# Perceptions on *ante-mortem* welfare, quantitation of pain and pregnancy biomarkers, muscular fibre architecture and quality of Dohne Merino offal

**By**

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*Together in Excellence*

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**Declaration**

I, FAYEMI Peter Olutope, hereby declare that this research is an outcome of my own investigation under the supervision of Prof. V. Muchenje. This thesis has not been previously presented in any application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of references.

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Approved as to style and content by:

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Supervisor..........................................................................Date...............................................

**Abstract**

**Perceptions on *ante-mortem* welfare, quantitation of pain and pregnancy biomarkers, muscular fibre architecture and quality of Dohne Merino offal**

**By**

**P.O. Fayemi**

The broad objective of the study was to investigate the importance of *ante-mortem* welfare of sheep, quantitate ovine biomarkers for pain and pregnancy, and characterize the quality of offal. A survey was conducted among 203 sheep farmers in Eastern Cape Province (ECP) to investigate their perceptions on the significance of *ante-mortem* welfare, slaughter indicators for sheep and preference for sheep meat. Blood samples were collected from the slaughtered Dohne Merino sheep (n = 60) and then assayed for the quantitation of pain and pregnancy biomarkers. The intestinal and non-intestinal offal (n = 138) were also collected to determine their eating quality, muscular fibre orientation and nutrient constituents. The results from the analysed data showed low awareness on the importance of *ante-mortem* welfare of sheep in majority of ECP municipalities. Most of the respondents (85.2%) were of the opinion that sheep should be consigned for slaughter during the winter season to produce lamb meat. The result further revealed that male farmers consume sheep meat because of personal interest (PI) but the female farmers, for traditional beliefs (TB). Significant relationships ($p < 0.05$) were observed between farmers' gender and their age group with regard their preference for mutton or lamb in each municipality.

The expression of the ovine Ubiquitin C-terminal hydroxylase (UCH L1mRNA) showed that the application of 110volts across the head did not induce immediate insensibility in 50% of the sheep. It is therefore evident from the current study that "head-only" stunning method is not a zero pain-free method and that the quantitation of UCH L1mRNA is a reliable biomarker for detecting pain in head-stunned sheep. Moreover, the expressions of ovine pregnancy-associated glycoprotein (ovPAG-1) within a range of $1.068E^{-09}$ to $8.977E^{-07}$ showed that 43.33% ewes with pregnancy ($\Delta$mRNA) signals at the point of slaughter. The assay validation further showed that half of these ewes were truly pregnant and the ewes that exhibited "true positives" were within 56-60kg live weight at an average age of 30 months. The offal from the slaughtered sheep manifested uniformity in fibre orientation (isotropy) for the lung, mouth muscle and fillet. Most of the offal with anisotropic orientation had higher Warner-Bratzler Shear Force (WBSF) values than those with isotropic orientation, indicating that isotropic offal are comparatively tender. Fibre length of the mouth muscle and the fibre thickness of the lung from the castrates differed significantly ($p < 0.05$). Higher ($p < 0.05$) crude protein ($70.0 \pm 4.10\%$) and digestible crude protein ($49.0 \pm 3.87\%$) contents were observed in the fillet of the castrates as compared to other offal. Comparing the relationships between ambient conditions and colour parameters of the offal, results revealed that ambient temperature (AT) was moderately correlated with the saturation index (SI) of the trachea ($r = 0.5087$) and heart ($r = 0.5315$). However, dew point indicated a negative correlation ($r = -0.5955$) with the total colour difference ($\Delta E^*$) for the fillet. Offal cooked at $100^{o}C$ for 30 minutes, recorded the least ($p < 0.01$) WBSF values except the heart. Expectedly the colour parameters were influenced by ambient conditions and cooking at $100^{o}C$ for 30 minutes in an enclosed temperature-controlled water bath produced the most tender offal.

It was concluded that only two municipalities were knowledgeable about the importance of *ante-mortem* welfare of sheep in ECP. Sheep farmers also considered age at slaughter, live weight and season as crucial slaughter indicators for sheep. Dohne Merino ewes demonstrated higher resistance to electric insults and consequently recorded lower traumatic pain in the brain. Findings from this study have provided evidence supporting the use of UCH L1 mRNA as a brain-specific neuronal biomarker for assessing pain in sheep that are subjected to "head-only" stunning method. The outcome of the energy dispersive x-ray spectroscopy showed variations in fibre orientations, Warner-Bratzler Shear Force (WBSF) values and nutritional compositions of the examined offal from Dohne Merino sheep.

**Key words**: Ambient conditions, energy dispersive spectroscopy, fibre anisotropy, offal, ovine Ubiquitin C-terminal hydroxylase, Sous vide cooking, welfare.

## List of Abbreviations

| | |
|---|---|
| DHM | Dohne Merino |
| $\Delta E^*$ | Total Colour Difference |
| $\Delta G$ | Gibbs free |
| $\Delta Rn$ | Baseline-corrected normalized reporter |
| 3' | Reverse Primer or an oligonucleotide that flanks the 3'end of the Amplicon |
| 5' | Forward Primer or an oligonucleotide that flanks the 5'end of the Amplicon |
| a* | Redness of meat/offal |
| AT | Ambient Temperature |
| b* | Yellowness of meat/offal |
| cDNA | complementary Deoxyribonucleic acid |
| CP | Crude Protein |
| CT | Threshold cycle |
| DCP | Digestible Crude Protein |
| DPER | Dorper |
| ECP | Eastern Cape Province |
| EDS | Energy Dispersive Spectroscopy |
| EDX | Energy Dispersive X-ray |
| GC | Guanine-Cytosine |
| GLM | General Linear Model |
| L* | Lightness of meat/offal |
| mRNA | Messenger Ribonucleic acid |
| MT | Muscle Temperature |

| NFQ-MGB | Non-fluorescent Quencher-Minor Groove Binder |
| OBRD | Other breeds |
| ovP4 | Ovine progesterone |
| ovPAG1 | Ovine pregnancy-associated glycoprotein |
| OvUCH L 1 | Ovine Ubiquitin Carboxyl terminal Hydrolase L1 |
| PCA | Principal Component Analysis |
| PDIFF | Probability Difference |
| PGP9.5 | Protein Gene Product 9.5 |
| $R^2$ | Coefficient of Determination |
| RH | Relative Humidity |
| RIA | Radioimmunoassay |
| Rn | Normalised Reporter |
| -Rn' | Derivative Reporter |
| RT-PCR | Reverse Transcription-Polymerase Chain Reaction |
| SAMM | South African Mutton Merino |
| SAS | Statistical Analysis System |
| SEM | Scanning Electron Microscopy |
| SI | Saturation Index |
| SYBR Green 1 | Synergy Brands |
| TBI | Traumatic Brain Injury |
| $T_m$ | Melting temperature |
| WBSF | Warner-Bratzler Shear Force |
| WI | Whiteness Index |

**Dedication**

I heartily dedicate this work to God, my family and my late mentor (Br Nwabueze Daniel).

"𝕭𝖑𝖊𝖘𝖘 𝖙𝖍𝖊 𝕷𝖔𝖗𝖉, 𝕺 𝖒𝖞 𝖘𝖔𝖚𝖑 𝖆𝖓𝖉 <u>𝖆𝖑𝖑</u> 𝖙𝖍𝖆𝖙 𝖎𝖘 𝖜𝖎𝖙𝖍𝖎𝖓 𝖒𝖊, 𝖇𝖑𝖊𝖘𝖘 𝖍𝖎𝖘

𝖍𝖔𝖑𝖞 𝖓𝖆𝖒𝖊.

𝕭𝖑𝖊𝖘𝖘 𝖙𝖍𝖊 𝕷𝖔𝖗𝖉, 𝕺 𝖒𝖞 𝖘𝖔𝖚𝖑 𝖆𝖓𝖉 𝖋𝖔𝖗𝖌𝖊𝖙 𝖓𝖔𝖙 <u>𝖆𝖑𝖑</u> 𝖍𝖎𝖘 𝖇𝖊𝖓𝖊𝖋𝖎𝖙𝖘

(𝕻𝖘𝖆𝖑𝖒 103:1-2)!"

