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Influence of conventional and Kosher slaughter techniques in cattle on carcass and meat quality

By

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DECLARATION

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

Babatunde Agbeniga
November, 2011

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DEDICATION

To my loving wife, Nancy and children, Shola and Victoria Agbeniga

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LIST OF ABBREVIATIONS

ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
ASPCA	American Society for Prevention of Cruelty to Animals
ACTH	Adrenocorticotrophic hormone
BL	Blood loss
BLT	Blood in the trachea
BS	Blood splash
BSE	Bovine spongioform encephalopathy
BVA	British Veterinary Association
BRBTF	Bright red blood tinged foam
C	Conventional
CST	Conventional Slaughter Technique
Ca^{2+}	Calcium ion
CL	Cooking loss
CP	Creatin phosphate
CPK	Creatin phosphokinase
CRF	Corticotropic releasing factor
DCB	Dark cutting beef
DFD	Dark firm dry
DL	Drip loss
dv	Dependent variable
ES	Electrically stimulated
EU	European Union
ECF	Exracellular fluid
FC	Fat code
FFA	Free fatty acid
FVE	Federation of Veterinarians of Europe
G	Gender
GLDH	Glutamate dehydrogenase
HPA	Hypothalamic- pituitary-adrenal axis
hr	hour
ICF	Intracellular fluid
ISF	Interstitial fluid
iv	Independent variable
LSD	Least significant difference

LWCC	Livestock Welfare Coordinating Committee
K	Kosher
KST	Kosher Slaughter Technique
kg	Kilogram
NES	Non-electrically stimulated
P	Phosphorus
PCV	Packed cell volume
pm	*Post mortem*
PSE	Pale soft exudative
SCF	Subcutaneous fat (thickness)
SM	Slaughter method
SR	Sarcoplamic reticulum
TCA	Tricarboxylic acid
TBW	Total body water
TF	Transcellular fluid
VER	Visual evoked response
VIP	Vasoactive intestinal peptide
vs	Versus

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ABSTRACT

The aim of this study was to evaluate and compare the influence of conventional and Kosher slaughter techniques in cattle on carcass and meat quality parameters. The conventional slaughter was done using a pneumatic captive bolt gun to stun the animals before sticking, while Kosher slaughter was done by sticking the animals and then stunning them with a 0.22 calibre cash special captive bolt gun, 20 seconds later. The animals (n=311) were randomly assigned into 4 treatment groups, namely; slaughter method (SM) (main group, sub-divided into: conventional slaughter technique (CST) and Kosher slaughter technique (KST)); electrical stimulation (ES), sub-divided into: electrically stimulated (ES) and non-stimulated (NES); gender (G), sub-divided into: male (M) and female (F); fat code (FC), sub-divided into: FC-2 and FC-3 (i.e. lean to medium fatness). The quality attributes evaluated were blood loss (BL), drip loss (DL), cooking loss (CL), presence of blood in the trachea (BLT), blood splash in the lungs (BS), shear force (SF), colour (L^*, a^* and b^*), pH and temperature profile over 24 hours and the effect of subcutaneous fat thickness (SCF).

Animals were mainly steers in the "A" age group with an average slaughter weight of about 400 kg. Different crossbreeds of Bonsmara, Brahman and Nguni cattle were used, which is typical of cattle slaughtered in South Africa. Blood loss, blood in trachea and blood splash were evaluated using 311 animals (CST, n=141; KST, n=170) but for the other parameters, smaller numbers were used because of the hectic nature of the trials. Evaluation of BL, BLT and BS were done in the abattoir and pH and temperature readings were also taken at 45 mins, 3, 6, 12 and 24 hrs *post-mortem* between the 10^{th} and 11^{th} rib on the *m. longissimus dorsi* which was where the carcasses were sampled for the meat quality analyses.

The results of this study revealed that there was no significant difference ($p > 0.05$) in % blood loss between the conventional and the Kosher group but the conventionally slaughtered group had a slightly higher bleed-out. Fat code had a significant ($p = 0.0475$) influence on %BL, with FC-2 (2.42%) group bleeding out slightly more than FC-3 (2.24%). For % drip loss, there were no significant differences ($p > 0.05$) between all the treatment groups except for fat code ($p = 0.0242$), with FC-3 (2.95%) samples exuding more water than FC-2 (2.42%) meat samples. In terms of % cooking loss, there was a significant difference ($p = 0.0004$) between the slaughter methods, with meat samples from the conventional method (22.11%) exuding more water than the meat from Kosher group (18.16%). For blood in trachea, there was a highly significant difference ($p < 0.0001$) between the slaughter methods, with the Kosher-slaughtered animals having significantly more blood in the tracheas than the conventional group. Similarly, for % blood splash, the Kosher-slaughtered group also had a significantly ($p < 0.0001$) higher amount of splash than the conventional group. In terms of shear force, analyses showed significant difference between the SM with the Kosher meat (42.99) appearing more tender ($p = 0.0005$) compared to the meat from conventionally slaughtered animals (53.54). Electrical stimulation also had a significant influence, with meat from the ES group (43.27) being significantly ($p < 0.0001$) more tender compared to the NES group (61.15). For subcutaneous fat thickness, there were no significant differences ($p > 0.05$) in all the treatments save for fat code, with FC-3 group (6.38) having thicker fat cover ($p = 0.0004$) than FC-2 (4.44) which was anticipated.

In terms of colour, there was a significant difference ($p< 0.0001$) between slaughter methods for the L^* value (lightness). Meat samples from the Kosher-killed animals (46.08) were lighter than the conventionally killed ones (35.40). Samples from the females (48.29) were also significantly lighter ($p= 0.0057$) than the males' (37.79). For a^* value (redness), there was a significant difference ($p< 0.0001$) between the slaughter methods. The conventional group meat (15.58), were redder than the Kosher meat (10.40). Gender effect was also significant ($p< 0.05$), with meat from the males (13.81) appearing redder than the females' (11.25). For b^* value (yellowness), significant difference ($p< 0.0001$) was also found between the slaughter methods. The Kosher meat samples (-6.49) appeared yellower than those from conventional slaughter (0.26). FC-3 samples (-2.36) were also significantly ($p= 0.0112$) yellower than the FC-2 samples (-3.05). For pH, analyses revealed a significant difference ($p< 0.0380$) at 45 minutes *post-mortem* (*pm)* between slaughter methods. The kosher carcasses (pH= 6.43) had a slightly higher pH compared to the conventionally slaughtered carcasses (6.33). The ES carcasses (pH=6.16) also had a significantly lower ($p< 0.0001$) pH compared to the NES carcasses (pH=6.89). At 3hrs *pm*, only electrical stimulation showed a significant influence ($p< 0.0001$). The ES carcasses (pH= 5.72) were lower than the NES group (pH= 6.49). At 6hrs, only electrical stimulation still showed a significant influence ($p< 0.0001$), with the ES carcasses (5.56) still having a faster decline compared to the NES group (pH= 6.01). At 12hrs, the ES group (pH= 5.61) still had a significantly ($p= 0.0008$) lower pH than the NES group (pH= 5.82). At 24hrs, only the slaughter method showed a significant influence ($p= 0.0314$) in carcass pH, with the Kosher carcasses (pH= 5.53) having a slightly lower pH compared to the conventionally slaughtered ones (pH= 5.56). The latter difference is probably not of any practical significance and could be ignored.

Temperature at 45 minutes *pm* showed a significant difference ($p= 0.0248$) between the slaughter methods with the carcasses from Kosher slaughter (36.50^0C) having a slightly lower temperature compared to those slaughtered conventionally (37.22^0C). At 3hrs *pm*, the carcasses from Kosher slaughter (30.06^0C) had a significantly higher ($p= 0.0005$) temperature than the conventional group (27.05^0C). The female carcasses (31.26^0C) also had significantly higher ($p< 0.05$) temperature compared to the male carcasses (27.89^0C). The FC-2 carcasses (28.19^0C) also had a significantly lower temperature ($p= 0.0149$) compared to FC-3 (30.11^0C) which was anticipated due to lower temperature decline in those with lower subcutaneous fat. At 6hrs *pm*, the conventionally slaughtered carcasses (14.71^0C) still showed a significantly faster decline ($p< 0.0001$) compared to those slaughtered by Kosher (20.16). FC-2 carcasses also showed a significantly ($p= 0.0104$) lower temperature (16.68^0C) compared to FC-3 (18.74^0C). At 12 hrs *pm*, the conventionally slaughtered carcasses (4.72^0C) still had a significantly ($p< 0.0001$) lower temperature compared to the Kosher group (10.24^0C). The FC-2 group (6.69^0C) also had a significantly ($p= 0.0011$) lower temperature compared to the FC-3 group (8.89^0C).
Finally at 24 hrs after slaughter, slaughter method still showed a highly significant influence ($p< 0.0001$) with the conventionally slaughtered carcasses having a much lower temperature (-0.42^0C) compared to the Kosher group (3.06^0C). The male' carcasses (0.62^0C) also had significantly lower ($p< 0.05$), ultimate temperature compared to carcasses from the females (2.99^0C). There was also a significant difference ($p< 0.05$) between the fat codes, with the FC-2 carcasses (1.01^0C) showing a lower temperature, compared to the FC-3 (1.71^0C) carcasses.

CHAPTER 1

INTRODUCTION

The problem of slaughtering of animals for food by various methods currently used in slaughterhouses, including the ritual slaughter (Shechita or Halal) has been discussed in many countries for more than a hundred years. It is still a topic surrounded by many controversies since no ideal method has been found to date (Levinger, 1995; Shragge *et al*., 2004).
These problems can be grouped into physiological, psychological, hygienic and technological problems and during the last eighty years, different systemic studies of this subject have been carried out (Miller, 1952; Levinger, 1995).

The purpose of this research was to evaluate and compare the influence of the conventional slaughter technique (using captive bolt stunner) and Kosher slaughter technique (Shechita) in cattle on carcass and meat quality parameters.
Various attacks have been launched on Shechita from the welfare and meat quality perspectives and efforts have been made to render it illegal in many countries like Great Britain, Spain, France, Germany and Denmark. Countries like Switzerland, Sweden, Norway and most recently, The Netherlands do not allow religious slaughter on conscious animals. All animals must be stunned before being bled while countries like Australia, Finland and New Zealand permit religious slaughter provided they are stunned post-cut (Levinger, 1995). In South Africa, the approach was to modify the technique rather than ban it. The major reason for these controversies is based on the welfare aspect of the slaughter of these animals (Gregory, 1998). At present in Europe, regulations are now being proposed and the European parliament's Agriculture and Rural Development Committee has voted in favour of plans to tighten up and improve on rules governing animal welfare at slaughter (The Veterinary Record, 164: 382 (2009)). Also in South Africa, there has been a long contention between the Livestock Welfare Coordinating Committee (LWCC) and the Jewish body for Kosher (Beth-din) about the manner in which animals are Kosher-killed in rotating box and the time of the post-cut stun which is presently 20 seconds. The LWCC is advocating that all Kosher animals (cattle) be slaughtered in the ASPCA pen with chin lift, rear pusher and belly plate, in the upright position and the time of the post-cut stun should be immediate (4 seconds) to reduce the pain of the animals (LWCC, 2010 unpublished; NSPCA, 2007; www.nspca.co.za). Part of this reason emanates from new evidences that these animals (cattle) do experience pain and distress for up to 60 seconds or longer after the incision, before they become unconscious (Mellor *et al*., 2009).

In the conventional slaughter method, a captive bolt gun is used to stun the animal before the cut and in Shechita, the animal's throat is cut without being stunned but a post-cut stun is administered 20 seconds after the cut (Protection of Animals Amendment Act, No 7 of 1991).

The parameters that were evaluated are: blood loss; pH and temperature profile of the carcasses within 24 hours after slaughter; water holding capacity in terms of drip loss and cooking loss; shear force which indicates tenderness; meat colour in terms of lightness (L), redness (a), and yellowness (b); presence of blood in the trachea, upper bronchi and lungs; presence of bright red blood-tinged foam in the trachea due to blood aspiration after the cut; presence of blood splash in the lungs and effects of subcutaneous fat thickness. Subcutaneous fat thickness was measured because of its possible effect on carcass chilling rate which can also affect enzyme activities in meat and meat tenderness (Smith et al., 1976). The effects of gender, electrical stimulation of carcasses and carcass fat code on these quality attributes were also evaluated and compared between the two slaughter methods.

This research was necessitated by the growing trend in consumer awareness of the type and quality of beef they consume and the growing awareness of the welfare aspects and humanness of slaughter of animals and the relationship between these two factors, which also has economic implications. Most of this awareness is set off by animal welfare pressure groups (Gregory, 1998).

Animal welfare is fast becoming a quality issue in the sense that some retailers are now imposing animal welfare standards in their product specifications for suppliers. The retailers want to have a "caring image" for animals for the companies'customers. Some of the retail outlets and major supermarkets are now setting a new standard on animal welfare within the market. These specifications on welfare and product quality by these supermarkets and retailers are aimed at securing their business and their economic benefits (Gregory, 1998).
Also in South Africa, the quality of meat has received much attention over the past decades. Viewing it from the producer's angle, much money is at stake in supplying good quality and consistent meat products to the market by the purchase and repurchase of products (Marais, 2007). Also from the consumer's angle, there is a growing trend of satisfying consumer preferences in supplying good quality meat with the best consistent eating qualities (Strydom, 1998).

In terms of the meat and carcass quality parameters, a number of reports also emphasize the importance of investigating these meat quality attributes. According to Miller (1952) and Levinger (1995), the gasping and the deep respiratory movement and muscle contraction after Shechita makes exsanguination better. They claim that the muscle contraction occurs towards the end of the bleeding, forcing the remaining blood out of the blood vessels. However, the work of Anil *et al*. (2006) on cattle by Halal without pre-stunning compared to the ones pre-stunned by captive bolt showed no significant difference in blood loss.
The study of Immonen *et al*. (2000) also reported lower shear force values in meat of high and intermediate glycogen contents compared to meats of low glycogen content, which could be caused by Shechita. Petty *et al*. (1994) also reported that plasma catecholamines were significantly higher in Kosher-killed cattle compared to the conventionally slaughtered cattle. The implication is that, meat from Shechita has the tendency to be tougher with high pHu, high cooking loss and darker colour, because of

the pre-slaughter stress (O'Neill, Webb, Frylinck and Strydom, 2006). Palatability could also be poor (Viljoen, De Kock and Webb, 2002). Several researchers have also found that high drip loss is due to rapid pH decline (Stoier, Aaslyng, Olsen and Henckel, 2001). The study of Onec and Kaya (2004) shows no significant difference in water holding capacity between groups of captive bolt stunned, electrically stunned and non-stunned cattle. They reported that the captive bolt stunned cattle are superior in terms of odour, flavour, chewiness and tenderness.

Concerning temperature, the findings of Veary (1991) on sheep shows that muscle temperature of stunned sheep were found to be lower than that of non-stunned sheep, but reports that there were variations in environmental temperatures. Lawrie (1966) and Troeger (2003) also reported that a combination of high initial muscle temperature and a low initial pH will result in protein deamination which reduces meat quality.

A recent study by Gregory *et al*. (2008) showed that Kosher-slaughtered cattle had blood in their respiratory tracts due to suffering from airway irritation and fine bright-red blood -tinged foam in their tracheas compared to the conventionally slaughtered cattle group.

Grandin and Regenstein (1994) also reported that blood splash is a severe problem in animals slaughtered by Kosher technique because of agitation and too much pressure on the body from belly lift and rear pusher especially in grain fed cattle.

All these calls for a comprehensive evaluation of slaughter methods of cattle since it will help us to better understand the ideal ways of slaughtering in order to produce the best quality meat and carcasses while observing the best welfare practices. It will also help South Africa to conform to international standards as far as meat production and quality are concerned.

CHAPTER 2

LITERATURE REVIEW

2.1 Slaughter

This could simply be described as the killing and bleeding out of animals. In the red meat industry, it is called sticking and in the poultry industry, it is called neck cutting. The scientific term for slaughtering is called exsanguination (Gregory, 1987). It is generally agreed that the two essentials in slaughter of food animals are that, they shall be dispatched without unnecessary suffering and that the bleeding of the animal shall be as complete as possible (Thornton, 1968; Swatland, 1984).

2.1.1 Purpose of bleeding: The purpose of bleeding is to remove its blood and to kill the animal with the aim of deflecting the blood away from the brain to stop the delivery of oxygen (Gregory, 1998). Bleeding is a major requirement for eating and keeping qualities and meat that is improperly bled can have unpleasant appearance and is an excellent medium for the growth of micro-organisms (Lawrie, 1966; Swatland, 1984). Good bleeding is ensured if an animal is healthy but is retarded in animals with heart, lungs and muscle infections, febrile conditions, severe indigestion and so on (Thornton, 1968).

2.1.2 Synopsis of bleeding: In cattle and sheep, bleeding is mostly effected by severing the carotid artery and the jugular vein, and in pigs by severing the anterior vena cava but if the knife penetrates too far, blood may collect beneath the scapula and cause taint by early decomposition (Thornton, 1949). Studies from humans have shown that when blood flow to the brain is arrested by occluding both carotid arteries, the time to loss of consciousness is on average of 7 seconds (Rossen *et al*., 1943). This may be preceded by a glowing sensation in the head, tingling sensation in the hands and feet, blurring of vision and mild respiratory stimulations.

When both carotid arteries of sheep are severed, the time to loss of consciousness is about 6 seconds if the animal had not been stunned. The time to loss of visual evoked responses (VERs) is 14 seconds. If only one carotid artery is cut, the time to loss of VERs is five times longer (70 seconds), and if instead, only the jugular veins in the neck are cut, it takes about 5 minutes for the VERs to disappear (Gregory and Wotton, 1984a).

When the jugular vein, which is the venous return from the brain is cut instead of arterial supply, the animal dies slowly by venous heamorrhaging and circulatory collapse. Pigs are bled with a chest stick and this aims at severing the common brachiocephalic trunk which gives rise to the carotid arteries. Cattle and calves are also bled by cutting the brachiocephalic trunk using a chest stick (Leigh and Delaney, 1987).

Sticking is usually done with the aid of sharp metal objects such as knives, in the red meat industry or by neck cutting machines in the commercial poultry industry. The metal of the sticking knife is usually about 40cm long for cattle, 25cm for sheep and about 12cm for poultry. The amount of blood obtained from a slaughtered sheep is about 4 to 5

pounds while that of cattle is on the average of 30 pounds but cows yield more blood than bullocks and in some cases, goes up to 50 pounds (Thornton, 1968). Clean knife should be used when sticking because a contaminated knife could pass bacteria to the red offal via the blood stream (Mackey and Derrick, 1979).

When an animal bleeds out, there is a fall in blood pressure and this activates the sympathoadrenomedullary nervous system. Noradrenaline is released from the sympathetic nerve endings and from the adrenal medulla along with adrenaline. During the initial stages of bleeding, this results in splenic contraction, cardiac acceleration and peripheral vasoconstriction. The adrenergic responses are followed by dilation of the pupil and eventually, relaxation of the jaw and other muscles of the carcass as nervous activity subsides (Gregory, 1998). Of the total amount of blood in the animal body, only about one half is extracted during bleeding at slaughter. The remaining blood is present throughout the carcass, especially in the viscera (Gregory, 1998). The weight of the blood in a live animal is usually about 8% of the live weight of the animal (Thornton, 1968).

2.1.3 Effect of circulatory stoppage on muscle tissue: When circulation of blood is stopped at death, a series of complex changes is initiated in the muscle tissue (Lawrie, 1966). The most important of these are outlined in Figure 2.1 below.

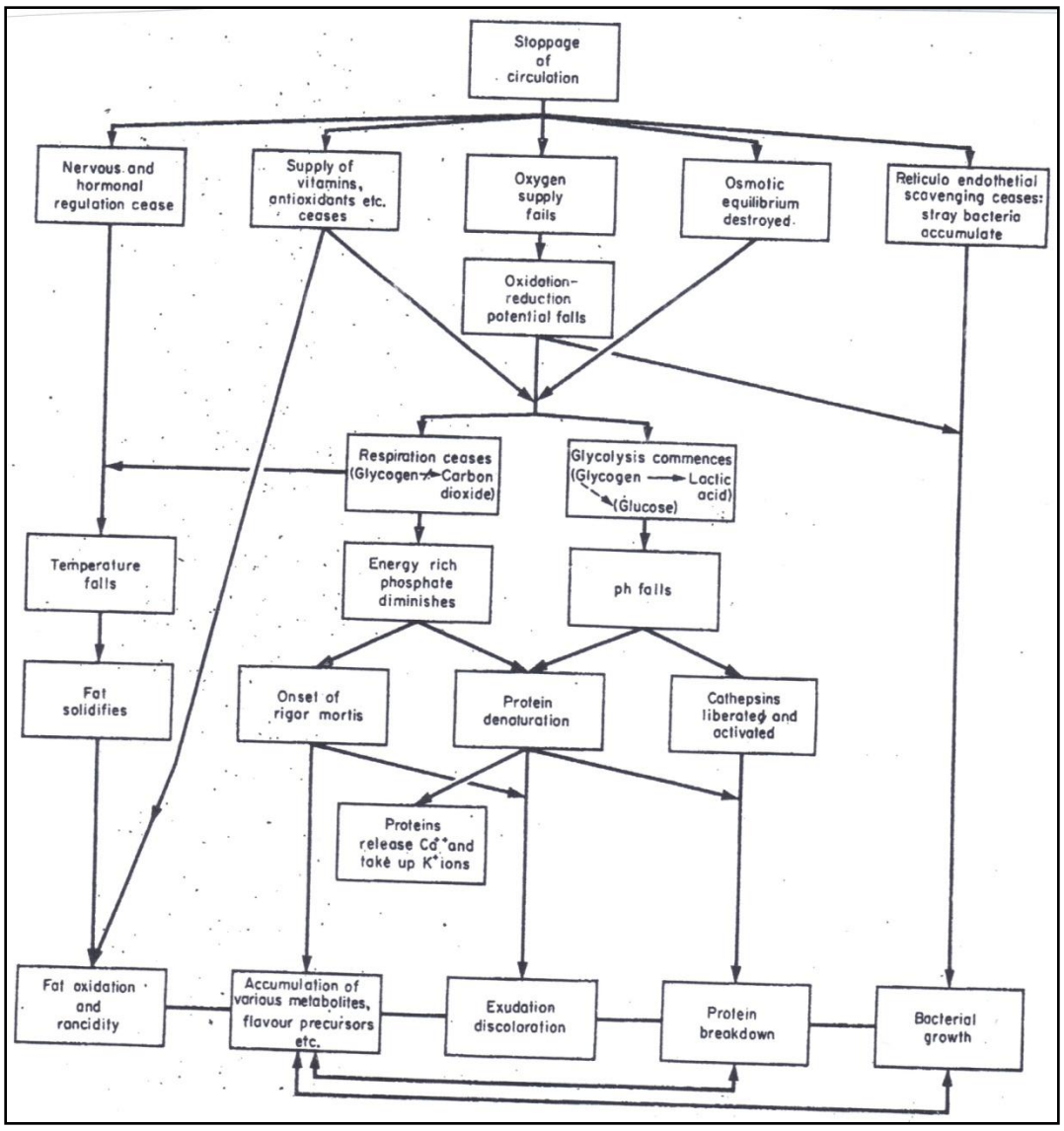


Figure 2.1 General consequences of circulatory failure in muscular tissue (Lawrie, 1966)

At the time of death of the animal, the various tissues go on with their metabolism under local control. Although muscle is not actively contracting at such a time, energy is being used to maintain its temperature and the organizational well being of its cells against the spontaneous tendency to breakdown. The ATP-ase of the sarcoplasm and not that of myosin is one of the enzymes involved in this case (Bendall, 1951). The first change caused by bleeding is the elimination of the blood-borne oxygen supply to the muscles and the consequent fall in oxidation production potential. As a result, the cytochrome enzyme system can no longer operate and the resynthesis of ATP from this source becomes impossible. The continuing operation of sarcoplasmic ATP-ase depletes the ATP level and at the same time producing inorganic phosphate which hastens the breakdown of glycogen to lactic acid. The ATP level can no longer be maintained due to its lack of resynthesis by anaerobic glycolysis and therefore it drops and actomyosin bond is formed, which leads to rigor mortis (Lawrie, 1966; Swatland, 1984).

As stated earlier, cattle and calves should be preferably bled by cutting the brachiocephalic trunk, using chest stick (Anil *et al*., 1995). The blade of the knife must be long enough to reach the brachiocephalic trunk to severe the anterior aorta and anterior vena cava. Care must be taken not to pass the knife too far so that the pleura is not punctured. If the pleura gets punctured, blood may aspire into the thoracic cavity and adhere to the parietal pleura, particularly along the posterior edges of the ribs. This contamination is known as back bleeding or over sticking and if not washed immediately may necessitate the stripping of the pleura (Thornton, 1968).

2.1.4 Alternative routes to the brain: Cattle have an anastomosis in their blood vessels which allows blood to flow to the brain through vertebral arteries, if the carotid arteries are cut instead (Bager, Devine and Gilbert, 1988). Consequently, the loss of brain function can be quite long if only the carotid arteries are cut when using gash stick (Newhook and Blackmore, 1982).

2.1.5 False aneurysm: When only the carotid arteries are cut, sometimes, their cut ends develop false aneurysms (ballooning). Ballooning is due to engorgement with blood, causing the artery or its connective tissue sheath to swell with trapped blood. There is partial occlusion of the severed artery orifice and impaired bleeding out (Anil, Mckinstry, Wotton and Gregory, 1995). This might increase the risk of resumption of consciousness. Carotid artery ballooning and a slow rate of blood loss can also extend the period of consciousness during slaughter without stunning (Blackmore, 1984).
Studies have shown that about 8% of cattle slaughtered by Shechita and Halal, without stunning develop false aneurysms in both carotid arteries (Gregory, Von Wenzlawowics *et at*., 2008).

2.1.6 Sticking methods: There are two neck-sticking methods in ruminants, they are: spear sticking and gash sticking. In spear sticking, the neck is pierced with a knife between the trachea and backbone, with the sharp edge of the knife pointing away from the backbone and knife is withdrawn along the path of entry. In gash sticking (ear-to-ear sticking), the knife is inserted as for spear sticking but all the soft tissues on the underside are cut as the knife is withdrawn (Gregory, 1998).
In sheep slaughter, bleeding is done by an incision in the jugular furrow and close to the head, severing both carotid arteries. After this, the head is jerked back sharply to rupture the spinal cord where it enters the skull. The purpose of this, as in the pithing of cattle, is to obviate reflex action when dressing the carcass. Bleeding of sheep carcass should last at least 5 minutes and in cattle about 6 minutes (Thornton, 1968).

2.1.7 Stunning and non-stunning methods: In most parts of the world, animals are either killed by stunning before slaughter to render the animal unconscious which is the standard (conventional) method or without stunning before bleeding, for religious reasons or purposes. The later could be “Shechita” which is used for producing Kosher meat for people of Jewish faith; “Halal slaughter” used in Islamic faith or “Jhatka” (decapitation) by the Sikhs (Thornton, 1968). The standard method, which is by stunning before bleeding, could be by electrical stunning, captive bolt, percussion bolt, carbon dioxide or free-bullet (Gregory, 1998; Swatland, 1984). Whatever the method of stunning that is

employed, it is desirable that the medulla oblongata in the brain should not be destroyed. This center which controls the heart and lungs should continue to function for some time since they help to pump blood out of the carcass when the blood vessel in the neck are cut (Lawrie, 1968).

2.1.8 Bleeding efficiency: Bleeding can be done with the animal in a vertical (standing), or horizontal or inverted position (as in Shechita). The most common technique in the past was to shackle a hind leg and to elevate the animal on the bleeding rail where sticking is done in a hanging position (Troeger, 2003).
Recent studies have shown that bleeding efficiency is not affected by the slaughter method i.e. the conventional slaughter methods or ritual slaughter (Anil *et al*., 2004 and 2006). *Anil et al*., 2004 and 2006 also showed no differences in the packed cell volume levels. However, the report of Gregory (2004) stated that bleeding rate varies with slaughter method. According to him, it can be influenced by delays between the development of a cardiac arrest and sticking and by the orientation of the carcass as it drains. In sheep and poultry, the initial rate of blood loss can be slow if the stunning method has stopped the heart. However, if the suspended animal is stuck within 3 minutes of stunning, the overall amount of blood voided from the sticking wound will be comparable to that from animals that have not experienced a cardiac arrest at stunning. We can also conclude that a cardiac arrest at stunning can slow down the rate of bleeding but if the carcass is stuck promptly and given enough time to bleed, the carcass will not be engorged with blood. When bleeding is delayed in the absence of heart beat, it allows the blood to clot within the main blood vessels, this holds back blood in the carcass and visibly impair bleeding out. Blood 'tears' or 'surface bleeding' in or on the fascia of the improperly bled carcass can also be an appearance defect (Gregory, 2004).

2.1.9 Residual Blood: Usually, only about 50% of the total blood volume is eliminated when bleeding an animal. Some of the remaining blood is removed during evisceration and some remains in the edible carcass. The residual blood content of lean meat usually varies between 2 and 9 ml/kg^{-1} (Gregory, 2004). The residual blood content of cattle hide is conventionally about $5.5ml/kg^{-1}$ fresh hide, but can be twice this amount in badly bled animals. Poor bleeding at sticking can cause additional blood being released during evisceration and this can create concerns about cleanliness in the slaughter house floor. Also, if the blood vessel of a piece of meat is engorged with blood, blood will smear over the cut surface, thereby impairing the appearance and visual acceptability of a carcass (Gregory, 2004)

It is often claimed that a carcass has to be well bled for meat to keep. This has not been confirmed by research. In an experiment, the rate of proliferation of bacteria in minced meat samples was not affected by the addition of blood in the meat. This tells us that additional blood in meat, which could arise from poor bleeding, is not important in influencing microbial spoilage or proliferation of food borne pathogens. In fact, there may be some residual anti-bacterial effects when blood is retained in a tissue. White blood cells have been observed to retain functional activity in terms of trypan blue exclusion for up to 10 days following death when carcass was stored at 4ºc (Gregory, 2004). Studies from Warris (1978) also shows that stress associated with stunning and

exsanguinations will conventionally produce vasoconstriction through the action of released catecholamines and this will result in minimal retention of blood in the skeletal muscles.

2.2 Treatment of animals prior to slaughter

The importance of reducing stress during and before slaughter is clear. In most animals, reducing stress, excitement, agitation and general discomfort will help improve welfare and preserve meat quality (Grandin *et al*., 1994). Voisinet *et al*. (1997) found that, cattle which becomes agitated during handling and restraint had tougher meat and more borderline dark cutters. Survey also shows that vocalization which is a stress indicator was reduced with good and adequate equipments and well trained personnel (Grandin, 1997b). Stress is also minimized by applying optimum pressure with the restraining device (Grandin, 1995).

The following factors should be considered before animals are presented for slaughter:

Resting: Animals should be rested adequately before slaughter. Improperly rested animals may show a reduction in the keeping quality of meat due to incomplete development of acidity and due to early invasion of the system by putrefactive bacteria from the intestinal tract (Thornton, 1968; Gallo *et al.*, 2003; Gardner *et al*., 2001).

Watering: Animals should receive enough drinking water during their lairage detention as this serves to lower the bacterial load in the intestine and facilitate pelt removal during dressing (Thornton, 1968).

Feeding: Animals intended for slaughter should be well fasted as this will facilitate better bleeding, easy dressing and a cleaner carcass. The risk of rupturing the digestive tract during evisceration is also reduced. Contamination of carcass with faeces and digesta is also reduced (Gregory, 1998; Thornton, 1968). Already in the 1960's, a 24 hour fast for sheep and cattle is recommended as optimum (Ziegler, 1967).

Cleanliness of animals: Animals must be presented in a clean condition as animals which are stained with dung, mud or dust could create a risk of spreading dirt which could force the hygiene officer to stop or slow down the line to avoid cross contamination (Swatland, 1984).

Freedom from blemishes: Animals must be free from blemishes as these could reduce carcass yield or value. If blemishes are severe, the meat will be rejected and used as pet food and in some cases they are used for mince, burger or lower value products with lower turnover (Gregory, 1998).

Freedom from stress: Animals must be unstressed when they are presented for slaughter as this can have adverse effects on yield by increasing drip loss, thawing loss and evaporative loss. Stress can also adversely affect pH, colour, temperature and WHC, which may downgrade the meat quality (Gregory, 1998; O'Neill *et al*., 2006; Lacourt and Tarrant, 1985). Pre-slaughter stress can also cause farm animals (especially cattle) to excrete more salmonella in their faeces, partly due to greater evacuation of the caecum

and large intestine. This can cause infection in other animals and increase hygiene risk (Berends *et al*., 1996). More of the adverse effects of pre-slaughter stress will be discussed as we progress in this work.

Freedom from disease: Ante-mortem inspection must be carried out as this aids in detecting animals suffering from scheduled or infectious diseases like anthrax, rabies, glanders, black leg which are communicable to man. Infected animals should be isolated and examined as this will help in preventing food poisoning and outbreaks (Thornton, 1968).

