricks et al., 1984; Williams et al., 1987; Moloney et al., 1991). The reduction in carcass fat occurs through a depressed lypogenesis and increased lypolysis in subcutaneous adipose tissue as demonstrated in heifers (Coleman et al., 1988; Miller et al., 1986). Swanek et al., (1999) conducted experiments to evaluate the use of dietary vitamin D_3 supplementation as an alternative method of elevating muscle calcium concentration to activate the calpains, to improve beef longissimuss tenderness.

They observed that steaks from steers that were orally administered with vitamin D_3 decreased Warner Bratzler shear force at all post mortem aging times, but they noted maximal improvement for those steaks post mortem aged for 14 days ($P \leq 0.05$). The purpose of the present study was to study the effect of a combined dietary β-agonist treatment and different levels of vitamin D_3. supplementation on the tenderness (shear force) of m. *longissimuss dorsi* from feedlot steers.

3.2 Materials and methods

3.2.1 Steers

Fifty Bonsmara type feedlot steers were fed a commercial feedlot ration (10,5 MJ ME/kg DM, 12% CP), supplemented with 0,15 mg Zilmax®/kg live weight in the feed for the last 35 days of fattening and with different levels of vit D_3 on a scale 1 to 5 X 10^6 IU Vit D_3 /day for five days prior to slaughter. The steers were randomly allocated to the different vit D_3 treatments and a control group that received no vit D_3. The steers were fed from ca.220-450 kg live weight, while Zilmax® was fed for the last 35 days to a target weight of ca.450 kg, including a Zilmax® withdrawal period of seven days.

The animals were slaughtered in a commercial abattoir in Witbank and m. *longissimuss dorsi* samples were collected 24 hours post-mortem.

3.2.2 Slaughter

All steers were slaughtered at a commercial abattoir after a Zilmax® withdrawal period of 7 days. Samples from the *m. longissimuss lumborum* (LD) were collected 24h post-mortem for shear force testing on an instron apparatus equipped with shear force blade. Cooking loss was determined by measuring the amount of fluid after cooking and expressing it as a proportion of the initial weight of the sample.

3.3 Results and Discussion

Table 1: Effect of level of inclusion of dietary vit D_3 supplementation on meat tenderness

Treatment	Shear force (N) (Mean)	± Std Dev
Control	63.01	12.407
1 X 10^6 IU of vit. D_3	63.20	14.162
2 X 10^6 IU of vit. D_3	62.12	10.514
3 X 10^6 IU of vit. D_3	60.41	12.666
4 X 10^6 IU of vit. D_3	55.25	17.294
5 X 10^6 IU of vit. D_3	54.33	14.835

Feedlot performance, carcass characteristics and drip loss of meat samples did not differ significantly between the different vit. D_3 treatments. The inclusion of 5 X 10^6 IU of vit. D_3 resulted in significantly lower shear force values ($P< 0.05$) compared to the steers in the control group. The results (Table1) suggest that dietary supplementation of 5 X 10^6 IU of vit. D_3 may significantly improve the tenderness of meet from steers fed 0, 15 mg Zilmax ®/kg live weight for the last 35 days in the feedlot. Similar results were observed by other authors, (Swanek et al., 1999; and Montgomery et al., 2000).

3.4 Conclusion

Dietary supplementation with vit D_3 significantly improves the tenderness of meat from animals fed Zilmax® in the last 35 days in the feedlot. This shows that vit D_3 and β-agonist can be used in combination effectively to improve the tenderness of meat while not compromising the lean content. More research needs to be done to identify the optimum period of Zilmax® supplementation and the quantity of vit D_3 for optimum results to be achieved.

Chapter 4

PART II

4. Literature overview: factors affecting meat tenderness

4.1 Electrical stimulation

Electrical stimulation (ES) of freshly slaughtered beef carcasses was first suggested by Harsham and Detherage (1974), principally as a means of rapid tenderisation of meat, but also to hasten the onset of rigor and the decline in muscle pH. De Fremery & Pool, (1960) have used ES on chicken and lamb carcasses respectively with the specific aim of accelerating the post-mortem changes and the method has been further developed by New Zealand workers for use in lamb slaughtering abattoirs.

Electrical stimulation of carcasses is usually done to reduce cold shortening in small carcasses and to facilitate the conversion of muscle to meat. It has been hypothesised that ES increases the activity of proteolytic enzymes that may degrade structural proteins in muscle cells and cause fractures and breaks in muscle fibres, which includes m-Calpain (Dransfield, 1992; Uytterhaegan et al, 1992). Titin in myofibrils is a long filamentous protein that extends from the z-lines, while Nebulin consists of inextensible filaments that are closely associated with or part of, thin filaments; ∝ actinin, an integral z- line protein; and desmin are the major components of

intermediate filaments that associate with z- lines (Robson et al., 1984, Robson and Huiatt, 1991).

Degradation of some of these structural proteins has been implicated in the loss of myofibrillar integrity thus increasing meat tenderness (Huff-Lonergan et al, 1995, Taylor et al, 1995). There has been mixed conclusions by different workers on which mechanism facilitate tenderness. Robson et al., (1984) and Koohmaraie et al, (1986), for example, suggested that post-mortem meat tenderness may be attributed, in part, to desmin degradation. Uytterhaegen et al, (1992) reported that ageing and ES increased the degradation of titin and troponin – T but that ES had no effect on sarcomere length.

In their study, Chiung-ying Ho et al., (1996) showed that electrical stimulation slightly accelerates the degradation of the cytoskeletal proteins titin and nebulin in post-mortem muscle.

They also found that ES increased the frequency of three types of myofibrillar I-band fractures (narrow, intermediate, and wide), and caused the wide I- band fractures to appear sooner post-mortem. They went on to suggest that ES may

improve meat tenderness by a combination of mechanical disruption and enhanced proteolysis, and that mechanical disruption seemed to be the predominant mechanism.