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**Table of Contents**

xi

**List of Tables**

## List of Figures

**CHAPTER 1:  Introduction**

**1.1 Background information**

Sheep farming is practiced extensively in South Africa for its significant contributions to the livestock, wool and meat industries. The sheep farming sector in the country has approximately 13,800 farmers with commercial and communal sheep farmers making up 58% and 42% of the entire work force (Directorate of Agricultural Information Services, 2008). An estimate of 28.8 million sheep and flock size ranging between $\leq 50$ and $\geq 1800$ exist in various South African provinces. Although the national herd size is unevenly distributed provincially most of the herds are found in the Eastern Cape (30%) followed by the Northern Cape (25%), Free State (20%) and the Western Cape (11%) respectively (Agriculture, Forestry and Fisheries, 2011).

Over twenty indigenous and locally developed sheep breeds are managed where about 69% of the land area is available for their grazing nation-wide (Campher et al., 1998; Palmer and Ainslie, 2006). Common among the indigenous breeds are the Afrikaner, Blackhead Persian, Blackhead Speckled Persian, Blinkhaar Ronderib, Damara, Karakul, Namaqua Afrikaner, Pedi, Redhead Persian, Redhead Speckled, Swazi and Zulu. The locally developed breeds include Dorper, Van Rooy and Merinos. The local breeds developed from Merinos consist of the Afrino, Dormer, Dohne Merino and South African mutton Merino (Hammond, 2000; Pranisha, 2004; Hinton, 2006; Sorma et al., 2012). All these sheep breeds are best suited for providing by-products such as wool, meat, hide, milk or a combination of products (Dave and Meadowcroft, 1996; Jensen, 2009). The indigenous and locally developed sheep were bred to meet the growing demand for its by-products (Peters et al., 2010). Expectedly, sheep farmers therefore, make use of the products from these sheep as a means of livelihood and sustenance of a viable local society (Cloete and Olivier, 2010).

Most sheep breeds are grazed extensively in the velds thus; they often receive low attention from welfare perspective (Dwyer, 2009). Many factors therefore constitute threats to the welfare of these animals prior to slaughter. Imbalance of the herd-men and herd-size ratio affects effective supervision of the stocks in the velds. Inadequate supervision by the stockowners often exposes sheep to cruelty from thieves (Palmer and Ainslie, 2006). In addition to the menace of theft, South African farmers lose a minimum of 10% of their stock to caracal predation on an annual basis (Smith, 2012). Merinos appear to be the most vulnerable due to their comparatively lower ability to defend themselves against the black-backed jackals (*Canis mesomelas*), caracal (*Caracal caracal*), leopards (*Panthera pardus*), baboons (*Papio ursinus*), brown hyaenas (*Parahyaena brunnea)* and cheetahs (*Acinonyx jubatus*) (McMaster, 2011).

In 2009 and 2010, the direct loss to predation from sheep and goats in the five major small stock producing provinces was in the range of R 1 to 1.39 billion per annum (de Wet, 2010; Smith, 2012). Intrinsically, unfavourable weather conditions in winter, poor immunity during the peri-parturient phase, attacks due to Rift Valley fever and other parasitic organisms affect the pre-slaughter welfare of sheep too (Dwyer and Lawrence, 2005; Kopke et al., 2008; Williams et al., 2010). A sharp decline in South African sheep population (from a range of 25 to 22million) was recorded between 2005 and 2010 due to adverse effects of these factors on their welfare (Palmer and Ainslie, 2006; Odenaal and Prozesky, 2011). Another major welfare concern is the slaughter of clinically healthy ewes for meat production. Welfare issues in this regard include non-compliance to ethics, which demands that livestock presented for slaughter at the abattoirs should be mainly males and reproductively inactive females (Opara et al., 2006; Muhammad et al., 2007; Alade et al., 2011).

The way these animals are handled at slaughter, methods used for restraining and stunning them, and sharpness of the knife for bleeding before the animal becomes unconscious, could either inflict pain on the animal or compromise the quality of its meat (Linares et al., 2007; Anil, 2012). This is the reason for focusing attention on minimizing animal's pain and suffering during slaughter in recent times (Zivotofsky and Strous, 2012). In order to enforce this idea and motivate stakeholders in the meat industry to respect pre-slaughter welfare of livestock, stunning was introduced as a legal pre-requisite before exsanguination (EU Council Directive 93/119/EC, 1993; Velarde et al., 2003). Among several stunning methods commonly employed in commercial meat production at the abattoirs, electrical stunning is the most used in sheep (Vergara and Gallego, 2000; Vergara et al., 2005). This is based on the assumption that loss of consciousness due to electrical stunning combined with exsanguination is a humane technique (Bórnez et al., 2010; Zivotofsky and Strous, 2012).

Recently, some shortcomings with this method of minimizing the animal's suffering have been noted indicating that effective stunning is difficult to achieve under practical conditions. Electrical stunning is perhaps more analogous to human electro-convulsive therapy (ECT) which queries the effectiveness of electrical stunning in the slaughter of animals (Zivotofsky and Strous, 2012). Besides, certain degrees of electronarcosis in form of cardiac dysfunction, circulatory arrest, petechial haemorrhages and blood splash from sheep during stunning, deterioration of meat quality have been observed (Vergara and Gallego, 2000; Linares et al., 2008). In addition, some stereotypic behaviours and elevated stress levels are also associated with the electrical stunning method (Nowak et al., 2007; Linares et al., 2008; Gruber et al., 2010).

Meat quality in this context is not limited to mutton or lamb but also include offal as well. This is because offal has been found to be one of the sources of nutrients or trace elements in human diets (Yilmaz and Gecgel, 2011). The quality of offal matters to the consumers if its consumption will serve the intended purpose of meeting the escalating demand for animal protein in human diet. In places where offal is acceptable, consumers refer to it as 'variety meat or as fancy meat' and its consumption is regarded as a way of keeping traditional heirloom recipes alive (Guerrero et al., 2009). Hence, this atypical meat makes a classic and frugal component of the cultural food basket for its affordability and unique taste (Magoro, 2007). This invariably leads to a momentous shift in its consumption pattern as a requisite meat type to combat malnutrition and enhance food security in many rural communities (Esenbuga et al., 2008; Agriculture, Forestry and Fisheries, 2011).


**1.2 Justification**

South Africa farmers engage in extensive sheep farming systems in all the provinces where sheep are raised. The possibility of neglecting basic animal care or pre-slaughter welfare ethics is very high in a profit-driven or extensively managed sheep enterprise (Morgan-Davies et al., 2006). Lots of sheep are lost to predators in various velds nationwide. Annually, about 4million sheep die yearly due to cold, hunger, Rift Valley fever, pneumonia and complications during pregnancy or birth (Smith, 2012). No information is available about the knowledge of farmers' on the importance of pre-slaughter welfare of ovine species in Eastern Cape Province where they are dominant. It is uncertain therefore if sheep farmers have adequate orientation towards the *ante-mortem* welfare of sheep to justify the enormity of pre-slaughter welfare threats facing the sheep industry.

Hitherto, there is no report indicating if the same is obtainable when the most slaughtered sheep genotype (Dohne Merino) are stunned prior to bleeding. The foregoing attests to the dearth of information, which is observed as pain, and anoxic signals that accompany the production of cytoxic cascade and activation of brain damaging processes when these indigenous sheep are stunned before bleeding. Moreover, farmers' knowledge or disposition to *ante-mortem* welfare of sheep is unknown considering the interconnectedness of their position in the meat production chain. It is also difficult to ascertain if the farmers are only producers or rather consumers of sheep meat as well. Paucity of information in this regards makes it difficult to understand why they consign their productive pregnant ewes for slaughter. It is even complicated to discern if farmers as producers consider it ethical to eat meat or offal from pregnant ewes raised on their farms. Nevertheless, no record is available on biomarkers that are used to quantitate ovine pain and pregnancy biomarkers from the sheep when they are electrically stunned before exsanguination. Despite a great demand for its offal, report on the meat quality of offal of Dohne Merino is unavailable. In the light of these issues, this study therefore attempted to fill in the observed knowledge gaps.