2.3 Effects of stress on meat quality

There are a number of environmental factors which can cause stress in animals, these includes; temperature, humidity, light, sound and confinement. Other intrinsic stressors include excitement, fatigue, pain, hunger, thirst, fear, exercise, aggression and so on (Grandin, 1997; Apple *et al*., 2005). Pre-slaughter stress can cause undesirable effects on the end quality of meat such as PSE meat, Dark firm dry (DFD) meat and Dark cutting beef (DCB) (Forrest, 2002; Bartos *et al*., 1993; Mounier *et al*., 2006). Animals experiencing stress will have physiological changes, including changes in heart rate, blood pressure, body temperature and respiration, stress hormones including epinephrine and norepinephrine, which are released into the blood stream. Epinephrine helps in breaking down glycogen into glucose which is converted to energy for the muscles in the presence of oxygen (O'Neill *et al*., 2006). When there is not enough oxygen for this conversion, glucose is still converted to energy, but the by-products are lactic acid and water, which would have effect on the meat pH and hence meat quality (Forrest, 2002).

2.3.1 Physiological responses that occur as a result of pre-slaughter stress: Different types of stress bring about different physiological and physical responses. There are different forms of stressors that can cause these responses and these include fasting, dehydration, exercise, motion sickness, aggression, fear/alarm, heat and cold. Most of these stressors emanate from bad pre-slaughter handling and consequently affect the overall meat and carcass quality attributes; like pH, tenderness, colour, and WHC (Gregory, 1998; Zhang *et al*., 2005; Silva *et al*., 1999; Kannan *et al*., 2002). For the purpose of this work, we will only look at fear, increased physical activity, fatigue and aggression as these are the likely factors that can affect the meat and carcass quality immediately pre-slaughter for both slaughter methods.

Table 2.1 Potentially useful stress indicators in plasma (Gregory, 1998)

Stressor	Plasma indicators
Fasting	↓ glucose, ↑ FFA, ↑glycerol, ↑ urea, ↑ GLDH, ↓ acetate (ruminants)
Dehydration	
- without feed	↑ Protein
- with feed	↑ Protein, ↑ osmolality ↑ PCV
Exercise	↑ PCV, ↑ adrenaline, ↑noradrenaline, ↑ K^+ ↑ β endorphin, ↑ lactate (if anaerobic), ↑CPK
Motion sickness	↑ cortisol, ↑ VIP
Fear/alarm	↑adrenaline, ↑noradrenaline, ↑ACTH, ↑cortisol, ↑glucagon, ↑prolactin, ↑β endorphin.
Heat	↑ACTH, ↑cortisol, ↑adrenaline, ↑β endorphin
Cold	↑ noradrenalin, ↑ cortisol, ↑PCV

ACTH: Adrenocorticotrophic hormone; CPK: Creatin phosphokinase;
GLDH: glutamate dehydrogenase; PCV: packed cell volume;
VIP: Vasoactive intestinal peptide; ↑: increase, ↓: decrease.

2.3.2 Physico-chemical aspect of stress: The part of the brain which makes animals to have exaggerated startle response is known as amygdala. It controls the effects of fear and can also activate many of the physiological stress responses in the body that are linked to fear. These include stimulation of the hypothalamic-pituitary-adrenal axis (HPA) which is integrated with autonomic nervous system, causing elevation of plasma cortisol or corticosterone concentrations (Gabr *et al*., 1995). Most frequent physiological changes include tachycardia, increased respiration rate, increased body temperature and redistribution of visceral blood volume towards skeletal muscles and brain (Ferguson and Warner, 2008). Behavioural changes is also evident in form of heightened alertness, immobilization, aggression, escape/avoidance. The sympatho-adrenal component of the autonomic response is mediated by catecholamines (epinephrine and norepinephrine). Activation of the HPA is manifested by the release of glucocorticoids (e.g. cortisol and corticosterone) from the adrenal cortex (Ferguson and Warner, 2008).

Adrenaline and noradrenaline are not as useful as corticostereoids as stress indicators, because they have a short half-life in the circulatory system (Lister *et al*., 1982). Noradrenaline has a half-life of about 2 minutes, whereas for cortisol, it is near 20 minutes. Notwithstanding, some of the metabolic and physiological effects of adrenaline and noradrenaline can be measured. For example, the heart rate response and PCV responses to stresses which involve these hormones can be very useful (Gregory, 1998). The adrenal cortex releases corticosteroids which have more delayed effects. During fear response, the paraventricular nucleus of the hypothalamus is activated to release corticotropic releasing factor (CRF). This neurotransmitter passes to the pituitary through

the hypophyseal-portal vessel, and it induces adrenocorticotropic hormone (ACTH) release into the general circulation.
The main functions of the corticosteroid hormone are: to stimulate proteolysis; to stimulate gluconeogenesis; and to bring about anti-inflammatory effects. Another common sign of fear in cattle is defaecation which is usually caused by the activation of the vagus nerve in the parasympathetic nervous system (Gregory, 1998). It is however worthy to note that cortisol indicates generalized stress while lactate indicates anaerobic muscle exercise.
During restraint stress and during bleeding out, most of the noradrenaline that is released into circulation comes from sympathetic nerve endings rather than the adrenal medulla (Young *et al*., 1984).
An important difference between noradrenaline and adrenaline is their effect on glycogenesis. Noradrenaline does not stimulate glycogenesis in muscle, but adrenaline does. This tells us that stress which stimulates the release of adrenaline is more likely to cause muscle glycogen depletion than stress which provoke a noradrenaline release (Gregory, 1998). There are no specific plasma indicators for pain, but if pain causes muscle activity or fear, some of the plasma indicators of these activities that causes fear, increased muscular activity and aggression immediately pre-slaughter, occurs when animals are being restrained for slaughter especially in the ritual slaughter method. The study of Dunn (1990) showed a high cortisol and heamatocrit in cattle restrained at Weinberg pen in which the cattle are inverted before the cut.

2.4 Types of muscles, their structure and composition, location and metabolism
Looking at the effects of pre-slaughter stress on meat quality, it would be wise to know the different types of muscles, their structure and composition and their metabolism, which will help to know the individual muscle details, their fibre type and situations when they are used most. This will help us to ascertain or anticipate which muscle could develop inferior quality when the animal performs particular behaviours (Gregory, 1998). The location of specific muscles in the carcass is shown in Figure 2.2.

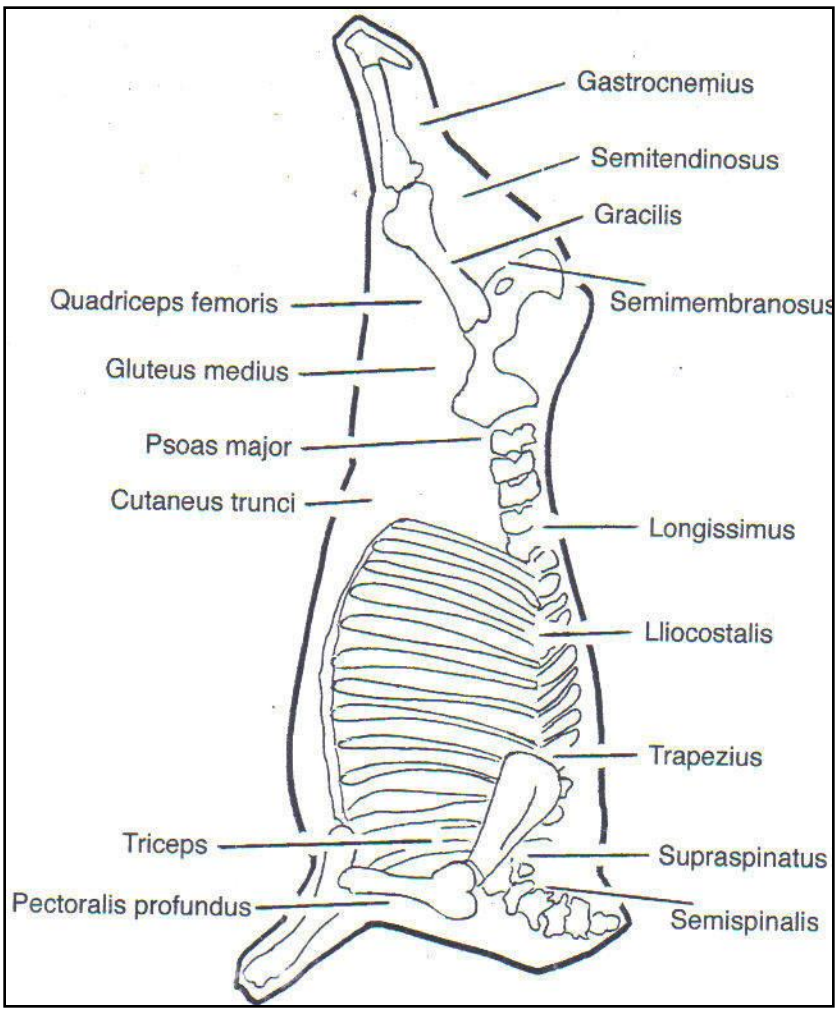


Figure 2.2 The location of specific muscles in the carcass (Gregory, 1998)

When animals are taken to slaughter, there are a number of factors which could cause muscle metabolism, starting from the journey, activity at the holding pen, fasting, movement, restraining and stunning (Apple *et al*., 2005; Fazio and Ferlazzo, 2003; Grandin, 1997; Mormede *et al*., 2003). All these activities use energy as ATP and CP in the muscle and this in turn affects meat quality (Gregory, 1998). Muscles are made of fibres which are held together by interconnecting connective tissue sheaths. The muscles are separated from each other and from other tissues by a fascia of connective tissue. This fascia contains pain receptors which gives signal to the brain when animals go through pain affecting the muscles (Gregory, 1998). The fibres are made up of myofibrils which are enveloped in tubules. The myofibrils contain two types of protein myofilaments; actin and myosin, which are the basic structural components that perform muscle contraction. The myosin filament have side branches which extend laterally to the actin filaments and they have an ATP molecule in the terminal position.

When a nerve impulse reaches the sarcolemma of the muscle, it extends through the tubule and depolarizes the sarcoplasmic reticulum of the cells. The SR releases calcium ions into the sarcoplasm and binds to troponin molecules in the actin myofilaments. This

binding allows the side branches of myosin to bind to actin to form actomyosin. During the binding process, the region of the myosin myofilament is exposed which bears an ATP enzyme and this enzyme causes the terminal ATP to be broken down to ADP + Pi, with large amount of energy being released. Some of these energy are used to re-arrange the myosin in side branches and the actin to slide over the myosin in one direction or the other. If it slides away from the z-line, the myofibre increases in length and vice-versa. These contraction process uses energy and ATP. As soon as the nerve impulses stops, Ca^{2+} is unbound from the troponin and passes back to the SR. The ATPase enzyme in the myosin then becomes inactive and no more ATP is broken down and the muscle relaxes. ATP is re-synthesized in two main ways: The first from an energy store, creatin phosphate (CP). CP passes on a high energy phosphate group to ADP, forming ATP. This reaction is catalysed by the enzyme, creatin phosphokinase (CPK). CP + ADP = ATP + C. CPK is present in muscles in large amount and its leakage into the bloodstream of live animals can be a good indicator of excessive muscular activity or damage to muscle membranes (Gregory, 1998).

The other way of ATP re-synthesis is through mitochondrial respiratory chain which involves electron transport system which is catalysed by NAD-linked dehydrogenases, flavoprotein dehydrogenases and cytochromes (Gregory, 1998).

2.4.1 Muscle contraction and Relaxation

Figure 2.3 shows muscle contraction according to the sliding filament theory –sarcomere shortening .

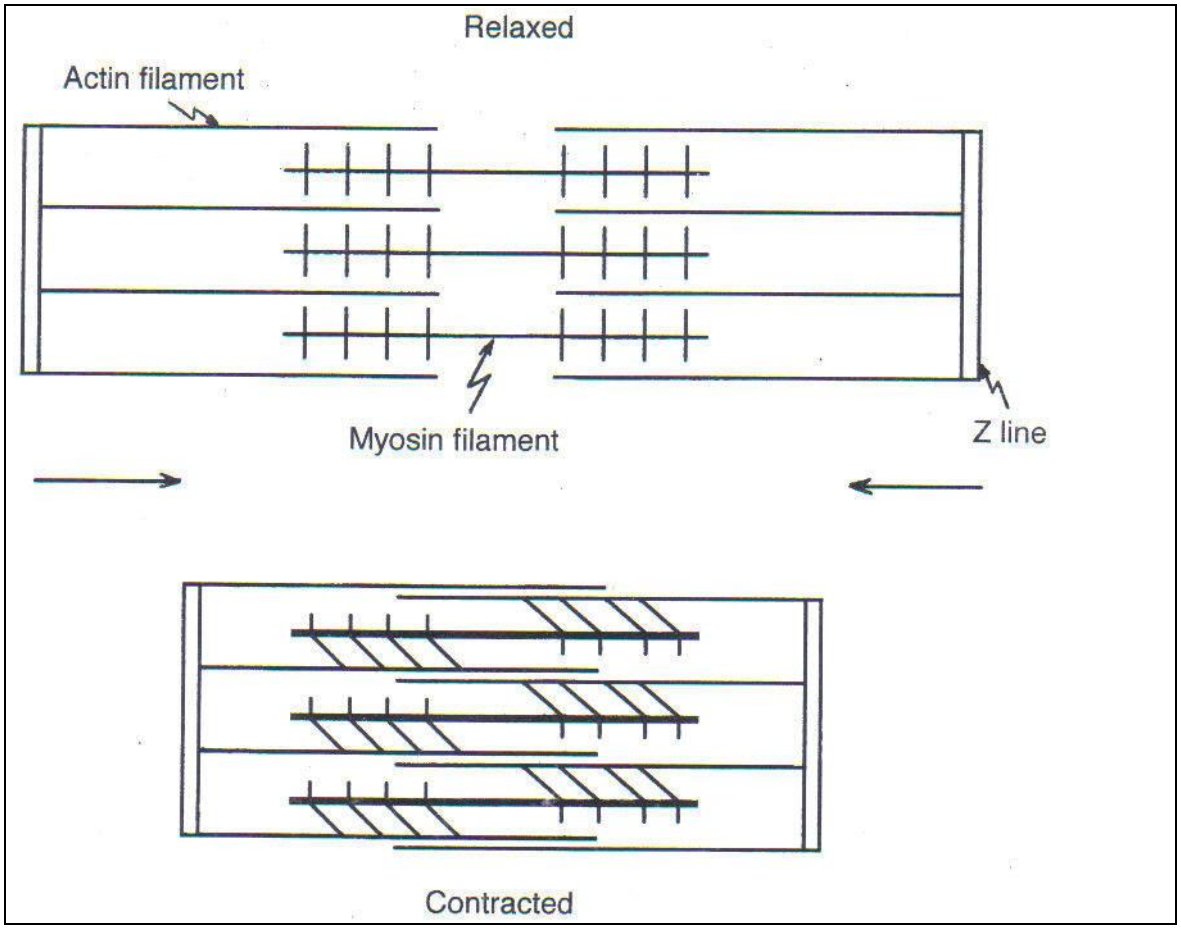


Figure 2.3 Muscle contraction according to the sliding filament theory-sarcomere shortening (Gregory, 1998)

During contraction, the actin and myosin filaments slide over each other and the z lines are drawn towards each other. This occurs through the myosin side-branches repetitively making and breaking contact with the adjacent actin filament. Each time an actomyosin bond is formed, myosin ATPase is activated and ATP is broken down. During relaxation, the myosin has to be reloaded with an ATP molecule. If no ATP is available (as it happens after slaughter) the side-branches remain attached to the actin filaments and the muscle stays in rigor (Swatland, 1984).

$$\text{Actin + myosin} \xleftrightarrow{Ca^{2+}} \text{Actomyosin}$$

$$\text{ATP} \xleftrightarrow{\text{Myosin ATPase}} \text{ADP + Pi}$$

Figure 2.4 also shows muscle contraction according to the sliding filament theory-actomyosin formation .

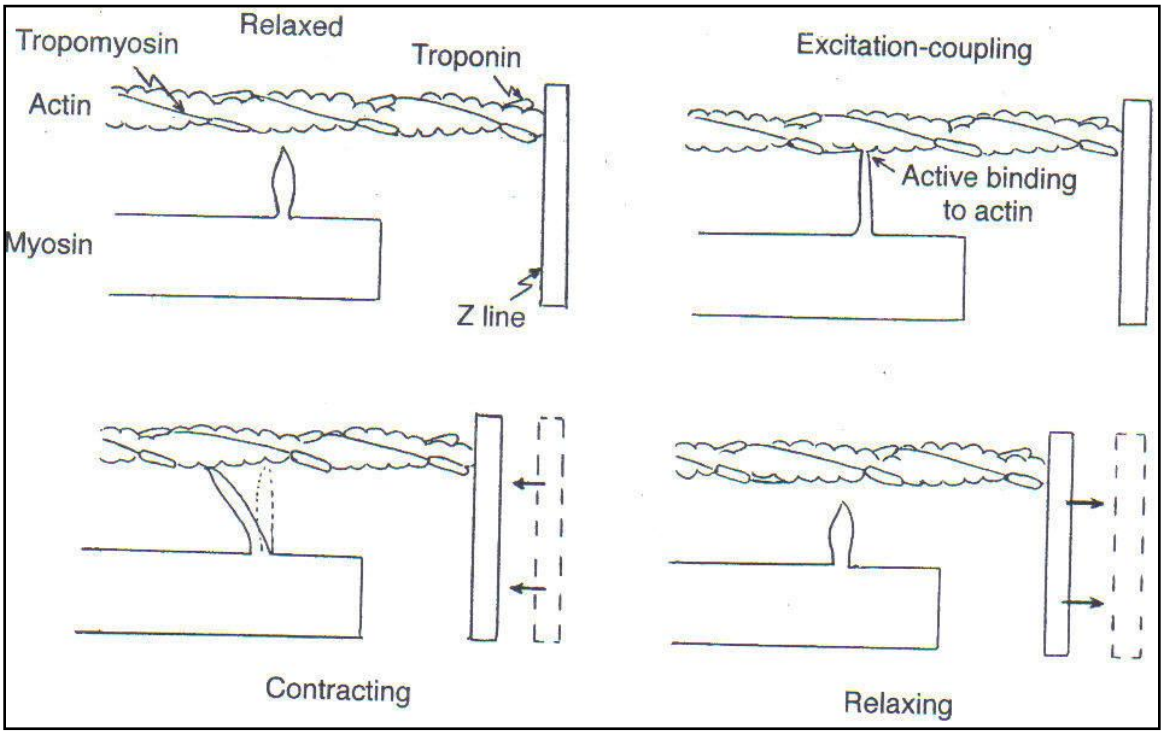


Figure 2.4 Muscle contraction according to the sliding filament theory-actomyosin formation (Gregory, 1998)

During excitation-coupling, Ca^{2+} is released from the sarcoplasmic reticulum and binds to troponin, which then activates an actin site to accept the terminal ATP molecule of the myosin side-branch. During contraction, the ATP molecule is broken down, releasing energy which swivels the side-branch, causing the actin to slide over the myosin. This creates tension and causes the muscle to contract. During relaxation, Ca^{2+} is released from the troponin and is taken up by the sarcoplasmic reticulum. The actomyosin bond is released and the muscle is restored to its original dimension.

Figures 2.5 and 2.6 shows the summary of the glycolytic pathway and TCA cycle respectively.

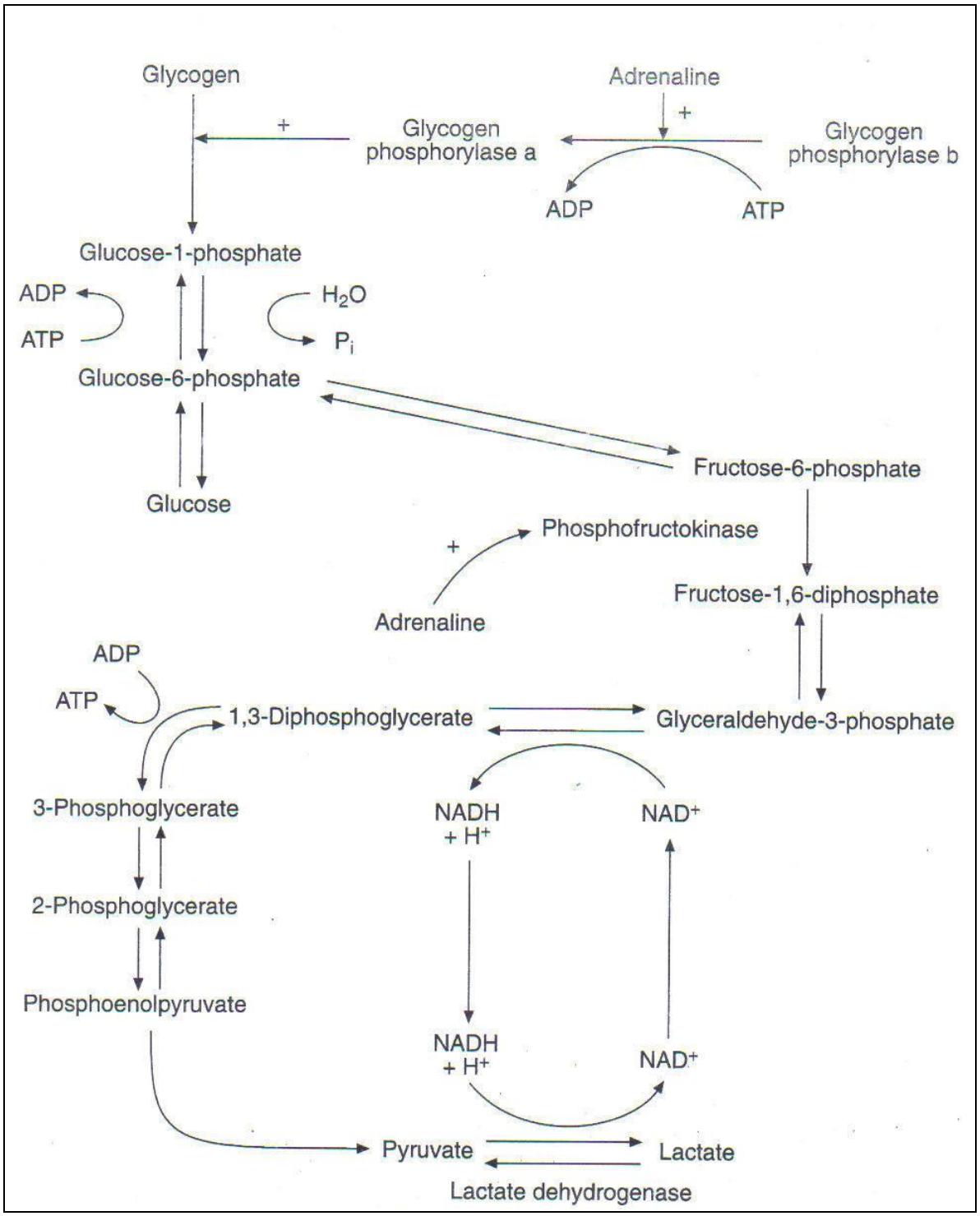


Figure 2.5 The glycolytic pathway (Gregory, 1998)

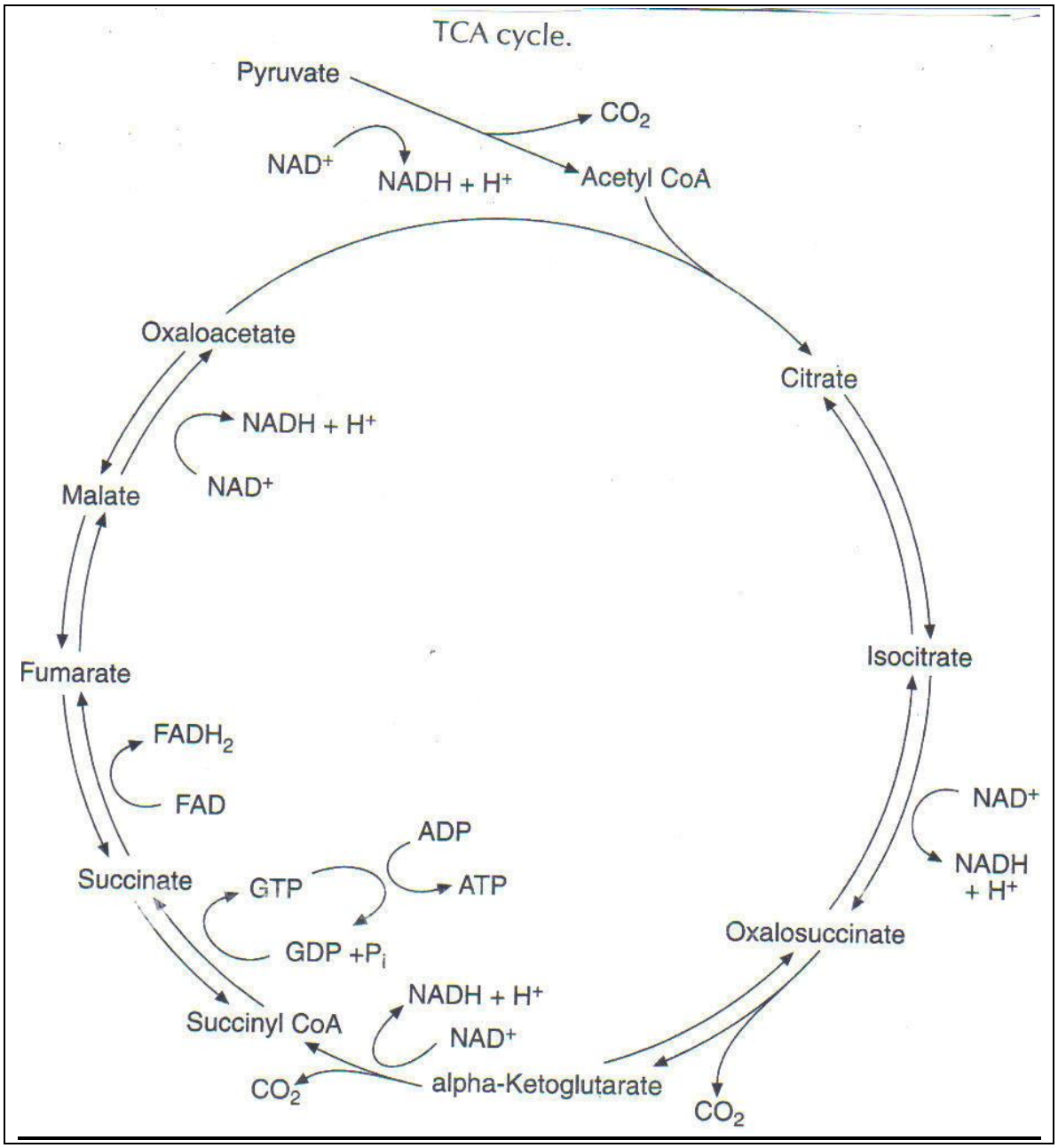


Figure 2.6 The TCA Cycle (Gregory, 1998)

The conversion of glucose to lactate occurs when the muscle is hypoxic or when there is an excess of pyruvate. Lactate formation from pyruvate does not require oxygen, whereas the conversion of 1 mol of glucose to CO_2 requires 6 mol of oxygen. Oxygen delivery from myoglobin in the muscle and haemoglobin in the blood is essential if the muscle is to continue to refuel its ATP reserves rapidly and in an energy-efficient manner using the TCA cycle. The oxygen consumption rate at which lactate starts being produced by exercising muscle is a useful indicator of physical fitness in humans. Physically fit people can maintain aerobic metabolism during exercise for longer period before their muscle changes to lactate production (Gregory, 1998). The works of Fogd Jorgensen and Hyldgaard-Jensen (1975) shows that pigs which were familiar with physical exercise and

training schedule before slaughter showed considerable lower blood lactate responses during exercise stress than untrained pigs (Grandin, 1997). They were also less prone to PSE meat after slaughter.

There are three ways in which the muscle glycogen reserves in an animal can be managed: 1. by reducing pre-slaughter exercise and stress; 2. supercompensation; 3. allowing repletion of glycogen. Supercompensation is used in some abattoirs where pigs are fed a sugar solution whilst in the lairage before slaughter. This solution provides an additional glycogen reserves in their muscle, thus preventing dark, firm, dry (DFD) meat (Gregory, 1998).

The way in which an animal is fed just before exercise can also affect the type of substrate that the muscle uses. Generally, circumstances that favour elevated plasma FFA help to spare the utilization of muscle glycogen (Costill *et al*., 1977).

The glycolytic pathway helps to fuel the TCA cycle and hence the electron transport chain. Exchange between glycolysis and the TCA cycle only occurs when pyruvate leaves the sarcoplasm, enters the mitochondria and is converted to acetyl CoA. This is a one-way process, which acts as a valve, maintaining the direction of flow of energy towards the TCA cycle. After slaughter, glycolysis is no longer fuelled by glucose derived from the blood-stream and instead it relies mainly on the glycogen stored in the muscle. FFA utilization post-mortem is greatly reduced in comparison with that occurring in the live animal, as there is limited ability to translocate FFA from the intramuscular lipid stores to the mitochondria *post-mortem*.

During death, there are three processes which cause the conventional metabolic process in living muscle to slow down and eventually stop: 1.depletion of oxygen; 2, depletion of substrate and 3, inhibition of enzymes. When an animal is stuck, the blood supply to its muscle stops. The muscle no longer receives oxygen and the respiratory chain instead depends on the reserve of oxygen in the tissue at the time of slaughter. When these reserves are used up, the electron transport chain stops and ATP resynthesis continues for a short period from the store of creatin phosphate.

ATP conventionally has two functions in muscle; It provides energy for muscle contraction, which occurs when an actin and myosin filaments interlock by sliding over each other. ATP also provides energy which operates the ionic pumps within the muscle cell (Gregory, 1998; Swatland, 1984; Lawrie, 1974).

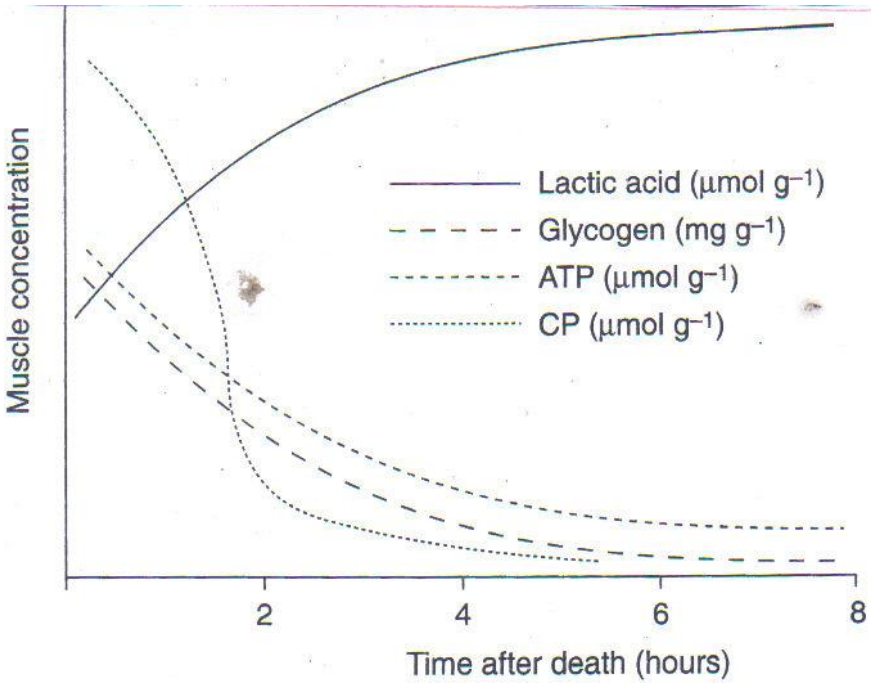


Figure 2.7 ATP, CP, glycogen and lactic acid changes during *post-mortem* muscle metabolism (Tarrant *et al*., 1972)

The intensity of the exercise, the duration and the muscle involved can all affect meat quality. During exercise, the TCA and the glycolytic pathways are activated and during short burst of exercise, there is rapid breakdown of glycogen and the production of large amounts of lactic acid through the glycolytic pathway (Gregory, 1998). During heavy exercise, the pH in the live animals' muscles may fall to 6.3 or in extreme cases, to as low as 5.9. Glycogenolysis in muscle is triggered by exercise and adrenaline released from the adrenal medulla. Glycogen is broken down in the glycolytic pathway to pyruvate which can either be used for generating ATP in the TCA cycle or is converted to lactate (Gregory, 1998). It is however worth noting that this relationship varies considerably between muscles, depending on their metabolic properties and their sensitivity to catecholamines (Larzul, Le Roy, Monin and Sellier, 1998). In cattle, repletion of muscle glycogen after exhaustive exercise takes at least 24 hours and can be up to 48 hours. The rate of repletion in a muscle depends on the main fibre types in the muscle. A potentially important issue in meat quality is whether the animals that are using FFAs in their muscle instead of glycogen are more prone to developing anaerobic metabolism and a pre-slaughter acidosis (Gregory, 1998; Swatland, 1984).

2.4.1 Muscle fibre types and their characteristics: Different muscles are made of different fibre types. There are three main types, based on their contractile and metabolic characteristics; β -red which are slow twitch oxidative; α-red which are fast twitch oxidative and α-white which are fast twitch glycolytic.
Table 2.2 shows the summary and characteristics of the three muscle fibre types.