In their study of the effect of ES on water holding capacity and protein denaturation of two bovine muscles, Hertog-Meischke et al, (1996) indicated that electrical stimulation effectively accelerated post-mortem glycolysis, especially in the semi-membranous muscle.

The effect of ES was also reported to be dependent on fibre type. Muscles with a higher percentage of slow twitch –oxidative (Type 1) fibres reportedly responding less intensively to electrical stimulation than muscles with a higher percentage of fast – twitch –oxidative –glycolytic (Type I1 A) or fast twitch –glycolytic (Type II B) fibres (Devine et al, 1994).

Many postulatoins on how tenderness is reached through electrical stimulation have been forwarded, these include reductions in cold shortening (Davey et al., 1976; Chrystall and Hagyard, 1976) and increased activity of acid proteases (Savell et al., 1977). In his study of the structural changes in electrically stimulated beef muscle Savell et al., (1978b), suggested that physical disruption of muscle fibres resulting from the massive contractions during stimulation may provide an explanation for the improved tenderness associated with electrical shock. They noted that in the electron micrographs of electrically stimulated samples removed at 20-24h post-mortem definite structural differences were apparent when compared to those from control sides.

Electrically stimulated samples had less well defined I- bands and Z- lines through the contracture bands while sarcomeres from either side of the contracture appeared to be stretched or even broken. Another study was undertaken by Sorinmade et al, (1982), to determine whether physical disruption as well as proteolysis could be detected in electrically stimulated muscle tissues obtained 48h post-mortem.

The damage in their samples was more compared to that of Savell et al., (1977). They attributed their pronounced differences to prolonged effects of proteolysis, with most filaments torn or fragmented around the Z-lines and empty vesicles observed in the fragmented areas. Other workers shifted the effects of electrical stimulation to include breeds. Crouse et al., (1987) reported that ES markedly improved tenderness from *Bos indicus* crossbred cattle. Wheeler et al., (1990) also noted that at 14d post-mortem in non-stimulated carcasses, fewer I- band fractures were present in muscles from *Bos indicus* than from *Bos taurus* cattle. Ho et al., (1996) confirmed Wheelers findings, they also observed that in Boss Taurus samples, ES not only caused the formation of CN and I- band fractures but also accelerated the appearance and increased the frequency of three types (narrow, intermediate, and wide) of I- band fractures. The incidence of narrow I- band fractures was greater in ES samples at each sampling time from 1d through 28d post-mortem, the difference was significant only at 3 d (Ho et al., 1996).

According to Hwang & Thompson, (2001a), the challenge for further development of electrical stimulation systems is optimising the activation of the enzyme systems. This he says could be done by chilling regimes to ensure *rigor-mortis* close to 15°c, within

the constraints of food safety concerns and taking into consideration the different fibre composition of muscles. Hwang & Thompson, (2001a) suggests a more targeted approach such as the use of regional simulation of certain muscle groups susceptible to rapid chilling effects, may be more beneficial for improving tenderness.

Hwang & Thompson, (2001a) propose the development of self-response stimulation units which first determine carcass resistance and decide on length of simulation time or strength of stimulation these they say would be an alternative approach for best practice in electrical stimulation. Hwang & Thompson (2001a) concluded that contribution of physical disruption to improvement in tenderness seemed to be a logical extrapolation, it is still subject to verification.

They also suggest that quantitative studies examining ultrastructure alteration and proteolysis simultaneously would assist in establishing the contribution of mechanical disruption to achieve improvements in tenderness.

4.2 Effect of high and low voltage electrical stimulation on carcass characteristics

Bendall, (1976) have shown that voltage of stimulation has a highly significant effect on the immediate pH fall during stimulation and on the subsequent rate of fall. It was noted that at 100V the *m. longissimuss dorsi* (LD) muscle for example, was scarcely affected since the times taken to reach pH 6.0 and 5.7 were almost identical compared

with the non-stimulated controls. In other muscles these pH values were reached in half the time

subsequent to stimulation, except for the *m. semimembranosus* (SM), which was rather more affected i.e. less time was required to reach these pH values.

When the voltage was raised to 300V, this caused a much larger immediate drop in pH and reduced the time to reach pH 6.0 and 5.7 drastically. An increase in voltage to 700V, further improved the time saving effect, and none of the muscles were

reported to have taken more than 1h to reach pH 6.0 or more than 2h to reach pH 5.7. The saving of time, at 700V, in reaching pH 5.7 was reported to be 8.5h in all four muscles, representing 82% of the time taken to reach this pH in unstimulated controls. Other authors have also supported the idea that electrical stimulation (ES) of muscles soon after slaughter hastens the onset of rigor mortis and provides the basis for the process to rapidly decrease muscle pH in lamb (Bendall, 1976;Carse, 1973; Chrystall, et al., 1984) and beef (Chrystall & Devine, 1978; Davey, et al., 1976), and thus avoid the toughening effects of cold shortening and thaw shortening. Many authors agree with the fact that low voltage is more practical (Fabianson & Reutersward, 1985; Hawrysh et al., 1987; Savell, et al., 1977). It is believed that for safety reasons a low voltage system is more attractive for application under commercial conditions (Polidori et al, 1999). In general, the lower the voltage the less danger to the operator and the less stringent requirements imposed by government regulatory agencies for preventing accidents to employees. Polidori et al., (1999) have shown that low voltage

electrical stimulation of lamb carcasses can reduce the deleterious effects normally associated with rapid chilling of the whole carcass.

Taylor and Tantikov, (1992), Dransfeild et al., (1991) and Taylor et al., (1994 a, b) reported a considerable tenderising effect with high voltage ES applied 20 minutes post- slaughter, with up to 30% improvement over non- stimulated controls. Taylor and Tantikov, (1992) also found that high voltage ES was more effective than low voltage ES which had been applied during debleeding.

A further advantage of high voltage is that it can be applied at a later stage in the dressing line; conveniently 20 minutes post- slaughter (Taylor and Martoccia, 1995).