**1.3 Objectives of the study**

The broad objective of the study was to investigate the importance of *ante-mortem* welfare of sheep, quantitate ovine biomarkers for pain and pregnancy and, characterize the quality of offal. Specifically, the study attempted to determine the following:

1. The perceptions of farmers on the importance of sheep *ante-mortem* welfare and the indicators for their slaughter.

2. The preference of Xhosa speaking farmers for mutton and lamb from the natural velds as producers and consumers.

3. The trauma gene quantitation of ovine ubiquitin C-terminal hydrolase L1 (UCH L1) in electrically stunned Dohne Merino sheep as a brain specific pain biomarker.

4. The pregnancy status of Dohne Merino ewes that are slaughtered for mutton using ovine progesterone (ovP4) and ovine pregnancy associated glycoproteins (ovPAG mRNA) as biomarkers for the diagnostic tests.

5. Muscular fibre architecture and x-ray microanalysis of offal from Dohne Merino sheep.

6. The effects of ambient conditions on muscle pH and colour development by offal from Dohne Merino sheep.

7. The effects of cooking regimes on Warner Braztler shear force values of Dohne Merino offal.

**1.4 Hypotheses of the study**

The specific hypotheses tested were the following:

1. The perceptions of sheep farmers on the importance of sheep *ante-mortem* welfare of and the indicators for their slaughter are not different from one another.

2. The preference for mutton and lamb by sheep farmers as producers are not different from mere consumers.

3. There are no differences in the real-time quantitation of a trauma gene biomarker in electrically stunned Dohne Merino sheep.

4. None of the ewes were pregnant at slaughter and no difference exists in the ovP4 concentration and ovPAG mRNA expressions in them.

5. Fibre orientations and micro-contents of offal Dohne Merino castrates are similar to those of the ewes.

6. The effects of ambient environment on pH and colour development by offal are not different in castrates and ewes of various age groups.

7. The effects of cooking regimes on the tenderness of Dohne Merino sheep offal are not different in castrates and ewes of various age groups.

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## 2.1 Introduction

South African sheep meat is produced from many breeds reared in a wide range of production systems in various agro-ecological areas of the country. Sheep are among the rustic small ruminants whose husbandry signifies a valuable asset to South African farmers. The sheep are unique among other livestock in that they exhibit stoical instinct, which makes them tolerant to any condition as well as able to withstand adverse stimuli (stressors) or forms of pain (Winter, 2008; Morris, 2009). Because of their stoical nature, little or no attention is accorded its *ante-mortem* welfare while on extensive grazing velds (Dwyer, 2009). This accounts for the reasons for seeing flocks of sheep genotypes in marginal places especially where farmers are not fully conscious of the importance of their *ante-mortem* welfare (Bradford et al., 2009; Stott et al., 2012).

Proper understanding of the *ante-mortem* welfare has multiple benefits. Substantive livestock husbandry enables farmers to rear quality animals for the production of quality meat (Adama et al., 2011). Among other reasons, poor knowledge about the pre-slaughter welfare of ovine species leads to gross violations of animal welfare such as presentation of breeding and pregnant ewes for slaughter. In light of the foregoing Garba and Hassan (2002) claimed that farmers often rush their animals for slaughter in order to meet urgent family needs, or to quickly earn profits or just to meet consumers' demand for meat. Consequently, successions of practices that infringe on *ante-mortem* welfare of the animals follow this decision at the slaughterhouse where the animals face series of traumatic experiences before exsanguination.

The aftermath of these, painful experiences therefore affect animal's welfare and alter the quality of meat (offal). With the foregoing in the background this review seeks to elucidate on ovine biomarkers coding for pain and pregnancy at slaughter and with special focus on farmers' perceptions about pre-slaughter welfare of sheep and sheep meat, and how ambient conditions influence the eating quality of ovine offal.

**2.2 General perceptions about the welfare of livestock**

Judging from consumers' viewpoint, Pethick et al. (2006) found that consumers expect premium quality and value from lamb meat. Similar to this, 41% of consumers in the EU are prepared to pay two to three times more for high quality beef (Hughes, 2008; Lyford et al., 2010). Unlike consumers, Te Velde et al. (2002) reported that farmers' perceptions on animal welfare is a bit different but depend on their frames of reference. This frame of reference is in turn influenced by convictions (opinions about the way things are), values (opinions about the way things should be), norms (translations of these values into rules of conduct), knowledge (constructed from experiences, facts, stories and impressions) and interests (economic, social and moral interests). Moreover, farmers place high premium of their opinions on *ante-mortem* welfare on optimizing production such as efficient growth and satisfactory food conversion (Lassen et al., 2006; Miele et al., 2011).

With respect to values, both farmers and citizens associate animal welfare to issues such as physical health, adequate protection, access to sufficient amount of food and drinking water (Te Velde et al., 2002; Vanhonacker et al., 2007). In a holistic manner, issues on animal welfare embrace high levels of biological functioning, absence of suffering, absence of prolonged pain, hunger, thirst, fear, discomfort and distress (Botreau et al., 2007; Gregory and Grandin, 2007).

14

Although these criteria are relevant to all animals yet as a sentient mammal the sheep species have the ability to express emotions or feelings (Veissier et al., 2012). Therefore, Scott (2007) suggested the need to monitor their welfare from birth until the point of slaughter. Indirectly, this assertion therefore implies that genetics and environment interaction (G*E) might produce undesirable physical outcomes when the animal's mental state is compromised or threatened (Hewson, 2003).

**2.2.1 Concerns about threats to pre-slaughter welfare of sheep**

The effects of poor shepherd-stock ratio and social isolation or boredom in the wild have shown to expose lambs and ewes to serious emotional and welfare threats (Chapter 1; Dwyer, 2009). Anecdotal evidence has shown that certain biological predators have constituted a nuisance to the welfare of sheep in South Africa. Small-stock farmers are suffering losses in the main small-stock farming areas of the Northern Cape, the Eastern Cape and the Free State Provinces (de Wet, 2010). In the Northern Cape where highest losses have been recorded 14% of total production loss is due to predation and stock theft. Report has indicated that about 6 500 sheep and goats are killed every day by jackals and caracals. This account literally implies losing 500g of meat from each animal and in the worst scenario, costing the small-stock industry huge amount of money annually (de Wet, 2011, Smith, 2012).

Under an extensive grazing system, compromises about the welfare of sheep have resulted in a number of neonatal deaths due to harsh weather conditions, starvation, insufficient colostrums intake, birth difficulties or increased litter size in the flocks (Animalia, 2007; Østerås et al., 2007; Holmoya et al., 2012). Caroprese (2008) has also expressed concerns over inactive behaviours in sheep due to negative impact of direct solar radiation and invasion of the mammary gland by environmental pathogens.

Skin irritation, wool loss, excessive panting, lameness, respiratory disorder breech, dirtiness of the ventral abdominal have been recorded prior to slaughter (Goddard et al., 2006). The implication therefore is that the factors (aversive environmental conditions, disease outbreak, theft and predation) posing some challenges to pre-slaughter welfare of sheep in the velds also affect consumers' perception about sheep meat (Botreau et al., 2007; Goddard, 2011).

**2.2.2 Perception on mutton, lamb and offal from ovine species**

Preference for mutton and offal has grown over the years since its consumption does not suffer from any racial discrimination, religious sentiment or traditional taboo (Corpet, 2011). The attitude of farmers towards lamb meat production or for ovine meat products is affected by their age and family composition. Unlike consumers, farmers base the quality of lamb meat on technical measurements and compare that with predetermined standards that vary annually. For instance in 2008, the peak mutton consumption in South Africa, reached 188 million kilograms (kgs) while its production peaked at 163 million kg the same year. In 2010, a total of 39% mutton production was recorded with the consumption slightly rising by 11% (Agriculture, Forestry and Fisheries, 2011).

The Xhosa people of South Africa for instance have demonstrated exceptional inclination towards meat due to their cultural belief (Dyubele et al., 2010). Comparing sheep meat with chevon, Tshabalala et al. (2000) found that sheep patties were tender, juicier, greasier and less chewy than goat patties. It has also been shown that Merinos have genes that enhance higher intra-muscular fat (IMF), unsaturated fatty acids and a propensity to produce tender meat (Fogarty et al., 2003; Hopkins et al., 2011).