Table 2.2 Properties of the three muscle fibre types (Gregory, 1998)

Properties	**β-Red**	**α –Red**	**α -White**
Colour	Red	Red	White
Contractile speed	Slow	Fast	Fast
Contractile action	Tonic	Phasic	Phasic
Myoglobin concentration	High	High	Low
Capillary density	High	Intermediate	Low
Fibre diameter	Small	Small/intermediate	Large
Number of mitochondria	High	Intermediate	Low
Glucogen storage	Low	Intermediate	High

The alpha fibres contract rapidly and are involved in phasic contraction during a burst of activity while beta fibres contract tonically in continous contractions. The red fibres contain a high proportion of the enzymes involved in oxidative metabolism and low levels of glycolytic enzymes. White fibres on the other hand have a high content of glycolitic enzymes and a low oxidative enzyme activity (Forrest *et al*., 1975). The red fibres have high myoglobin concentrations and a relatively high capillary blood flow which helps in maintaining oxygen supply. Red fibres can utilize fatty acids more effectively than white fibres and they store more lipids (Gregory, 1998).

Different muscles contain different proportions of the three types of fibre. The ones for cattle are shown in Table 2.3.

Table 2.3 Proportion of fibre types in some different muscles (Gregory, 1998)

Muscle	**β-Red**	**α –Red**	**α- White**
Semitendinosus	8	26	66
Cutaneus trunei	7	28	65
Semimembranosus	13	31	56
Gluteus medius	26	22	52
Longissimus	25	25	50
Triceps longus	22	32	46
Psoas major	52	15	33

Postural muscles (e.g. trapezius) have a high proportion of beta red fibres, while muscles involved with movement such as running (e.g. semitendinosus) have a higher proportion of alpha white fibres. White-fibred muscles are better equipped to obtain energy by anaerobic metabolism than are red-fibred muscles. They tend to hold energy in a readily utilizable form, like CP and ATP and during exercise and *post-mortem* metabolism, they produce more lactate and a lower pH_{ult} than red fibres (Gregory, 1998).
Postural muscles which are rich in red fibres are continously active and their sustained activity in live animals makes them more susceptible to depletion of energy reserves when energy supply from the bloodstream declines. Also, during prolonged activity, the intracellular pH of the muscle slowly declines, as the muscle becomes more acidic. The

force and velocity of contraction also declines and fatigue sets in. The fast-contracting muscle fibres are more sensitive to this effect than slow-contracting fibres. White fibred muscles are more likely to exhibit rapid post-mortem glycolysis than muscles that have a mainly oxidative type of metabolism. White fibred musles can exert strong tensions and are able to contract and relax rapidly while red fibres can maintain a sustained but weaker contraction for a long time. White-fibred muscles usually have shorter sarcomere length when in rigor than re-fibred muscles (Gregory, 1998).

Concerning metabolism, there are three important energy substrates for muscle; they are glycogen, glucose and fatty acids. If a fasted animal is unable to mobilize body fat to supply muscle with FFAs, the muscles turns to glycogen and blood glucose for its energy. These carbohydrates only last for a little period and hence the risk of high PH_{ult} meat increases as the fasting lengthens (Gregory, 1998). FFA utilization within muscle is controlled by the rate of the entry of the FFA into the mitochondria. FFA transfer from the sarcoplasm into the mitochondria is controlled by an enzyme, carnitine acyl transferase located in the mitochondrial walls. Physical training helps to increase its activity and also reduce the likelihood of developing high blood lactate (Gregory, 1998).
An adrenaline-type stress would be expected to lead to more generalized dark-cutting in the carcass, while exercise stress is more muscle specific and within the muscle, the fast-acting muscle fibres are more likely to be affected by pre-slaughter exercise than slow types (Gregory, 1998).

2.4.2 Chemical composition of a typical mammalian muscle: Before going further, it would be thoughtful to look at the chemical composition of a typical mammalian muscle after *rigor-mortis* but before degradative changes *post-mortem*. This is presented in Table 2.4.

Table 2.4 Chemical composition of a typical mammalian muscle (% weight) (Lawrie, 1996)

WATER		75.5%
PROTEIN		18.0%Broken down to↓
Myofibrillar	→myosin,tropomyosin, x protein	7.5
	→Actin	2.5
sarcoplasmic	→myogen, globulins	5.6
	→myoglobin	0.36
	→haemoglobin	0.04
mitochondrial	Cytochrome C	Ca 0.002
sarcoplasmic reticulum	collagen elastic	
sarcolemma	"reticulin"	2.0
connective tissue	insoluble enzymes	
FAT		3.0%
SOLUBLE NON-PROTEIN SUBSTANCES		3.5% Broken down to↓
Nitrogenous	creatinin	0.55
	Inosine monophosphate	0.30
	di-and tri-phosphopyridine nucleotides	0.07
	amino-acids	0.35
	carnosine, anserine	0.30
carbohydrates	lactic acid	0.90
	glucose-6-phosphate	0.17
	glycogen	0.10
	glucose	0.01
inorganic	total soluble phosphorus	0.20
	potassium	0.35
	sodium	0.05
	magnesium	0.02
	calcium	0.007
	zinc	0.005
Traces of glycolytic intermediates, trace materials, vitamins, etc.		Ca 0.10

The essential unit of muscular tissue is the fibre which consists of formed proteins, the myofibrils, between which is a solution, the sarcoplasm and a fine network of tubules, the sarcoplasmic reticulum; the fibre being bounded by a very thin membrane called sarcolemma, to which connective tissue is attached on the outside (Lawrie, 1966). The principal amino acids in fresh muscle are α-alanine, glycine, glutamic acid and histidine. (Tallan, Moore and Stein, 1954).

2.5 Blood and body fluid

It would be thoughtful to look into blood and body fluid and its constituent, as this is the carrier and medium of exchange of all these chemical substances that affect the quality of meat (Phillis, 1976).

The total body water (TBW) can be divided into fluid inside the cells, the intracellular fluid (ICF), and that outside, the extracellular fluid (ECF). The ECF in turn can be divided into three main subdivisions:

1. Blood plasma: which is the rapidly circulating fluid contained within the vessels. To obtain plasma volume from the blood volume, we simply subtract the volume occupied by the blood-corpuscles. Plasma volume generally constitutes 50-60 percent of the circulating blood volume and about 7-8 percent of the TBW.
2. Interstitial fluid and lymph: interstitial fluid (ISF) is the fluid that penetrates the small spaces between the cells and through which the interchange of raw material and waste products between blood capillaries and cells take place. The ISF is slowly and continuously formed by ultrafiltration from the plasma. It circulates through the tissue slowly, and is ultimately drained back into the blood through the lymphatic system. The fraction of the TBW occupied by ISF is about 20 percent.
3. Transcellular fluid (TF): is a special form of extracellular fluid which is secreted by specialized cells other than capillary endothelium. Examples of TF are intraocular, cerebrospinal, synovial, peritoneal and pleura fluids. The fraction of the TBW contributed by this fluid volume is insignificant.

Table 2.5 Typical concentration of major ions (mEq/L) in the three fluid compartments (Phillis, 1976)

	Na	K	Ca	Mg	Cl	HCO_3	PO_4	Organic Anions	Protein
Plasma	155	5	5	3	105	27	2	6	17
ECF	150	45	3	2	110	29	1	6	-
ICF	8	150	>0.2	28	5	10	100	4	65

The various cellular components are suspended in the plasma. The blood cells are generally classified as platelets, red cells (erythrocytes), and white cells (leukocytes). The cells constitute about 4 percent of the total blood volume in meat animals. The primary function of the erythrocytes is to carry oxygen. The function of the leukocytes are not well known but outside the vascular system, some are known to be phagocytic (i.e. they digest foreign matter) and others function in immune reactions. Platelets take part in blood clotting mechanism. The erythrocytes contain haemoglobin which gives blood its red colour, and is directly responsible for transport of oxygen to the cells. The colour of meat is largely due to the presence of the sarcoplasmic protein called myoglobin (muscle pigment), but haemoglobin also contributes to the colour because, even with ideal slaughtering technique, a small amount of blood remains in the muscle (Forrest *et al*., 1975).

2.6 Slaughter methods in international abattoirs

Table 2.6 Slaughter methods used internationally in abattoirs for different livestock (Gregory, 1998)

	Stunning methods					Slaughter Methods		
Species	Electrical stunning	Captive bolt	Percussion bolt	Carbon dioxide	Free bull-et	Sticking After stunning	Cardiac Arrest with bleeding	Sticking without stunning
Cattle	+	+++	+			+++		+
Pigs	+++			++	+	+++		+
Sheep	+++	+				+++	+	+
Poultry	+++			+		++	+	+

* includes religious slaughter

+++, main method; ++, common method; +, uncommon method used in some abattoirs.

2.7 Legislation and regulations guiding animal slaughter

At present in Europe, welfare at slaughter is governed by an EU directive which has not been amended since 1993 despite advances in methods of stunning and slaughter. Standards vary between countries and the fact that a regulation is now being proposed is significant. The European parliament's Agriculture and Rural development committee has voted in favour of plans to tighten up and improve on the rules governing animal welfare at slaughter. They agreed that animals must be slaughtered using only methods that ensure death instantly or after stunning but that the current blanket exemption for religious rituals should be preserved, rather than allowing exemptions to be agreed at national level. An amendment calling for special labelling of products of religious slaughter was rejected as were calls for stunning to be made compulsory before animals' throat are cut.

The committee also approved the introduction of indicators that could be used to detect signs of consciousness or sensibility in animals during killing to ensure that stunning had been properly conducted. It also ensures that animals should be restrained only when the person responsible for stunning was ready to do so and that bleeding should start as soon as possible after stunning, to ensure that animals do not regain consciousness before death.

The Federation of Veterinarians of Europe (FVE) is also concerned about the fact that in certain EU countries, a high proportion of animals are killed without stunning. They stated that, meat from these animals are sold on domestic and export market which is contrary to animal welfare and consumer's right to be informed. They said operators should ensure that only animals destined for a religious market are killed without pre-stunning and in case slaughter takes place without prior stunning, the animals should be stunned immediately after the cut, while specific information (labelling) should always be available to consumers if animals were slaughtered according to this procedure.

The British veterinary Association (BVA) also supports the FVE's amendments as a pragmatic means of enabling a better balance between animal welfare and religious requirements (The Veterinary Record, 164: 382 (2009).

Some countries have introduced legislation that the animals for Jewish slaughter should be rendered unconscious before the throat cut. These countries are Norway, Sweden, Australia and in Switzerland, it is prohibited (Levinger, 1995). In Stockholm, a type of electrical stunning apparatus known as elther is used to anaesthetize cattle prior to slaughter (Thornton, 1968).

In South Africa, there has been a long contention between the Livestock Welfare Coordinating Committee (LWCC) (which includes Abattoir, feedlot, SPCA and animal auction associations) and the Jewish body for Kosher (Beth-din) about the manner in which animals are Kosher-killed in rotating box with the use of the devils fork which is inhumane and causes unnecessary suffering to the animals. They also said that no abattoir will be permitted to undertake Kosher slaughter unless an upright restraining box with chin lift is installed. That battle appears to have been won but argument still lies in the amount of time it takes to stun the animal post-cut which is 20 seconds. LWCC insists that animals should be stunned at most 4 seconds after the cut because it reduces the pains and stress of the animal and bleeding out is even more effective.

2.8 The standard slaughter methods and their effects on meat and carcass quality parameters

These are the slaughter methods that take cognisance of the humaneness of killing and welfare of animals. They are non-religious slaughter methods that ensure that animals are not subjected to unnecessary pains or distress while they are slaughtered. They are internationally agreed and practiced procedures of slaughter, where the animals are rendered unconscious by stunning before they are actually slaughtered. (Gregory, 1998; Swatland, 1984).

There are five main stunning methods that are used internationally in abattoirs, these are: electrical stunning, captive bolt method, percussion bolt, carbon dioxide and free bullet (Gregory, 1998; Swatland, 1984). Apart from electrical stunning, the other methods usually cause permanent insensibility when applied correctly. The animals are less likely to regain consciousness and there is less need to bleed immediately.

Table 2.7 Different stunning methods used internationally for different livestock species (Gregory, 1998)

Species	Stunning Methods				
	Electrical stunning	Captive bolt	Percussion bolt	Carbon dioxide	Free bullet
Cattle	+	+++	+		
Pigs	+++			++	+
Sheep	+++	+			
Poultry	+++			+	

+++, main method; ++, common method; +, uncommon method used at some abattoirs.

For the purpose of this research, we are going to focus more on the captive bolt stunning which is the main method of stunning cattle in most parts of the world.

2.8.1 Electrical stunning: This is a method in which a current is passed through the brain with the intention of inducing epilepsy (Gregory, 1991a). This method is the commonest and most widely used standard method. The required current varies with species. For calves, 1.0A; sheep, 0.5A and pigs, 1.25A are the recommended minimum current which will stun 98% of the animals (Gregory, 1998). The instrument most commonly employed resembles a pair of tongs. The voltage required can be calculated from Ohm's Law, (V=IR). However, some animals may have a high resistance and in practice, it is not possible to control all the variations in resistance. Instead, most equipment is designed to supply sufficiently high voltage to stun all animals. Very high stunning currents could cause unnecessary safety hazard and could lead to carcass and meat quality problems such as blood splash. This is a defect where blood capillaries in the carcass have ruptured, resulting in bleps of blood appearing in the meat. The rise in arterial blood pressure following stunning is brought about by vaso-constriction of the vessels but this does not occur in capillaries, which are virtually passive tubes. When the vessels of the arterial system are in a state of constriction, the capillaries contain relatively little blood, but when the stimulus which causes vaso-constriction ceases, as when the tongs are removed from the head of the animal, the arterioles undergo immediate vaso-dilatation which cause the rush of blood into the capillaries. The effect of this sudden diffusion of the capillaries, which is already weakened by anoxia or oxygen lack is to cause rupture of the capillary wall and haemorrhage into the surrounding tissues (Thornton, 1968). The higher the arterial blood pressure, the more severe will be the impingement of blood on the capillary wall when vaso-dilatation occurs. Conversely, any blood pressure following stunning will reduce the incidence of splashing. Examples are immediate bleeding and pithing in cattle which destroys the medulla oblongata (Thornton, 1968).

An alternative system is to use a constant voltage-current limiting stunner in which the excess current above a predetermined level is dissipated through a choke resistor in the control box. In this way, the current delivered to each animal is regulated on an individual basis. With most electrical stunning systems, the aim should be to place the electrodes on or across the head, otherwise there is a risk that the current will not pass through the brain. For pigs, it is not always easy to place the electrodes across the head, instead, it is common to put it across the neck and that is why it requires more current.

Effectiveness of electrical stunning: The bleeding of an electrically stunned animal is better in the sense that the heart continues to function and the arterial blood pressure are at a higher level than that obtained when other methods like captive bolts are used. The nature of the muscular contraction also expels the maximum amount of blood (Thornton, 1968). This agrees with the work or Velarde *et al*. (2000) on lambs-in which the blood loss in electrically stunned lambs (head-only) was higher than non-stunned lambs.

Electrical stunning equipment requires routine maintenance. Electrodes often develop a layer of charred material where saline water is not used and this causes electrical resistance. They can be removed with powered wire brush. The operator should also be able to see the amount of the administered current from an ammeter. Stunning an unrestrained animal can lead to misapplication of the tongs and the animal may experience an electric shock which leads to welfare problems (Gregory, 1998).
When head-only electrical stunning is used, it is important to stick the animal immediately. Sheep and pigs usually start regaining consciousness at about 60 seconds after applying the current but a general recommendation is to stick within 20 seconds of head-only electrical stunning to avoid regaining of consciousness (Gregory, 1998).

There are two approaches to assessing the effectiveness of electrical stunning; the first is to inspect the equipment to ensure they are delivering the recommended current for the species, to check if the electrodes are applied in appropriate position and to check that the animal is stuck at the right time. The second approach is to check and observe the behaviour of the animal. Signs of epileptic fit as well as absence of responsiveness to particular stimuli should be looked out for. Usually, when an animal is stunned by the head-only method, the hind legs immediately go into flexion and the animal collapses if it is not supported. The forelegs usually go into extension, this is called the tonic (rigid) phase, breathing is absent and the eyeballs may be obscured. At the end of this phase, the body relaxes and rhythmic breathing resumes, a quiet phase follows, which is linked to exhaustion of the nervous system. This phase is followed by the clonic (kicking) phase which can be galloping, cantering or erratic kicking action. After the kicking, the animal is alert and responsive (Gregory, 1998). A common way of checking the effectiveness of a stun is to check the corneal reflex which indicates whether the brainstem is responsive. Absence of reflex shows that the animal is unconscious. Sheep regains responsiveness to a smack on the snout before the regain of responsiveness to a threatening gesture. Responsiveness to an ear pinch and other painful stimuli takes even longer to recover (Gregory and Wotton, 1988a). Short current durations have been linked with quicker recovery of consciousness in sheep and on the other hand, high stunning currents are also more likely to act instantaneously and if the stun is not instantaneous, the animal will experience an electric shock before the stun is induced.

Other methods of avoiding welfare problems, is to use a cardiac arrest stunning method. By stopping the heart, the brain function is halted. The common methods used on sheep are head-to-back stunning and head-to-foreleg stunning (Gregory, 1998). The benefits of inducing a cardiac arrest at stunning are: 1, there is less risk of the animal regaining a consciousness after the stun. 2, from the welfare perspective, it's not important to bleed the animal immediately. 3, physical convulsions are usually reduced. 4, there is less risk of blood splash and bruising in the carcass. Some Halal authorities accept electrical stunning before the cut but they will not accept stunning methods that simultaneously induce a cardiac arrest.

Doubts on the effectiveness of electrical stunning on cattle: There have been doubts about the effectiveness of electrical stunning on large cattle, especially when it has been accompanied by a current that induced a cardiac arrest. Gregory (2008), examined 67

cattle stunned with 2.3A applied between the neck and nose for 2.2 seconds, followed by a current delivered to a brisket electrode to stop the heart. The animals were stuck at 90sec after the end of electrical stunning. The depth of the stun was assessed from brainstem reflexes and spontaneous behaviours. Just under half of the animals showed no positive features following electrical stunning, and 8% showed three positive features at both 20 and 90 seconds after the stun. These positive brainstem reflexes and behaviour patterns might have been due to residual activity in the brainstem and the stun was not deep and they had to be shot. Twelve out of the animals (18%) were shot with a captive bolt gun because the slaughter man was concerned about the effectiveness of the electrical stun. The prevalence of cases that are shot needs to be reduced in order to improve confidence in this stunning method (Gregory, 2008).

Effects of electrical stunning on meat and carcass quality parameters: Concerning the effects of electrical stunning on meat quality parameters, it is generally believed that head-only electrical stunning of sheep can increase the rate of *post-mortem* glycolysis due to increased muscle activity and elevated release of cathecolamines into the blood (Peterson and Blackmore, 1982). This also conforms to the findings of Pearson, Kilgour, De Langen and Payne (1977) who noted that electrical stunning caused a secretion of noradrenaline and adrenaline up to 20 and 14 times more than in unstunned lambs. However, sticking non-stunned animal also produced a marked drop in pH in lamb muscles (Devine, Ellery, Wade and Chrystall, 1984). The findings of Peterson and Blackmore (1982); Vergara and Gallego, (2000) and Velarde *et al*. (2003) confirms that the rate of *post-mortem* muscle glycolysis in electrically stunned cattle is not markedly different from that observed in non-stunned cattle (there were no significant differences in ultimate pH).

Effect of electrical stunning on WHC: Regarding water holding capacity, the rate of pH decline in muscle plays a significant role in water holding capacity and cooking loss of meat (Lyon and Buhr, 1999). It is also well known that cooking losses are much smaller in high pH meat compared to meat of lower pH, likewise, several researchers have suggested that high drip loss is due to rapid pH decline (Stoier, Aaslyng, Olsen and Henckel, 2001). The findings of Onec and Kaya (2004) shows that there was no statistically significant differences in water holding capacities of electrically stunned cattle compared to non-stunned group, that had higher WHC than those electrically stunned. More water was also exuded from meats of the electrically stunned cattle compared to the non-stunned cattle. The same work of Onec and Kaya (2004) also shows that cooking loss was not significantly different between treatments at 24 hours and 4 days *post-mortem*, however, significant differences were found at 7 and 14 days *post-mortem* between the electrically stunned and non-stunned group, with the electrically stunned group having more cooking loss than the non-stunned group.

Effect of electrical stunning on meat texture: Concerning electrical stunning and meat textural parameters, the work of Onec and Kaya (2004) shows that textural attributes of non-stunned cattle appeared lower than those of the electrically stunned group. Hardness decreased with time in all groups due to ageing which agrees with the findings of Jeremiah, Tong and Gibson (1997) and Vergara and Gallego (2000). The research of

Vergara and Gallego (2000) generally shows that meat from non-stunned animals appeared more tender than the electrically stunned but the differences were not significant. The difference in pH may be the cause of the higher values of shear force in meat of the electrically stunned group, due to decreased activity of calpain (Dransfield, 1994).

Effect of electrical stunning on meat colour: Concerning electrical stunning and meat colour, the same findings of Onec and Kaya (2004) shows that L^* value of beef gradually increased from day 0 to day 3. L^* and b^* values from day 3 onwards, shows a significant difference between the electrically stunned group and the non-stunned group. However, the findings of Velarde *et al*. (2000) and Vergara and Gallego (2000) on lamb showed no significant differences in colour parameters between electrically stunned lambs and non-stunned lambs.
The same research of Onec and Kaya (2004) shows that electrically stunned cattle are more acceptable than non-stunned cattle in the sensory attributes of odour, flavour, chewiness and tenderness but the Percussive stunned group had the best overall acceptability in sensory attributes ($P< 0.05$) at all ageing times than the others.

2.8.2 Percussion bolt: This is an uncommon method. It is mainly used to stun cattle before Halal slaughter for workers' health reason. It has a blunt end which looks like mushroom and it's designed to concuss without penetrating the brain (Gregory, 1998).

2.8.3 Carbon dioxide: This is widely and commonly used in the Scandinavian countries to stun pigs. It is also used on poultry but not so common (Gregory, 1998). Except in Britain and most of the continental countries, it was a common practice to slaughter pigs without rendering them unconscious but as time went on, carbon dioxide was increasingly used for anaesthetisation and this new method is permissible under the slaughter of pigs regulation of 1958 (Thornton, 1968). In Europe, the most commonly used apparatus was the oval tunnel type and is designed for killing rates of up to 240 bacon pigs per hour, though not capable of dealing with larger boars and sows. In the 1990's, the most common system was the compact stunner in which pigs were individually loaded into a chair which was then lowered into a well filled with carbon dioxide. The combi system also emerged as a modification of this, where two or more pigs are loaded into each chair and recently, a high throughput dip-lift has been developed (Gregory, 1998). On exposure to CO_2 for about 45 to 50 seconds, the pigs become rapidly anaesthetized and become completely unconscious for 1½ to 3 minutes during which they are bled (Thornton, 1968). This method has been criticized from the welfare outlook because it inevitably leads to a sense of breathlessness before the animal becomes unconscious and studies have also demonstrated that pigs and poultry shows aversion to CO_2 and they develop laboured breathing before the collapse (Gregory, 1998). However, for poultry, an acceptable system is to use a low concentration of CO_2 in combination with anoxia (less than 2% O_2) (Mohan Raj *et al*., 1992) which is less averssive. CO_2 is advantageous in that it leaves no undesirable residues in the meat.

2.8.4 Free bullet: This also depends on concussion, it is usually used on breeding pigs and horses in abattoirs when an animal runs amok. For human safety, it is advisable not

to use an unnecessarily high velocity bullet because of the risk of the bullet penetrating the animal's head and endangering personnel and equipment. Nevertheless, the velocity must be sufficient to stun the animal. For bulls, a low velocity, $0.4m/s^{-1}$ have been recommended. Alternatively, bullets which fragment inside the skull could be used (Gregory, 1998).

2.8.5 Captive bolt: This is the main method used in stunning cattle. It was developed in the 1920's and in those days it was probably more valued for its ability to reduce struggling and increase processing speeds than for any perceived improvement in welfare (Shragge *et al*., 2004). The aim is to concuss the animal by firing a bolt against the head. The bolt is held captive inside the gun, hence the name captive bolt (Gregory, 1998). The effectiveness of the blow depends on the kinetic energy imparted on the head which in turn depends on the velocity of the bolt. The bolt velocity is largely determined by gun design and how it is applied to the head, choice of cartridge and bolt friction or jamming due to inadequate cleaning of the gun. Captive bolt gun could be penetrative or non-penetrative and it is important to ensure that the bolt is fully returned between each shot. It is also important to clean the chamber when it fails to return to its prime position due to jamming, which is caused by the build up of carbon particles and gases, which are released after each explosive shot. There are four types of captive bolt gun; these are, cash special, cox universal, cash cow puncher and pneumatic captive bolt gun, which uses compressed air to shoot the bolt. The cash special and cox universal have bolts that protrudes from the muzzle when in prime position, while the cash cow puncher have bolt that is recessed within the muzzle of the gun. The pneumatic bolt gun uses compressed air to push out the recessed bolt. Guns with protruding bolts should be held slightly (up to 5mm) away from the animal's head to reach its maximum velocity while the guns with recessed bolts can be pressed against the head. Bolt diameter also affects the transfer of energy to the skull. The larger the diameter, the more the energy, that is transferred to the skull. The ideal shooting position is often considered to be the cross-over point between imaginary lines drawn between the base of the horns and the opposite eyes (Gregory, 1998) and (Anil *et al*., 2002). Figure 2.8 shows the pictures of the types of captive bolt gun and Figure 2.9 shows the diagram of the correct shooting position.

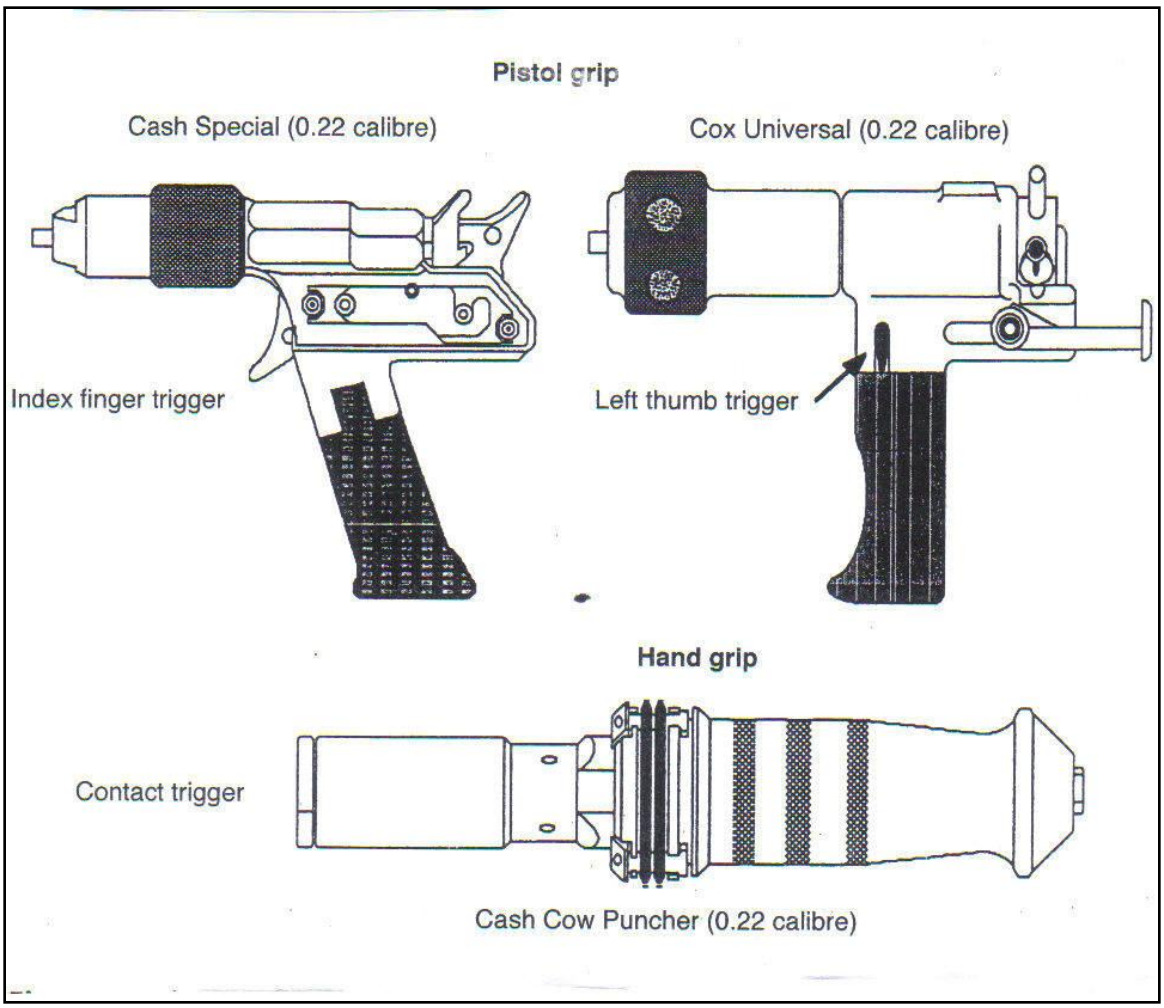


Figure 2.8 Types of captive bolt guns (Gregory, 1998)

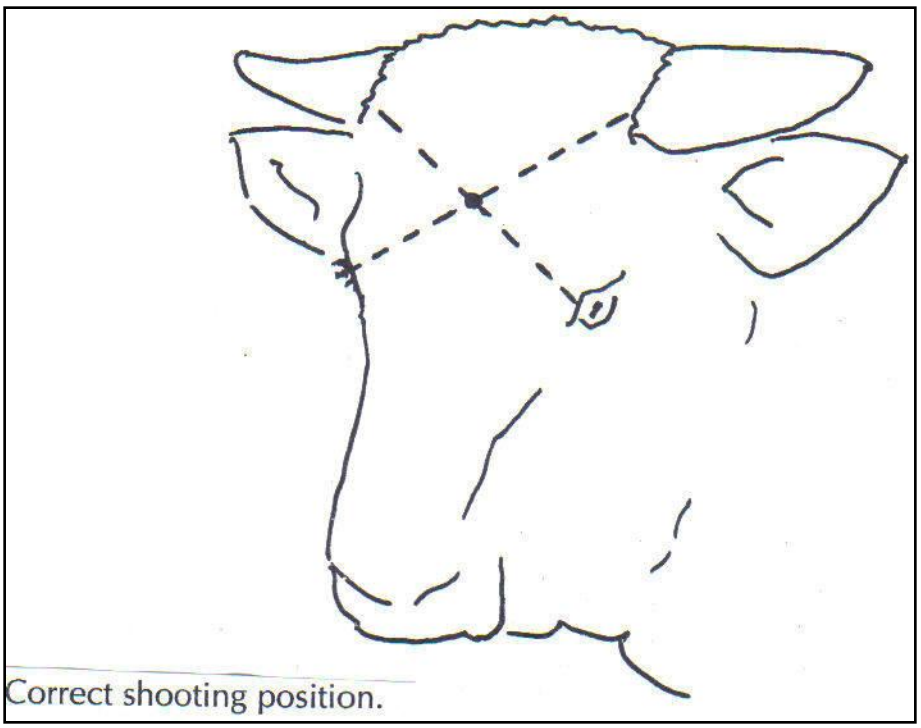


Figure 2.9 Correct shooting position (Gregory, 1998)

There are four stages (or depths) of concussion. In stage 1, the subject is slightly disorientated and the memory is affected. In stage 2, the subject has poor coordination and impaired memory. In stage 3, the subject is on the ground and breathing is maintained and in stage 4, the subject is prostrate on the ground and breathing is absent. Stage 4 concussion is dangerous in humans and if respiration is not restored, the blood will progressively be deoxygenated and brain function will eventually fail altogether. For animals, stage 4 concussion should be targeted every time and absence of breathing should be used as a measure of success (Gregory, 1998).

Much of the studies on captive bolt stunning have been based on analysis of their effect on evoked responses in the brain. This is the electrical activity in the brain which occurs in response to external stimulus such as clicking noise, flash light or mild electrical pulse applied to the limb. Recommendations on minimum bolt velocities have been based on the velocity that will blot out the visual evoked responses in the brain. For steers, heifers and cows, it is $55m/s^{-1}$ and for young bulls, it is $72m/s^{-1}$. The following list of signs, indicate an effective stun:

1. The animal must immediately collapse.
2. Breathing must be absent.
3. Muscles in the back and legs should go into spasm, forelegs and hindlegs should be flexed and after about five seconds, forelegs will straighten and become extended.
4. The eyes should not be rotated. If it rotates, it shows that the stun is not deep enough and there is risk of the animal regaining consciousness (Gregory, 1998).

It has been observed in sheep killed by captive bolt that the epithelial lining of the intestine is shed and this could have microbial implications (Badawy *et al*., 1957). Captive bolt stunning, like other stunning methods, causes an increase in blood pressure,

violent muscular contractions, and elevated levels of catecholamines (Troeger, 2003; Petty *et al*., 1994). These factors can also result in blood splash. In some European countries where cases of bovine spongioform encephalopathy (BSE) have occurred, the use of captive bolt is under debate and there is a clear risk to public health because of the destruction of brain tissues and blood vessels which might disperse these microbial organism into the blood stream and consequently to the organs and muscles (Troeger, 2003; Bowling *et al*. (2007) and Buncic *et al*. (2002)). Anil *et al.* (2002) recommends the use of non-penetrative captive bolt guns to minimise the risk of haematogenous dissemination of the central nervous system (CNS).