4.3 Effect of electrical stimulation on meat pH

Electrical stimulation (ES) has been shown to be a variable that can improve meat quality, colour, quality grade and sensory qualities and may facilitate hot boning (Cross and Siedman, 1985, Smith, 1985). This has also been confirmed by Bray et al, (1994), who reported that the rate of *post mortem* decline in muscle pH and the level at which it ultimately reaches ultimate pH, influences the storage life, attractiveness to purchasers, and eating qualities of meat.

In his study on the biophysical aspects of meat tenderness, Tonberg, (1996) found that the pH, 1 hour after debleeding was lowered by ES, but not significantly for LD. He

suggested that one possible explanation for higher tenderness in the electrically stimulated meat could be that by an earlier attachment of lower pH in the rigor process, the inhibitor calpastatin is released and thereby greater Calpain activity is achieved. It is however important that rigor temperature stays low because the enzymes are more susceptible to denaturation at a lower pH, and autolysis of the calpains is favoured at higher temperatures.

4.4 Meat tenderness

Tenderisation is a generalised term for the process that leads to an improvement in tenderness and in reality can only be measured post rigor, (Hwang et al., 2002). A measure of tenderness is the subjective consumer appreciation of the meat and a high score is desirable. Connective tissue and the amount of intramuscular fat influence tenderness. Hwang et al., (2002) states that, an objective measure of tenderness is the force required to shear a standardised piece of meat with low shear values being desirable. According to Devine & Graafhuis, (1995), the process affecting meat tenderness starts at slaughter, but changes may not be significant at the time and also measurement of tenderness at this stage is also meaningless. The endogenous enzymes responsible for tenderisation will be active throughout the rigor process. During proteolysis, significant tenderness changes are not evident until most of the muscle fibres are in rigor.

The tenderisation process is estimated to begin soon after slaughter, perhaps as soon as 3 hours, but it is highly variable among individual carcasses (Veiseth, et al., 2001).

Current evidence suggests that proteolysis of key myofibrillar proteins is the cause of meat tenderisation. These proteins are involved in the following processes (i) inter-myofibril linkages (e.g. desmin and vinculin), (ii) intra- myofibril linkages (e.g. titin, nebulin, and possibly troponin-t), (iii) linking myofibrils to sarcolema by costameres (e.g. vinculin and dystrophin), and (iv) the attachment of muscle cells to the basal lamina (e.g. laminin and fibronectin). It is pointed out that the function of these proteins is to maintain structural integrity of myofibrils (Price, 1991). Proteolytic degradation of these proteins is believed to be the cause of weakening of myofibrils and, thus, tenderisation.

According to Koohmaraie et al., (2002), sarcomere length, connective tissue content, and petreolysis of myofibrilar proteins account for most, if not all, of the explainable variation observed in tenderness of aged meat (after post mortem storage). However, the relative contribution of each of the above components of tenderness is muscle dependent.

While sarcomere length is the major determinant of *Psoas major* muscle tenderness, proteolysis is the major determinant of *Longissimuss* muscle tenderness, while

connective tissue content is a major contributor to the tenderness of muscles such as the *Biceps Fermoris* and *Semimembranosus.*

4.4.1 Effects of rigor mortis temperature on tenderisation

Devine et al., (1999), pointed out that initially it was thought that the minimal shortening, which occurs when *rigor mortis* is attained at 15°C, was the explanation why such meat had the lowest shear force. However, it has been shown in beef that when tight wrapping prevented shortening and rigor mortis occurred at a range of temperatures from 15-35°C, that shear force was greater at the high *rigor mortis* temperatures. This difference was maintained with ageing at 4°C (Devine et al., 1999).

Of some interest is the finding by Hwang & Thompson (2001b) that the most tender beef after 14 days of ageing was achieved when the temperature at pH 6.0 was 29-30°c under *in situ* conditions. Hwang et al., (2002) highlights that this means that caution is needed when extrapolating from the *in vitro* to *in situ* states. This means that a long duration at elevated *post mortem* temperature and low pH therefore may be critical in terms of calpain inhibition.

4.4.2 Optimum tenderisation

It has been noted that the tuning of the amount of stimulation with chilling rate to reach rigor mortis at 15°C resulted in optimum tenderisation in several studies. If there is a rapid pH fall resulting from stimulation within two minutes of slaughter, the meat is not as tender after three days of ageing at 4°C as when stimulation takes place 30 minutes after slaughter. Walgren et al., (1997) stresses that, both are more tender than non-stimulated meat.

It is said that in time, after 4 days ageing, the meat all becomes tender, but the meat stimulated at 30 minutes is still the most tender. Hwang and Thompson (2001b), noted that temperature records showed that the meat stimulated at 30 minutes attained *rigor mortis* close to 15°C. In his study on the biochemical and physical effects of ES on beef and sheep meat tenderness, Hwang et al., (2002) reported that meat attaining rigor mortis at 15°C will be more tenderer than meat entering *rigor mortis* at other temperatures, but the results of Hwang and Thompson, (2002) suggest that further studies are required to determine the precise processing situations required to achieve optimum tenderisation.

4.5 Carcass quality and composition

The ideal carcass can be described as one that has a minimum amount of bone and maximum amount of muscle. The amount of fat regarded as optimum can vary according to consumer preference and according to the purpose for which the carcass is to be used (Barwick &Thompson, 1996)

A certain proportion of fat is desirable to reduce drying out of the carcass.

McClelland & Russell (1972) compared purebred Scottish Blackface and finish Landrace lambs and found that the latter deposited less fat in the carcass and more in the body cavity. In their work on the estimation of sheep carcass composition from fat and muscle thickness measurements taken by probes, Kempster et al., (1986) indicated that fat thickness can predict carcass composition as precisely as the visual fat score used in the MLC Sheep Carcass Classification Scheme and that, visual score and fat measurements are complementary to some extent. Kempster et al. (1986) further adds that a re-examination of the prediction relationships is also especially important at the moment because of concern about diet and health and the need to provide accurate information on the fat content of meat and meat products.