As found in other ovine species according to Simmons and Ekurius (2001); Simmons (2005) mutton, lamb, offal, wool, milk, fleeces, bio-fuels, sausage casings, gelatine, surgical sutures, lanolin and cosmetics are products from different sheep genotypes. In South Africa, Dohne Merino is one of the greatest sources of these products.

**2.3 Dohne Merino sheep and meat production**

At national level, the Merinos add up to 55% of the total sheep population in South Africa (Cloete et al., 2012). Dohne Merino sheep is a well-known Merino genotype in the country that was developed in the 1930s at the Dohne Research Station in Eastern Cape Province (Agriculture, Forestry and Fisheries**,** 2009). The driving force behind its breeding programme at inception was the need to explore the genetic traits between the Peppin-style Merino ewes and German Mutton Merino rams for fine wool production (Kotzé, 1951). Sequel to the growing demand for meat from the top progeny from each generation of Dohne Merino, finding a balance between production of fine wool and capacity for efficient utilization of the sour grassveld soon became the next priority (Mortimer et al., 2009; McMaster, 2011). This is responsible for one of the reasons why its farming is positioned within the dual-purpose livestock system where it holds the centre stage in Southern Africa and beyond (Daetwyleret al., 2010; Merino South Africa, 2011).

As discussed in Chapter 1, the Dohne Merino is the largest ovine genotype among the registered composite sheep in South Africa. They are more in number (34.3%) than the moribund Merinos (32.6%), Dorper (25.4%) and other registered studs which are predominantly found in the Western Cape (10.0%), Free State (20.8%), Northern Cape (27.2%) and Eastern Cape (29.8%) respectively (Abstract of Agricultural Statistics, 2008).

Its prominence over other breeds is attributed to its hardiness, high feed conversion efficiency, good mothering ability and body conformation (McMaster, 2011; Cloete et al., 2012). It has been reported to be highly prolific (110-150%) and found to have a steady growth rate of 350g per day until weaning. As an efficient meat converter, the lambs attain market weight of $\geq 40$ kg between 4-6 months of age and its wool has high competitive demand in international markets (Olivier and Roux, 2007; Olivier et al., 2010). The per capita income of 3-4kg per annum generated from its meat is stable, resulting in 60.6% income that is higher than 31.4% from wool and 0.2% from karakul pelts (McMaster, 2011). Thus, Cloete et al. (2012) found that its *M. longissimus dorsi* to be tender at first bite than Dormer and South African mutton Merino. The desirable meat and wool traits in the top progeny of Dohne Merino (Mortimer et al., 2009; Refshange et al., 2010; Thornber, 2010) thus guarantees market security and profitability (Kopke et al., 2008).

**2.3.1 Biomarkers for diagnosing traumatic brain injury and pregnancy**

Habitually, Dohne Merinos are also subjected to similar stunning and slaughter procedures as applicable to other breeds of sheep. It has thus been suggested that the elicitation of post-stunning behaviours that accompany electrical stunning also requires an integrative approach for explaining the interplays of pathways or processes influencing their behaviours (Gruber et al., 2010). With advances in genomics, a major shift towards genomics in which specific markers are linked with specific attributes, behaviours or experiences has been reported (Papa et al., 2008; FDA, 2010). This shift leads to further recognition of substances, which appear to be specific to the cells found in the brain that could act as biomarkers (Land, 2002). These biomarkers have thus been defined as "a characteristic that objectively measures, evaluates or indicates normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group, 2001).

Biomarkers provide a dynamic and powerful approach to understanding the spectrum of neurological conditions with applications in observational clinical trials, screening, diagnosis and prognosis (Mayeux, 2004). According to FDA (2005, 2010), translational biomarkers had been measured in blood or urine in both experimental animals and man (Fitzpatrick et al., 2006; Liu et al., 2010). Vaidya and Bonvente, (2010) therefore gave examples of biomarkers which include proteins, lipids, genomic, metabolomic or signals and cells present on urinarylysis. Thus, biomarkers have been used in man for quantitation of traumatic brain injury (TBI) and the TBI has been defined as an insult to the brain caused by an external force that may produce diminished or altered states of consciousness, which results in impaired cognitive abilities or physical functioning (Ghajar, 2000). The Centers for Disease Control and Prevention (CDC) further defined TBI as an injury to the head arising from blunt or penetrating trauma or from acceleration/deceleration forces. In both cases, these forces are associated with one or more of the following: decreased level of consciousness, amnesia, objective neurologic or neuropsychological abnormality (ies), skull fracture(s), diagnosed intracranial lesion(s), or head injury leading to death (CDCP, 2010; Li et al., 2010).

**2.3.2 Ovine ubiquitin C-terminal hydrolase-L1 (UCH L1) synthesis in stunned sheep**

During electrical stunning, the impact of these forces make sheep to be vulnerable to pain as earlier discussed in Chapter 1. According to Fitzpatrick et al. (2006), pain signals are processed from the periphery and conducted to the spinal cord to the brain where pain is perceived. It has been established that sheep experience hyperalgesic pain on-farm, during transportation to the lairage, at the lairage and in the process of stunning at the abattoir (Nolan, 2000). Pain associated with some or all of these processes obviously violates international welfare standard codes and consequently alters the quality of mutton, lamb or ovine offal (Goddard, 2006).

Concerns about the forms of pain these animals (pregnant and non-pregnant) experienced during electrical stunning and slaughter calls for attention from animal welfare view point. In literature, ovine ubiquitin carboxylase terminal hydroxylase (UCH L1) has been identified as a possible biomarker based on recent proteomic study (Yajun et al., 2012). UCH-L1 also called neuronal-specific protein gene product (PGP9.5), is a 223-amino acid protein with a molecular mass of approximately 24 kDa (Yajun et al., 2012). Among several related enzymes of this class (UCH-L1, UCH-L2, UCH-L3 and UCH-L5), UCH-L1 consists of about 5-10% cytoplasmic protein. Among these classes, UCH-L1 is the most highly expressed and is one of the most abundant pain biomarkers detected as a brain-specific protein, which is present and localised in the neurones and neuroendocrine cells in vertebrates. Brain specificity of this biomarker simply implies its concentration in the brain is more than other tissues (Day and Thompson, 2010).

The best-known function of this biomarker is its deubiquitinating enzyme activity that catalyses hydrolysis of C-terminal esters and amides of ubiquitin to produce monomric ubiquitin (Hurst-Kennedy et al., 2012). Elevated levels of UCH L1 have thus been observed in human tumour-derived cell lines from lung, prostrate and osteosarcomas that suggest its capacity in oncogenic cancer pathogenesis (Leiblich et al., 2007). Moreover, under practical conditions, traumatic brain injury has been reported leading to the expression of this protein in the victim (Papa et al., 2010). During electrical stunning at the abattoir, current passed through the brain causes substantial depolarisation of the neurons resulting in a brain status similar to a grand mal epileptic (Terlouw et al., 2008). Sometimes at the slaughterhouse, electrical stunning might not result in instant death of the animal, but avoidable with attendant tonic or clonic seizures and cardiac fibrillation (Wotton et al., 2000).

All manners of serious physical post-stunning convulsions, epileptiform activity, groaning, vocalization, increased rate of the *post mortem* muscle glycolysis and elevated release of cathecolamines have been observed (Velarde et al., 2003; Gruber et al., 2010). Although often ignored, before electro-immobilisation is done on poorly stunned animals certain degrees of pain which compromise the essence of stunning occur and consequently affect the meat-offal quality (Rusen, 1986).

### 2.3.3 Slaughter reforms and conversion of livestock to meat

In South Africa, it is expected that livestock meant for meat production be slaughtered at registered abattoirs that have met certain statutory requirements (Meat Safety Acts, 2000). These requirements are similar to the long exsting one made during the 1880's and early 1890's by the animal-protectionists, veterinarians and anti-Semitic societies in Saxony when the Germans lobbied for slaughterhouse reforms (Judd, 2003). The reforms were based on the concern expressed by the Saxon animal protectionists with regards the traditional ways in which animals were slaughtered for food (Metcalf, 1989; Lavi, 2007). They sought the licensing of slaughterers and the restriction of the abattoir operations to men only. They also proposed the implementation of stricter inspection procedures and the stunning of animals into a state of unconsciousness before exsanguination. These groups as well, called for change because they were convinced that the then state of affairs in the municipally run slaughter houses posed a risk to the public's health (Judd, 2003).