Effects of captive bolt on blood loss and meat quality parameters: In studies carried out by Anil *et al*. (2006), on cattle to determine the effects of the stunning methods (captivate bolt) and slaughter without stunning on blood and quality parameters; they found that there were no significant differences between the two groups in terms of packed cell volume, amount of blood loss and time taken to loss their blood and meat quality parameters. However, the study of Petty *et al*. (1994) reports that catecholamine levels were significantly higher in cattle slaughtered by Shechita than in cattle slaughtered conventionally but the application of a stun after the throat has been cut in the Shechita slaughter, abolished the increase in blood variables associated with Shechita in the absence of stunning.

The study of Anil *et al*. (2004) on sheep to compare blood and meat quality parameters on slaughter without stunning and captive bolt stunning were also carried out. They found no significant differences in the blood parameters but meat pH and colour were affected. Muscle pH at 24 hours (ultimate pH) post-sticking was significantly higher ($P<0.001$) in the captive bolt-stunned group than the non-stunned group. The colour of the captive-bolt-stunned group was also significantly different from the unstunned group ($P<0.05$). The captive bolt stunned group had the darkest colour when looking at the L^* and b^* values. Daly *et al*. (1988) found that the time to loss of brain responsiveness was significantly longer in cattle following Shechita (55 seconds) than following captive bolt (immediate). This also agrees with the findings of Kallweit *et al*. (1989) on cattle which registers no evoked potential following captive bolt stunning whereas, these potentials lasted for 77 seconds (somatosensorically evoked potentials) and 55 seconds respectively (visually evoked potentials) after ritual slaughter cut.

The study of Onec and Kaya (2004) which compared the effects of electrical stunning (ES), percussive captive bolt stunning (PS) and ritual slaughter (NS) on meat quality parameters in cattle reveals that, for all sensory attributes (odour, flavour, tenderness and overall acceptability) at all ageing times (24hr, 4, 7 and 14 days), the percussive captive bolt stunned group was significantly superior to the non-stunned group. In this study, the rate of muscle pH decline was significantly faster in PS group at 24 hours (5.75) compared with NS (5.99) and ES (5.96). For colour, the L^*, a^* and b^* values were significantly better for the PS group compared to the NS and ES group at the different storage times. For texture, the PS group (9.12kg) produced significantly more tender meat then the NS group (9.80kg). However, the water holding capacity was less in the PS group compared with NS and ES groups at 7 days, there was no significant differences.

Cooking loss was not significantly different between treatments at 24 hrs and 4 days *post-mortem*, however, significant differences were found at 7 and 14 days with PS having more loss than NS and ES groups. These results indicate that the captive bolt stunning of cattle improved meat quality compared with cattle electrically stunned using head only tongs and those non-stunned before slaughter.

2.9. Kosher slaughter method and its principles

Kosher slaughter is carried out by people of the Jewish faith. It is the slaughter of animals without prior stunning before sticking. Although the Muslims also slaughter in a similar way, they do not forbid stunning of the animal prior to bleeding provided the stunning instrument have never been used on pigs (Thornton, 1968). Regulations for the slaughter of animals for Jewish consumption have existed since A.D. 500 and according to the Mischna of the Talmud, a blow on the head of an animal intended for Jewish consumption is forbidden, as perforation of the brain membranes constitutes one of the eight mutilations which renders meat "terepha" or unfit for food (Thornton, 1968; Jennifer *et al*., 2002). Slaughter animals which are fit for Jewish consumption are described as "Kosher". For animals flesh to be considered Kosher, it must be a ruminant and have split hooves. Pigs for example have cloven hooves but are not ruminants, and thus they are non-Kosher (Leviticus 11:3-8). Poultry such as chicken, ducks, turkeys and geese lack front toes, have claws and double-lined stomach and are potentially Kosher depending on how they are slaughtered and except for those species that are specifically forbidden (birds of prey), (Leviticus 11:13-20), (Jennifer *et al*., 2002). For fish to be suitable, they must have scales and fins (Leviticus 11:9-12).

The actual reference to the slaughter of food animals occur in Deuteronomy 12:21. "Thou shall kill of thy herd and of thy flock, which the Lord hath given thee, as I have commanded thee, and thou shall eat within thy gates, after all the desires of thy soul". It is generally interpreted from this passage that there existed in biblical times a canon of oral law (Hebrew: halacha) that was to be used as an exegetical guide for understanding and executing the written Torah (Shragge and Price, 2004).

Kosher slaughter must be done according to a prescribed ritual by a "Shochet" or religious butcher, who is specially trained in these laws. The animal is rendered unconscious as quickly as possible while fully conscious and restrained by way of an expertly wielded, razor-sharp blade which causes cerebral hypoxia by the severance of the trachea, oesophagus, carotid arteries and jugular veins in one swift and instantaneous stroke (Shragge *et al*., 2004). In most countries where Kosher slaughter is practiced, secular statutes require that it be done in approved premises (usually a government inspected abattoir) and be subject to governmental hygiene and welfare regulations in addition to the religious requirements.

The Talmud identifies five rules which must be scrupulously observed during the act of Shechita, these are: (1) Shehiya (delay); the throat must be cut with one rapid continuous motion from start to finish. There shouldn't be any pause. (2) Derassa (Pressing); no upward or downward pressure may be exerted on the knife beyond that which is absolutely required to create the incision. (3) Chalada (digging); the incision must not

close back upon itself and cover the surface of the blade, which must be visible at all times. There should be no burrowing or stabbing action. (4) Hagrama (slipping); the incision must occur laterally across the throat between the larynx and the top of the inflated upper lung. (5) Ikkur (tearing); the oesophagus and trachea must be cleanly cut and not torn.
The animal must then be examined immediately after sticking to determine whether any damage was incurred that would lead to declaration of "neveila" (animal that dies before slaughter) on the carcass. The ritual knife (chalaf) usually have a broad, rectangular blade with length of at least twice the width of the animal's neck, 6 inches long for the slaughter of fowl and 18 inches for cattle (Shragge *et al*., 2004).

2.9.1 Bedika: After a successful sticking, the Shochet or his assistant will then carry out the next level of the process called "bedika". This involves an examination of various internal organs, which is made to ensure that the animal's health at the time of death met Talmudic standards of wellness. The Talmud defines eight pathological conditions in food animals including missing organs, torn organ walls, bone fractures and perforated organs that would render them unfit for Kosher consumption. Lung examination is the focal point of bedika. This examination seeks to identify evidence of pleural adhesions that might, for example, indicate a punctured lung. The lungs are filled with air and submerged in water as a test of pulmonary integrity. Air-tightness, indicating an absence of organ wall damage is sufficient evidence for Kosher certification. This process is predicted on the belief that any systemic mobidity in an animal would be evident in the lungs (Shragge *et al*., 2004).

The laws of Shechita are designed to secure maximum freedom from pain and many Jewish and non-Jewish physiologists of world re-known have said that, Shechita is a most humane way of killing animals. However, various attacks have been launched since the end of the nineteenth century on Shechita and efforts have been made to render it illegal in many countries like Great Britain, France and USA. In Switzerland and recently in The Netherlands, it is prohibited. The Jews claim that Shechita is an essential part of Jewish religion and legislation and any interfering with it inflicts hardship on Jewish citizens and in effect, amounts to religious persecution (Grunfeld, 1972). Levinger (1995) also indicates that Shechita has been discussed in many countries for more than hundred years, and is still a matter of discussion since no ideal method was found up to this day. Some countries have however introduced legislation that the animals for Jewish slaughter should be rendered unconscious before throat cutting. These countries are Norway, Sweden, Austria and Switzerland (Levinger, 1995).

In some countries, like New Zealand and Finland, Shechita procedure have allowed the use of a stun after sticking. Cattle are shot with captive bolt pistol within 10 seconds of the Shochet's stroke. In South Africa, the present regulation allows stunning, 20 seconds after the cut, although this practice does not address all of the perceived welfare deficiencies of conscious slaughter. Despite the lack of its widespread adoption, post-slaughter stunning of bovines does appear to represent a significant improvement in both the humaneness and efficiency of Shechita. It is a procedure that will probably receive greater study in the future (Shragge *et al*., 2004).

In South Africa, there have been a long contention between the livestock welfare coordinating committee (LWCC) and the Jewish body for Kosher (Beth-din) about the rotating restraining box (Weinberg pen) to be replaced by the ASPCA pen, which gives less stress to the animal before slaughter. That battle appears to have been won but argument still lies in the amount of time it takes to stun the animal post-cut, which is 20 seconds. The LWCC insists that the animals (cattle) should be stunned at most 4 seconds after the cut because it reduces the pain and stress of the animals and bleed-out is even more effective. The final conclusion on this is yet to be made.

Another recent concern with Shechita is the pain sensitivity of the animals. The findings of Mellor, Gibson and Johnson (2009) on calves, reveals that consciousness and ability to perceive pain and experience distress after the incision may persist for 60 seconds or longer in cattle. This research was done by quantitative analysis of electroencephalogram (EEG) which allows the experience of pain to be assessed more directly than has been possible before now.

2.9.2 Restraint methods: When ritual slaughter is to be performed on an animal, it becomes necessary to restrain the animal so that its neck can be presented to the knife and held relatively still until the stick is complete. Until the twentieth century, the common method was to first 'cast' the animal to the ground, using ropes and/or chains and most commonly on adult bovine. Sheep, goat and calves are small enough to be held manually or placed in an inverting cradle. In 1906, the United States federal law did away with this casting procedure entirely, so as to prevent disease causing filth from gaining entrance into the carcass while animals are on the ground. As a result, Shochetim were forced to adopt the practice of shackling and hoisting cattle directly to the rail. A single hindleg is shackled by a chain (or rope) connected to an overhead rail system. As the hoist was engaged, the animal's hind quarters were gradually lifted off the floor as the entire weight of the animal now hangs on the shackled leg. Both the cast and rope and shackle and hoist methods were slow and awkward, and could be perilous to the abattoir staff. They were also stressful to the animal and could lead to carcass damage sufficient for a ruling of 'terefah'.

The first holding pen specially designed to improve both humaneness and efficiency for Shechita and Al-dhabh (Muslim slaughter) was developed in the United Kingdom in 1927. It is called the Weinberg casting pen which consist of adjustable enclosure, large enough for adult cattle that could be rotated on circular rails about its longitudinal axis. With a 180º turn, the animal became inverted, thus allowing for easier neck extension and a more rapid incision. This pen was hailed by many as a great step forward and in the United Kingdom, it became mandatory for religious slaughter in 1958 (Shragge *et al*., 2004).

However, the Weinberg casting pen was never embraced as a permanent solution to the problems associated with conscious restraint. Inverting any animal, particularly, large ruminants like cattle, causes distress as well as potential suffocation through pressure of the abdominal contents on the thoracic cavity. These problems were addressed in the

design of the first upright slaughter pen in 1963, known as the ASPCA (American Society for Prevention of Cruelty to Animals) or 'Cincinnati' pen or 'The kill Pen' or 'Elizabeth Pen' manufactured by Schmidt & Co. It provided for the gentle restraint of both the head and body of a still, standing animal with the body supported ventrally and cowdally within the enclosure. The neck is also extended by a hydraulic chin lift. The physical force applied to the animal was also greatly reduced (Shragge *et al*., 2004). The study of Dunn (1990) shows that cattle slaughtered with the Weinberg pen showed more stress reaction than the ones slaughtered with the ASPCA pen. The cortisol, haematocrit and the pHu were much higher in the cattle slaughtered with the Weinberg pen.
Figure 2.10 and Figure 2.11 shows the Weinberg and the ASPCA pens respectively.

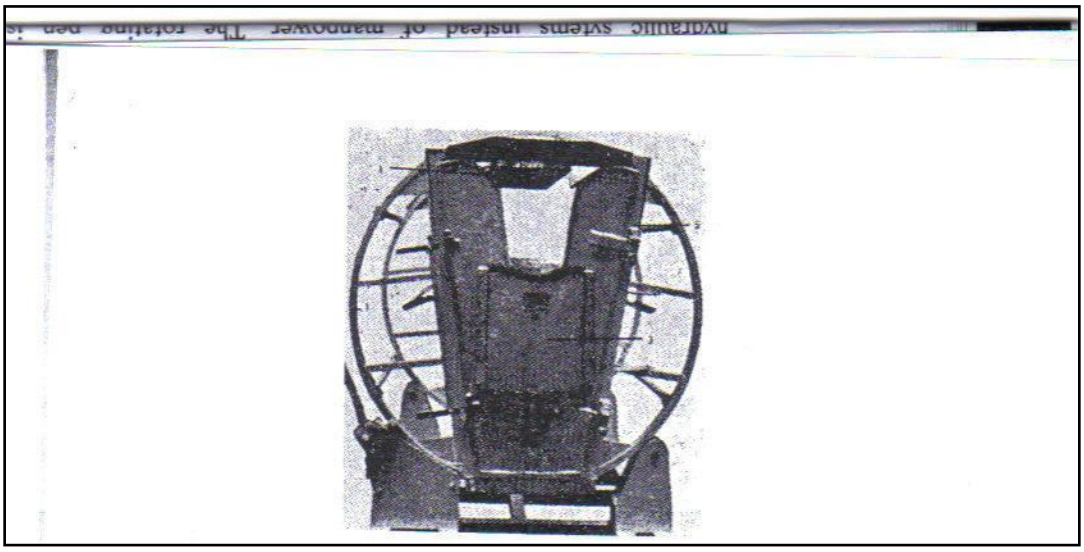


Figure 2.10 The Weinberg casting pen (Levinger, 1995)

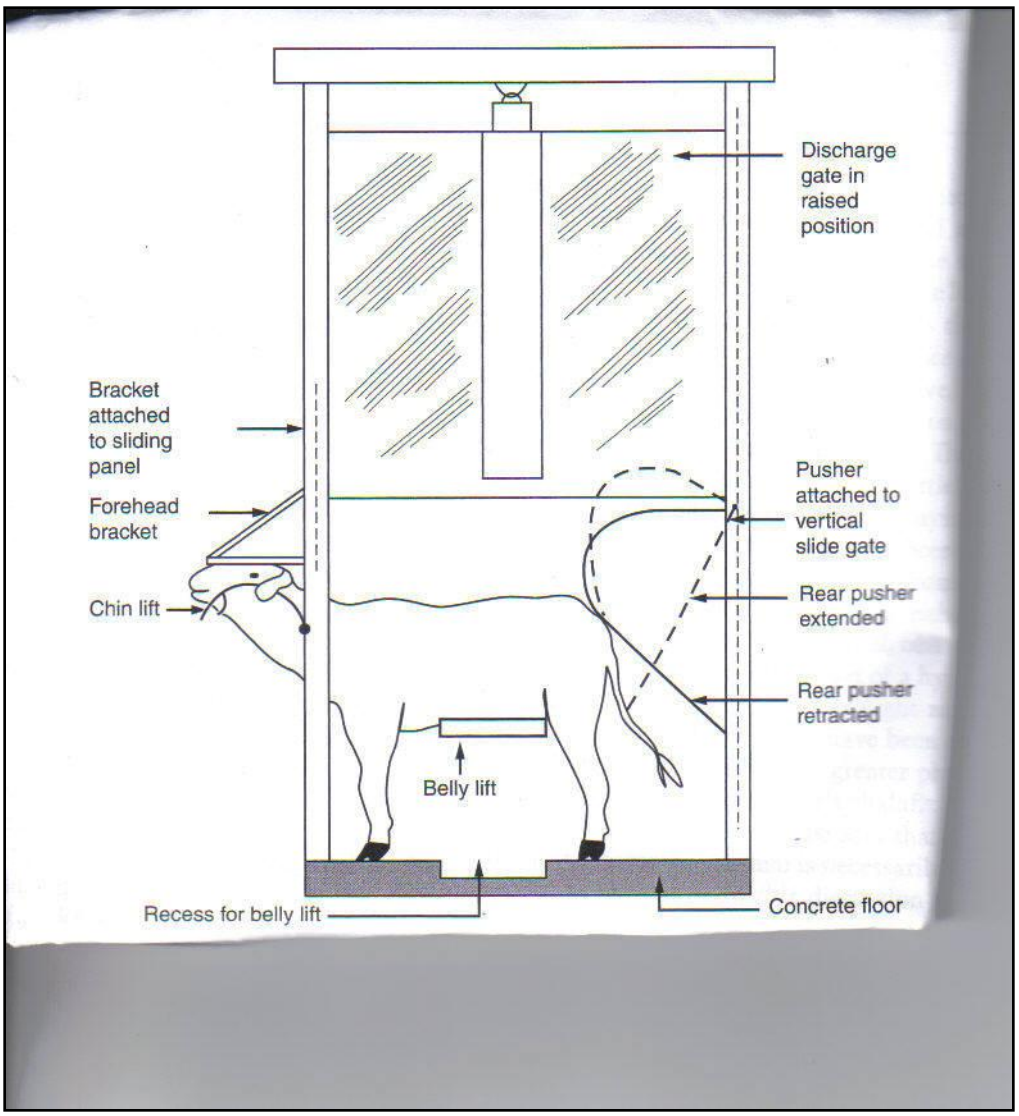


Figure 2.11 Modified ASPCA pen showing the animal in the correct position for low stress restraint. Both the forehead and the back should be levelled. Excessive pressure must be avoided. (**Grandin and Regenstein, 1994)**

2.10 Effects of Kosher slaughter technique on meat and carcass quality parameters

2.10.1 Bleeding efficiency/blood loss: The residual blood content of lean meat usually varies between 2 and 9 ml/kg^{-1}. It is often claimed that a carcass has to be well bled for the meat to keep but this view has not been confirmed by research. However, poor bleeding can affect the appearance and visual acceptability of a carcass (Gregory, 2004). The findings of Anil *et al*. (2004 and 2006), on sheep and cattle respectively, shows that bleed out is not affected by electrical or captive bolt nor is it improved by slaughter without stunning. There were also no significant differences in packed cell volume and meat quality parameters. The findings of Sahlstedt (1928) and Dukes (1958) shows that most animals in their experiments, after Shechita, lost over 80% of their blood within the first 3 minutes of the cut, although small amounts of blood kept dripping for 6 to 7

minutes. Dukes (1958) also showed that blood pressure in the brachial and vertebral artery (in calves) dropped to 45-50mmhg/sec for the first three seconds. He said with the rapidity of blood loss following Shechita, it is very unlikely that blood could reach the brain even during the first few seconds after the severance of the carotids. No blood flow can also be measured in the internal maxillary artery and no arterial blood supply reaches the brain immediately after Shechita (Levinger, 1995). Levinger (1995) also says that the deep respiratory movements and muscle contractions occurring after Shechita, improves exsanguination. Kallweit *et al*. (1989) found a non-significant difference between the haemoglobin and myoglobin pigment content in the muscle of cattle slaughtered conventionally and by Shechita. Kallweit *et al*. (1989) also found a non-significant difference in plasmacortisol concentration in a similar experiment. Catecholamine concentrations were significantly higher in Shechita compared to conventional slaughter (Petty *et al.*, 1994).

However, during Shechita, some animals take longer to lose brain function and to die. This has been shown using somatosensory evoked response in the brain and from the delay before the animal collapses (Blackmore, 1984 and Daly *et al*., 1988). It is thought that the delay is due to a combination of false aneurysm in the severed carotid arteries plus sustained blood flow to the brain through the vertebral arteries (Baldwin, 1960). Eight percent of cattle slaughtered by Shechita develop false aneurysms in both carotid arteries (Gregory *et al*., 2008). When this happens, the animal takes longer to lose consciousness before they die.

Another recent concern about Shechita is the aspiration of blood into the upper respiratory tract and lungs which causes suffering during slaughter (Von Wenzlawowicz and Von Holleben, 2007; Webster, 1994). Others believe that there will be no suffering because afferent signals activated by lung irritation are conveyed by neurones within the vagus nerves and these are severed in ritual slaughter (King, 1999). A recent research done by Gregory *et al*. (2008) shows that 19% of 123 cattle slaughtered by Shechita had substantial amount of blood in the trachea (covering >10% of the inner surface area), and 36% had blood in the bronchi. These frequencies were similar to those for 103 cattle shot by captive bolt and stuck (secular slaughter) whilst in the same upright position (21% and 31%, respectively). Ten percent of the Shechita cattle had bright-red blood-tinged foam lining the trachea whereas none of the captive bolt-shot cattle had blood-tinged foam lining their trachea. This calls for concern during Shechita slaughter.

2.10.2. Effect of Shechita on pH: The research of Kallweit *et al*. (1989) found no significant difference in the pH_{24hr} of *musculus longissimus dorsi* of cattle slaughtered by captive bolt and Shechita. This agrees with the work of Onec and Kaya (2004), who found no significant difference in pH_{24hr} of cattle slaughtered with captive bolt and non-stunned cattle. Although the rate of pH decline is significantly faster in captive bolt compared to others. It also agrees with the findings of Vegara and Gallego (2000) and Velarde *et al*. (2003), who found no significant difference in ultimate pH of electrically stunned lambs and non-stunned lambs. This indicates that rate of *post-mortem* glycolysis in stunned cattle and lamb is not markedly different from non-stunned groups. However, the observation of Anil *et al*. (2004) on sheep slaughter to compare pH_{24hr} on ritual

slaughter group and captive bolt-stunned group, found a higher pH_{24hr} (6.2) with sheep slaughtered by captive bolt compared to 5.7 for non-stunned sheep.

2.10.3 Effect of Kosher slaughter on meat colour: The study of Kallweit *et al*. (1989), which compared the haemoglobin and myoglobin pigments in cattle slaughtered by captive bolt and cattle slaughtered by Kosher technique, found no significant difference between both. The research of Anil *et al*. (2004) on sheep (*M.Trapezius*) also found no significant difference ($P< 0.05$) in colour between groups slaughtered by electrical, captive bolt and Kosher method. Although meat from the captive bolt stunned sheep was the darkest, followed by that from Kosher, whilst meat from electrically stunned sheep was the lightest. Velarde *et al*. (2003) also found no significant difference in colour (L^*, a^* and b^* values) at 24 hours between lambs slaughtered by electrical stunning and non-stunned lambs. However, the study of Onec and Kaya (2004) to determine the meat quality differences between groups of electrically stunned, captive bolt and non-stunned cattle, from one to fourteen days of blooming, found significant differences between groups. The L^* values of the meat (*m. longissimus thoracis*) gradually increased from day 0 to day 3. L^* and b^* values on day 3 were significantly different ($P< 0.05$). During blooming, samples from the captive bolt stunned group were brighter and redder than those from non-stunned and electrically stunned groups but differences were not statistically significant. Likewise b^* values were higher in the captive bolt stunned group than the other groups during blooming. After 5 days of blooming the electrically stunned group had significantly higher colour tone compared to non-stunned groups, while the captive bolt-stunned group had the lowest colour tone and after 9 days of blooming, the differences became sharper but were not statistically significant.

2.10.4 Effect of Kosher slaughter on meat textural parameters: The most important physical attribute of meat is its degree of tenderness when eaten after some degree of cooking (Swatland, 1984). However, the exact mechanism involved in *post-mortem* tenderisation process is complex and still not fully understood (Fritz and Greaser, 1991; Koohmaraie, 1994; Odeh, 2003 and Swatland, 1984).There are quite a number of factors that affect the textural attributes of meat such as; breed, age, gender, anatomical location of muscle, electrical stimulation, temperature, pH, water holding capacity, myofibrillar proteins, cooking method, proteolytic enzymes, connective tissues, sacomere length, pre-slaughter stress and so on (Scheepers, 1999; Lee, 1986; Thompson, 2002; Bouton *et al.*,1973b and 1973a; Locker and Daine, 1975b; Monin, 1998). The research of Onec and Kaya (2004) on *m.longissimus thoraci* of cattle slaughtered by captive bolt stun (PS), electrically stunned (ES) and non-stunned (NS) shows that, PS (9.12kg) and NS (9.80kg) groups produced significantly more tender meat than ES (13.83kg) at 24 hour *post-mortem* ($P< 0.05$). These result shows that PS prevents glycogen loss in cattle muscle and so improves meat texture. Immonen *et al*. (2000) also reported lower shear force values in meats of high and intermediate glycogen contents compared to meats of low glycogen content. Hardness decreased with time in all groups agreeing with Jeremiah, Tong and Gibson (1997) and Vergara and Gallego (2000). With longer period of ageing (14 days), the meats from the three groups reached similar tenderness.

2.10.5 Effect of Kosher slaughter on water holding capacity (WHC): Much of the water in a muscle cell is bound to various proteins and if the proteins are not denatured, they will continue to bind water during the conversion of muscle to meat and even through the cooking process. The changes that occur in water binding during the conversion of muscle to meat depends on the rate and extent of the pH drop and amount of protein denatured (Forrest *et al*., 1975). At early *post-mortem* when the pH is still high, the water binding properties remains relatively high but as the pH falls rapidly during conversion of muscle to meat, a low water binding capacity becomes evident (Forrest *et al*., 1975). Meat has its poorest WHC at the iso-electric point of the principal protein in muscle at pH 5.4 to 5.5 (Veary, 1991). From these findings, we could deduce that DFD meat will have low WHC while PSE meat will have a high WHC.

The findings of Onec and Kaya (2004) shows no significant difference in WHC between groups of captive bolt stunned, electrically stunned and non-stunned cattle. However, more water exuded from meats of the captive bolt stunned group than the non-stunned and electrically stunned group. Significantly higher cooking losses were also found in meats of the electrically stunned and percussive stun groups at 7 and 14 days *post-mortem*. This may be caused by the faster pH decline in the PS group. Several researchers have found that high drip loss is due to rapid pH decline (Stoier, Aaslyng, Olsen and Henckel, 2001). These researchers also found that WHC decreased with stunned ones. Vergara and Gallego (2000) also found no significant difference in WHC of stunned and unstunned lamb. There could be variations in WHC in different muscles in the carcass irrespective of glycogen depletion, because not all muscles in the carcass acidify at the same rate (Gregory, 1998). For example, neck muscles tend to have a high ultimate pH (e.g. 5.8) which is well above the iso-electric point of its proteins.

2.10.6 Effect of Kosher slaughter on muscle temperature: Studies have shown that stress associated with the sequence of slaughter or even the day of slaughter affects initial temperature of *m.longissimus dorsi, m. semimembranosus and m. semitendinosus* (Gregory, 1998). The muscle temperature of stunned animals were found to be lower than that of non-stunned animals in experimental work performed on sheep but there are variations which lies on the level of environmental temperatures (Veary, 1991). The enzyme non-contractile ATP-ase of myosin is involved in maintaining meat temperature and muscle tone as it maintains the integrity of the muscle cells against spontaneous breakdown (Veary, 1991). This uses energy and causes a steady ATP depletion. The temperature at which a muscle goes into rigor influences the amount of contraction and therefore the final tenderness of meat (Aberle *et al*., 2001; Herring *et al*., 1965). The time required for a muscle to reach its ultimate pH is also temperature dependent and as a result, the rate of pH decline decreases with decreasing temperature in a complex relationship (Veary, 1991). The combination of a low pH_{45} with a high muscle temperature is vital in the formation of PSE meat (Gregory, 1998). Too high temperature *post-mortem* also causes denaturation, however rapid chilling should be avoided as this causes thaw rigor and cold shortening. Proteins are usually denatured at temperature above 25ºC or below 0ºC (Lawrie, 1966). The findings of Onec and Kaya (2004) shows a higher temperature at 15 minutes and 24 hours *post-mortem* in captive bolt stunned cattle compared to non-stunned cattle. The figures were not statistically, significantly different.

2.10.7 Effect of subcutaneous fat thickness on meat and carcass quality: According to Swatland (1984) and Gregory (1998), adipose tissue serves its proper function only when an animal uses the energy and insulation provided by this fat to survive a period of inadequate feed intake or adversely cold weather. It also gives a kind of finished appearance to a carcass and without at least some subcutaneous fat, a carcass could be judged to be unattractive by traditional standards (Swatland, 1984). The systemic deposition of fat in carcass influences the dressing percentage which is indicated as:

$$\frac{\text{Carcass weight}}{\text{liveweight}} \times 100$$

On a high energy ration, cattle deposit subcutaneous fat at a greater rate than they deposit intermuscular fat (Fortin *et al*., 1981). Breeds of cattle also differ in the way they deposit fat, for example, Herefords produces more subcutaneous fat and less perirenal and pelvic fat than Angus, Fresian and Charolais cross breeds (Charles and Johnson, 1976).

Animals have a high proportion of saturated fatty acids (ca.50%) and their fat is solid or semi-solid at room temperature. When a beef carcass is cooled from body temperature down to 0^0C after slaughter, subcutaneous fat passes from a liquid to solid state (Swatland, 1984). This could have an effect on the rate of cooling down in individual carcasses depending on their subcutaneous fat deposit. In addition, other meat quality parameters like tenderness and meat colour could be affected by subcutaneous fat cover (Swatland, 1984). Increased thickness of subcutaneous fat was found to improve tenderness by allowing the carcass to chill slowly and to increase enzyme activity (Smith *et al*., 1976). Fat cover also play a significant role in reducing cold shortening during chilling of beef (Doleza, Smith and Carpenter, 1982) and lamb (Smith *et al*., 1976). This could also bring about a faster pH decline (Koohmaraie *et al.*, 1988). The fat colour, for example, the yellow fat is also sometimes traditionally perceived by consumers that the beef was from old animals and that it might be tough. This has no scientific backing because the yellow carotene pigment originates from the animal's feed such as corn or grass (Palmer and Eckles, 1914).

2.11 Electrical stimulation of carcasses

This is the post-slaughter application of electrical current to the animal carcass. It is used as a way of accelerating *post-mortem* muscle metabolism and so allowing rapid chilling without producing tough meat (Gregory, 1998). It accelerates their conventional decline in pH and may enhance tenderization during conditioning (Harsham and Deatherage, 1951). This process has been adopted by the meat industry as a means of countering cold shortening in beef and lamb. Pearson and Dutson (1985), provides an excellent review of electrical stimulation thus; in the living animal, the signal to contract is provided by a membrane depolarization from the nervous system. The physical application of electric current stimulation simulates this system and results in accelerated muscle metabolism and serves to hasten the onset of *rigor mortis*. It brings about rigor onset before the muscle reaches low temperature at which cold shortening takes effect (Kinsman *et al*., 1994).

When low voltage ES is used, the current is applied during bleeding but when high voltages are used, they are often applied either after the bleeding tunnel or on the dressing

line (Gregory, 1998). Electrical stimulation could be applied up to 1 hour after slaughter to permit rapid chilling but in some cases, it is now routinely applied to control fall of pH value (Swatland, 1984). The general principle is to apply pulsed voltage to the carcass. Either high voltages of 500-1000v or low voltages of 90v may be applied, but a typical stimulation process involves 600v at a current of 5-6A applied in 16-20 pulses of 2 seconds duration (Varnam and Sutherland, 1995). ES exerts its greatest tenderising effects when carcass cooling is sufficiently rapid to provoke appreciable cold shortening. Some findings show that ES significantly increased the rate of pH decline in beef (Fjelkner-Modig and Ruderus, 1983; Kastner *et al*., 1993) and also in veal (Smulders *et al*., 1989) and in lamb muscles (Polidori *et al*., 1999). The readings were taken at various intervals within 20 hours *post-mortem* during which the ultimate pH were attained.

The findings of Wiklund *et al*. (2001) on red deer also shows that shear forces in electrically stimulated deer is lower during the first 3 weeks of ageing than in unstimulated venison. Electrical stimulation also resulted in more tender meat when measured as shear force at 7 days *post-mortem* in veal (Smulders *et al*., 1989) and 2 and 7 days *post-mortem* in lamb (Polidori *et al*., 1999). Accelerated tenderisation stimulated carcasses are attributed to more rapid proteolysis that follows from both earlier onset of *rigor mortis* and higher carcass temperatures in the early post-rigor period compared with non-stimulated controls (Dutson, 1981; George *et al*., 1980). This accelerated tenderization offers advantages for product that is intended to be frozen or likely to reach retail market within a short time. It has also been reported that apart from guarding against cold shortening, the beneficial effects of ES on meat tenderness are probably a simple consequence of muscle fibre fracture (Marsh *et al*., 1981; Sorinmade *et al*., 1982). Electrical stimulation (ES) also affects meat colour in beef (Cross, 1979; Bendal, 1980), although the extent of the changes will depend on the chilling rate and the observed muscles. In general, it improves a paler colour (Hector *et al*., 1992; Martin *et al*., 1983; Sleper *et al*., 1983) because of increased protein denaturation and myofibrillar lattice shrinkage (Offer and Trinick, 1983; Swatland, 1993). In carcasses from stressed cattle it has no effect on tenderness. Carcasses from stressed cattle would not be prone to cold shortening if the ability to regenerate ATP has been reduced by the stress (Gregory, 1998). This could be particularly helpful in Kosher slaughter. Also, if a carcass is over stimulated, it will lose more weight as drip (Gregory, 1998). Cooking loss and colour stability may also be negatively affected by electrical stimulation (Bouton *et al*., 1980a; Marsh *et al*., 1983; Unruh *et al*., 1986; Laak and Smulders, 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 Pre-slaughter processes

The research was conducted at Chamdor Meat Packer's abattoir, which is located in Krugersdorp in the western part of Johanesburg, in Gauteng Province of South Africa. This abattoir is regarded as a high throughput abattoir with a slaughter capacity of over a thousand heads of cattle per day. The trial took place towards the end of July, during winter, when daytime temperature ranged from 10^0C to 17^0C while nighttime temperature ranged between 3^0C to 6^0C.