According to Stanford et al., (1998), in order to reverse the downward trend in lamb consumption in some countries, the needs of the modern consumer have to be addressed. Ward et al., (1995) outlines that consumers require meat with more lean; less fat i.e. the minimal fat required is that which can maintain juiciness and flavour.

Before lamb carcasses can be changed to better meet consumer demand, carcasses must be evaluated using two equally important categories quality attributes such as tenderness, cut size, fat cover, marbling, meat and fat colour. Hopkins et al., (1998) suggested that extremely rapid methods capable of evaluating a carcass in 6 seconds or less would be relevant for outline use, provided that damage to the commercial value of the carcass is minimal.

4.6 Carcass classification system

Most carcass evaluation work is carried out with an economic objective in mind and is concerned with those characteristics, which have most influence on carcass retail value (Kempster, 1984). It is suggested that consideration should be given to a homogeneous method. Nicol and Parrat (1984) and Edward et al., (1989) found that trained livestock evaluators have been able to estimate lamb carcass composition in different breeds with accuracies superior to that of ultrasound.

Carcass composition assessment is used to serve three functions: -

(1) Assigns carcass value (2) allows sorting carcass for processing or fresh meat merchandising and (3) Transfers information back to the production sector, hopefully ensuring that carcasses meet consumer demand (Stanford et al., 1998).

The various methods of assessing carcass composition *ex vivo* should be precise, accurate over time and distance and across lambs of varying breeds, sexes and ages. In addition, cost, ease, and speed of measurement are crucial.

4.6.1 Carcass classification system in South Africa

Changes occurred in the grading of lamb and mutton carcasses in 1981 (Bruwer et al., 1987). These changes were based on the preliminary investigations on lamb and mutton carcasses (Bruwer et al., 1984). The main difference between the carcass grading systems of 1972 and 1981 is that the 1972 system was merely a grading system while the 1981 system was a carcass classification system (Bruwer et al., 1987).

The South African carcass classification system is said to include the following: Carcasses are classified according to fatness in six different fatness classes (1- very lean, 2- lean, 3- medium, 4 – fat, 5- over fat, 6- excessively over fat) in each of the three age groups

(A age group-0 permanent to incisors (p.i); 1-6 permanent incisors and more than 6 permanent incisors).

Carcasses are divided in five different conformation classes in each of the three age groups:

1- emaciated; 2-flat; 3- medium; 4- round and 5- very round. Carcasses are then graded according to the classified principles in the different grades as shown in table below (Bruwer et al., 1987)

Table 1: The South African Meat Classification System

Grade	Fatness Class	Age Group	V3 (mm)2	Confirmation Class	Kg/cm[b]
Lamb 3,B3,C3	1	A,B and C	‹1.0	2-5	1=‹ 0.13
LAMB 1,B1,C1	2	A, B and C	1.0- 4.0	2-5	2=0.13- ‹0.10
Super lamb prime B and top C	3 and 4	A, B, and, C	4.1- 7.0 7.1- 9.0	2-5	3=0.16-‹0.18 4=0.18-‹0.19
Lamb2,B2 and C2	5 and 6	A, B, C	9.1- 11.0 › 11.0	2-5	5=0.19

(a) Measured between the third and the fourth lumber vertebrae, 25mm of the midline on he intact carcass.

(b) Cold mass as recorded divided by the carcass length as measured from the most distant end of the hind leg up to the lower surface of the neck next to the vertebrae.
(c) Exceptions- Carcasses with code 2 conformation and code 3 fatness have to be classified as grade 1: and code 2 conformation with code 4 fatness have to be cassified as grade 2 within the relevant age groups.

In their study on the evaluation of the lamb and mutton carcass grading system in South Africa, (Bruwer et al., 1987) indicated that, subcutaneous fat thickness measurements are useful predictors of carcass composition, these measurements, not only serve as a description of the deposition of the fatty tissue at the point where it is measured, but also as an indirect measure of the development of the fat as well as the muscle tissue in the whole carcass. The choice of most practical measurement is said to be more dependent on the precision with which the fat is measured. Measurement is expected to predict the carcass lean content (ii) the cost of predicting these prediction measurements as this will reflect ease, speed and accuracy with which the measurements can be recorded and the carcass depreciation involved (iii) the stability of prediction equations in indicating treatment differences or differences between the type of lamb being compared (Kempster, 1981; Hendrick 1987).

The objective of the Study by (Bruwer et al., 1987) was to find the most practical and accurate fat measurement for the prediction of carcass composition to be applied in a

classification of grading system. His results support the findings of Kempster & Cuthbertson (1977), Kirton & Johnson (1979) and Thomson & Atkins (1980) which states that a combination of fat measurements with carcass mass provided the best prediction of percentage carcass composition. (Bruwer et al., 1987) adds that the practical situation in the South African market presently does not allow the use of more than one fat measurement where sheep are slaughtered at 600 an hour on one line, It is further suggested that fat thickness measured between the third and the fourth lumber vertebrae, 25 mm from the midline of those fat thickness evaluated 'seems to be the best single measurement to be used in the classification system for prevailing conditions in South Africa.

4.7 Effect of growth and development on carcass quality

Two processes that occur during animal growth include (1) an increases in weight until mature size is reached (referred to as growth), (2) changes in body conformation and shape, and its various functions come into full being (referred to as development) (Hammond 1940 quoted by Lawrie, 1992).

The relationship between live weight and age is shown by an s-shaped curve and has similarities in sheep, cattle and pigs (Lawrie, 1992).

In the study of environmental and maternal effects on early postnatal growth, of lambs of different genotypes, (Peeters et al., 1996), demonstrated that birth weight, litter size, age of ewe, number of suckling lambs and sex of lambs have an important influence on early postnatal growth. This is supported by other workers (Ekje, 1972) who adds that these influences can cause remarkable differences in growth rate, they can be responsible for 50% and 40% of the variation, respectively in early postnatal growth and weaning weight.