In their view, the activities at the abattoir purportedly encouraged brutal behaviour, attracted unsavoury characters from employees and aided the accumulation of contaminants from its dirty and bloody surfaces (Collis et al., 2004).

While the prevention of cruelty to animal acts were revised regularly to keep pace with the time, it became apparent in the latter half of the twentieth century that these anti-cruelty acts did not provide sufficient protection for animals. This is because many people find the subject of animal slaughter and their pre-slaughter welfare to be very unpleasant. According to Grandlin (2004), people rather prefer not to know the details of what goes on inside a slaughterhouse. Because of this, different species of pregnant livestock are slaughtered indiscriminately at the abattoirs for meat. The scenario at the abattoir has even shown that different breeds of pregnant livestock are routinely slaughtered without stunning (Abdulkadir et al., 2008; Thornber, 2010).

Sheep however have more than a fair share of the reported cases on maternal slaughter (Sanusi et al., 2006; Addass et al., 2010; Thornber, 2010). In their report, Cadmus and Adesokan (2010) indicated that one-out-of-every-three sheep with single, twin or multiple foetuses are being slaughtered for meat. The pragmatics that sees this practice as incongruent with the original code of conduct on slaughter reforms refer to it as 'anthroparchy'. The term anthroparchy has thus been defined as the structure of attitudes, practices and institutions, by which humans dominate, exploit and abuse members of other species (Cudworth, 2008). This manner has been described as a drift from the original code of conduct on public abattoir operation (Judd, 2003). It is therefore considered as non-conformity to the rules for which only unproductive, infertile, sterile, old or accidentally injured animals are allowed to be slaughtered (Biggs and Blackwell, 2005; Butterworth and Wotton, 2005).

**2.3.4 Cases and evidence on the conversion of pregnant livestock to meat**

An assertion has been made in that the wastage of conceptus through the slaughter of pregnant livestock is one of the most destructive practices man has ever used against his production endeavour (Umar et al., 2006). By implication, the indiscriminate slaughter of pregnant livestock could be a constraint to future livestock population which might consequently result in protein malnutrition (Nwakpu and Osakwe, 2007; Cadmus and Adesokan, 2010). A typical example was the incidence in an abattoir at the south of England where 26.9% of the slaughtered cows were in the third trimester of pregnancy (Ernst, 2009). Approximately, 27.3% of the farmers who consigned these animals for slaughter were ignorant of the pregnancy status of the cows and at least 6% of the culled cows were "discarded" for fertility reasons when they were actually pregnant (Singleton, 1996; Ernst, 2009).

In 2003, out of 70.1% of the pregnant Ethiopian Highland sheep consigned for slaughter, 24% of these ewes were found carrying twins (Mukasa-Mugerwa and Tekelye, 2003). According to Muhammad et al. (2009), 34.3% pregnant ewes from 0.26 million sheep that were annually slaughtered at the semi-arid abattoir in Nigeria were converted into meat on daily basis. Earlier in the same region, Sanusi et al. (2006) had reported that 26.1% of female goats (does) from 0.21 million that are annually converted to meat were also pregnant at slaughter. A lower range of 1.50-2.1% pregnant cows were slaughtered in Iran (Sepehr et al., 2012) and higher range of 6.39-10.27% for sheep and goat were reported being pregnant at slaughter in Western part of Nigeria. As a foreseeable consequence, efforts directed to meet the dietary protein requirements of people in many developing nations might be affected unless for those that meet protocols intended for "emergency slaughter" (Grandlin, 1998; Ademola, 2010).

**2.3.5 Permissible reasons for slaughtering pregnant livestock**

Few authors have however given the case of "emergency slaughter" otherwise known as euthanasia" as an outstanding circumstance that supports the slaughter of pregnant animal (Biggs and Blackwell, 2005; Butterworth and Wotton, 2005; Riehn et al., 2010). When emergency slaughter remains the only alternative, the farmers are expected to contact the slaughter operators. As a *modus operandi*, the Official Veterinarian (OV) must be present at the slaughterhouse during the *ante- mortem* and *post-mortem* inspection to declare the meat either fit or unfit for human consumption (Grandlin, 1998). This is the only permissible situation in the USA when maternal slaughter will not be counted as anthroparchy otherwise; the culprit will pay fines (Perera, 2006; Becker, 2010; Singleton, 2010).

The depressions in the beef market in the United States of America have sometimes compelled farmers to retain fewer heifers on their farms as breeding replacements (Gregory and Grandlin, 2007). In the 1980's and 1990's, the recurrences of spontaneous abortion by pregnant heifers within 3 weeks of arrival at the feedlots also inform farmers' decision to present their pregnant heifers for slaughter. Due to the disruptive bulling behaviour of pregnant cows amounting to 11% during the peak period in autumn and winter most farmers in the USA prefer to cull their injured pregnant cows even from early stage of pregnancy (Gregory and Grandlin, 2007). This factor has been noted being a contributor to the increasing rate of maternal slaughter at the abattoirs. In addition, poor economic status of the farmers and ignorance of the physiological state of the animals were reasons advanced for this maternal slaughter in Nigeria (Muhammad et al., 2009; Cadmus and Adesokan, 2010).

Since sheep flocks are vulnerable to the risk of limited production if the teeth are in poor condition, veterinarians recommend the culling of old sheep from the flock as a measure of curbing dental disorder or abnormality (Farquharson, 2009; Ridler and West, 2010). Abortion is sometimes induced in pregnant Karakuls to get the lustre and smoothness of the pelts from their foetuses, which attracts high auction prices (Starkov, 1990). Lowe (2008) therefore associated the motive behind slaughter of pregnant horses for meat to the exploitation of conjugated equine oestrogen (CEE) for the treatment of menopausal syndrome in women. The utilization of this hormone for alleviating age-related mnemonic decline or for improving spatial reference memory and for the prevention of scopolamine-induced amnesia is connected with the reason for massive slaughter of pregnant horse (Frick et al., 2002; Creidi et al., 2005). The biochemical benefits of enhancing the facial skin of ageing post-menopausal women has been reported as one of the drives for the slaughter of pregnant animals at the equine abattoirs or Premarin farms by Williams (1994) and Frick et al. (2002).

**2.4.1 Early pregnancy diagnosis in slaughtered livestock**

Studies on pregnancy diagnosis have given substantial evidence of the physiological status of female animals that are consigned for slaughter (Beg et al., 2001; Okoli et al., 2006; Ladds et al., 2008). The earliest signs of pregnancy of these animals have been detected in livestock by observing non-return to oestrus, laparoscopy and laparotomy and by a marked reduction in the endometrial oxytocin receptor numbers (Wani et al., 2003, Jerome, 2012). The determination of estradiol-17β concentrations in serum and follicular fluid have been used to confirm that some cows brought for slaughter were reproductively active and were being sold for reasons other than infertility (Opara et al., 2006).

Radioimmunoassay of steroid hormones (based on the ability of antibodies) directed against a steroid determinant to react preferentially with that particular steroid has also been successfully used in the assay of protein hormone in slaughtered animals (Beg et al*.,* 2001). Visual assessment and the palpitation of the uterus, ovaries and oviduct have confirmed the pregnancy status of 61% cows in Pakistan at the point of slaughter. Critical observation of the reproductive tracts from the right and left side showed the presence of single, twin or triplets foetuses respectively (Khan and Khan, 1989). A range of 21.28% in sheep and 19.22% in goats and in buffaloes, pregnancy distribution of 51.11% on the right and 48.88% on left side in buffaloes in studies by  Khan and Khan (1989); Ladds et al. (2008). Ovaries harvested from the slaughtered animals have shown evidence of current and past reproductive states of such animals. Follicular developments, corpus haemorrhagicum, corpus albicans, *corpus luteum* (CL) characteristics during pregnancy and progesterone secretion have given substantive evidence elsewhere (Addass et al., 2010).