The pre-slaughter conditions were assessed and they were satisfactory. Animals were usually transported from different feedlot farms of an average of about 150 kilometers from the abattoir and it takes about two and a half hours for the animals to arrive at the abattoir. They were transported by trucks in the evenings and the animals had about 12 hours of lairage time, with access to water but without feed. The animals were treated humanely and animals from the same farms were penned together to avoid fight and stress. The animals were a homogenous group that are accustomed to handling.

Three-hundred-and-eleven crossbreeds of Bonsmara, Nguni, Africaner, Brahman and Tuli which is typical of cattle slaughtered in South Africa, were randomly assigned and used in this trial. It was a completely randomized control study. There were four treatment groups based on slaughter method (SM), electrical stimulation (ES or NES), gender (G) and fat code (FC). Animals were predominantly steers in the "A" age group and a very few AB group. Their fatness was also predominantly two or three (lean-medium) according to the current South African Beef Classification System (Meat Classification Regulations No 863 in Government Gazette of September 2006). All the animals were weighed before going into the slaughter box and their average slaughter weight (WBS) was about 400kg.

3.2 Slaughter processes

The slaughter process and data collection were done over a period of two weeks in which animals were randomly assigned in two batches, each batch for each week. Random allocation occurred across batches and across slaughter days and each animal had equal chances of being in the treatment groups.
In the first week (first batch), 77 animals were assigned to Kosher slaughter while 66 animals were assigned to conventional slaughter. In the second week (second batch), 93 animals were assigned to Kosher slaughter while 75 animals were assigned to conventional slaughter and these comprised the two main treatment groups, i.e. Kosher slaughter technique (KST) and conventional slaughter technique (CST). Prior to slaughter, the live weights of the animals were recorded in kilograms.

3.2.1 Kosher Slaughter: Kosher slaughter was performed by a trained and experienced Shochet (Rabbi J Miller and his assistant, Rabbi Y David). Animals were restrained in an

ASPCA (American Society for Prevention of Cruelty against Animals) box in which cattle stand upright with a hydraulic chin lift to lift the head upwards and to extend the neck for the cut; belly plate to raise the animal a up for stability and a rear pusher to reduce the struggle of the animal with a firm restraint (Shragge *et al*., 2004). The Shochet then gash stuck the animals with a sharp knife of about 40cm long. The cut was made upwards and across the ventral part of the neck whilst the animal was restrained. This was done swiftly within the space of one second and in accordance with the Talmudic standards (Levinger, 1995). Each animal was then stunned with a 0.22 calibre cash special captive bolt gun in the frontal position of the head, after 20 seconds post-cut to render them unconscious. This was done in accordance with the present South African Animal Protection Amendment Act, No 7 of 1991.

Post-cut stunning was done to reduce the pain and suffering of the animals during exsanguinations. Figures 3.1 and 3.2 are the pictures to demonstrate the Kosher slaughter procedure:

Figure 3.1 The Shochet as he gash stuck an animal

Figure 3.2 The Shochet as he gash stuck the animal (2)

3.2.2 Post-slaughter processes (Kosher slaughter procedure): After sticking and stunning, the carcasses were then hoisted by the hindleg and allowed to bleed for about 8 minutes on the bleeding rail before they were electrically stimulated. Electrical stimulation (ES) lasted for about one minute for each carcass and it was done at 810volts. Stimulation was done to accelerate the carcass pH decline and to also enhance tenderisation during conditioning (Swatland, 1984). Forty of the Kosher carcasses were exempted from electrical stimulation (NES) so as to be able to compare the effect of electrical stimulation on the meat and carcass quality parameters.
After stimulation, the weight of each carcass (WAS) was then recorded in kilograms to be able to determine the blood loss and bleeding efficiency. Each of the carcasses then proceeded to the dressing line where the head and limbs were removed, followed by evisceration and carcass splitting.

Following carcass splitting and removal of organs, the lungs went through the conventional bedika procedure. During this procedure by the Bodek, each lung was inflated by clamping the severed end of the trachea to a nozzle that delivered compressed air to the lungs whilst operating a foot switch. The inflated lungs were then examined by the Bodek for any adhesions, holes or infections in the pleura cavity according to Shragge *et al*., 2004 and Gregory *et al*., 2008.
Following the bedika procedure, the lungs and trachea were then removed to subjectively score the amount of blood in the trachea (BLT) and upper bronchi (BLUBR). Blood splash (BS) was also quantified in each set of lungs. The presence of bright-red blood-

tinged foam (BRBTF) was also observed and recorded in each trachea. This was done by cutting out about 25cm of the trachea and opening it along its length up to the upper bronchi with a sharp knife to observe and to quantify the amount of blood and the bright-red blood-tinged foam (Gregory *et al*., 2008). **The scoring was done in the following way**: 0, no blood; 1, less than 10% blood present in the inner surface of the trachea; 2, blood covered 11% to 50% of the inner surface area; 3, blood covered more than 50% of the inner surface area (Gregory *et al*., 2008). Blood in the upper bronchi was also observed and recorded. When it was present, it was denoted as P and when it was absent as A. The presence of bright-red blood-tinged foam in the trachea was also observed and recorded. When it was present, it was denoted as P and when absent as A. **Blood splash** was also visually and subjectively scored according to the amount of splash, from 0% to over 50%, similar to BLT scoring, i.e. 0, no splash; 1, 1% to 10% splash present; 2, 11% to over 50% splash present. The amount of blood splash in the lungs could easily be seen as dark patches as they were scattered over the two lobes of lungs. Some of the lungs were also split opened with a knife to observe the extent of the splash. In total, one hundred and seventy sets of lungs were observed for Kosher. Figure 3.3 to Figure 3.7 shows the pictures of opened tracheas with or without blood and pairs of lungs showing the presence or absence of blood splash in the Kosher- and conventionally slaughtered carcasses.

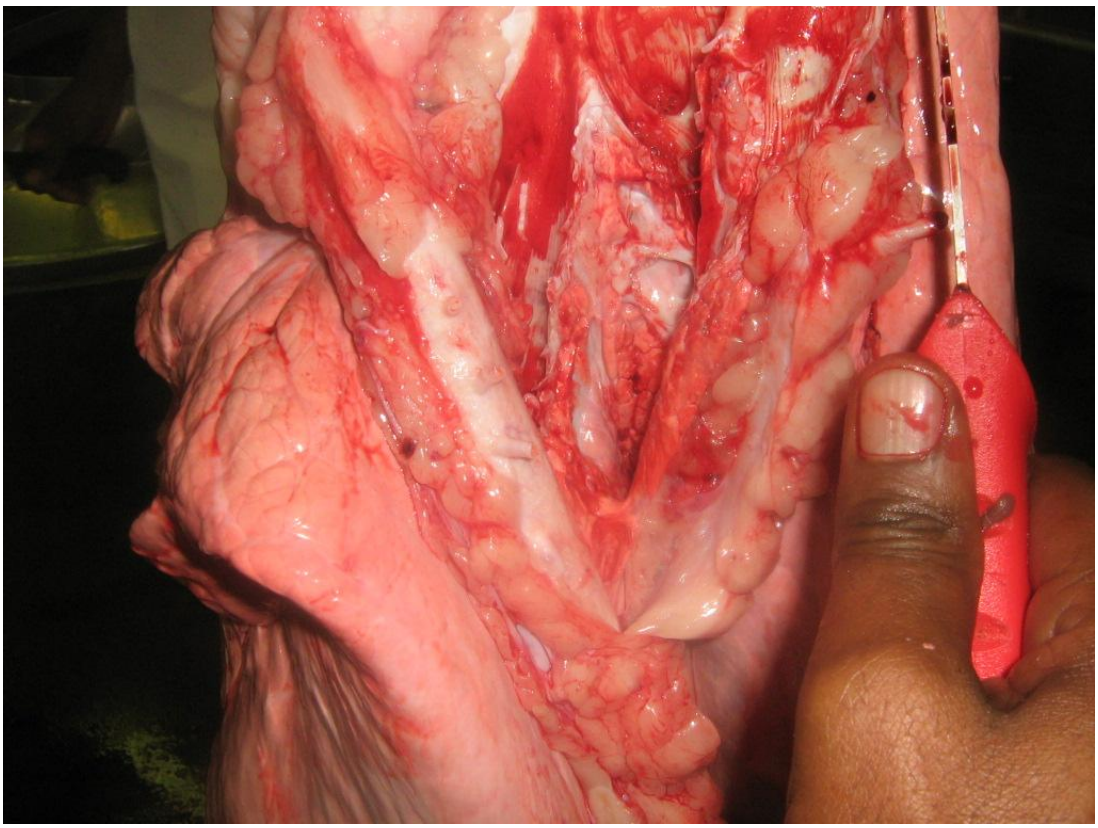


Figure 3.3 An opened trachea showing the bright-red blood-tinged foam in the trachea of cattle slaughtered by Kosher slaughter procedure

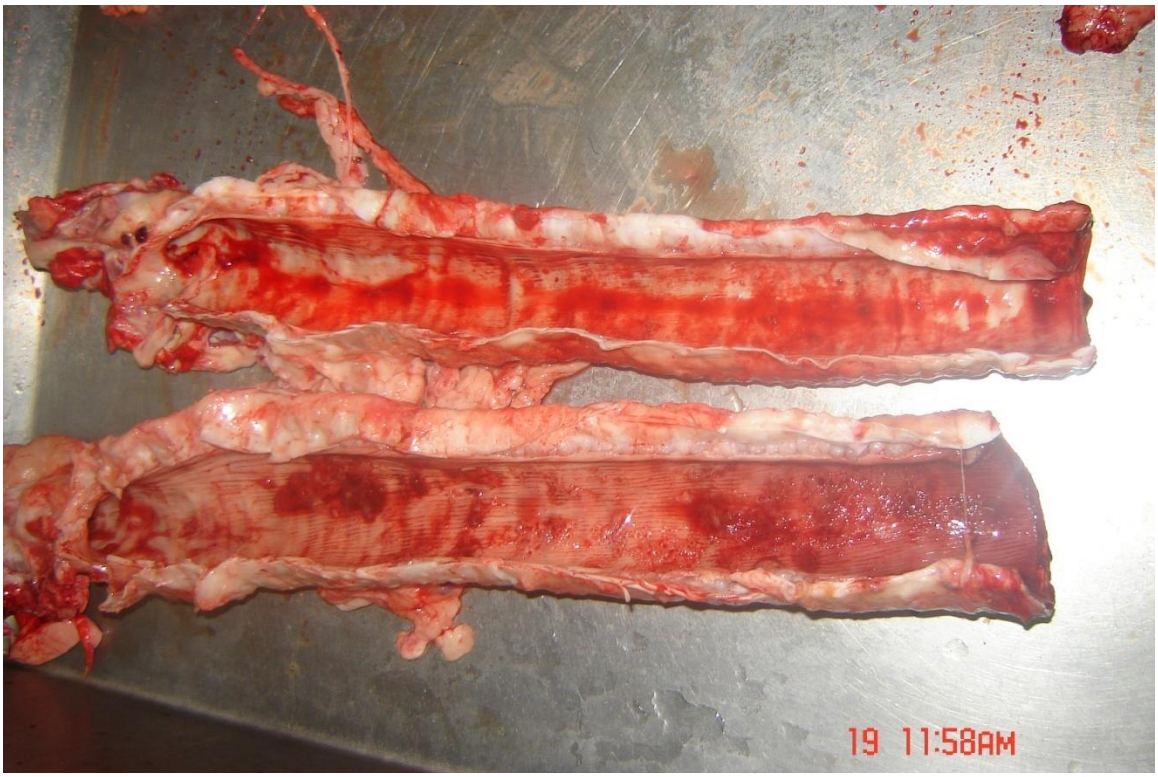


Figure 3.4 The presence of blood in the tracheas of cattle slaughtered by Kosher slaughter procedure

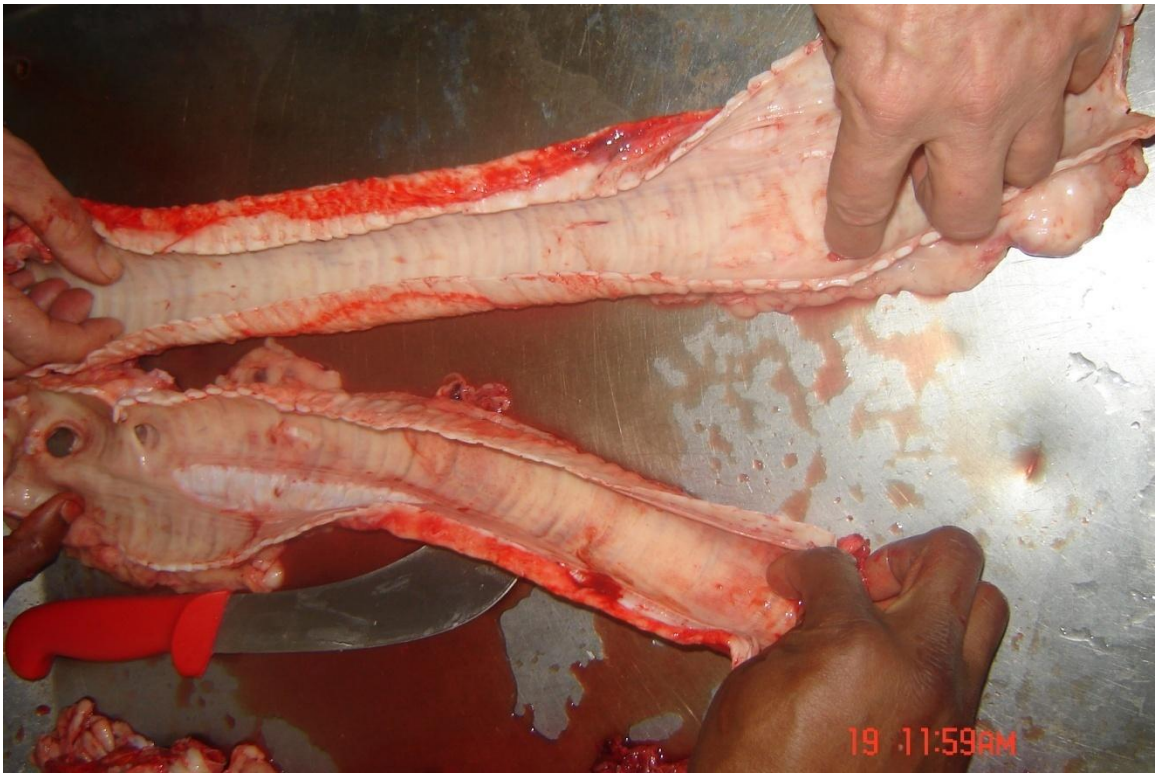


Figure 3.5 The absence of blood in the tracheas of conventionally slaughtered cattle

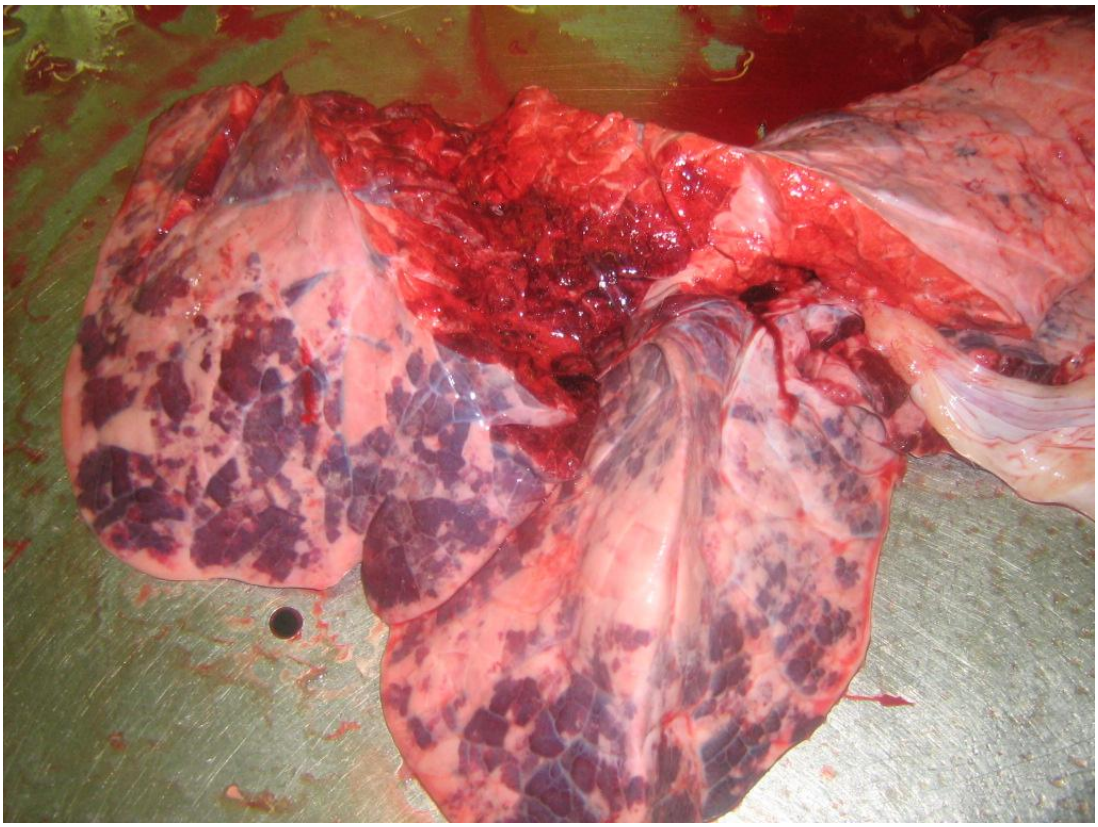


Figure 3.6 Lungs showing the presence of blood splash in a carcass slaughtered by Kosher slaughter procedure

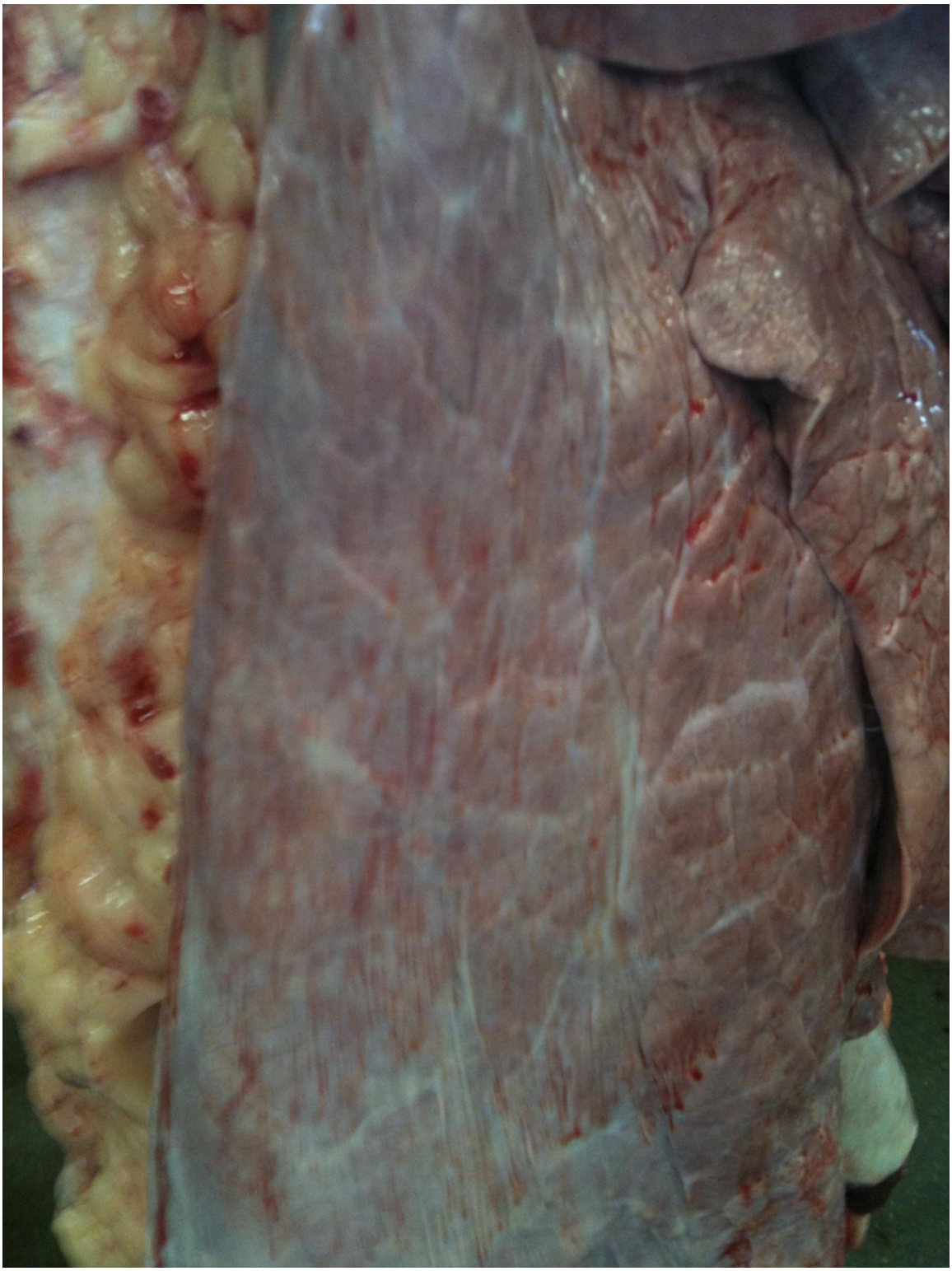


Figure 3.7 The absence of blood splash in lungs of cattle slaughtered by conventional slaughter procedure

Following carcass splitting and organ removal, the carcasses were moved to a compartment where the warm and cold carcass weight, sex, and conformation (bone to

muscle ratio) grade were recorded. Damaged and infected carcasses were also removed at this point. After this point, the carcasses were then moved to the chilling rooms where the initial temperature was 5^0C and air speed of about 1.5m/second.

3.2.3 pH and temperature measurement: The pH_{45min} and temperature at 45 minutes were recorded with a portable pH meter from Hanna instruments (code-H18424N), with temperature probe and a special glass electrode (visc smicrokynar electrode, code-FC 200B), which is specifically suitable for measuring meat pH. This was done by poking 2 holes of about 3cm deep with a sharp metal and inserting the temperature probe and the glass pH probe between the 10^{th} and 11^{th} rib in the *m.longissimus dorsi*. This muscle was chosen because it is the standard reference muscle in meat science but in future studies, we might consider other muscles that could be affected by the slaughter process.
Following this, the same instruments were used to measure the pH and temperature at 3 hours**,** 6 hours, 12 hours and 24 hours post-slaughter.
However, it was noticed that the temperature of the chilling rooms dropped close to zero from 12 hours post-slaughter.

3.2.4 Conventional slaughter: The conventional slaughter procedure was performed by a trained slaughter man. This was done with a pneumatic captive bolt gun which uses compressed air to shoot a recessed bolt (Anil *et al*., 2002). One-hundred-and-forty one animals were assigned to this treatment. The animals were restrained in a v-shaped metal box with an open top and with a rear gate to prevent them from struggling or moving backwards after entering the box. Cattle were restrained in an upright position and once the animal is locked up in the box in a stable position, the shot was delivered by the gun to the forehead. After the shot, each animal went into spasm while it collapsed, the fore and hind legs were flexed while the head is lowered. The animal immediately became unconscious while breathing was absent and the eyes were not rotated in most of them. This showed that the animal was deeply stunned and this procedure was carried out in accordance with the South African Protection of Animals Amendment Act, No 7 of 1991.

Figure 3.8 is a picture of the conventional slaughter procedure showing how the animal is stunned by the slaughter man with the pneumatic captive bolt.

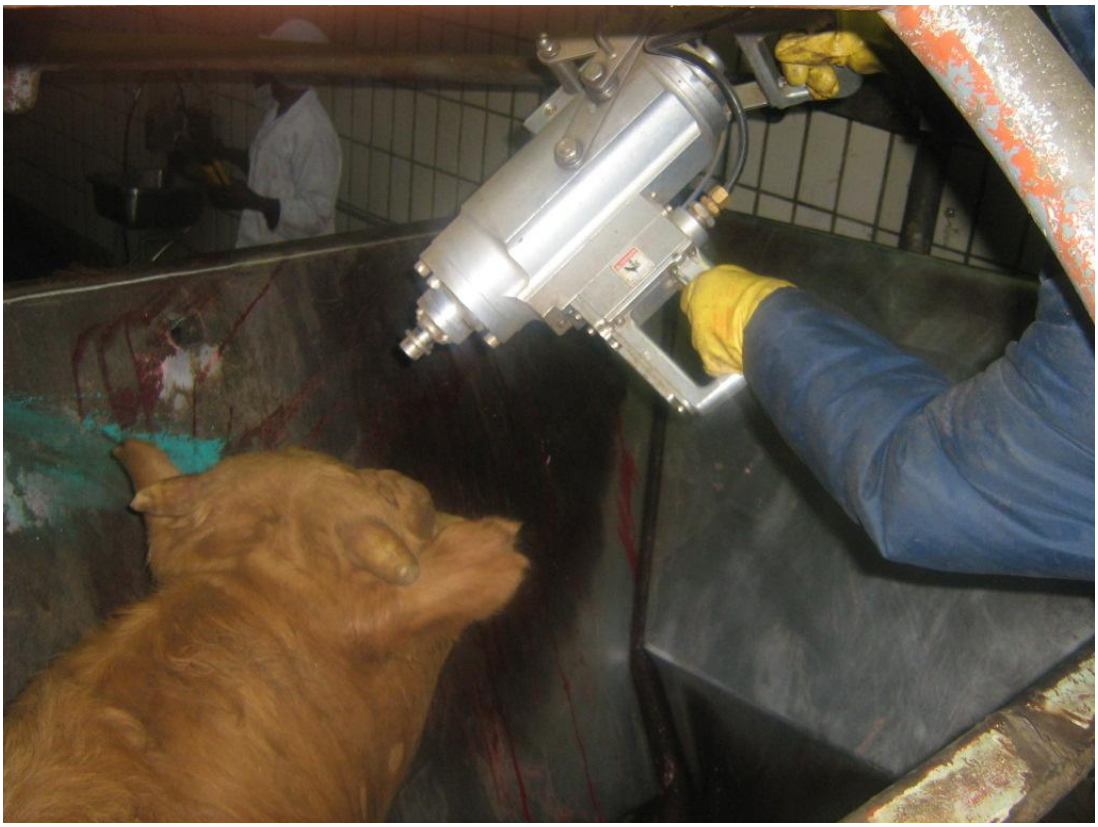


Figure 3.8 Conventional slaughter procedure, showing how the animal is stunned with a pneumatic captive bolt gun by the slaughter man

Figure 3.9 also shows the conventional slaughter procedure, indicating the correct stunning position on the head of the animal.

Figure 3.9 The correct shooting position, indicated by the black arrow

Following the stunning, the box was then rotated at about 110^0 to release the animal and after this, each animal was hoisted by the hind leg and moved to the bleeding rail, where it was gash-stuck and allowed to bleed for about 8 minutes before being electrically stimulated. Electrical stimulation (ES) lasted for about one minute for each carcass at 810volts.
As in the case of the Kosher group, forty of the carcasses were also exempted from electrical stimulation (NES) so as to be able to compare the effect of electrical stimulation on the meat and carcass quality parameters. After the stimulation, the weight of each carcass was recorded just like for Kosher, to determine the blood loss and after this, the carcasses proceeded to the dressing line where the head and limbs were removed. This was followed by evisceration and carcass splitting and the removal of organs.
In this case (conventional slaughter), the lungs did not go through the bedika procedure but the same procedure was followed; to observe and to score the blood in the trachea, blood in the upper bronchi, presence of bright red blood tinged foam and blood splash in the lungs. In total, one hundred and fifty nine sets of lungs and tracheas were observed for conventional slaughter.

After the carcass splitting and removal of organs, just like for Kosher, the carcasses were removed to a line where the warm and cold weight, sex and conformation grade were recorded. Damaged and infected carcasses were also removed at this point.
The carcasses were then moved to the chilling rooms with the same conditions as for Kosher and the same pH and temperature instrument as for Kosher were used to measure

the pH and temperature of the carcasses at 45 minutes, 3 hours, 6 hours, 12 hours and 24 hours respectively post-slaughter.

3.3 Sample collection

For both slaughter methods, i.e. Kosher and conventional slaughter, samples of *M. longissimus dorsi* were collected between the 9th and 12th ribs but specifically on the 10th and 11th rib for further analysis after the 24 hour pH and temperature measurements. Samples were taken from either the right or left side of the carcasses.

The sample collection was done with a knife and saw by cutting deep (about 6cm) on one side of the carcass. An average of about 450g of muscle sample was extracted from each carcass for further meat quality analysis.

The meat and carcass quality parameters that were analysed are: subcutaneous fat (mm), drip loss, colour (L, a and b), cooking loss and mean shear force. The position of the carcass from which the meat samples were extracted could be seen on Figure 2.2.

The meat samples were then transported to the university laboratory, which was about 80km from the abattoir and further analysis commenced at 36 hours post-slaughter. In total, 57 samples were analysed for Kosher while 66 samples were analysed for conventional slaughter.

3.4 Methods

3.4.1 Blood loss (BL) and percentage blood loss (%BL): This was obtained by subtracting the weight after bleeding (WAS) from the weight before slaughter (WBS) (Anil *et al*., 2006);

$$BL = WBS - WAS$$

$$\%BL = \frac{BL}{WBS} \times \frac{100}{1}$$

3.4.2 Subcutaneous fat thickness (mm): This was obtained using a vernier caliper. It was measured directly by placing the jaws of the caliper on the fat under the skin (without hide), which was separated from the skin by a small knife. The jaws of the caliper were inserted to measure the fat thickness in millimeter (Swatland, 1984). This measurement was done at 36 hours after slaughter for both treatments.

3.4.3 Drip Loss: The samples for these analyses were taken from the big sample (*M.longissimus dorsi*) between the 9th and 12th rib. They were beta (β)-red fibre with parallel and longitudinal fibrillation. These samples are regarded as slow-twitch oxidative muscle. They are low in connective tissue. The fibre direction could also be regarded as lateral (Kinsman *et al*., 1994). The cut was made at right angle to the fibre direction at about 36 hours *post-mortem* at chill temperature of 4^{0}C and average ultimate pH of 5.5. The weight of the samples ranged from between 15grams to over 50grams.

Each sample was suspended with a thin wire from the lid of a sealed, transparent plastic container. This was done by drilling two thin holes through the lid and passing the thin wire through the meat and through the holes to suspend the meat without touching the container. By so doing, the meat was allowed to release the drip directly to the floor of the container. The samples were then stored at a temperature of 4^{0}C for 24 hours. After 24 hours, the samples were taken from the containers, gently blotted dry and weighed (Knight, 1988) and Honikel (1998).

The drip loss was expressed as a percentage of the initial weight:

$$\% \text{ drip loss} = \frac{\text{weight loss after drip}}{\text{Initial sample weight}} \times \frac{100}{1}$$

3.4.4 Cooking loss: Meat samples of about 200g to 400g were cut from the *longissimus dorsi* samples that were extracted. The properties of the meat, i.e. fibre orientation, position of samples, e.t.c. were similar to the samples used for drip loss. Meat temperature was 4^0C and average pH of 5.5 at 36 hours *post-mortem* and at the same position of the muscles i.e. between 9th and 12th rib. Each sample was cut in a rectangular shape of about 8cm x 6cm x 5cm. They were then placed in thin walled transparent plastic bags and placed in continuously boiling water-bath, with the opening extending above the water surface on a metal rack. This was done to prevent water from entering the plastics. The temperature of the boiling water was fluctuating between 75^0C and 80^0C and they were boiled for one hour.
After boiling for one hour, the plastic bags were removed from the water bath and cooled in a bath of ice-cold water. They were then stored in chill condition at 4^0C for 12hours. After the 12hour storage, the meat samples were then taken from the bag, blotted dry and weighed (Offer, 1984; Ham, 1977; Honikel, 1988).
The cooking loss was expressed as a percentage of initial weight:

$$\% \text{ cooking loss} = \frac{\text{weight loss after cooking}}{\text{Initial sample weight}} \times \frac{100}{1} \quad .$$

3.4.5 Shear force: The meat samples that were used to determine the cooking loss were also used to determine the shear force, which is a measure of tenderness. After the samples were blotted dry and cooled, the shear force was analysed. A hollow metal probe of 1cm in diameter and 8cm in length was used to take 10 samples from each block of meat along the length of the fibre arrangement. An instron shear force apparatus was attached to an instron machine, model 1101. Ten shear force values were measured on each core sample obtained from each block of meat. The shear was made perpendicular to the fibre arrangement (Honikel, 1998). The setting of the Instron Model 1101 for the compression test was as follows:

Load transducer	: 500N
Operating unit	: N (Newton)
Gage length	: 38mm
Testing Speed	: 500mm/min
Load range	: 40%
Specimen type	: Round.
Specimen dimension	: 1cm

The average of the ten values from each meat sample was now calculated to get the mean shear force value. A standard deviation for each mean value was also calculated.