4.7.1 Effect of birth weight on carcass quality

The factors that influence birth weight were studied in detail by Villette and Theriez (1981). It was found that as birth weight increased by 1 kg, an augmentation 39 ±1 of daily weight gain during the total lactation period (42) was observed. This increase is said to be related to an augmented intake of dry feed of heavier lambs, and not to a better feed conversion (Villete and Theriez, 1981), Literature data states that, higher birth weights and feed intakes, combined with more efficient feed conversions, seem to be responsible for superior growth results of ram lambs (Lativ and Owen, 1980, Galbraith and Topps, 1981). According to Casey, (1982), a one-way analysis of varience showed highly significant differences among the Doper, South African mutton merino and Boer goat. The highest average birth mass was recorded in the merino, followed by the Doper and Pedi respectively.

A study at Melbourne University suggests that carcass weight is the main factor affecting the composition of a carcass. As the carcass gets heavier, the proportions of muscle and bone decrease and the proportion of fat increases. The yield of saleable cuts from a carcass decreases as the carcass gets heavier, this is supported by Casey, (1981) who suggested that, composition is the most important characteristic of any carcass, while tenderness is the major parameter of eating quality of the meat, he also adds that the worth of a carcass depends on its yield of saleable or unsaleable meat.

4.7.2 Effect of sex on carcass quality

According to Fourie (1970), sex of an animal influences carcass composition. Sheep of different sexes and weights were dissected and it was concluded that ewe lambs are fatter than ram lambs, and the difference became greater as carcass weight increased, (table 2).

Table 2 Percentage carcass composition and muscle to bone ratio for ewe and ram lambs over a range of carcass weight. (from Fourie et al., 1970).

Carcass Weight (kg)	Sex	Muscle	Percentage Bone	Fat	Muscle/bone Ratio
5	Ram	58.2	14.9	16.3	3.91
	Ewe	57.6	14.1	17.5	4.09
10	Ram	56.5	12.4	21.3	4.56
	Ewe	54.3	11.1	24.5	4.89
20	Ram	54.8	10.3	28.0	5.32
	Ewe	51.2	8.7	34.2	51.8
30	Ram	53.8	9.2	33.9	5.85
	Ewe	49.5	7.6	41.6	6.51

It is said that when comparison is made at the same weight, genotypes which are heavier at maturity generally grow faster, contain less fat and more protein and bone in their whole bodies and carcasses than do animals of smaller mature size (McCelland, et al., 1976, Searle and Griffiths, 1976 a, b Thompson et al., 1979, Wood et al., 1980,Theriez, et al., 1981). Griffiths (1976a) observed that the differences due to sex do not become apparent until the commencement of the fattening phase of growth and also that, the rate of change in body fat with body weight during both the

pre-fattening phases of growth were the same for the entire males and females, but the transition to the fattening phase was greater in males.

4.8. Factors affecting mutton/lamb flavour

According to Missek et al., (1976), aroma and flavour are most important in determining palatability of cooked lamb. The aroma and odour of meat are the sensory quality attributes ascribed to the perception of certain volatile substances by the olfactory organ (Cross et al., 1986).

Flavour is described as a complex sensation obtained from the combination of the olfactory and gustatory attributes perceived during tasting, which may be influenced by tactile, thermal and even kinaesthetic effects (Ford & Park, 1977).

A major portion of the characteristic odour/aroma of lamb, when heated, is said to be contributed by carbonyl compounds, these flavour compounds, or their precursors are apparently present only in trace amounts in the fat (Vesely, 1971). The South African consumer is said to be unique, in respect to the red -meat consuming world, in that these consumers are prepared to pay appreciably more for sheep meat than for either beef or pig meat on a market where there is usually a shortage of both lamb and mutton (Schonfeldt et al., 1993). It is deduced that species with specific flavour of sheep meat is not considered less desirable by the South African consumer.

Consumers in New Zealand are also said to find the odour and flavour of lamb attractive (Wood & Fisher,1990). In a study comparing the tenderness and flavour of goat and sheep meat, (Schonfeldt et al., 1993) found that according to the sensory panel *m. longissinuss thoracis et laborium* and *m. semi-membranous* cuts of sheep carcasses were significantly more tender than those of Angora and Boer goat carcasses. This difference in tenderness was further supported by the sheer force measurements.

Other workers have also reported similar differences. Kirton (1970) found meat of New Zealand feral goats to be significantly less tender ($P \leq 0.001$) than that from sheep. Smith et al 1974 reported that meat from Angora and Spanish goats was less tender than lamb beef or pork.

These results are similar to those of Heinze et al., (1986). These workers have reported the muscle Collagen content of the South African mutton merino. Significant differences ($P \leq 0.05$) in muscle Collagen solubility were found between breeds. The muscle Collagen of the South African mutton Merino was significantly more soluble than that of the Pedi and Dorper sheep breeds, with the Boer goat and Merino.

4.9 Influence of the age of the animal on meat flavour

Age according to Sink and Caporaso (1976), it is a generally accepted fact that meat flavour intensity increases with chronological age. Hammond (1932) observed in sheep that muscles of older animals are more highly flavoured than those of younger ones .On the contrary Schonfeldt et al., (1993) found that the results of the sensory evaluation regarding the *m. longissimus thoracis et lumborum* cuts, age had no effect ($P \leq 0.05$) on either the species specific flavour, aroma, or general flavour. Crouse, (1983) ascribed the variation found in the lamb flavour age association, to the interactions of age with breed, sex condition or diet.

4.10 Influence of long-chain fatty acids on carcass and meat quality

Fatty acids are the most important lipid fraction .It is known that linolenic acid is essential for almost every animal species at a dietary level of about 1% of energy, although there are some insects and protozoa that are able to produce this fatty acid (Gurr & Harwood, 1991). Fatty acids have a particular role in the immune function, prevention of inflammation and as energy sources (Egan 1976; Wan et al., 1989). Schmidt, (1994) further indicated that unsaturated fatty acids, linolenic, linoleic, and arachidonic appear to be essential in reducing the occurrence of coronary diseases, rheumatoid arthritis, hypertension and cancer. The 18 carbon acids cannot be made in the body and must be supplied by foods.