## 2.4.2 Ovine progesterone and pregnancy-associated glycoproteins (PAG 1)

Progesterone (P4) is synthesised from a precursor known as pregnenolone, which is a derivative of cholesterol (Schumacher et al., 2007). Under the enzymatic influence of 3-beta-hydroxysteroid dehydrogenase and cytochrome P450, cholesterol is converted into pregnenolone (Meffre et al., 2005). As one of the neurosteroid hormones, P4 is secreted in the nervous system where it performs various physiological actions as guided by de novo synthesis (Locke, 2008). In ovine species, P4 production from the *corpus luteum* occurs in early pregnancy in sheep and therefore supports functional placentation (Al-Gubory et al., 2004; Ayad et al., 2007; Grazul-Bilska et al., 2010).

During placentation in ruminant species, the trophoblast binucleate cells migrate from the trophoblast and fuse with the maternal endometrial epithelial cells. The initiation of this process results in the release of maternal secretory products like the placental lactogen and pregnancy associated glycoproteins (Green et al., 1998; Gonzalez et al., 2001). Otherwise known as ovine pregnancy-specific protein B (PSPB), the ovine pregnancy-associated glycoproteins (PAG) are multi-genes that originate from the maternal placenta and are detectable from the third week of gestation (Ranilla et al., 1994). As a member of the aspartic proteinase family, they exhibit high homologous sequence that is similar to pepsin, pepsinogen, chymosin, cathepsin D and the cathepsin E (Verberckmoes et al., 2004).

These genes are encoded in distinct extra-embryonic chorionic membranes (trophoblast, trophectoderm) in pregnant livestock (Jerome, 2012) and are not less than 100PAG genes in bovine and ovine species (Green, 1998). As confirmed in various cDNA libraries, the PAG gene family is regarded as a novel genetic biomarker that is useful in early pre-selection and conservation of endangered animals (Szafranska et al., 2006). These have therefore been identified immunologically or characterised under an enzymic condition from saccharide units or the nascent polypeptide chain in pregnant ruminants (Lucy, 2001). Although several techniques have been used for ascertaining the pregnancy status of animals at slaughter, the motive behind the practice of slaughtering pregnant animals is the production of meat products in form of beef, mutton, chevon and their offal (Cadmus and Adesokan, 2010; Ngbede et al., 2012).

**2.5.1 Offal as a source of atypical meat**

In practical terms, offal are a culinary term used to refer to the entrails and internal organs of butchered animals or the edible parts of slaughtered animals that are gathered after evisceration or cleaning (Olomu, 1995; Fernandes et al., 2010). Literally, the word offal shares its etymology with the German words "*Abfall*" (*offall* in some Western German Dialects), *afval* in Dutch and Afrikaans. These Germanic words all mean 'garbage' or 'off-fall', referring to that which has fallen off during butchering or any part of slaughtered animal that is excised from the carcass in the process of evisceration and dressing. Specifically, offal include the liver, heart, kidneys, trachea, spleen, brain, pancreas, trotters (feet), tongue, tail, thymus glands or sweetbreads and tripe or stomach lining, (Olomu, 1995; Aberle et al., 2002; Magoro, 2007).

In some cuisines, the head and eyeballs, natural binder, blood plasma, cow-heels, gut (casings), omentum, pluck (oesophagus, trachea, lungs), pericardium, associated lymph nodes, spleen, trotters, udder, pillars of the diaphragm and rind are also regarded as offal (Fernandes et al., 2010; Warriss, 2010). In recent time, offal is prized meat sources and are no longer discarded as waste by-products (Toldrá et al., 2012). Although studies have shown that 40% are edible and 20%, inedible there are common ingredients in the cuisine which are widely consumed as important nutritious food sources (Ockerman and Basu, 2007). The impact of urbanisation, population growth and increase in earnings are collectively responsible for consumer preference for offal. Industrially, various parts of meat species are converted into value added products as pet food, livestock feeds, fertilizers, biofuels and nutraceuticals (Toldrá et al., 2012).

In communities where the worth of offal is not less than that of the popular skeletal muscle, pâté from the liver and blood of goat offer an alternative for the economic benefit as a 'variety meat' (Damez and Clerjon, 2008). These can be viewed as what constitutes variety components as they provide multiple types of nutrients from different anatomical parts of an animal. Bioavailability of heme iron in the blood of animals serves as food source that prevents anaemia in developing nations (Griffin et al., 1992). The exploitation of comminuted meat products brings more economic returns, and provides food security in form of value added by-products (Florek et al., 2007). Food manufacturing industries produce peptides from the offal through hydrolytic intervention with commercial bromelain, papain, pronase, thermolysine and proteinase K. Protein hydrolysates derived from offal are used as flavour enhancers and nutritional additives (Vercruysse et al., 2005). Consumers therefore benefit from the antihypentensive, antioxidative and opioid potential of these bioactive paptides derived from offal (Arihara et al., 2001).

**2.5.2 Muscular fibre architecture and nutritive components of sheep offal**

According to Pearce et al. (2011), muscle tissues form the basic component in meat or offal from any meat species. The muscle however consists of individual cells referred to as fibres that are fused together by connective tissues. According to Gilbert and Napadow (2005), muscle fibres are typically arranged in parallel arrays, allowing them to work together effectively. Three types of muscle tissue have been identified histologically: skeletal muscle, cardiac muscle and smooth muscle. The fibres of skeletal muscle and cardiac muscle exhibit cross striations at the light microscope level and they are both referred to as striated muscle. The muscular system of the sheep primarily has much in common with other small ruminant species and closely resembles the musculature of all quadruped (four legged) mammals which has been used for fibre classification  (Suzuki, 2002).

As applicable to most muscles in domestic animals, muscle fibres types such as IIA and IIB have been classified considering the nature of their ATPase (Peinado et al, 2004; Strbenc et al., 2009). The type of fibres found in the muscle thus defines its muscle architecture. By a way of definition, muscular architecture particularly describes the geometric design of a muscle (Alegre et al., 2005). An understanding of muscular architecture is critical to grasping the functional properties of different sized muscles and the complex array of fibres orientations exhibited by muscles such as the tongue, oesophagus and heart (Van et al., 2001; Gaige, 2007). The thickness of individual muscle fibres differs depending on location in the body and exercise (Lieber and Fridén, 2000).

Skeletal muscle, according to Bandman and Rosser (2000) is not only highly organized to function at the microscopic level; the arrangement of the muscle fibres at the macroscopic level rather demonstrates a striking degree of organization. Skeletal (or striated) muscle differs in microstructure from the smooth muscle that is present in internal organs such as intestinal walls, blood vessels and so on. The most obvious feature in longitudinal sections of skeletal muscle is the alternating pattern of dark and light bands, referred to as the A (anisotropic) and I (isotropic) bands. Analysis of cross-fibre anisotropy indicates a basic contrast of design between the extrinsic and the intrinsic fibres (Wedeen et al., 2001). Whereas the extrinsic muscles exhibit a uniaxial architecture typical of skeletal muscle, the intrinsic core muscles, comprised of the verticalis and the transversus muscles, show strong cross-fibre anisotropy. This pattern is consistent with the theory that the tongue's core functions as a muscular hydrostat in that conjoint contraction of the transverse and vertical fibres enables the tissue to expand at right angles to these fibres (Richard et al., 2006).

30

Muscles also differ in their constituent myofibre populations and, vascular and nerve supply which affects the speed and force developed during muscle contraction (Valberg et al., 1999). For instance, the myoarchitecture of the tongue is believed to consist of a complex network of interwoven fibres, which function together to produce a near limitless array of functional deformations. These deformations contribute mechanically to speech production and to oral cavity food handling during swallowing (Gilbert and Napadow, 2005). Aside from these mechanical actions, the meat tenderness is greatly influenced by the muscle characteristics and its fibre orientations (Maltin et al., 2003; Muchenje et al., 2009).

Notably, tenderness is one of the most important organoleptic characteristics by which consumers judge meat quality (Jurie et al., 2007). Meat tenderness is influenced by muscle characteristics at slaughter and by the *post-mortem* changes induced by ageing. Beyond tenderness, meat structure is associated with meat eating like texture, pastiness, crusting, palatability, chewiness, juiciness and of course tenderness. These sensory properties are associated with several objective physical properties of the product: fat content, fat spatial organization, collagen content, collagen spatial organization, myofibre spatial organization, myofibres type, size, shape and density, sarcomere integrity and other qualities exhibited by different muscles (Damez and Clerjon, 2008).