3.4.6 Colour: The meat colour parameters which are L^*(lightness), a^* (redness), and b^*(yellowness) were obtained using the Konica Minolta colour sensing Chroma meter CR-400. The instrument was first calibrated on a white standard and a reading of Y(L)=

93.7, X(a)= 0.3135 and y(b)= 0.3194 was obtained. These were the actual figures of the white calibration that came with the instrument.
The same meat samples that were used for cooking loss and shear force measurements were used to obtain the colour measurements before they were cooked. After cutting out the meat samples, the samples were allowed to bloom for 1 hour and this was done at 36 hours after slaughter and at 4 ^{0}C. Prior to recording the meat colour, the meat samples were stored in transparent plastic bags in the chilling room under a fluorescent light bulb (Muchenje *et al*., 2008; AMSA, 1991).
The colour was taken by placing the point of the chroma meter with the lense on the meat sample and pressing the illuminating knob. The lense was placed at right angle to the meat (Velarde *et al*., 2003; Honikel, 1998; Onec and Kaya, 2004).

3.5 Statistical analyses

Analyses of variance was done using the general linear models (GLM) procedure to analyse the effects of the independent variables (slaughter methods, electrical stimulation, gender and fat code) and their interactions on; pH and temperature at the different times (0.75, 3, 6, 12 and 24 hours) *post-mortem*; % blood loss; % drip loss; % cooking loss; shear force; subcutaneous fat and colour. Percentage blood in the trachea and % blood splash were analysed using the Proc Logistic procedure and using Type ш analyses of variance and Chi square to determine the p values. Data processing was performed using SAS (version 9.2) under Microsoft Windows XP, service pack 3. (SAS Institute INC., Cary, North Carolina, USA). Correlations were done using the Proc correlation procedure. Fisher's protected t-test, least significant difference (LSD) at 5% level of probability were used. Although the statistically significant differences were taken at the 5 % level of probability, the numerical differences were also highlighted because of its practical importance. The frequencies of percentage blood in the trachea and percentage blood splash in the lungs were calculated using Proc frequency procedure (also from SAS).
However most of the raw data set had to be transformed using special statistical procedure to facilitate easy analyses. This was done because they did not have normal distribution when the normality check was done. The data set for all the parameters were transformed except for blood in the trachea and blood splash in the lungs.

CHAPTER 4

RESULTS

As pointed out previously, the primary aim of this research was to evaluate and compare the influence of conventional and Kosher slaughter techniques in cattle on carcass and meat quality parameters. Some of the data were transformed (perturbed) to facilitate easy analysis of the data. As stated earlier, the transformation of the raw data was done because some of the data set did not have a normal distribution when the normality check was performed. The data set was transformed for all the parameters, except for the presence of blood in the trachea and blood splash in the lungs. Numerical differences were also highlighted in some of the results because of their practical importance.
The effects of slaughter method, electrical stimulation of carcasses, gender, and fat code on % blood loss (%BL), % drip loss (%DL), and % cooking loss (%CL) are summarised in Table 4.1 below.

Table 4.1 Effects of slaughter method, electrical simulation, gender and fat code on %BL, %DL and %CL

TREATMENT		%BL Mean±SD	%DL Mean±SD	%CL Mean±SD
SM	K	2.3±0.58	2.4±1.15	18.2±4.81[a]
	C	2.4±0.67	2.7±1.34	22.1±3.56[b]
ES	ES	2.4±0.59	2.7±1.37	20.1±5.02
	NES	2.4±0.71	2.3±0.92	20.8±3.51
G	M	2.4±0.62	2.7±1.30	20.7±4.66
	F	2.3±0.65	2.3±1.09	18.9±4.27
FC	2	2.4±0.65[a]	2.4±1.25[a]	20.1±4.68
	3	2.2±0.50[b]	2.9±1.23[b]	20.9±4.43

SM= Slaughter method; K= Kosher; C= Conventional; ES= Electrical stimulation; NES= Non-electrically stimulated; G= Gender; M= Male; F= Female; FC= Fat code; %BL= % Blood loss; % DL= % Drip loss; % CL= % Cooking loss
[a, b] Means in the same column and treatment group with different superscript letters differ ($p < 0.05$)

4.1 Percentage blood loss (%BL)

A total of 311 beef cattle were analysed for the effect of slaughter method on blood loss. One-hundred-and-seventy cattle were assigned to the Kosher method while 141 were assigned to the conventional slaughter method. Two-hundred-and-thirty-seven carcasses were electrically stimulated (ES) (103 for CST and 134 for KST) while 74 were non-electrically stimulated (NES) (38 for C and 36 for K). Forty-eight cattle were females (5 for CST and 43 for KST) while 263 were males (136 for CST and 127 for KST). Two hundred and forty-five cattle had a fat code 2 (112 for CST and 133 for KST), while 66 had a fat code 3 (29 for CST and 37 for KST). Proc GLM was used to compare between SM, ES, G and FC treatments and also the interactions between SM and ES, SM and FC, while gender was nested within SM (G(SM)) because of the very small number of

females. According to our results, there was no significant difference (p= 0.0643) between the two slaughter methods but the conventional slaughter group (2.4±0.67%) had a slightly higher bleed-out compared to the Kosher slaughter group (2.3±0.58%). There were no significant differences within the other treatments and their interactions using GLM and LS means except for fat code (FC) (p= 0.0475). Although the statistics showed a significant difference at the 5% level of significance between the fat codes, this could be ignored in practical terms because the difference between the means of the fat codes was marginal (FC-2 (2.4±0.65 %) and FC-3 (2.2±0.50 %)).

4.2 Percentage drip loss (%DL)

A total of 123 *m. longissimus dorsi* samples were analysed for percentage drip loss. Fifty-seven samples were obtained from the Kosher slaughter group while 66 were obtained from the conventional slaughter group. Eighty-six (46 for CST and 40 for KST) were electrically stimulated while 37 (20 for CST and 17 for KST) were non-stimulated. Thirty of the samples were from females while 93 were from males. The number of samples from animals with fat code 2 were 90 (51 for CST and 39 for KST) while the ones with fat code 3 were 33 (15 for CST and 18 for KST). Proc GLM was used to analyse and compare the means and percentages of the ranked, transformed data and their interactions. The results set out in Table 4.1 show that there is no significant difference (p= 0.1092) in drip loss between the Kosher- and the conventionally slaughtered groups. However, meat samples from the conventionally slaughtered group (2.7±1.34%) exuded more water than the Kosher group did (2.4±1.15%). The effects of electrical stimulation and gender and their interactions were not significant but there was a significant difference between the two fat codes (p= 0.0242), with meat samples from FC-3 group (2.9±1.23%) exuding more water than FC-2 (2.4±1.25).

4.3 Percentage cooking loss (%CL)

The same number of samples as for percentage drip loss and from the same meat samples were also analysed for percentage cooking loss. The same number of samples as for percentage drip loss analyses were also obtained from the Kosher- and the conventional slaughter groups. Proc GLM was used to analyse and compare the means and percentages and their interactions (SM, ES, G, FC, SM and ES, G (SM), SM and FC) for both the raw and the ranked, transformed data. Similar results were obtained for both. The analyses revealed a significant difference (p= 0.0004) between the slaughter methods for cooking loss (Table 4.1). The conventional slaughter group lost more water (22.1±3.56%) than the Kosher group (18.2±4.81%). However, electrical stimulation, gender, fat-code and their interactions did not influence (p> 0.05) the percentage cooking loss.

4.4 Percentage blood in the trachea (%BLT)

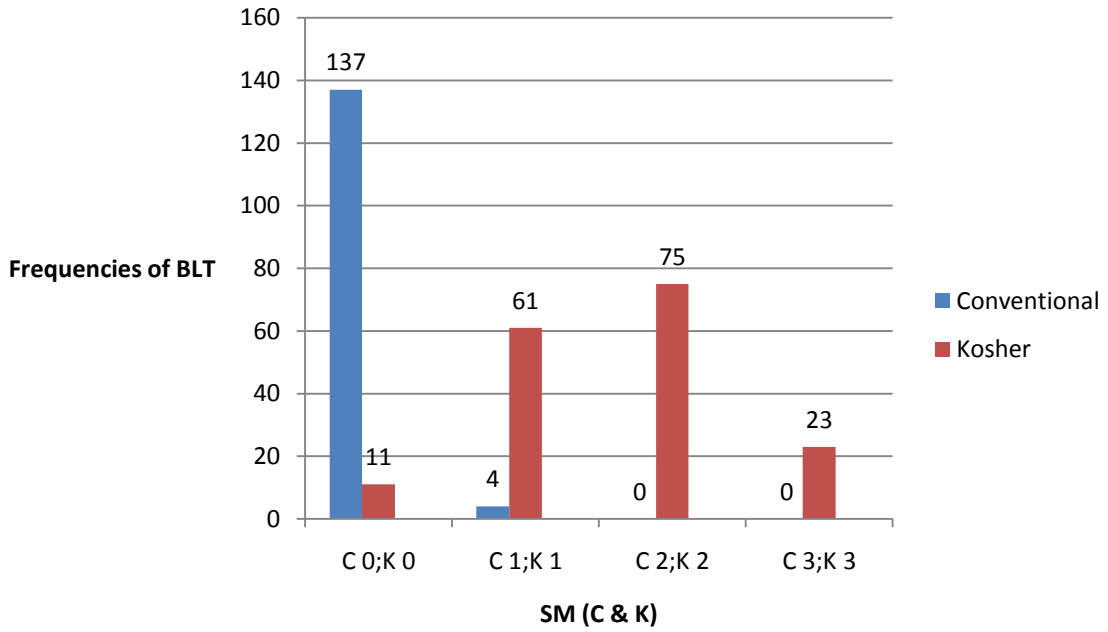
The same carcasses and numbers as for %BL were used to quantify the amount of blood in the trachea. One-hundred-and-seventy tracheas were observed and scored for Kosher while 141 were observed and scored for the conventional group. The amount of blood in the trachea was scored according to the method described in section 3.2.2 (Gregory *et al*., 2008). The presence of bright-red blood-tinged foam in the trachea was also quantified and recorded but the researchers decided not to correlate this parameter with the independent factors (treatments) because the foam was present in almost all the tracheas

that had blood, especially for the Kosher group. The presence of the bright-red blood-tinged foam was almost directly proportional to BLT in its quantification. Most of the tracheas that were examined for the Kosher group (44.12%) fell under code 2; i.e. blood covered between 11% and 50% of the trachea, while almost all the tracheas examined from the conventional slaughter group (97.16%) were categorised as code 0 (i.e. no blood) (frequency summary tabulated in Table 4.2). In addition, 76% of the tracheas examined for Kosher had blood in the upper bronchi and about 32% of them had bright-red blood-tinged foam in the trachea. In the conventionally slaughtered group, only 2.84% of the tracheas had blood lining under code 1 (i.e. less than 10%). Proc Logistic procedure was used to do Type ш analyses of effects, using Chi square within SM, ES, G and FC. Analysis revealed a highly significant difference ($p < 0.0001$) between the slaughter methods. Analysis of maximum likelihood estimate was also performed. SM still had a highly significant effect ($p < 0.0001$). Odds ratio estimate was also carried, out using the same Proc Logistic procedure for SM, i.e. conventional versus Kosher and the result was 177.307 to > 999.999 at the 95% confidence level. Almost all the tracheas from the Kosher group had blood lining while almost all those from the conventional group had clean tracheas. The differences were obvious (see pictures in Figure 3.4 and 3.5). Electrical stimulation also showed no significant effect ($p > 0.05$), likewise gender and fat code. Table 4.2 shows the summary of the frequencies of BLT for slaughter method.

Table 4.2 Summary of the frequencies of BLT for slaughter method

Code	Frequencies	**Conventional**	**Kosher**	total
0	Freq	137	11	148
	Row %	92.57	7.43	
	Column %	97.16	6.47	
1	Freq	4	61	65
	Row %	6.15	93.85	
	Column %	2.84	35.88	
2	Freq	0	75	75
	Row %	0.00	100	
	Column %	0.00	44.12	
3	Freq	0	23	23
	Row %	0.00	100	
	Column %	0.00	13.53	
Total		141	170	**311**

A bar chart of the frequency of occurrences of BLT for conventional and Kosher slaughter is presented in Graph 4.1.



Graph 4.1 Bar chart for frequency of BLT occurrences for SM

Key: code 0 (no blood); code 1 (blood covered 1% to 10% area); code 2 (blood covered 11% to 50% area); code 3 (blood covered over 50% area)

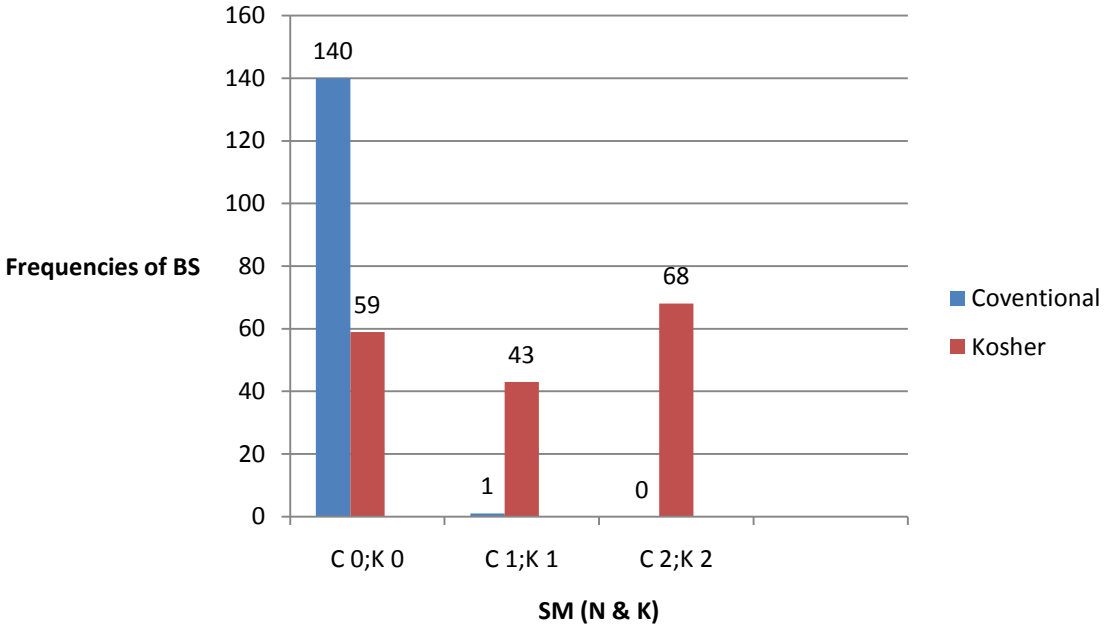
4.5 Percentage blood splash (%BS)

The same carcasses and numbers as for BLT were used to quantify the amount of blood splash in the lungs of the carcasses. One-hundred-and-seventy pairs of lungs were scored for Kosher- while 141 were scored for the conventional slaughter group. The lungs were visually and subjectively scored according to the amount of splash from 0% to over 50% (as described in Chapter 3); i.e. 0, no splash present; 1, 1% to 10% splash observed; 2, 11% to over 50% splash observed. Proc Logistic procedure was also used to analyse the %BS. Type ш analyses of effects was carried out using Chi square to determine the p value. Only the slaughter method showed a significant difference ($p < 0.0001$). Walds confidence interval was also done for odds ratio at the 95% confidence limit within SM and the other treatments. Only the slaughter method showed a highly significant effect at 33.437 to > 999.999. Generally, analysis revealed a highly significant difference ($p < 0.0001$) between the two slaughter methods. Sixty-five % of the lungs from the Kosher slaughter group had blood splash ranging from 5% to over 50% while those from the conventional group had 0.71% occurrence of blood splash. Between the males and the females, there was a large numerical difference but there was no significant difference in terms of %BS. This difference could be due to the high disparity between the number of males and females. The standard deviation was also very high. The females were too few compared to the males. Electrical stimulation and fat code did not show any significant influences. Table 4.3 presents a summary of the frequencies of %BS occurrences for slaughter method.

Table 4.3 Summary of the frequencies of blood splash for slaughter method

Code	Frequencies	**Conventional**	**Kosher**	total
0 (no blood)	Freq.	140	59	199
	Row %	70.35	29.65	
	Column %	99.29	34.71	
1 (1- 10% blood)	Freq.	1	43	44
	Row %	2.27	97.73	
	Column %	0.71	25.29	
2 (11+ % blood)	Freq	0	68	68
	Row %	0.00	100.00	
	Column %	0.00	40.00	
Total		141	170	**311**

Graph 4.2 shows the frequencies of occurrence of blood splash for both slaughter methods



Graph 4.2 Frequencies of blood splash in conventional and Kosher slaughter

Key: code 0 (no blood splash); code 1 (blood splash covered 1% to 10% of lungs); code 2 (blood splash covered 11% to over 50% area).
Table 4.4 shows the effects of treatments on shear force of meat, subcutaneous fat, and meat colour (L^*, a^* and b^* values).

Table 4.4 Effects of treatments on shear force of meat, subcutaneous fat thickness and colour (L, a and b values)

TREATMENT		SF(N)	SCF(mm)	L	a	b
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
SM	K	43.0±15.08[a]	5.2±2.96	46.1±6.42[a]	10.4±2.58[a]	-6.5±4.01[a]
	C	53.5±12.30[b]	4.7±2.35	35.4±2.86[b]	15.6±1.78[b]	0.3±1.67[b]
ES	ES	43.3±11.93[a]	4.9±2.79	40.1±6.97	13.1±3.48	-2.5±4.34
	NES	61.2±12.49[b]	5.2±2.30	40.9±7.79	13.3±3.19	-3.8±4.81
G	M	48.4±15.08	4.8±2.61	37.8±5.52[a]	13.8±3.38[a]	-1.4±3.88
	F	49.5±13.18	5.5±2.75	48.3±5.93[b]	11.3±2.61[b]	-7.3±3.22
FC	2	49.1±15.83	4.4±2.34[a]	40.2±7.33	13.2±3.12	-3.1±4.64[a]
	3	47.4±10.63	6.4±2.94[b]	40.7±6.95	13.1±4.06	-2.4±4.13[b]

SM= Slaughter method; K= Kosher; C= Conventional; ES= Electrical stimulation; NES= Non-electrically stimulated; SCF(mm)= Subcutaneous fat (millimetre); G= Gender; M= males: F= females; FC= fat code; SF(N)= Shear force (newton); L= Lightness; a= redness; b= yellowness; SD= Standard deviation.

[a,b] Means in the same column and treatment group with different superscript letters differ ($p < 0.05$)

4.6 Shear force of meat (Newton)

The same samples and numbers that were used for CL determination were used to determine the shear force. Shear force determination was done about 18 hours after cooking (section 3.4.5). Proc GLM was used to analyse the data. The LS means were also analysed and similar results were obtained. The analyses of effects of treatments (Table 4.4) revealed a significant ($p = 0.0005$) difference between the slaughter methods, with meat from the conventionally slaughtered group (mean and SD of 53.5±12.30) appearing less tender than the meat from the Kosher slaughter group (43.0±15.08). There was also a significant difference ($p < 0.0001$) between the meat samples from the ES (mean 43.3±11.93) and the NES group (61.2±12.49), the NES samples appearing tougher than the ES samples. There was no significant difference between the two genders in terms of shear force. Likewise, the fat code did not have a significant effect on the shear force. Also, the interactions between SM and ES, G (SM) and FC vs SM were not significant.

4.7 Subcutaneous fat thickness (SCF) (mm)

The same samples and numbers that were used for %CL and SF were also used to determine the fat thickness under the skin. Determination of SCF thickness was done before extracting the samples for drip loss and cooking loss. The thickness of SCF was a direct measurement with the aid of a vernier calliper and, although this parameter should have been grouped as an independent variable because the researchers did not have control over it. The researchers decided to group it with the dependent variables because it would be like a duplication of the fat code, since both parameters are directly proportional. Proc GLM was used to analyse this variable within the treatments and their interactions; i.e. SM and ES; G (SM); SM and FC. The analyses were done on both the raw and the transformed data and similar results were obtained. Table 4.4 shows that the treatments and their interactions have no significant ($p > 0.05$) influences on SCF but there was a significant difference ($p = 0.0004$) between the two fat code groups (FC-2 and

FC-3) which was anticipated. According to the South African beef carcass classification system (Meat Classification Regulations No 863 in Government Gazette of September 2006), class 3 is expected to have more fat than class 2. In this case, the mean value for FC-2 is 4.4 mm while that of FC-3 is 6.4 mm.

4.8 Colour (L*, a* and b* values)

The same meat samples and numbers that were used to determine cooking loss were used for colour determination before samples were cooked. Colour determination was done according to the procedure in section 3.4.6. GLM procedure was performed to analyse these variables on both the raw and the transformed data. Similar results were obtained. Repeated measures analysis of variance was also done to further affirm the results. When the LS means were compared, similar results were also obtained for both raw and transformed data. Table 4.4 shows the following results:

L* value: There was a significant difference ($p< 0.0001$) between the slaughter methods in the lightness of the meat, with the samples from Kosher slaughter group (46.1±6.42), appearing lighter than samples from the conventional group (35.4±2.86). There was no significant ($p> 0.05$) difference between the ES and NES samples but there was a significant difference ($p= 0.0057$) between the two genders in terms of L^* value, with the meat samples from the females (48.3±5.93), appearing lighter those from the males (37.8±5.52). There was no significant ($p> 0.05$) difference in meat lightness between the two fat code groups. The interactions between SM and ES; SM and FC and G (SM) were also analysed but there were no significant differences.

a* value: Analyses shows there was a significant difference ($p<0.0001$) between the two slaughter methods in the redness of the meat, with the meat from the Kosher slaughter group appearing less red (10.4±2.58), compared to the samples from the conventional group (15.6±1.78). There was no significant ($p> 0.05$) correlation between the ES and NES group, but there was a significant ($p< 0.05$) difference between the two genders, just like for L value. The meat samples from the males (13.8±3.38) appeared redder than those from the female group (11.3±2.61). Also, like the L value, there was no significant difference ($p> 0.05$) in meat redness between the two fat codes. The interactions between SM and FC was also analysed and found to be significant ($p= 0.0033$). Likewise, when the SM was nested within gender because of the large disparity in number between the males and the females, there was a significant difference between the two genders. Table 4.5 shows the interactions between gender and slaughter method for a^* value.

Table 4.5 Interaction of gender and slaughter method for a* value

Gender	and	SM	P value
Male(CST)	**and**	KST(Male)	<0.0001
Male(CST)	**and**	KST(Female)	<0.0001
Male(KST)	**and**	KST(Female)	0.0031

Table 4.6 shows the interaction of slaughter method and fat code for a* value.

Table 4.6 Interaction of slaughter method and fat code for a* value

SM	and	FC	p value
Conventional(FC-2)	**and**	**FC-3(CST)**	0.0079
Conventional(FC-2)	**and**	**FC-2(KST)**	<0.0001
Conventional(FC-2)	**and**	**FC-3(KST)**	<0.0001
Conventional(FC-3)	**and**	**FC-2(KST)**	<0.0001
Conventional(FC-3)	**and**	**FC-3(KST)**	<0.0001
Kosher(FC-2)	**and**	**FC-3(KST)**	0.1283

From Tables 4.5 and 4.6, we can see that the various interactions were highly significant.

b* value: Just like the L* and a* values (Table 4.4), analyses showed a significant difference ($p < 0.0001$) between the two slaughter methods in the meat yellowness. Meat samples from the conventional slaughter group (0.3) appeared more yellow than the samples from the Kosher group (-6.5). There was no significant difference ($p > 0.05$) between the ES and the NES groups in terms of meat yellowness, likewise the two gender groups, but there was a significant difference ($p = 0.0112$) between the two fat codes. The FC-3 (-2.4) samples appeared yellower than the FC-2 (-3.1) samples; although, this difference is small when we look at it from a practical point of view.

It is, however, worth noting that, in all cases where there were significant differences in the meat quality attributes (most especially the shear force), the standard deviations for the Kosher slaughter group were higher than for the conventional slaughter group and this results show that there are high inconsistencies in the Kosher meat.
Table 4.7 shows the effects of treatments on pH.

Table 4.7 Effect of treatments on pH

TREATMENT		pH45min	pH3hr	pH6hr	pH12hr	pH24hr
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
SM	K	6.43±0.39[a]	6.01±0.53	5.74±0.43	5.66±0.39	5.53±0.39[a]
	C	6.33±0.44[b]	5.90±0.47	5.66±0.35	5.68±0.35	5.56±0.24[b]
ES	ES	6.16±0.26[a]	5.72±0.38[a]	5.56±0.35[a]	5.61±0.37[a]	5.54±0.35
	NES	6.89±0.22[b]	6.49±0.29[b]	6.01±0.29[b]	5.82±0.31[b]	5.55±0.21
G	F	6.57±0.41	6.18±0.51	5.77±0.35	5.64±0.25	5.47±0.20
	M	6.31±0.40	5.88±0.48	5.67±0.40	5.68±0.40	5.57±0.34
FC	2	6.39±0.43	5.96±0.50	5.73±0.41	5.71±0.40	5.57±0.34
	3	6.34±0.40	5.94±0.51	5.61±0.33	5.55±0.20	5.47±0.21

SM= Slaughter method; K= Kosher; C= Conventional; ES= Electrical stimulation; G= Gender; NES= Non-electrically stimulated; Gender: M= males; F= females; SD= Standard deviation; FC= Fat-Code.

[a,b] Means in the same column and treatment group with different superscript letters differ ($p < 0.005$).

4.9 pH

A total of 123 carcasses were analysed for pH. Fifty-seven were sampled for the Kosher slaughter group while 66 were sampled for the conventional group. Eighty six were electrically stimulated while 37 were exempted from electrical stimulation. Thirty of the carcasses were from females while 93 were from males. Ninety carcassses fell under fat code 2 while 33 fell under fat code 3.

The same SAS software was used to analyse the pH. Residuals provided by the GLM procedure were investigated using the normal and plot options in the univariate procedure in SAS and were found to be abnormally distributed, which justified the transformation of the raw data. Similar results were obtained for both data sets but the transformed data was used for the analyses apart from the means. The analyses were done using the GLM procedure.

pH 45 minutes: From Table 4.7, analyses revealed a significant difference ($p < 0.0380$) between the slaughter methods at 45 minutes after slaughter, although the difference in their means was small (KST= 6.43; CST= 6.33). This difference could be ignored in practical terms. The Kosher group had a slightly higher initial pH. The electrically stimulated group (ES) also had a significantly lower ($p < 0.0001$) initial pH (6.16) compared to the NES group (6.89). This rseult shows that SM and ES had significant effects on the initial pH of the carcasses. Looking at gender and fat code, there were no

significant differences (p> 0.05) between their sub-groups; i.e. gender and fat code did not affect the initial carcass pH.

pH 3 hours: Table 4.7 shows that SM did not affect pH at 3 hours post-slaughter. However, the ES carcasses still had a significantly lower (p< 0.0001) pH (5.72) compared to the NES carcasses (6.49) after 3 hours of slaughter. Again, gender and fat code showed no significant influence on pH at 3 hour *post-mortem.*

pH 6 hours: At 6 hours after slaughter (Table 4.7), we could see that slaughter method did not affect the pH but the Kosher slaughtered carcasses still had a marginally higher pH (5.74) compared to the conventionally slaughtered carcasses (5.66). Again, the ES carcasses had a significantly lower (p< 0.0001) pH (5.56) compared to the NES carcasses (6.01). Also, at this time, gender and fat code did not have any significant influences.

pH 12 hours: At 12 hours after slaughter, SM did not have any significant effect (p> 0.05) on the carcass pH. However, the ES carcasses still had a significantly lower pH (p= 0.0008) compared to the NES carcasses. This result shows that the effect of electrical stimulation persisted for up to 12 hours after slaughter, with the ES group having lower pH at all times. Again, gender and fat code did not have any significant influences on the pH at 12 hours.

pH 24 hours: At 24 hours after slaughter (Table 4.7), analysis shows that the ultimate pH was significantly affected (p= 0.0314) by the slaughter method. The conventionally slaughtered carcasses had a slightly higher pH than the carcasses from Kosher slaughter. Although the numerical disparity was very low (0.03), this could be ignored in practical situation. The other treatments did not have any significant effects on the ultimate pH. Time profile analysis was also carried out to determine the effect of time on pH decline. Time had a significant effect (p< 0.0001), likewise the interaction of time with slaughter method and the interaction of time and electrical stimulation (p< 0.0001). LS means were also compared between the two SM and ES and NES groups to further establish our statistical results. Similar p values were obtained. We also did a time profile comparison of the slopes of the transformed data between each time level; i.e. 45 minutes to 3 hours; 3 hours to 6 hours; 6hours to 12 hours and 12hours to 24 hours. We found significant (p< 0.0001) difference between the ES and NES groups as the pH fell from 45 minutes to 12 hours *post-mortem*, the ES group declined faster than the NES group. A point worth noting is that, there was a slight rise in pH from 6 hours (5.56) to 12 hours (5.61) in the ES group as the effect of electrical stimulation subsided, but at 24 hours, the pH came down to 5.54, as seen in Table 4.7.
Table 4.8 shows the time profile contrast within treatments and their interactions for pH.

Table 4.8 Time profile contrast within treatments and their interactions for pH

Profile level	Source (Treatment)	DF	F	P
$T_{45min}$to T_{3hr} (Time 1)	SM ES	1 1	0.00 1.01	0.9834 0.3175
T_{3hr} to T_{6hr} (Time 2)	SM ES	1 1	0.02 13.83	0.8921 0.0003
T_{6hr} to T_{12hr} (Time 3)	SM ES	1 1	2.75 34.55	0.1001 <0.0001
T_{12hr} to T_{24hr} (Time 4)	SM ES	1 1	3.78 27.54	0.0543 <0.0001

The contrast between the initial pH profile was also compared to the other time level differences; i.e. 3 hours to 6 hours; 6 hours to 12 hours and 12 hours to 24 hours between the ES and the NES carcasses and slaughter methods using repeated measures ANOVA. Table 4.9 shows the summary of the time profile comparison for pH.

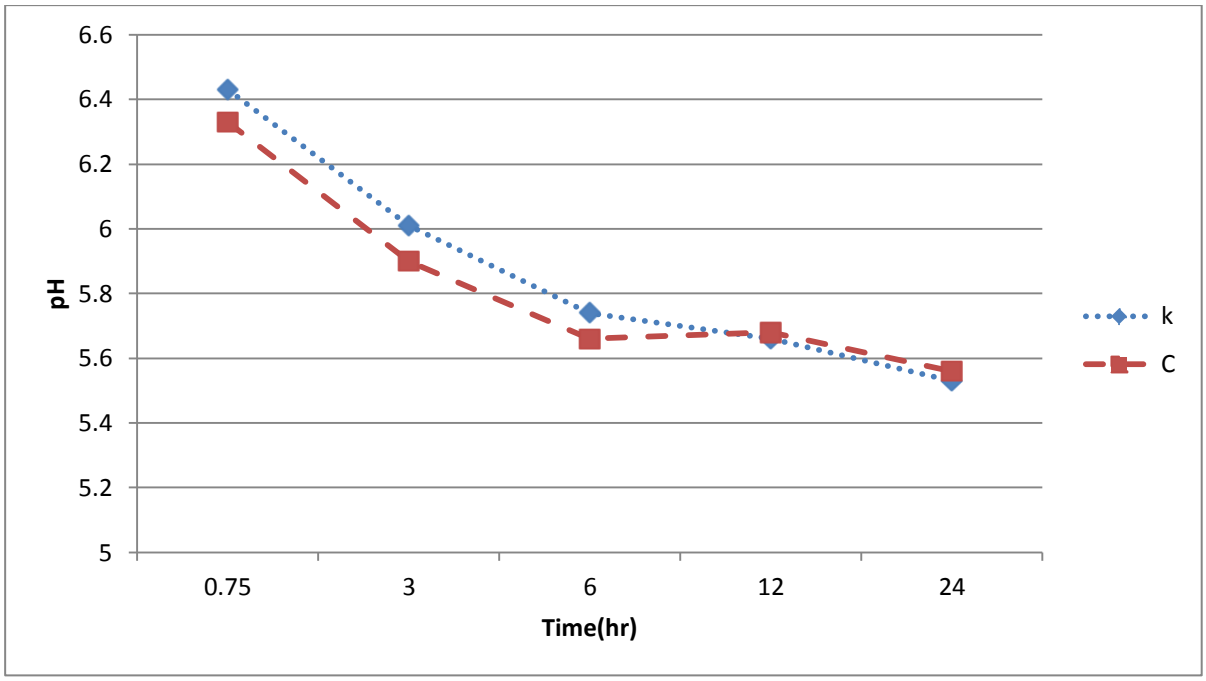
Table 4.9 Time profile comparison for pH

Profile contrast	Treatment	df	F	P
Time 1 x Time 2	SM	1	2.32	0.1308
	ES	1	7.11	0.0088
Time 1 x Time 3	Mean	1	6.68	0.0110
	SM	1	1.22	0.2720
	ES	1	22.69	<0.0001
Time 1 x Time 4	ES	1	19.27	<0.0001

There were significant differences in the time profile, with the ES group showing a lower pH at all the profile levels. SM was not significant at all levels but the mean difference was significantly affected when time 1 interacted with time 3. The other parameters (treatments) were not affected.