A limited consumption of both saturated and unsaturated fatty acids is recommended by the National Cholesterol Education program (1988).

The quality and composition of ruminant fat may be affected by nutrition, in particular the kind and nature of the cereals and the kind of presentation of roughage (Orskov et al., 1974; Duncan et al., 1974; Miller et al., 1980; Casey & Van Niekerk, 1985; Cazes et al., 1990). In their study, Webb et al., (1993), suggested that a high-energy treatment significantly changes the fatty acid profiles of subcutaneous fat in wethers otherwise fed a medium energy diet. They observed significant increases in the concentration of C15: 0, C17:0, C17: 1 and C18: 1 in the subcutaneous fat of wethers fed the H- diet, while that of wethers fed the M- diet contained significantly higher concentrations of C16:0, C18:0, C18: 2, and C18: 3.

Chapter 5

EFFECT OF ELECTRICAL STIMULATION ON TENDERNESS AND CHEMICAL COMPOSITION OF CLASS AB- SHEEP CARCASSES

Abstract:

In this study, the effect of electrical stimulation on meat tenderness, fatty acid profile and consumer acceptance of class AB- lamb was investigated. Amongst all meat quality characteristics, tenderness is considered by consumers to be the single most important component of meat quality. Electrical stimulation of the carcass shortly after slaughter reduces cold shortening and provides an alternative method of increasing meat tenderness. A total of 22 wethers of class AB weighing between 45 and 50kg, were selected from a homogeneous group of Dorper wethers. The carcasses were assigned to two treatment groups, of which group one was electrically stimulated (denoted as ES) and the other group was not electrically stimulated (NES). The results revealed that electrical stimulation does not have a significant effect on the fatty acid composition of the meat or crude fat content. Treatment however, significantly ($P \leq 0,038$) affected the moisture content of the samples. There was a variation in shear force values between the two treatment groups; samples from the ES group were less compared to that of the NES group. This suggests that ES can be successfully applied to reduce the variation in tenderness within the class- AB mutton.

5.1 Introduction

The texture of meat is of outmost importance to consumer acceptance as a result, much research effort has been put into this issue in order to be able to control and understand it (Tonberg, 1996). According to (Swatland, 1981), electrical stimulation was historically developed as a means to accelerate post-mortem glycoysis so that muscle entering rigor would be prevented from shortening excessively, shortening occurs when action potential is transmitted from he sarcolemma to the interior of the fibre along the T tubles. It causes bound calcium to be released from the sarcoplasmic reticulum into the sarcoplasmic fluid. Shortening phenomena, resembling contraction in the living animal, can be observed in post-mortem muscle before and during rigor development. These contractions can cause a marked toughening of meat, or loss of natural meat juices (Forest et al., 1975). The potential effects of electrical stimulation on post- mortem muscle are described as either physical disruption of the myofibrillar matrix (Ho et al., 1997) or the acceleration of proteolysis (Uytterhaegen et al., 1992). Consensus has not been reached as to which of these effects is important in reducing the toughness of meat. Electrical stimulation can also be used to reduce the percentage of meat in a population that would otherwise have been unacceptable in tenderness (Lee et al., 2000). There are numerous other benefits of electrical stimulation, which include: increased shelf life, bright red (crimson) colour and reduced bacterial growth (Menday, 1979).

Meat tenderness can be measured objectively (using instruments) or subjectively (sensory analysis e.g. taste panel) Webb and Bosman, (2003). It is noted that tenderness assessment by laboratory instruments can, however, not match the complex and multifaceted actions that occur during biting and chewing (Perry, 2002). On the other hand, Combes et al., (2003) regards tenderness assessment using a taste panel as time consuming.

This study was carried out to determine the effect of electrical stimulation on the tenderness of class AB-mutton.

5.2 Materials and methods

A total of twenty-four Dorper wethers weighing ca. 45kg were used in this study. The sheep were slaughtered in a commercial abattoir by severing the jugular vein, suspended from the hind legs and dressed down. The carcasses were randomly allocated to two treatments, i.e. electrically stimulated (ES) and non- electrically stimulated (NES). The carcasses were clearly marked to indicate ES or NES. Twelve wethers were subjected to the ES procedure and twelve wethers the NES procedure immediately post-mortem. The carcasses in the ES treatment were electrically stimulated with alternating current (ca. 20 v, 45 Hz, 45 s) and chilled for 24 h at 2 °C. The initial carcass temperature and pH at the beginning of cold storage was ca. 30 °C

and pH 6.8 respectively. The result was a decrease to an average carcass temperature of 3 °C and pH of 5.8 within 12 hours post-mortem.

5.2.1 Sampling and Preparation

Samples that were reserved for shear force determination were removed from the cold room and thawed (2°C) for 1h. Chops taken from the longissimuss et lumborum, were roasted on a metal tray at an oven temperature of 180°C to an internal temperature of 73°C (monitored with thermocouples), according to the procedure of Riley et al. (1981). Total cooking loss was determined according to procedure described by (Webb, Bosman & Casey, 1994).

5.2.2 Chemical analysis

Samples for the chemical analysis were prepared from the right side of each carcass. The frozen meat samples were thawed and used to determine % dry matter. The freeze-dried samples were ground homogenised, and then bottled and stored until required for analysis of the percentages of protein (%CP), water (%W), fat (%F) and the profile of medium and long-chain fatty acids.

5.2.3 Dry matter determination

Percentage dry matter (%DM) was determined by means of the standard procedure (AOAC, 1990) on thawed meat samples. We used 10g of meat sample and weighed it into a pre weighed cubicle dish. The samples were consequently incubated in an oven at 100°C overnight. The crucible dishes with dry matter were cooled in a desiccator, and the weights recorded.