### 2.5.3. *Post-mortem* ambient conditions and pH of sheep meat

The *post-mortem* changes that take place when muscle is converted into meat have a marked effect on its quality (Bender, 1992). Following slaughter, glycogen continues to be degraded but in the absence of blood circulation, protons and lactate accumulate locally in the muscle cell, causing a decline in pH (Bendall, 1972).

This decline is initially fast, then slows and stabilises at a value called ultimate pH that is reached at 24h *post-mortem*. Terlouw et al. (2008) therefore showed that the ultimate pH for land animals varies between 5.4 and 6.0 but higher than 6.0 for fish. A high ultimate pH having low amplitude for pH decline indicates that the animal had reduced glycogen reserves, probably due to increased activity and possibly psychological stress prior to slaughter (Terlouw, 2005). The ambient conditions play vital roles in this regard by altering the muscle temperature. High muscle temperature and a fast pH decline during the minutes or hours following slaughter indicate increased activity and possibly psychology stress just before slaughter (Morzel et al., 2003).

Few authors have indicated some support for the glycolytic potential model which is an index of the muscle's capacity for *post-mortem* glycolysis or a measure of all compounds present in the muscle to be converted into lactic acid (Monin and Sellier 1985; Hamilton et al., 2003; Daly et al., 2006). In their report (Ferguson et al., 2008), indicated that the M. *longissimus thoracis et lumborum* from Merino wethers showed the greatest mean pH decline rate (~0.4 units per hour), the *M. semitendinosus* showed the least (~0.25 units per hour) and the *M. semimembranosus* was intermediate (a little over 0.3 units per hour). This led to the conclusion that the rate of pH decline in muscles comprising predominantly type 1 (mitochondrial-rich) oxidative fibres is generally faster than muscle built from predominantly type IIB (mitochondrial-poor) glycolytic fibres. When the muscle glycogen has been used up rapidly during the pre-slaughter period, it results in limited lactic acid production and this is the condition that is measured by an L* coordinates (Commission International De I'Eclairage, 1976).

Thus, production of meat with inferior quality as the less pronounced taste and the dark colour, which is less acceptable to the consumers and a short shelf life due to abnormally high pH value, that is suitable for bacterial growth (Priolo et al., 2001). Zhang et al. (2005) found that high pH meat had lower lightness (L*), redness (a*), yellowness (b*), hue angle (degrees) and chroma (saturation) values than normal pH meat, indicating that high pH meat was darker than normal pH meat. Hence, the rate and extent of *post-mortem* pH decline is an important determinant of meat quality (Hudson, 2012) because high pH meat is a persistent quality defect found in meat from all animal species. Merinos particularly have shown to be susceptible to high pH meat when compared to first-and second-cross lambs (sired by Border Leicester and Poll Dorsets) and consequently producing darker colour in the loin and the leg (Sheep CRC, 2008).

### 2.5.4. *Post-mortem* ambient conditions and colour of sheep meat

In lamb consuming countries, consumer perceptions of meat quality are associated with both lamb breed and rearing system. For consumers, meat colour is on one of the most important parameters influencing purchase decisions (Rodríguez et al., 2011). Nevertheless, meat does not have a homogenous surface for diversity of its structure, connective content and intramuscular fat. Colour occurs due to emission of electromagnetic radiation within the visible range or a reflection of atoms or molecules (MacDougall, 1983). It is not a characteristic proper to the object under observation but a subjective psycho-physical characteristic as it exists only in the observer's eyes and brain. Meat colour therefore depends on the concentration of pigments (such as myoglobin and hemoglobin), their chemical states and the light-scattering properties of the meat (MacDougall, 1983; Lawrie and Ledward, 2006). Enzymes, diet, age of the animal and even the activity done by the animal also influence the meat colour (Brewer, 2004).

Furthermore, ambient conditions also exert some effects on meat colour. For example, Bendall (1972) and Rosenvold and Wiklund (2011) indicated that colour may be associated with rigor temperature when meat is fresh and not after an extended ageing period. Besides, oxygen consumption due to mitochondria remaining active *post-mortem* influences the depth of the oxymyoglobin layer, hence blooming time and the depth from the surface at which metmyoglobin forms (Bendall, 1972; Faustman et al., 1998). Under laboratory condition, many options are available for instrumental colour analysis. For example, several types of instruments (colorimeters and spectrophotometers) are available. Each instrument offers a variety of options that allow researchers to choose from several: colour systems (Hunter, CIE, and tristimulus); Illuminants (A, C, D65, and Ultralume); observers ($2^O$ and $10^O$); and aperture sizes (0.64–3.2 cm) (Mancini et al., 2005).

Other work has reported no effects of illuminant and angle of observer on lightness measures from the CIE L* a* b* and Hunter Lab systems (Garcia-Esteben et al., 2003). The use of computer vision (Zhang et al., 2005) or reflection-based optical fibre measurement (O'Farrell et al., 2004) is other methods of measuring meat colour. Currently, food colour is measured in terms of CIE L*, a*, b* values, hue angle and chroma. The L* a* b*, or CIE color space is an international standard for colour measurement, adopted by the Commission International d'Eclairage (CIE) in 1976. The L* is the lightness component, which ranges from 0 to 100 (from black to white) and the parameters a*(from green if negative to red if positive) and b* (from blue if negative to yellow if positive) are two chromatic components which range from −120 to +120 (Oleari, 2004).

## 2.5. 5 Cooking regimes and tenderness of sheep meat

Mancini et al. (2005) and King and Whyte (2006) have reported that heat treatment during the cooking process influences colour change in meat. Of all the attributes of eating quality, tenderness is rated the most important factor affecting beef palatability and much research has been focused on improving tenderness (Vasanthi et al., 2007). Cooking has a major influence on meat tenderness as myofibrillar and connective tissue proteins undergo several temperature and time dependent structural changes during cooking which impact directly on product yield, texture and overall eating quality (Wattanachant et al., 2005; Walsh et al., 2010).

During heating process, different meat proteins denature, and they cause structural changes in the meat, such as the destruction of cell membranes, shrinkage of meat fibres, gel formation of myofibrillar and sarcoplasmic proteins or solubilization of the connective tissue (Tornberg, 2005). Thus, the effect of cooking on meat product has received considerable attention, as it is essential to achieve a palatable and safe product (Tornberg, 2005; Vasanthi et al., 2007). Cooking has a major influence on tenderness as myofibrillar and connective tissue proteins undergo several temperature and time dependent structural changes during cooking which impact directly on product yield, texture and overall eating quality (Walsh et al., 2009). Nevertheless, tenderization or toughening might result as the net effect based on heating method, the inherent composition and characteristics of the muscles, the method of heating and the time-temperature combination employed (Christensen et al., 2000; Obuz and Dikeman, 2003). A guarantee for eating quality can only be given if the links that effectively affect tenderness are controlled along the meat production chain (Thompson, 2002).

It has been shown that internal temperature during the meat cooking has a significant effect on all the meat texture profile parameters (cohesiveness, springiness, chewiness, hardness, elasticity) (Barbera and Tassone, 2006). These reach their optimum level in the 70–80 $^{o}$C range but increasing the temperature to 100$^{o}$C causes the meat structure to become more compact due to a significant decrease in fibre diameter (Palka and Daun, 1999). During heating, at varying temperatures (37-75 $^{o}$C), meat proteins denature and cause structural changes such as transversal and longitudinal shrinkage of muscle fibres and connective tissue shrinkage. Two phases of meat shrinkage have been noted namely: at a temperature of about 45-60 $^{o}$C the shrinkage is primarily transverse to the fibre axis and at 60-90 $^{o}$C primarily parallel (Offer et al., 1984). At a higher temperature of about 121 $^{o}$C there may be a third shrinkage of meat, which is transversal to the fibre axis (Barbera and Tassone, 2006).

## 2.6 Conclusion

The challenges faced in meat production are enormous. Although, meat production is the goal, yet crucial issues associated with the pre-slaughter welfare of the livestock, their meat quality and consumers' acceptability should be addressed. Decisive steps are expected taken to protect valuable meat species from extinction through indiscriminate slaughter of pregnant species. As suggested by Velarde and Dalmau (2012), technical efficiency of stunning device and stunning voltage should be revisited to keep pace with international standards on animal, welfare quality codes and meat quality.