As we can see from the Table 4.9, there were significant differences for electrical stimulation from 45 minutes to 6 hours and the differences became highly significant as time progressed from 12 hours to 24 hours *post-mortem*. However, the graph did not follow a linear pattern as we can see from Figure 4.3 below. From 6 hours to 12 hours, there was a slight rise in pH for the electrically stimulated group (from 5.56 to 5.61) and this was the time the effect of electrical stimulation was losing its grip on the meat, and at 24 hours *post-mortem*, it came down again to 5.54.

Graph 4.3 shows the interaction between pH and time for Kosher- and Conventional slaughter methods.

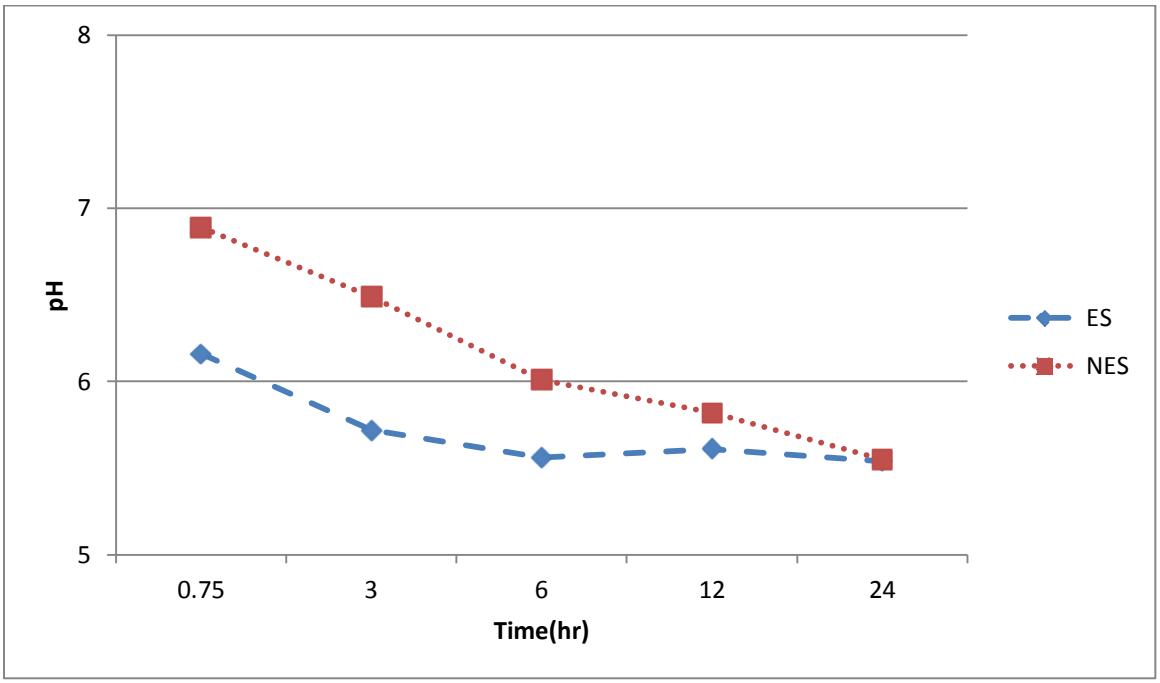


Graph 4.3 Graph of slaughter methods showing pH and time interaction for Kosher and conventional slaughter

Key: K= Kosher; C= Conventional slaughter; SM= slaughter method.

The slopes of the pH graph were also compared between each time of measurement from 45 minutes to 24 hours and compared between the sub-groups of each treatment; i.e. SM, ES, G and FC. From 45 minutes to 3 hours, there were no significant differences between the sub-groups of all the treatments. From 3 hours to 6 hours, only electrical stimulation showed a significant difference ($p < 0.0001$) between the stimulated and the non-stimulated groups. The NES group displayed a sharper decline than the ES group. All other treatments did not show any significant differences. Similar effects were obtained between 6 hours and 12 hours *post-mortem* for the pH, with the NES group showing a sharper decline ($p < 0.0001$). Again, at this time, other treatments did not show any significant influence except the interaction of slaughter method (SM) and electrical stimulation (ES), which gave the following results: KST (NES) and KST (ES) ($p = 0.0005$); KST (NES) and CST (ES) ($p < 0.0001$); CST (NES) and CST (ES) ($p < 0.0001$); KST (ES) and CST (NES) ($p = 0.0005$). From these outputs, we could clearly see that all the ES differed significantly from NES, whether Kosher or conventional slaughter. From 12 hours to 24 hours after slaughter, SM showed a significant influence ($p = 0.0308$). The Kosher group had a slightly steeper slope compared to the conventional group. Electrical stimulation also showed a significant influence ($p < 0.0001$) on pH at this time (12 hours to 24 hours), with the NES group still showing a sharper decline compared to the ES group.

Graph 4.4 shows pH and time interaction between the ES and NES groups.



Graph 4.4. Graph of Electrical Stimulation showing pH vs time for ES and NES

Key: ES= Electrically stimulated; NES= Non-electrically stimulated.

Table 4.10 shows the effects of treatments on the carcass temperature at different times of measurements.

Table 4.10 Effect of treatments on temperature

TREATMENT		Temp45min	Temp 3hr	Temp 6hr	Temp 12hr	Temp 24hr
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
SM	K	36.50±1.69[a]	30.63±2.64[a]	20.16±2.56[a]	10.24±2.63[a]	3.06±0.95[a]
	C	37.22±1.61[b]	27.05±2.50[b]	14.71±2.72[b]	4.72±1.70[b]	-0.42±0.23[b]
ES	ES	36.96±1.70	28.86±3.05	17.53±3.76	7.44±3.58	1.20±1.85
	NES	36.71±1.64	28.36±3.30	16.54±3.82	6.90±3.38	1.19±1.92
G	F	36.61±1.73	31.26±2.55[a]	20.27±2.07	10.12±2.00	2.99±0.82[a]
	M	36.97±1.67	27.89±2.84[b]	16.26±3.71	6.36±3.41	0.62±1.74[b]
FC	2	36.93±1.64	28.19±2.84[a]	16.68±3.64[a]	6.69±3.04[a]	1.01±1.77[a]
	3	36.76±1.80	30.11±3.46[b]	18.74±3.85[b]	8.89±4.20[b]	1.71±2.05[b]

SM= Slaughter method; K= Kosher; C= Conventional; ES= Electrical stimulation; NES= Non-electrically stimulated ; G= gender; M= males; F= females; FC= Fat-code; SD= Standard deviation.

[a,b] Mean in the same column and treatment group with different superscript letters differ ($p< 0.05$).

4.10 Temperature

The same carcasses and numbers that were used for pH were also used to determine and analyse the temperature profile over 24 hours. The same software (SAS) and the same comparisons were used for temperature. The temperature readings were taken simultaneously with the pH on the same portion (*m. longissimus dorsi*) of the carcasses (section 3.2.3). The data was analysed using the transformed data. Although the graph of temperature against time showed a linear pattern, the data had to be transformed because they were not normally distributed when the normality check was carried out.

Temperature at 45 minutes: Table 4.10 revealed that the initial temperatures were significantly affected ($p= 0.0243$) by the slaughter methods. Although the Kosher-slaughtered carcasses had a slightly lower mean initial temperature (36.50^0C) compared to the conventionally slaughtered carcasses (37.22^0C), the difference was statistically significant. In practical situation, however, the difference could be ignored. The initial temperature was not significantly affected ($p> 0.05$) by electrical stimulation. The ES group (36.96^0C) had a slightly higher initial temperature compared to the NES group (36.71^0C) and this was not statistically significant. Likewise for gender, there was a slight increase in the initial temperature for males (36.97^0C), compared to the females (36.61^0C) but this increase was also not statistically significant. The possible reasons for these results will be discussed in the next Chapter. The FC sub-groups also had no significant ($p> 0.05$) effects on the initial temperature.

Temperature at 3 hours: From our analyses (Table 4.10), it was revealed that the slaughter methods affected the carcass temperature at 3 hours after slaughter. The Kosher- slaughtered carcasses exhibited a higher mean temperature (30.63^0C), compared to the conventionally slaughtered carcasses (27.05^0C) and this difference was statistically significant ($p= 0.0005$). The effect of electrical stimulation was not significant at this temperature. Gender had a statistically significant effect ($p< 0.05$) on the temperature at 3 hours, although there was a wider difference between genders compared to the initial

temperature. Carcasses from the females (31.26^{0}C) had a higher temperature compared to those from the males (27.89^{0}C). When SM was nested within gender (G (SM)), because of the relatively smaller number of females, there was no significant difference ($p > 0.05$) between the two treatments. Again, the possible explanations for this effect will be discussed in the Chapter 5. Fat code also showed a statistically significant effect ($p = 0.0149$) at this temperature between the two sub-groups, with FC-2 (mean= 28.19^{0}C) cooling down faster than FC-3 (mean= 30.11^{0}C) group.

Temperature at 6 hours: At 6 hours after slaughter, analysis reveals a statistically significant ($p < 0.0001$) difference between the slaughter methods. The Kosher-slaughtered carcasses (mean= 20.16^{0}C) had a higher temperature than the conventionally slaughtered ones (mean= 14.71). This result shows that the conventionally slaughtered carcasses were progressively cooling down faster than the Kosher-slaughtered carcasses. There were no statistically significant ($p > 0.05$) differences between the sub-groups of electrical stimulation and gender. Fat code, however, had a significant effect ($p = 0.0104$) between the two groups. The FC-2 carcasses (mean= 16.68^{0}C) cooled down faster than the FC-3 carcasses (mean= 18.74^{0}C). None of the interactions of the treatments were significant at this time.

Temperature at 12 hours: At 12 hours after slaughter, slaughter method had a statistically significant ($p < 0.0001$) effect on the carcass temperature. The Kosher-slaughtered carcasses (mean= 10.24^{0}C) had a wider increase over the conventionally slaughtered carcasses (mean= 4.72^{0}C). Again, we could see a progressively wider gap between the two SM. ES and G and their interactions did not have any significant effect at this time. Again, fat code had a significant influence ($p = 0.0011$), with the FC-2 carcasses (mean= 6.69^{0}C) cooling down faster than the FC-3 carcasses (mean= 8.89^{0}C). The interaction of SM and FC was also significant ($p = 0.0378$) at this time.

Temperature at 24 hours: At 24 hours after slaughter, slaughter method significantly ($p < 0.0001$) affected the temperature, with the Kosher-slaughtered carcasses exhibiting a higher mean temperature (3.06^{0}C) compared to the conventionally slaughtered group (-0.42^{0}C). At this temperature there was no significant difference ($p > 0.05$) between the ES and NES groups. However, gender and fat code significantly ($p < 0.05$) affected the carcass temperature at this time. The carcasses from the females (2.99^{0}C) had a higher mean temperature than those from the males (0.62^{0}C). The FC-3 carcasses (1.71^{0}C) had a significantly higher temperature than the FC-2 group (1.01^{0}C). This was also anticipated because the carcasses with less fat are expected to cool faster than the ones with more fat due to the insulation effect. None of the interactions had any significant influence at this time.

GLM repeated measures ANOVA was carried out using Wilk's Lambda for time and its interactions with other parameters. The effect of time and SM was significant ($p < 0.0001$). The interaction of time and G (SM) was also significant ($p = 0.0424$). When a univariate test of hypothesis for within subject effects was carried out for time and its interactions using Greenhouse-geisser epsilon and Huynh-Feldt epsilon to further affirm our results, similar results were obtained.

The slopes of the initial temperature were contrasted and compared between the different treatments with the other time levels; i.e. 3, 6, 12 and 24 hours using repeated measures ANOVA. For temperature 45 minutes to 3 hours, it was significant between the slaughter methods ($p< 0.0001$). The conventionally slaughtered group showed a faster decline compared to the Kosher group. FC was also significant ($p= 0.0444$), with the FC-2 showing a faster decline. From 45 minutes to 6 hours, there was also a significant difference between SM ($p< 0.0001$). FC was also significant ($p= 0.0488$). From 45 minutes to 12 hours, SM was significantly different ($p< 0.0001$). The Kosher slaughtered carcasses displayed a slower rate of temperature decline. FC was also significant ($p= 0.0235$), with FC-2 showing a faster rate of temperature decline. From 45 minutes to 24 hours, SM was still significantly influenced ($p< 0.0001$). The Kosher-slaughtered carcasses had a higher temperature. Fat code was not significantly affected ($p= 0.1086$) at this time. The Interaction of gender and slaughter method was also significant ($p= 0.0322$) between 45 minutes and 24 hours after slaughter. Table 4.11 presents the summary of the contrast variables and their p values.

Table 4.11 Contrast variable and their p values for temperature

Contrast variables	Source (Treatments)	DF	F	p
T_{45mins} to T_{3hr}	Mean	1	3.91	0.0502
	SM	1	37.93	<0.0001
	FC	1	4.13	0.0444
T_{45mins} to T_{6hr}	Mean	1	5.55	0.0202
	SM	1	60.23	<0.0001
	FC	1	3.96	0.0488
$T_{45mins}$to T_{12h}	Mean	1	8.27	0.0048
	SM	1	76.63	<0.0001
	FC	1	5.27	0.0235
$T_{45min}$to T_{24hr}	Mean	1	7.68	0.0065
	SM	1	65.42	<0.0001
	G (SM)	1	4.70	0.0322

Note: Only parameters that showed significant differences are listed in Table 4.11. Other factors and interactions like ES, G, SM x ES and SM x FC did not show significant differences at $p< 0.05$.

Profile analyses of the slopes were also carried out using GLM procedure and compared within treatments and their interactions at different time intervals; i.e. 45 minutes to 3 hours; 3 hours to 6 hours; 6 hours to 12 hours and 12 hours to 24 hours. At 45 minutes to 3 hours, significant difference was revealed for SM ($p< 0.0001$), with the conventionally slaughtered carcasses showing a steeper decline. FC also showed a significant influence ($p= 0.0213$), with the FC-2 carcasses having a faster decline. Other treatments did not show any significant influence at this time. For 3 hours to 6 hours, SM also showed a significant difference ($p=0.0002$). The conventionally slaughtered carcasses' temperature declined faster than the Kosher- slaughtered carcasses. Other parameters were not

influenced by the rate of temperature decline at this time. From 6 to 12 hours, there was no significant difference (p= 0.2388) between the slaughter methods. The interaction between SM and FC was significant (p= 0.0234) at this time, although SM (P= 0.2388) and FC (p= 0.4963) were not significant, their interaction was significant. The other parameters did not influence the rate of temperature decline at this time. From 12 hours to 24 hours, there was a significant difference (p< 0.0001) between the slaughter methods. The Kosher slaughtered carcasses had a steeper decline. The influence of FC was also significant (p= 0.0040); the FC-3 carcasses showed a faster decline than the FC-2 carcasses at this time. There was also a significant (p= 0.0474) interaction between SM and FC.

This result shows that the effects of SM, FC and their interactions with time really affected temperature decline. Repeated measure ANOVA for univariate tests of hypothesis for within subject effect was also carried out using Greenhouse-Giesser epsilon and Huynh-Feldt epsilon, SM still had significant effect (p< 0.0001), likewise FC (p= 0.0175) and the interaction of SM and FC (p= 0.0498). Table 4.12 shows the summary of the profile analyses and comparisons.

Table 4.12 Temperature profile analyses between treatments

Profile levels	Source (Treatments)	DF	F	P
T_{45min} to T_{3hr} (T1)	SM FC	1 1	44.51 5.39	<0.0001 0.0213
T_{3hr} to T_{6hr} (T2)	SM	1	14.48	0.0002
T_{6hr} to T_{12hr} (T3)	G(SM) SM x FC	1 1	4.12 5.51	0.0446 0.0234
T_{12hr} to T_{24hr} (T4)	SM FC SM x FC	1 1 1	39.35 9.06 3.95	<0.0001 0.0040 0.0474

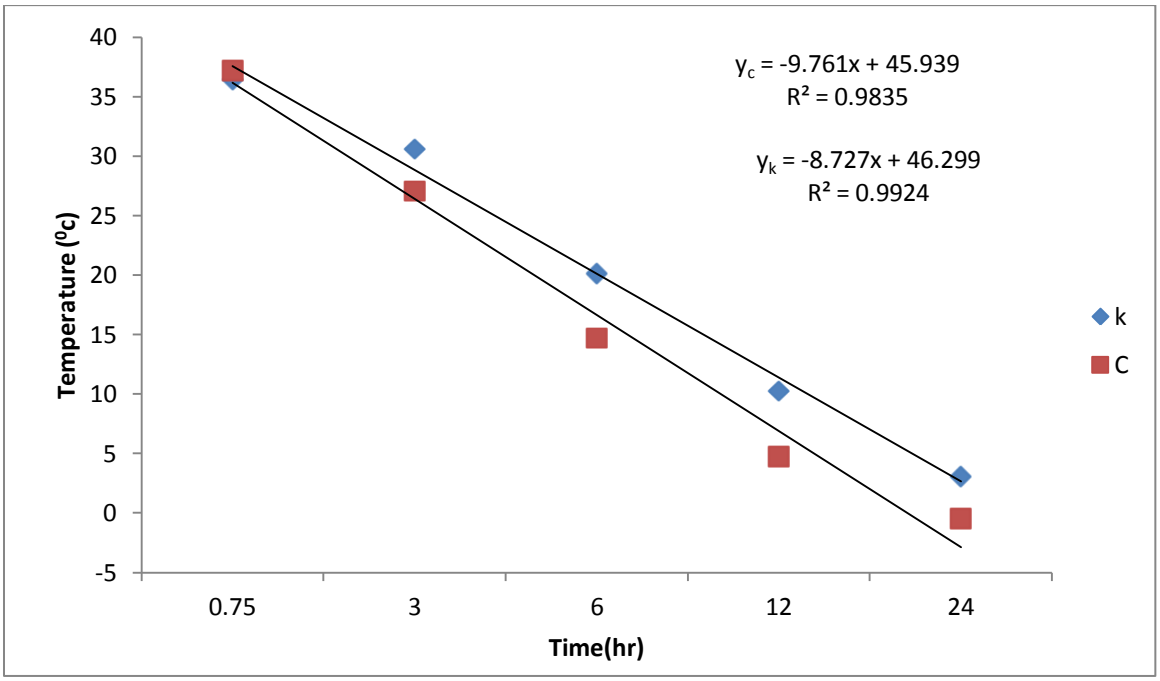
Note: Only treatments that showed significant differences are listed in Table 4.12. Other treatments and their interactions were not significantly different at p< 0.05.

A contrast of the profile differences from the first profile (i.e. 45 minutes to 3) hours was also analysed using repeated measure ANOVA; i.e. from 45 minutes to 3hours (1); 3 hours to 6 hours (2); 6 hours to 12 hours (3) and 12 hours to 24 hours (4), for slaughter method and other treatments. Table 4.13 shows the temperature profile comparisons.

Table 4.13 Temperature profile comparison between treatments

Profile Comparison	Source (Treatment)	DF	F	P
Time 1 vs Time 2	NIL			
Time 1 vs Time 3	SM	1	9.98	0.0020
Time 1 vs Time 4	Mean SM FC	1 1 1	4.48 64.05 10.93	0.0365 <0.0001 0.0013

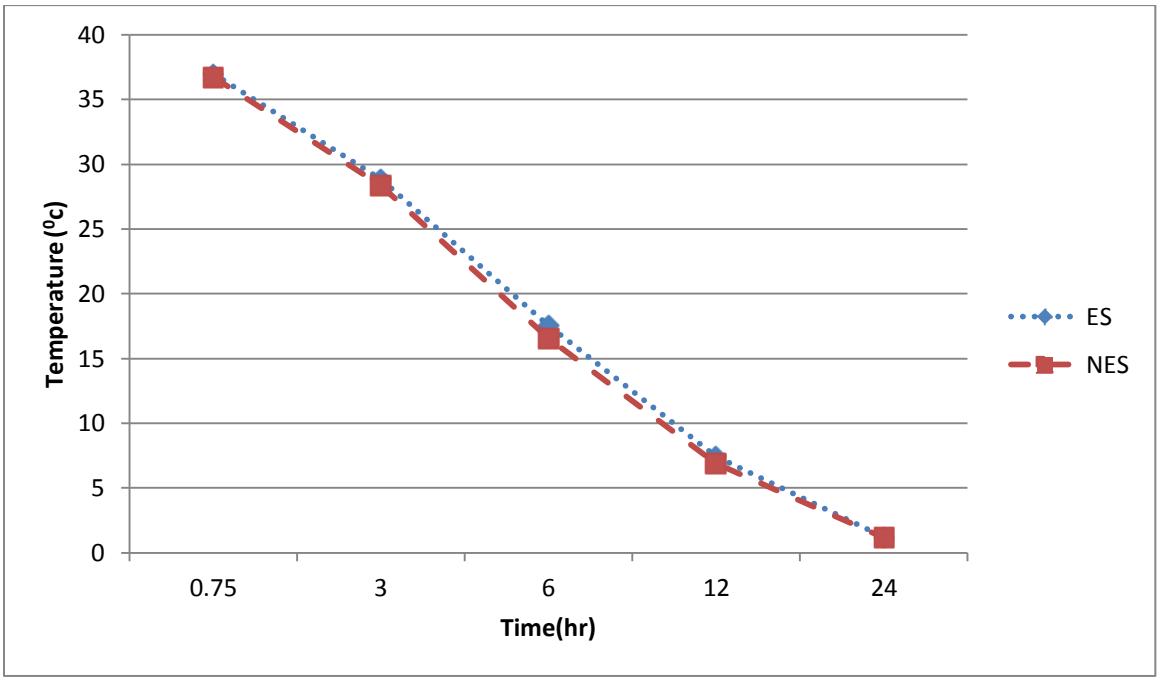
Graph 4.5 shows the interaction between temperature and time for Kosher- and conventional slaughter methods.



Graph 4.5 Graph of slaughter method showing temperature vs time for Kosher and conventional slaughter

K= Kosher slaughter; C= Conventional slaughter.

Graph 4.6 shows the interaction between temperature and time for the ES and NES groups.



Graph 4.6 Graph of electrical stimulation showing the interaction between temperature and time for ES and NES groups

Key: ES= Electrically stimulated; NES= Non-electrically stimulated.

4.11 Correlations

Some of the parameters were correlated against the shear force for the purpose of further ascertaining the effects of the main treatments; i.e. slaughter methods and electrical stimulation on meat and carcass qualities. These parameters were correlated against the shear force because it determines tenderness, which is the most important physical attribute of meat when eaten after some degree of cooking (Swatland, 1984). Shear force is also the most important factor when it comes to consumer demands and preferences (Naude, 1985; Huffman *et al*., 1996; Park *et al*., 1997). Parameters like Subcutaneous fat thickness, drip loss, cooking loss, blood in the trachea, blood splash in lungs, pH and temperature decline were correlated against the shear force using Pearson correlation coefficients. Point biserial correlation was also done.

Table 4.14 presents the summary of the statistically significant correlations.

Table 4.14 Correlations between dependent variables (dv) and shear force in relation to slaughter method and/or electrical stimulation

Variables	n	r	p
CL vs SF	123	0.39874	<0.0001
CL vs SF (KST)	57	0.44290	0.0006
CL vs SF (NES)	37	0.55978	0.0003
CL vs SF (ES)	86	0.47514	<0.0001
CL vs SF (KST) (NES)	17	0.82431	<0.0001
T45min to 3hr vs SF	123	0.33221	0.0002
T12hr to 24hr vs SF	123	-0.29525	0.0009
T45min to 3hr vs SF (CST)	66	0.38147	0.0016
T6hr to 12hr vs SF (CST)	66	-0.33718	0.0056
T12hr to 24hr vs SF (CST)	66	-0.30013	0.0143
T45min to 3hr vs SF (NES)	37	0.40207	0.0136
T45min to 3hr vs SF (ES)	86	0.37072	0.0004
T12 hr to 24hr vs SF (ES)	86	-0.32789	0.0021
T45min to 3hr vs SF (CST) (NES)	20	0.44606	0.0487
T45min to 3hr vs SF (CST) (ES)	46	0.31086	0.0355
T6hr to 12hr vs SF (CST) (ES)	46	-0.48212	0.0007
pH3hr to 6hr vs SF	123	0.19236	0.0330
pH6hr to 12hr vs SF	123	0.20188	0.0251
pH12hr to 24hr vs SF	123	0.32779	0.0002
pH12hr to 24hr vs SF (CST)	66	0.48588	<0.0001
pH3hr to 6hr vs SF (KST)	57	0.31525	0.0169
pH6hr to 12hr vs SF (KST)	57	0.54751	<0.0001
pH6hr to 12hr vs SF (CST) (NES)	20	-0.46464	0.0390
pH12hr to 24hr vs SF (CST) (NES)	20	0.52118	0.0184
pH45min to 3hr vs SF (KST) (ES)	40	0.42900	0.0057
pH6hr to 12hr vs SF (KST) (ES)	40	0.31669	0.0465

Key: CL= Cooking loss; SF= Shear force; BLT= Blood in trachea; T= Temperature; KST= Kosher slaughter technique; CST= Conventional slaughter technique; ES= Electrically stimulated; NES= Non-electrical stimulated; n= numbers; r= coefficient of correlation; p= p value.

Point biserial correlations were also carried out between SF and the treatments and the following results were obtained: for SM and SF (r= -0.4525; p= 0.0004); for ES and SF (r= -0.7404; p= <0.0001). For gender and fat code, there were no statistically significant correlations. This result shows that SF strongly correlated negatively with ES; i.e. as we moved from NES to ES, the SF decreases, which was anticipated. Similarly for SM, as we moved from conventional to Kosher, the SF decreased. This effect was reflected in our results, the Kosher meat was more tender.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Percentage blood loss (%BL)

Quite a number of studies have tried to address the issue of blood loss in the past without reaching any firm conclusions, although there have been reports of more efficient exsangiunation after Kosher slaughter compared to using captive bolt (conventional slaughter) (Miller, 1952; Levinger, 1976 and 1995). These researchers claim that blood loss can be impeded by stunning, as a result of neurological, muscular and cardiovascular changes caused by this practice. They also claim that the deep respiratory movement and gasping after sticking makes exsanguination better, because the muscle contraction occurs towards the end of the bleeding, forcing the remaining blood out of the vessels. Levinger (1995) also claims that the heart continues to pump blood for more than one minute after sticking, towards the opening of the carotids and that ensures better exsanguination.

However, the results obtained in this study (section 4.1 and Table 4.1) reveal that there is no significant difference ($p= 0.0643$) in blood loss between the two slaughter methods. The percentage blood loss in the conventionally slaughtered group was slightly higher (2.42%) than for the Kosher group (2.34%). This result agrees with the reports of Anil *et al*. (2004 and 2006), which shows that there is more bleed out in the captive-bolt stunned group compared to the non-stunned group, on sheep and cattle respectively. Anil *et al*. (2004 and 2006) also found no significant differences in their analyses. Although the Muslim method was used in these studies, it could be likened to Kosher. In both studies, the captive bolt group (conventional) still lost more blood than the non-stunned group. These researchers also found no significant difference in the percentage packed cell volumes between the two slaughter methods.

Kalweit *et al*. (1989) also found no significant difference in the amount of haemoglobin in the different muscles of sheep and calves subjected to conventional slaughter and Kosher. Similarly, the research of Kotula and Helbacka (1966) on broilers also found no significant differences in blood retention in the different cuts of broilers subjected to different slaughter methods. From these results and analyses, it can be concluded that blood loss is not significantly affected by the slaughter method. In fact, there is more bleed out when cattle are stunned with captive bolt before sticking compared to Kosher slaughter technique.

From the results of this study and from the literature, it can therefore be concluded that there is no significant difference in blood loss between Kosher-slaughtered and conventionally slaughtered cattle.

5.2 Percentage drip loss (%DL)

As stated in Table 4.1, there was no significant difference ($p= 0.1092$) in drip loss of meat samples from the Kosher- and the conventionally slaughtered group, but the meat

samples from the conventionally slaughtered cattle (mean= 2.70±1.34%) exuded more water than the samples from Kosher slaughtered cattle (2.40±1.15%). The other treatments, i.e. ES and G and their interactions did not show any significant influences on drip loss. However, there was a significant difference (p= 0.0242) between the two fat codes. Meat samples from the FC-3 group (2.95±1.23%) exuded more water than the samples from the FC-2 group (2.42±1.25%). From the literature, we know that a number of *pre* and *post-mortem* factors influence the water holding capacity of meat. For example, genotype and diet affects WHC in the growth and developmental stages of animals (Cheng and Sun, 2008). Stress and slaughter method can also influence WHC at the immediate pre-slaughter period and, at the post-slaughter period, chilling regime, ageing and tumbling can have important influences on WHC (Cheng and Sun, 2008).

The results from this study partially agree with those from the study of Bertram *et al*., 2002 on pig *longissimus dorsi*, in which captive-bolt-stunned group (8.6%) exuded more water than an electrically stunned group (8.3%), and CO_2 stunned group (6.4%). It also agrees with the findings of Onec and Kaya (2004) that show no significant difference in water holding capacity at 24 hours and 7 days *post-mortem* between groups of captive-bolt-stunned, electrically stunned and non-stunned cattle. Onec and Kaya (2004) also reported that more water exuded from meat of the captive-bolt-stunned group, 7 days *post mortem*. Vergara and Gallego (2000) also found no significant difference in WHC between electrically stunned and non-stunned lambs. The research of Dreyer *et al*. (1972) compared fibre diameters in pigs slaughtered with or without captive bolt (non-stunned). In Dreyer *et al*. (1972), the captive-bolt stunning caused a decrease in fibre diameter and, with the lower pH, led to loss of extra-cellular water and fibre shrinkage.

Although the reason why different stunning methods affect WHC is complex and not clearly understood (Cheng and Sun, 2008); from our pH profile (Table 4.7) we could see that the pH drop from both the Kosher- and conventionally slaughtered groups were virtually similar and that the differences were slight between each group at each time of measurement (especially the initial pH) *post-mortem*. This similar pH drop could be a possible reason why there was no significant difference in drip loss, as pH is a key factor when it comes to drip loss (Offer and Knight, 1998; Cheng and Sun, 2008; Bendall and Swatland, 1988). The initial temperatures were also similar, as the differences between the slaughter methods were low. Although, after 3 hours *post-mortem*, the differences became higher as the conventionally slaughtered carcasses cooled down faster and the pH dropped faster, and this is strongly suspected as the reason why the meat samples from the conventional slaughter exuded more water than the Kosher meat samples did (Cheng and Sun, 2008). From the results obtained in this study, we can conclude that there is no significant difference between the two slaughter groups in terms of meat drip loss.

A significant difference (p= 0.0242) was also found between the two fat codes groups in terms of drip loss. The FC-3 meat samples (mean= 2.95%) exuded more water than the FC-2 samples (mean= 2.42%). This result is also a complex one to explain and it is contrary to the findings of Olivan *et al*. (2004), who reported that lower fat (intramuscular and subcutaneous) could lead to higher drip loss in meat. In this study, the reverse is the case because FC-2 samples were expected to exude more water than the

FC-3 samples. Forrest (2004) also reports that intramuscular and subcutaneous fat reduces evaporation as drip and cooking loss. More studies needs to be carried out to ascertain the cause of this difference.

5.3 Percentage cooking loss (%CL)

In this study (Table 4.1), analysis revealed a significant difference (p= 0.0004) between the two slaughter methods in terms of cooking loss of meat. Meat samples from the conventionally slaughtered group (22.11%) lost more water than the meat samples from the Kosher-slaughtered carcasses (18.16%).

This result partially agrees with the findings of Onec and Kaya (2004) in which the captive-bolt group had significantly more cooking loss (29.04% and 30.81%) at 7 and 14 days *post-mortem* respectively, compared to the non-stunned group (23.01% and 23.68%) at the same days *post-mortem*. Although at 24 hours and 4 days *post-mortem*, meat samples from the non-stunned group had higher cooking loss than the captive bolt sunned group, the difference was not significant.

As stated earlier, the reason why different stunning methods affect WHC is a complex one and not clearly understood (Cheng and Sun, 2008). From Table 4.7, we can see that the rate of pH decline and the ultimate pH which are key factors in WHC of meat, were almost the same for both slaughter methods, although faster in the conventionally slaughtered group. Just as for drip loss, the rate of temperature and pH decline, which were faster in the conventionally slaughtered group, are strongly suspected to have played a major role in this case. Properties of water in muscle tissues are known to be temperature dependent. Meat temperature could affect the chemical/physical state and distribution of water in muscles (Savel *et al*., 2005; van der Wal *et al*., 1995; Bertram *et al*., 2003). The stress that is experienced at stunning could also have played a role in this result, because it could cause physiological changes, which include redistribution of visceral blood volume towards the skeletal muscles and brain among others. In this way, more water is redirected to the muscles (Ferguson and Warner, 2008). Other intrinsic factors such as genotype of the different breeds could also have played a role in this case (Uytterhaegen *et al*., 1994).

Furthermore, as reported earlier in Section 3.4.4 of this study, there was a temperature fluctuation in the water bath (between 75^0C and 80^0C) when heating the meat. This could also be a possible cause of more cooking loss in the meat samples from the conventionally slaughtered cattle (Aaslyng *et al*., 2003). More protein (e.g. collagen) could have been denatured in meat samples from the conventionally slaughtered group at over 75^0C, which causes less water to be entrapped within the protein structures held by capillary forces (Davey and Gilbert, 1974; Cheng and Sun, 2008).