The weights of the crucible dishes with ash were determined. The formulae used to determine %DM was as follows.

%DM = Mass of dried sample/Mass of fresh sample ×100

5.2.4 Crude Protein determination (Kjeldahl Technique)

The standard Kjeldahl procedure (AOAC, 1990) was used to determine the protein content of samples. A sample of 0.5g of freeze-dried meat was weighed into a Kjeldahl flask. Potassium sulphate together with a pinch of selenium, glass beads and 25ml sulphuric acid were added to the flask which was then fitted to the Kjeldahl digestion rack and digested for 1 hour. The mixture was then cooled and 350 ml distilled water, 100ml sodium hydroxide (NaOH) and beads of metallic zinc were added and the flasks coupled to the distillation apparatus. The discharge tubes were

submerged in the boric acid/ indicator solution, which was contained in a 500ml Erlenmeyer flask. The colour of boric acid was blue and as distillation continued, a green colour was assumed. Distillation continued until the volume in the Erlenmeyer flask reached 200ml. The solution was then titrated against 0.714 M sulphuric acid to a blue colour that marked the end point.

The titre volume was reached. The percent nitrogen was calculated using the formula below.

% N = B (ml) ×M× 14 × 100/ 1000 × weight of sample

Where: B= the titre volume minus blank titre

Volume M = the molarity of the acid used.

The following formula was used to calculate the protein content:

% Crude protein =% N × 6.25

5.2.5 Crude fat determination (Soxhlet method)

The fat content was determined on freeze-dried samples using the soxhlet (AOAC, 1984) method. Flat-bottomed soxhlet flasks were cleaned, labelled and dried in the oven at 100°C over night. The following morning the flasks were cooled in the dissector and their weights were measured and recorded. Approximately 2g of sample material were weighed onto a filter paper. The sample was rapped and pushed into a numbered thimble. Exactly 375ml petroleum ether was added. The extraction took 16 hours. The amount of extractable fat was determined by incubating the flask over night in an oven pre heated to 105°C to allow all the traces of ether to evaporate followed by cooling and weighing the flasks.

The following formula was used in the soxhlet method.

% Ether extract = Mass of fat × 100/Mass of sample

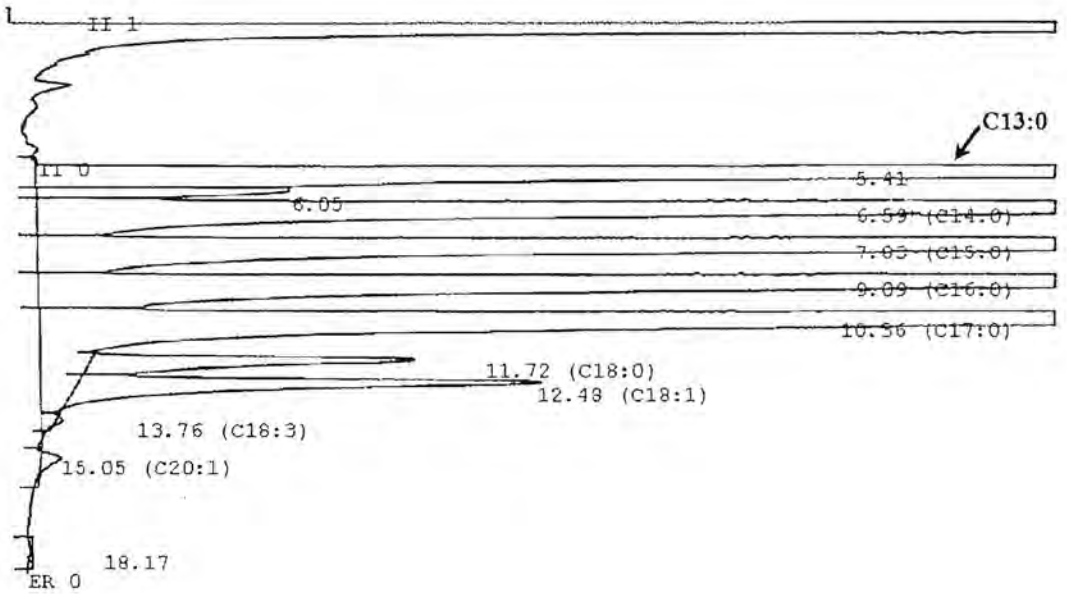
5.2.6 Fatty acid Profile

Here, freeze- dried sample material was used to analyse the composition of fatty acids. The method includes three stages, the extraction stage, esterification (preparation of methyl esters) and gas chromatography. One gram of sample material

was weighed into an Erlenmeyer flask. Ten millilitres of chloroform ($CHCl_3$) and Butylated Hydroxytoulene (2.6 DI- tert- BUTYL-P- CRESOL) (0.1g Butyl Hydroxytoulene dissolved in 100 ml chloroform) was added to the flask. Butyl Hydroxytoulene was included as an antioxidant.

The flasks were shaken vigorously and stored at 4°C overnight. The clear liquid was separated and transferred to a test tube. The methyl esters of the fatty acids were prepared by adding 1ml NaOH/methanol solution, 5 ml sample chloroform and 0.5 ml sample extract to a centrifuge tube (AOAC, 1975). The mixture was shaken to mix and heated in a water bath for 30 minutes at 55°C after which it was allowed to cool. The cold mixture was centrifuged for 15 minutes at 5000 rpm using a Beckman model TJ-6 centrifuge. The clear supernatant (isolated esterified lipid) was separated and refrigerated until required (within two days) for subsequent fatty acid analysis on a Varian 3300 gas chromatograph (see Figure 2 below).

Figure 2



Long-chain fatty acid standard (from Webb, 1994)

5.3 Results and discussion

Sensory Samples tested

Data for the samples was categorical data. The chi-squared method was used to analyse the data. It was measured on a scale of 1-5 between two treatments ES and NES.

There was no significant difference in juiciness between treatments. Juiciness is often associated with increased slaughter weight and carcass dressing and is also dependent on the proportions of unsaturated fatty acids (Webb et al, 1994). There were no significant differences in tenderness. The results suggest that ES does not have a positive effect in terms of the variation in meat tenderness. This was partly because the muscle used was more tender and also because low voltage electrical stimulation was utilised which did not significantly improve the tenderness of meat samples, this is emphasised by other authors (Epley, 2002; Webb, 1998). It is however possible that tenderness of other muscles will be affected more positively by ES as indicated by (Bray et al., 1994). There were no significant differences in flavour between ES and NES. Frequency was significantly affected by treatment.

Table 4: Long-chain fatty acid content of loin samples *(m. longissimus thoracis et lumborum)*

Fatty acid	ES (n=6)	NES (n=6)
C14:0	0.27 ± 0,281	0.24 ± 0,215
C16:0	14.6 ± 1,523	14.4 ± 1,73
C16:1	5.2 ± 0,346	4.4 ± 3,533
C17:0	2.4 ± 1,419	0.2 ± 0,210
C18:0	10.7 ± 0,923	9.6 ± 1,896
C18:1	38.7 ± 2,232	41.3 ± 0,944
C18:2	22.0 ± 6,409	20.1 ± 4,431
C20:0	4.4 ± 3,641	6.2 ± 5,628
C18:3	0.9 ± 1,669	2.8 ± 4,892
C20:4	0.2 ± 0,259	0.3 ± 0,415

Test between subjects showed no significant treatment effects on the composition of fatty acids in samples of the *m.longissimus thoracis et lumborum.*

The most abundant fatty acids present in the loin samples of sheep in proportions higher than 10% were c16: 0, c18: 0, c18: 1, and c18: 2 respectively. Similar results were reported by (Steenkamp, 2000; Schwagert and Price, 1987).

The table reveals that linolenic acid (c18: 2) was present in highest proportions in the *m. longissimuss et lumborum* in this experiment. The extent of saturation was high in all samples that were analysed, polyunsaturated acids showed lowest mean values. Low saturated to polyunsaturated ratio or high oleic content is reported to be significant in reducing the risk of cardiovascular diseases (Martin et al., 1999). Treatment (ES) had a tendency to affect the c17: 0 as depicted in the analysis. Webb et al., (1994) suggests that a higher gravimetric concentration of oleic acid C18: 1 in the subcutaneous fat may intensify the aroma of lamb. The characteristic mutton aroma is reportedly associated with volatile acidic components, which include even numbered fatty acids containing 6-12 carbon atoms and branched chain acids (Wong et al., 1975). It is also noted that breed differences affect the molar concentration of saturated fatty acids and were limited to margaric acid C17: 0 and heptadecenoic acid C17: 1 (Webb & Casey, 1995). Dorpers tend to contain slightly higher proportions of unsaturated fatty acids in comparison with the later maturing SA Mutton Merino breed. A greater proportion of C14: 0 is also associated with lower initial juiciness scores. Webb et al., (1997) also found that increasing concentrations of total fatty acids in the adipose tissue may also result in greater total cooking loses. A relation was also noted between the proportion of carcass fat, the thickness of subcutaneous fat and the proportion of myristic acid C14: 0, palmitoleic acid C16:1 and stearic acid C18:0.

Table 5: Proximate composition of samples from the m. *longissimus thoracis et lumborum*

Treatment (n=6)	Crude fat (ether extract) (CF)	Crude Protein (CP)	Moisture Content
ES	8.9±3,91	26.8±0.693	82.1±1.105
NES	7.8±3,86	27.6±0.536	85.5±1.528

Treatment did not significantly affect the crude fat content of m. *longissimus thoracis et lumborum* samples for sheep in this experiment. Treatment significantly affected (P>0.038) the moisture content of the samples.

Table 6: Muscle pH (pH_{45} ; pH_4 P; pH_u ; measured in *m. longissimuss lumborum*) and shear force values (measured on an Instron apparatus in Newtons)

Treatment (n=11)	Shear Force (N)	pH_{45}	pH_4	pH_u
ES	32.6±5.03	6.2±0.282	6.1±0.506	6.0±0.543
NES	37.7±10.57	6.3±0.0.021	6.2±0.636	5.9±0.585

5.4 Conclusion

It is concluded that there were no significant differences between ES and NES from the class AB-mutton. Variances between standard deviations indicated that the variation in tenderness within ES samples were significantly less when compared to the NES samples. It is therefore apparent that ES can be applied in older animals or within the same age group to reduce possible variation in tenderness influenced by factors such as age, animal history, and nutrition. ES can be applied successfully to reduce the variation within the class AB- mutton group.

GENERAL DISCUSSION AND CONCLUSIONS

6. General discussion and conclusions

It was noted that electrical stimulation was able to reduce variation in tenderness within the class AB mutton. This was indicated in that the variation in tenderness within the ES samples was significantly less when compared to the NES samples.

It is apparent that ES can be applied in older animals or within the same age group to reduce possible variation in tenderness as influenced by factors such as age, sex, and nutrition.

Electrical stimulation can increase shelf life, reduce bacterial growth and also enhance the red colour of meat, it can also be applied successfully to reduce the variation within the class AB- mutton group.

Supplemental feeding of vitamin D_3 $5x10^6$ IU significantly improved the tenderness of meat from steers fed 0,15 mg Zilmax®/kg. This suggests that β-agonists can be used successfully in combination with supplementation of vit D_3 to improve both tenderness and lean content of animals.

Chapter 7

7. Critical evaluation

It was observed that electrical stimulation did not significantly improve tenderness in mutton samples, it however improved the variation in tenderness in the class AB-sheep. This will assist this class of sheep to obtain a better scoring in the South African sheep classification system and also gain a better perception from consumers. On the other hand it was noted that adding vit D_3 to feed of steers, improved tenderness significantly, it should also be noted that β- agonists improve the lean in beef. Caution must be taken that a balance should be reached since fat is also positively associated with acceptability. Fat improves the aroma and juiciness of meat samples.

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