From these findings and from the literature, we can conclude that the captive-bolt stun resulted in more cooking loss in meat, even though other factors may be involved.

5.4 Percentage blood in the trachea (%BLT)

The method for subjectively scoring the percentage of blood in the trachea was clearly stated in Section 3.2.2 and was based on Gregory *et al*. (2008). In this study, there was a

very clear distinction between the animals slaughtered by Kosher and the ones slaughtered by pre-stunning with a captive bolt (Table 4.2 and Graph 4.1). The difference was highly significant ($p < 0.0001$). Our analyses also revealed that other treatments did not have any significant effect ($p > 0.05$) on the amount of blood in the trachea. This result agrees with the work of Gregory *et al.* (2008), except for differences in frequencies. Gregory *et al*. (2008) recorded a lower percentage (19%) of cattle with blood in the trachea (covering >10%) for Kosher slaughter, while in this study we recorded 44.12% of the tracheas with a code 2 (i.e. 11% to 50%) for Kosher slaughter. Gregory *et al*. (2008) also recorded 21% of the tracheas containing blood for captive-bolt-slaughtered group (covering <10%) while we recorded 3% under code 1 (covering <10% area) and none under codes 2 and 3. We also recorded a higher percentage of animals with blood in the upper bronchi (76%) and bright-red blood-tinged foam (32%) in the Kosher group compared to the findings of Gregory *et al*. (2008) who recorded 36% and 10% respectively. For the incidence of bright-red blood-tinged foam in the conventionally slaughtered group, both sets of researchers recorded none.

In the past, there have been concerns about blood aspiration in cattle slaughtered in the inverted position for ritual slaughter when the cut is made (Blokhuis *et al*., 2004). This study shows that animals slaughtered in the upright position, in the ASPCA pen without pre-stunning, exhibited the same behavior as the ones that are slaughtered in inverted position. The difference was very clear between the two treatments, with the Kosher group having 93% of the tracheas lined with blood while the conventionally slaughtered group was only 3%. This result is also similar to the findings of Tsokos *et al*. (2004) when animals were decapitated without prior stunning. Blood was found in the respiratory tracts, presumably due to aspiration. The findings of Gregory *et al*. (2008) indicated the likelihood of activation of laryngeal receptors as a culprit. They excluded coughing which would not normally occur during Shechita because of the severed end of the trachea. Gregory *et al*. (2008) also reported that the fine blood-tinged foam in the respiratory tract indicates that blood has been expelled from the lungs following churning in the alveoli through breathing actions before reaching the exsanguinated state. The same authors further reported that the redness of the foam could be caused by the blood entering from the pulmonary capillary bed if there is rupture of the alveolar capillary barrier or by aspiration with air taken in through the severed trachea. The research of Slade *et al*. (2001) also points out that raised capillary blood pressure in combination with negative airway pressures can lead to the rupture of the alveolar-capillary barrier and the formation of blood-tinged foam in the lower respiratory tract.

Another possible reason for the presence of the blood-tinged foam, provided by Gregory *et al*. (2008) was that, during slaughter without stunning, much of the caudal venous return has to pass through the lungs before it goes out through the severed carotids and in this case, the lungs may not be able to drain rapidly and this may increase the chances of blood-tinged foam formation from a ruptured alveolar-capillary network.
My personal observation at the abattoir is that blood was inspired through the cut end of the trachea due to agonal respiration in the Kosher group. Most of the animals struggled to continue breathing before the captive bolt stun 20 seconds after the cut. We also observed some agonal spasms before the stun.

From these findings, we can conclude that Kosher slaughter caused a high incidence of blood in the trachea, compared to conventional slaughter, which is a meat quality and welfare concern.

5.5 Percentage blood splash (%BS)

The blood splash was subjectively scored as for BLT, but in this case we had only code 0 to 2 (Section 3.2.2). As it was for BLT, we also found a very distinctive difference between the Kosher slaughter and the conventional slaughter group (Table 4.3 and Graph 4.2). The difference was highly significant ($p< 0.000$). In fact, only one animal in the conventional-slaughter group had a 10% splash in the lungs. Three of the animals slaughtered by Kosher had over 50% blood splash and they were condemned.
These results agree with those from the research of Grandin and Regenstien (1994) and Grandin (1994) who reported that blood splash is a severe problem in Kosher slaughter because of agitation and too much pressure on the body, like belly lift and rear pusher, especially in grain-fed cattle compared to grass-fed cattle. These researchers also reported that in a regular plant, where cattle are stunned with captive bolt, blood splash levels are usually less than 0.5%, whereas in Kosher slaughter the levels can reach 30%.

The factors that cause blood splash in meat are not yet fully understood (Gregory, 1998; Swatland, 1984). Blood splash is usually commonly noticeable in the diaphragm, forequarters, heart, lungs and gall bladder (Meat and livestock Australia, 1997) but in this study we detected the splash only in the lungs. Our findings are similar to the findings of Gregory *et al*. (2008), but these researchers refer to blood splash as lobular haemorrhage. Gregory *et al*. (2008) found 63% lobular haemorrhage in 35 animals (Halal) and 47% of the lungs affected. In this study, we also split-opened some of the lungs to ascertain that the blood was fresh. As Gregory *et al*. (2008) reported in their research and in my own view, the blood splash could have been caused by the rupture of the alveolar-capillary interconnections in the lungs, or blood was drawn into the lungs from the severed trachea as the animals tried to breathe (Swatland, 1984). Furthermore, as Gregory *et al*. (2008) indicates in their research, it is also possible that the lungs were unable to drain the blood from the caudal venous return because at that time they were progressing to the exsanguinated state as respiratory agonal spasms sets in. The blood splash in the lungs of the Kosher-slaughtered animals calls for concern because, apart from the welfare aspects, there is also economic concerns especially in Africa where a lot of people consume lungs because it is cheaper than lean meat. There were no statistically significant influences between the other treatments (i.e. electrical stimulation, gender and fat code).

From these findings, we can conclude that Kosher slaughter caused a high incidence of blood splash in the lungs compared to conventional slaughter, which raises concerns about meat quality, welfare, and economic perspectives.

5.6 Shear force of meat (Newton)

The shear force results show a significant difference ($p= 0.0005$) between the slaughter methods. The meat samples from the Kosher-slaughtered cattle had lower shear force

values (i.e. were more tender) compared to the meat samples from the conventionally slaughtered cattle.
There could be a number of possible explanations for the differences in shear force; the first is the water content of meat. The amount of water that is bound within muscle fibres may have an effect on the tenderness (Currie and Wolfe, 1980; Scheepers, 1999; Bertram *et al*., 2000; Lawrie, 1998). In terms of drip loss and cooking loss, our analyses revealed that meat from the conventionally slaughtered group lost significantly more water than the meat from Kosher slaughter. This higher water loss could explain why the meat from the conventionally slaughtered cattle had a significantly higher shear force value, since the same samples were used for shear force analysis.
Another possible factor in the shear force difference is the ultimate temperature at 24 hours and the rate of temperature decline which could have caused cold shortening (Honikel *et al*., 1982; Lochner *et al*., 1980; Petaja *et al*., 1984). From our raw data and analyses, temperature decline in the conventionally slaughtered carcasses was significantly faster than the Kosher-slaughtered carcasses from 3 hours up to 24 hours *post-mortem* and, in fact, the mean temperature at 24 hours for the conventionally slaughtered carcasses was negative (sub-zero). The faster temperature decline happened while the meat was still in rigor and it could have influenced sacomere shortening which could have led to a significantly higher shear force value in the conventionally slaughtered carcasses (Locker and Daines, 1975b; Scheepers, 1999; Locker and Hagyard, 1963; Swatland, 1984).

Electrical stimulation also affected the meat shear force significantly ($p < 0.0001$). The electrically stimulated samples had a significantly smaller value compared to the non-electrically stimulated samples. This effect was anticipated, according to Smulders *et al*. (1989); Scheepers (1999); Polidori *et al*. (1999); Dutson (1977) and George *et al*. (1980). The accelerated tenderisation could be attributed to more rapid proteolysis that follows from both earlier onset of rigor and carcass temperatures in the early *post-rigor* period, compared to the non-stimulated ones. The effect of electrical stimulation on shear force in this case may also be due to muscle fibre fracture, which enhances tenderness (Marsh *et al*., 1981; Sorinmade *et al*., 1982).

From the result of this study, we could conclude that meat samples from the animals slaughtered conventionally were less tender because of the poorer water holding capacity and rapid temperature decline, which affected the myofibrillar properties of the meat as reported earlier in this chapter (Sections 5.2 and 5.3). The effect of electrical stimulation was anticipated according to the literature stated above (Smulders *et al*., 1989; Scheepers, 1999; Swatland, 1984; Smulders, 1994; Wiklund *et al*., 2001).

5.7 Subcutaneous fat thickness (SCF) (mm)
Our analysis showed a significant difference ($p = 0.0004$) between the two fat codes (2 and 3). Samples from the FC-3 group had significantly more subcutaneous fat compared to samples from the FC-2 group.
This result was anticipated because, according to the present beef and carcass grading system in South Africa which has a six point scale (from 1 to 6), FC 3 is expected to have more fat deposit compared to FC 2 (Meat Classification Regulation No 863 in

Government Gazette of September, 2006). According to this grading system, the leanest carcass starts with score 1 while the fattest carcasses gets a score of 6. Increased thickness of subcutaneous fat cover was found to improve tenderness by allowing the carcass to chill more slowly and to increase enzyme activity (Smith *et al*., 1976; Doleza *et al*., 1982). This effect was observed in this study, as the FC-3 meat samples had lower shear force (47.44), compared to the FC-2 samples (49.09), although this difference was not statistically significant.

As stated earlier in the literature review, this parameter should have been an independent variable because it is an intrinsic factor and there is no known evidence that it is influenced by slaughter method, electrical stimulation or gender. However, as stated earlier in Chapter 1 and 2, it affects the carcass chilling rate (due to insulation) and enzymatic activities which can affect meat tenderness. The researchers treated it as a dependent variable because they did not want to duplicate fat code. In this study, there were no significant differences in subcutaneous fat between slaughter methods, electrical stimulation, gender and their interactions.

5.8 Colour

In this study, the meat colour was measured from its L^*, a^* and b^* values. L^* represents the lightness, a^* redness and b^* its yellowness (CIE, 1976).

Starting with the **L* value**: The results revealed a significant difference between the meat samples from Kosher- and the conventional slaughter. The samples from Kosher slaughter appeared lighter (46.08±6.42) than meat samples from the conventionally slaughtered cattle (35.40±2.86). Although the reason for this could be a complex one to explain, it disagrees with the findings of Onec and Kaya (2004), who compared the L^* coordinates of non-stunned cattle with percussive (captive bolt) stunned ones. At 48 hours *post-mortem*, their results (on *m. longissimus thoracis et lumborium*) showed a higher L* value (41.00) for meat from the captive bolt group compared to the non-stunned group (36.79). This result also disagrees with the findings of Anil *et al*. (2006) on cattle *m. trapezius*, which showed no significant difference in meat colour between groups slaughtered by captive bolt and the un-stunned group (Halal). However, a similar previous study on sheep *m. trapezius* by the same authors reported a significantly darker meat colour in groups pre-stunned by captive bolt compared to the un-stunned group (Halal). This results further shows how variable and complex meat colour could be (Mancini and Hunt, 2005). A possible explanation for this is the rate of temperature decline and initial pH decline, which was faster in the conventionally slaughtered carcasses (Sammel *et al*., 2002; Hood, 1991; Ledward, 1985; Farouk and Swan, 1998; Farouk and Lovatt, 2000). Another possible reason could be dietary effect on subcutaneous fat (Bruce, Stark and Beilken, 2004). Housing system may also affect beef colour through changes in physical activities, which could influence muscle type and metabolism (Vestergaard *et al*., 2000). Breed of animals could also cause variation in meat colour (Lynch *et al*., 2002). In this study, breed, housing, diet and feeding regime were not properly ascertained and any of these or their combination could have been responsible.

Some authors also reported a possible correlation between glycolytic potential and free glucose content to muscle darkening in the *m. logissimus dorsi* (Hamilton *et al*., 2003b). These authors suggest that L* value increases at 0.99 and 1.32 units for every one standard deviation in *ante* and *post-mortem* glycolytic potential respectively, which could cause paleness (lower L* value) in pork (Hamilton *et al*., 2003b; Moeller *et al*., 2003; Meadus and MacInnis, 2000). This correlation could have possibly affected our results because meat samples from the conventionally slaughtered carcasses were expected to have more free glucose and glycolytic potential than the meat samples from non-stunned group (Peterson and Blackmore, 1982; Gregory, 1998). From the result obtained in this study, it can be concluded that meat from cattle slaughtered by the Kosher technique appears lighter than the meat from cattle slaughtered conventionally, although a number or combination of other factors may have played a role in this.

Our results also revealed a significant difference between the two genders in meat L^* value. The meat samples from the females (48.29±5.93) had a higher L^* value than the meat samples from males (37.79±5.52). In our raw data, the ratio of males to females was 60% to 40% for the Kosher group while the conventionally slaughtered group was almost 100% male. This big disparity could possibly affect the result but when the gender was nested in the slaughter method (G (SM)), similar results were obtained. More work needs to be done on this to be able to better understand the effects of these treatments on the L* value of meat.

a* value : The results showed a significant difference ($p< 0.0001$) in the redness of the meat between the Kosher (10.40±2.58) and the conventionally slaughtered cattle (15.58±1.78). Meat samples from the conventional group appeared redder. A possible reason for this is more pigment (myoglobin) retention in the meat samples of the conventionally slaughtered cattle, which enhances the red colour (Lindahl *et al*., 2001). This result partially agree with the work of Onec and Kaya (2004) on *m. longissimus thoracis et lumborum***,** although the differences were not statistically significant at 48 hours, their results show that the captive-bolt-stunned group (16.51) is redder than the non-stunned group (14.87). Immonem, Ruusunen and Puolanne (2000) also reported that increasing residual glycogen concentration within the *longissimus* beef decreased redness and increased yellowness. In this study, the rate of pH decline in both treatments was similar, which indicates similar glycolysis rate, although the carcasses from Kosher slaughter had higher pH from 45 minutes to 6 hours *post-mortem*. At 12 hours and 24 hours *post-mortem*, there were little insignificant differences between the pH. More research work is needed to further explore the effects of stunning and non-stunning on the redness of meat. The faster rate of temperature decline in the conventionally slaughtered carcasses could have also made it redder according to Farouk and Lovatt (2000). It can then be concluded that meat samples from cattle slaughtered conventionally are redder than the ones slaughtered by Kosher, although with reservations.

There were also significant differences in meat redness between the two genders, with meat samples from the males (13.81±3.38) displaying a redder colour than those from females (11.25±2.61). Although the difference is not much, it was statistically significant. Also, when slaughter method was nested in gender (G (SM)), the effect was also statistically significant and this further confirms the strong effects of gender and slaughter

method on the a* value. A possible reason for this could be a higher rate of exercise in the young bulls, which enhances higher pigmentation and hence, a higher rate of oxymyoglobin formation which gives a redder appearance (Ramsgaard, Jensen and Oksama, 1996). The interaction of slaughter method and fat code was also found to be significant (p= 0.0033). This is difficult to explain and more work needs to be done on this. Electrical stimulation and fat code did not affect the a^* value in our analyses.

b value: For the yellowness of the meat, the result (Table 4.4) revealed a significant difference between the meat samples from cattle slaughtered by the Kosher method and the conventionally slaughtered group. The meat from the conventional slaughter group (0.26±1.67) appeared yellower than that from Kosher slaughter (-6.49±4.01). This result agrees with the findings of Onec and Kaya (2004), who reported a higher but not statistically significant b* value (15.70) for captive bolt stunned group of cattle compared to a non-stunned group (13.16). The researchers reported that the meat colour was more vivid and the tone was more stable in the meat from captive-bolt-stunned group compared to the non-stunned group. Researchers like Chanon *et al*. (2000); Vergara and Gallego (2000) and Velarde *et al*. (2003) report that there are no statistically significant differences in meat colour between slaughter methods at 24 hours and 5 days *post-mortem*. Although the reason for this could be difficult to explain, we could infer that it has more to do with some of the above mentioned factors (like increased residual glycogen concentration, level of oxymyoglobin, rate of temperature decline, e.t.c.) than the slaughter method. More work needs to be done in this area to ascertain the real cause.

The results also revealed a significant difference between the meat samples from the two fat codes, with the FC-3 (-2.36±4.13) appearing yellower than FC-2 (-3.05±4.64). Although there was a high variability based on the high standard deviations, the result indicates that meat samples from FC-3 which are fatter according to the grading system (South African Meat Classification Regulation No 863 in Government Gazette of September, 2006) are yellower than those from FC-2, which are less fat. Again, the reason could be due to a number of factors, such as the greater amount of β-carotene (which increases yellowness) in the feed of the FC-3 group (French *et al*., 2000). These same authors also report that there is a significant correlation between b* value and fat score and, according to our analyses, FC-3 has more subcutaneous fat cover. Increasing residual glycogen concentration in the *m. longissimus* also increased meat yellowness according to Immonen, Ruusunen and Puolanne (2000). Also, according to Ringkob (2003) and Swatland (1984), fat colour affects the yellowness of meat. Feeding regime and housing could also be responsible for this difference in yellowness between treatments (Lynch *et al*., 2000).

5.9 pH

From the pH table (Table 4.7, Chapter 4), the results revealed a significant difference in pH 45 minutes and pH 24 hours between the slaughter methods. From a practical point of view, the differences are numerically small. At 45 minutes, the difference in pH between slaughter methods was 0.10 and at 24 hours it was 0.03. However, the effect of time and the interaction of time and slaughter method was significant ($p< 0.0001$) when repeated measure of ANOVA was performed. The pH of the conventionally slaughtered carcasses

declined faster from 45 minutes to 6 hours *post-mortem* but at 12 hours, the pH of the Kosher carcasses became slightly lower, likewise at 24 hours. Looking at it from a practical angle, this result agrees with the findings of Anil *et al*., 2006 who reported no significant differences in initial and ultimate pH of captive bolt and non-stunned cattle. Channon *et al*., 2002 also report that muscle glycogen concentration in the LT muscle at 24 hours *post-mortem* is not influenced by the stunning method in pigs (head only, head to brisket and CO_2 stunned). Immonen *et al*. (2000) also report that the effect of residual glycogen concentration on physical and sensory attributes of beef was complex. The result of this study differ from the findings of Onec and Kaya (2004) on cattle. These authors reportedly found a significant difference for initial pH between the captive-bolt-stunned group (6.77) and the non-stunned group (6.50). In their study, at 24 hour *post-mortem*, there was no significant difference. However, the non-stunned group had a much higher pH (5.99) compared to the captive-bolt-stunned group (5.75).

This result could also be said to be in agreement with the findings of Vergara and Gallego (2000) and Velarde *et al*. (2003) who found no significant difference in ultimate pH between different slaughter methods.
From this study, it can be concluded that captive-bolt stun caused a slightly faster acidification due to glycolysis from 45 minutes to 12 hours and at 24 hours the mean values for both slaughter methods (Table 4.7) were almost the same. Pre-slaughter stress which is a major factor in glycogen depletion could be excluded in this case because the animals were not unnecessarily stressed before slaughter and in fact, most reports on stress responsiveness and meat quality are largely speculative and do not quantify the magnitude of the relationship between stress hormone levels and meat quality (Mota-Rojas *et al*., 2006; O'neill *et al*., 2006; van Schalkwyk *et al*., 2000).

5.10 Electrical stimulation of carcasses

This treatment showed a markedly significant difference at 45 minutes, 3, 6 and 12 hours *post-mortem* but at 24 hours, there was no significant difference between the ES and the NES groups in terms of pH decline. As we moved from 45 minutes to 12 hours *post-mortem*, from the statistical analyses, the F values decreased steadily and the p values increased. This shows that the effect of electrical stimulation decreased with time and this agrees with the work of Wiklund *et al*. (2001). Electrical stimulation is usually done to accelerate *post-mortem* glycolysis and rigor onset, so that rapid cooling or freezing of carcasses can be done without cold shortening (Davey and Chrystall, 1980). Electrical stimulation has also been adopted in commercial slaughtering to tenderise beef, lamb and goat carcasses (Geesink, van Laak, Barnier and Smulders, 1994). This was also confirmed in this study as the ES group were significantly ($p< 0.0001$) tender compared to the NES group. When the slopes were analysed and compared for the combined effect of electrical stimulation and slaughter method from 45 minutes to 12 hours *post-mortem*, we also noticed that electrical simulation affected both slaughter methods i.e. caused significantly faster decline whether it was Kosher or conventional slaughter. Electrical stimulation can also negatively affect drip loss (Martin *et al*., 1983; Unruh *et al*., 1986; van Laak and Smulders, 1990). This effect was also confirmed in this study, as the ES group (2.68%) had more drip loss compared to the NES group (2.28%).

5.11 Temperature

Effect of slaughter method on temperature*:* The results reveal significant differences in temperature between the slaughter methods from 45 minutes to 24 hours *post-mortem*. The result differs from the findings of Onec and Kaya (2004), who report higher temperature at 15 minutes and 24 hours *post-mortem* in captive bolt stunned cattle compared to non-stunned group.

In this study (Table 4.10), there was a consistent temperature decline in both slaughter methods, showing a linear relationship, but the conventionally slaughtered carcasses declined at a significantly faster rate until 12 hours *post-mortem*. Between 12 hours and 24 hours *post-mortem*, the temperature of the Kosher-slaughtered carcasses declined at a faster rate even though the mean ultimate temperature of the conventionally slaughtered carcasses was sub-zero (-0.42^0C) at 24 hours compared to the carcasses from Kosher slaughter (3.06^0C). When the temperatures were contrasted from the initial temperature with the other time levels between the slaughter methods, similar results were obtained. The profile analysis was also done and compared between the slaughter methods. Again, similar results were obtained, with the conventionally slaughtered carcasses showing a faster temperature decline up till 12 hours *post-mortem*. A point worth mentioning is that the carcasses from both Kosher and conventional slaughter were managed in the same way and at similar times of the day. From a practical point of view, the researchers had to fit into the normal slaughter schedule of the abattoir. Kosher slaughter is done separately at this abattoir.

These results are difficult to explain but a possible cause could be that the Kosher-slaughtered animals were physically and/or psychologically stressed during or before slaughter and, according to Ferguson and Warner (2008), stress could cause elevated body temperature as a result of shunting of more blood into the muscle. There were also lots of muscular (tonic and clonic) contractions in the Kosher animals after sticking and this could also be a possible cause of these differences in the rate of temperature decline.
In conclusion, more work needs to be done in this area to be able to properly ascertain the cause of this difference.

Effect of gender on temperature: The results show statistically significant differences at 3 hours and 24 hours *post-mortem*, with carcasses from the males showing a faster temperature decline than those from females. This could be because the females weigh more than the males and, hence have more fat cover and bigger conformation compared to the males (Klont *et al*., 1999). At 6 hours and 12 hours *post-mortem*, there were no statistically significant differences but the carcasses from the females still had substantially higher temperatures with strong tendencies to be significant.

Effect of fat-code on temperature: The results reveal significant differences ($p< 0.05$) between the two fat codes (2 and 3) from 3 hours to 24 hours (Table 4.10) after slaughter, with carcasses from FC-2 cooling down faster than those from FC-3. The rate of temperature decline was faster in the FC-2 group up till 12 hours *post-mortem* and between 12 hours and 24 hours, it was faster in the FC-3 carcasses just like for SM. This result was anticipated because when carcasses are cooled down from body temperature to

0^0C or less, after slaughter, the subcutaneous fat passes from liquid to solid state (Swatland, 1984). This could have effect on the rate of cooling down in individual carcasses depending on their subcutaneous fat deposition. In this study, the FC-3 carcasses had more subcutaneous fat cover than FC-2 and this is the reason for the difference in the rate of temperature decline. Furthermore, according to the South African beef classification system, FC-3 carcasses are expected to have more fat cover than the FC-2 carcasses.

From Table 4.10 and Graph 4.6, it is clear that electrical stimulation did not affect the rate of temperature decline. The initial and ultimate temperatures were almost the same for the ES and NES groups.

5.12 Correlations

From the results of correlations (Table 4.14), **cooking loss** generally correlates (r= 0.39874) positively (p< 0.0001) with meat shear force, with the samples from Kosher slaughter (NES) having the highest correlation (r= 0.82431) (p< 0.0001). This shows that, as cooking loss increases, the shear force also increases. The ES and NES groups of samples from both Kosher- and conventionally slaughtered animals also showed significantly positive correlation (r= 0.47514 (ES) and r= 0.55978 (NES)).
However, the meat samples from conventionally slaughtered carcasses, unexpectedly, did not show any significant correlation between cooking loss and shear force. The reason for this is not clearly understood because in this study, meat samples from the conventionally slaughtered carcasses exuded more water as cooking loss and had higher mean shear force values. Nevertheless, the general positive correlation of shear force and cooking loss was anticipated because WHC influences tenderness (Offer and Night, 1989; Currie and Wolfe, 1980; Swatland, 1984). Several researchers also suggest that sarcomere shortening which could be caused by cooking loss is a major causative factor when it comes to tenderness (Savell *et al*., 2005).

The slope of the initial **temperature** profile for both Kosher- and conventionally slaughtered carcasses (45 minutes to 3 hours) correlates significantly positively (p= 0.0002) (r= 0.3321) with meat shear force. Although, this was a moderate correlation, it was significant. As the temperature decreased, the shear force decreased. This effect was expected because at that point in time, the meat conversion of muscle to meat had just begun and the meat was yet to go into rigor (Wheeler and Koohmaraie, 1994). At this stage, evidence points to the action of µ-calpains, which is the primary enzyme of *post-mortem* proteolysis. The enzyme is thought to be the first to be activated *post-mortem* as the pH declines to 6.02 and below and, at that time, intracellular calcium concentration rises from 0.1to 0.2 µM (Vidalence *et al*., 1983; Jeacocke, 1993 and Dransfield, 1994). From 12 hours to 24 hours *post-mortem*, the temperature profile for Kosher- and conventionally slaughtered carcasses correlated negatively with the meat shear force (r= -0.29525) (p= 0.0009). This was also a weak correlation and it means that, at this time, the actin/myosin interaction was moving from a weak binding state to a strong binding state (Goll *et al*., 1995a). At this time, the meat was proceeding to a full rigor state (Wheeler and Koohmaraie, 1994). This toughness is regarded as myofibrillar toughness (Marsh and Leet *et al*., 1996; Quali, 1991). Meat samples from the conventionally slaughtered

carcasses also exhibited a similar effect with a higher correlation with shear force at this profile time (12hrs to 24hrs) (r= -0.30013) (p= 0.0143). This effect was due to a faster chilling process that occurred in the conventionally slaughtered carcasses because when carcasses chill too fast, they have the potential to be affected by cold-shortening and toughening (Savell *et al*., 2005). Enzymatic degradation may have also been affected by the faster chilling in the conventionally slaughtered carcasses and this makes the meat tougher (Smulders *et al*., 1992). The theory behind this is that the more contracted the sarcomere due to faster chilling, the larger the fibre diameter due to sliding of the filaments over one another and this also goes back to explain the more cooking loss in the conventional slaughter group, as meat of larger fibres is tougher (Herring, Cassens and Briskey, 1965).

Meat from the conventionally slaughtered carcasses also displayed a negative correlation with meat shear force (r= -0.33718) (p= 0.0005) from 6 hours to 12 hours. Again, a similar explanation applies to this because, that was the time the rigor was starting, as the rate of glycolysis increases and storage temperature and pH declines (Wheeler and Koohmaraie, 1994). The toughness begins to rise at this stage until it gets to its peak around 24 hours to 36 hours *post-mortem* (Goll *et al*., 1995a). At this stage, the temperature went from around 15^0C to 5^0C and that reduces the functioning of the sarcoplasmic reticulum, leaving the Ca^{2+} to be unbound and, because there is ATP left in the muscle, it contracts at maximum level (Aberle *et al*., 2001). This makes the toughness increase. The temperature profile from 45 minutes to 3 hours for both the ES and NES groups also correlates positively (r= 0.37072 and 0.40207 respectively) with the meat shear force. Again, the initial explanation applies, because the µ-calpains have just been activated and calcium concentration rises from 0.1to 0.2µM to over 100µM (Vidalence *et al*., 1983; Jeacocke, 1993 and Dransfield, 1994). At this time, the carcass is still warm and the enzymes can act without inhibition.

There were positive correlations between **pH** profile with meat shear force as time went from 3hrs to 6 hrs; 6hrs to 12 hrs and12hrs to 24 hrs *post-mortem* (r= 0.19236; 0.20188 and 0.32779 respectively) for all the 123 samples, although, they were moderate correlations. This means that, as pH declines due to glycolysis, the meat shear force also drops. This is contrary to the findings of Howard and Lawrie (1956) who suggest that the rate of pH decline is inversely related to tenderness. As pH falls near the iso-electric point, all the negatively and positively charged amino-acid side chains equal, which causes the maximum attraction between the two. These attractions hold the filaments together and do not allow water to get in, greatly reducing the water holding capacity which in turn reduces tenderness (Smulders *et al*., 1992). These positive correlations in this study could be due to a number of factors like high calpastatin activity which results in decreased calpain activity and thus decreased tenderness (Boehm *et al*., 1998; Sazil *et al*., 2003). The µ-calpain is regarded as the primary enzyme of *post-mortem* proteolysis and is thought to be activated as the pH declines to 6.02 and below, and intracellular calcium concentration rises from 0.1 to 0.2µM (Vidalence *et al*., 1983; Jeackock, 1993; Dransfield, 1993). It has also been suggested that 50% of the tenderisation occurs before 24 hours, after which tenderisation continues exponentially with time (Dransfield, 1994).

Generally, the physiological and biochemical mechanisms of meat tenderness and tenderisation are based on theory (Jiang, 1998). Tenderisation has been known to be a variable process depending on a number of biological factors such as sex, age, muscle type, breed, rate of glycolysis, rate of pH decline, osmolarity of muscle cells, temperature and so on (Geesink, 1993). The only negatively significant correlation we obtained for pH and shear force was for the non-electrically stimulated, conventionally slaughtered carcasses which were 20 in number.

The point biserial correlation also confirmed our results, with electrical stimulation showing a significantly negative correlation with the shear force ($r= -0.7404$ and $p <0.0001$). This means as we moved from NES to ES the meat tenderness increased. The slaughter method also correlated with shear force ($r= -0.4525$ and $p= 0.0004$). This also confirmed what we obtained in the result; i.e. as we moved from conventional to Kosher slaughter, the shear force decreased. Gender and fat code did not show any significant correlations with the meat shear force.

CHAPTER 6

RECOMMENDATIONS

Blood loss, blood splash in the lungs and blood in the trachea: Regarding these parameters, I would like to recommend that cattle slaughtered by Kosher be stunned by captive bolt before the cut if possible or, at most 4 seconds after the cut to get a more effective bleed-out and to minimise blood splash in the lungs and the presence of blood in the trachea which are also animal welfare concerns due to painful agonal respiration and rupture of alveolar capillary wall (Gregory *et al*., 2008). I also want to recommend the use of non-penetrative captive bolt guns for Kosher, if the animals have to be stunned pre-cut because of mutilation, which is prohibited by the Kosher law (Levinger, 1995).

Drip loss and Cooking loss: I would like to recommend more research work on these parameters using NMR (Nuclear magnetic resonance) effect to better understand the effects of stunning and stress on water holding capacity, membrane integrity and amount of myofibrillar water in meat. This will also help to better understand the relaxation and contraction patterns of meat (Bertram *et al*., 2004; Cheng and Sun, 2008). More research needs to be carried out on these parameters so as to know exactly why the captive bolt (conventional) meat samples exuded more water, which eventually, in combination with other factors led to decreased tenderness in this study.

Shear force, pH and colour: I would like to recommend the use of NIR (Near infra-red reflectance) machines which gives high accuracy of shear force measurement (Park *et al*., 1997), because of the inconsistencies and high standard deviations we got for the Kosher meat samples. It will also be worthwhile to repeat some of the trials using specific breeds and to monitor their growth, nutrition and developmental stages because, breed for example can affect glycogen depletion because of how different breeds respond physiologically to pre-slaughter stress which can affect parameters like pH, colour, tenderness and cooking loss (O'Neill, Webb, Frylinck and Strydom, 2006; Lanier *et al*., 1995; Muchenje *et al*., 2008; Grandin, 1997). It will also be interesting to conduct trials on different muscles that are directly put to work at the time of slaughter, whether Kosher or conventional because, different muscles differ in their metabolic properties and sensitivity to catecholamines (Larzul, Le Roy, Monin and Selliers, 1998). I would also recommend sensory evaluation for tenderness.

Temperature: More trials should be done on temperature decline rate, to properly ascertain why the conventionally slaughtered carcasses' temperature declined faster than carcasses from Kosher slaughter.

Generally, I would like to recommend the labeling of meat from animals killed without stunning because the EU (European Union) parliament also voted in June 2010 in favour of this (Christopher Barclay, 2010) and if South Africa is to be globally competitive in meat trade, we also have to comply with these rules.

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