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# CHAPTER 3: Farmers' perception on *ante-mortem* welfare of sheep and slaughter indicators in Eastern Cape Province, South Africa

*(Submitted to Scientific Research and Essays)*

**Abstract**

A survey was conducted among 203 sheep farmers to investigate their perceptions on the significance of pre-slaughter welfare and slaughter indicators for sheep. Descriptive statistics were computed for seasonal indicators, farmers' age groups, gender and their distribution within the Eastern Cape Province (ECP). The $X^2$-tests were also computed to determine the associations between the farmers' attributes and their perceptions on pre-slaughter welfare for sheep breeds. The results revealed that older male farmers (51-60 years old) had the least (1.6%) knowledge about the importance of *ante-mortem* welfare of sheep. Most of the respondents (85.2%) were of the opinion that sheep should be culled during winter season to produce lamb meat. However, live weight was perceived to be a highly significant ($p < 0.001$) slaughter indicator for Dormer sheep and age was found a significant ($p < 0.05$) slaughter index for common sheep breeds in ECP. It was concluded that only two municipalities were well informed about the importance of *ante-mortem* welfare of sheep in Eastern Cape Province of South Africa. Nevertheless, sheep farmers considered age at slaughter, live weight and season as crucial slaughter indicators for sheep.

**Key words:** Pre-slaughter welfare, pin mapping, sheep farmers, winter slaughter

**3.1 Introduction**

Sheep, like other livestock are sentient creatures that are endowed with the ability to feel pain or distress and experience a sense of wellness. Wellness in this context implies that their welfare requires a major consideration and that man has the moral obligation to respect, and to safeguard those that are intended for slaughter from avoidable suffering (Manteca, 1998; Belk et al., 2002). As previously indicated in Chapters 1 & 2, sheep are among the extensively managed livestock that have received little welfare attention because they are seen to be rustic creatures (Dwyer, 2009). This disposition is responsible for the inadequate attention accorded to the pre-slaughter welfare of various ovine species where they are extensively managed in the grassvelds or natural pastures. Rising public concerns have therefore been registered in countries like Chile (Schnettler et al., 2008), France (Deiss et al., 2009), Argentina (Zimerman et al., 2011), South Africa (Mahlangu, 2009; Ndou et al., 2011) and elsewhere.

In reality, the pre-slaughter phase includes the circumstances and practices that apply when the animal are mustered on-farm for slaughter. During this period, meat-producing animals are exposed to a range of avoidable stressors, which trigger their homeostasis and distort their adaptive responses in a bid to restore balance (Grandlin, 2007; Muchenje et al., 2009; Zimerman et al., 2011). These conditions typically occur because of human cruelty, improper pre-slaughter handling (Daly et al., 2006; Ferguson and Warner, 2008), inappropriate loading, reprehensible driving of the animals to the lairage (Miranda-de la Lama et al., 2010; Vimiso et al., 2012), unpleasant abattoir environment and inhumane slaughter methods (Schaefer et al., 2001; Terlouw et al., 2008). Consequently, the relationships among these pre-slaughter conditions usually alter the biophysical status of the muscle.

One of such is the depletion in muscle glycogen and the inability of muscle to accumulate adequate lactic acid concentration which results in high ultimate pH (pH > 5.9) level in the meat (Grandlin, 2007; Muchenje et al., 2008). Sometimes due to ignorance, negligence or the quest for immediate financial gain essential slaughter indicators are compromised. Thus the age of the animal (Cifuni et al., 2000; Okeudo and Moss, 2007); its health condition (Green et al., 1995); body conformation (Green et al., 1995; Yardimei et al., 2008); slaughter season (Panella et al., 1995; San˜udo et al., 1996) and live weight (Ermias et al., 2006; Strydom et al., 2009) are not given due attention.

These compromises result in the production of meat with inconsistent quality such as colour aberration, poor carcass grade, low market value and low keeping quality or reduced shelf life (Grandlin, 2007). Due to utilization of grassvelds in the Eastern Cape Province for extensive sheep farming, it becomes necessary to investigate farmers' opinions on pre-slaughter welfare and slaughter indicators of sheep. Therefore, the objective of this study was to determine the perceptions of farmers about the importance of pre-slaughter welfare of sheep and the indicators for their slaughter.

## 3.2 Materials and Methods

### 3.2.1 Study site

This study was conducted in the Eastern Cape Province (ECP) of South Africa. The province, as a frontier of diversity that encompasses all seven of South Africa's biomes, offer unrivalled range of climates and landscapes which support livestock husbandry. The provincial land mass of 16958 km$^2$ (which forms 14% of South African's land area) accommodates 28% of the country's sheep; 21% of its cattle and 40% of its goats (Eastern Cape Development Corporation, 2011).

Breeds such as fine-woolled Merino, the South African mutton Merino, Dohne Merino, Dormer, Dorper and the Karakul are reared in various parts of the country and particularly in ECP (Cloete, and Olivier, 2010; National Department of Agriculture, 2010; Eastern Cape Development Corporation, 2011).

### 3.2.2 Data collection and statistical analysis

A total of 203 sheep farmers (both commercial and smallholder producers that were either full-timers or part-timers in sheep farming) participated in this study during the winter period of 2010. The survey questionnaires were administered to all of them through personal contacts in their respective farms. Questions asked were focused on their demographic characteristics (such as age group, gender and farm location or municipality); their perceptions on the importance of pre-slaughter welfare and slaughter indicators of sheep. In order to achieve the objectives of the study, a group of experienced field-workers that could effectively communicate to these farmers in both vernacular (IsiXhosa) and English Languages were used to administer the questionnaires.

Having been screened for suitability as interviewers, they were also trained in order to minimise problems that might affect the reliability of the results. Each trainee was afterwards instructed to administer the questionnaire to each respondent and the expected responses were generated by guiding the respondents on how to objectively answer to all the questions raised. The collected data was analysed using the PROC FREQ and PROC CHISQ procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007).

**3.2.3 Pin Mapping for the pre-slaughter welfare of sheep in Eastern Cape Province**

A database pinmap was generated from the survey above (section 2.2.2) using the Geospatial data and TNT product (TNTmips® and TNTview®) concepts (Skrdla, 2010). This database made it possible to visualise and represent farmers' perceptions on pre-slaughter welfare of sheep on spatial coordinates within the study area.

**3.3 Results**

**3.3.1 Sheep farmers' demography from the Eastern Cape Province**

Result from the current study has shown that sheep husbandry in the ECP consists of approximately 86% male farmers (Figure 3.1). A glaring inequality was observed between female and male farmers with the male sheep farmers making up a population of proportion six-times more than that of their female counterparts in all the municipalities. Results further showed that sheep farmers are not distributed evenly in the ECP but highest concentrations were found in Mzimvubu, Mhlontlo and Elundini and, the least from Mbashe and O.R. Tambo municipalities (Figure 4. 1).

While most of the male farmers were within 41 to 50 years of age in the study areas, the youngest ones ( ≤ 30 years) were the least in population (Figure 3.2). It was observed that farmers belonging to different age categories were found at Mzimvubu where majority of farmers were situated. As presented in Figure 3.3, the youngest sheep farmers (≤ 30 years) were only available at the GreatKei, Mhlontlo and Mzimvibu municipalities. The Mbashe, OR Tambo and Sakhisizwe municipalities however, are the only municipalities with the unique feature of having only the oldest cadre of farmers on their sheep farms.

## 3.3.2 Farmers' perceptions on pre-slaughter welfare of sheep in Eastern Cape Province

Results on perceptions about *ante-mortem* welfare showed that female respondents (within 31-40 years old) from Mzimvubu were the most enlightened farmers on the importance of pre-slaughter welfare of sheep (Figure 3.4). On the contray, the opinions of older farmers from Mbashe, Mnquna, OR Tambo and Sakhisizwe municipalities were the least in the entire province. As shown in the map below, data collection from the brown shaded portion that covers 34% of the geographical footprint of ECP was not possible for bureaucratic constraints. This implies that the local municipalities within the Cacadu District were not captured and this includes Baviaans, Blue Crane, Camdeboo, Ikwezi, Kouga, Kou-Kamma, Makana, Ndlambe and Sundays River Valley respectively.
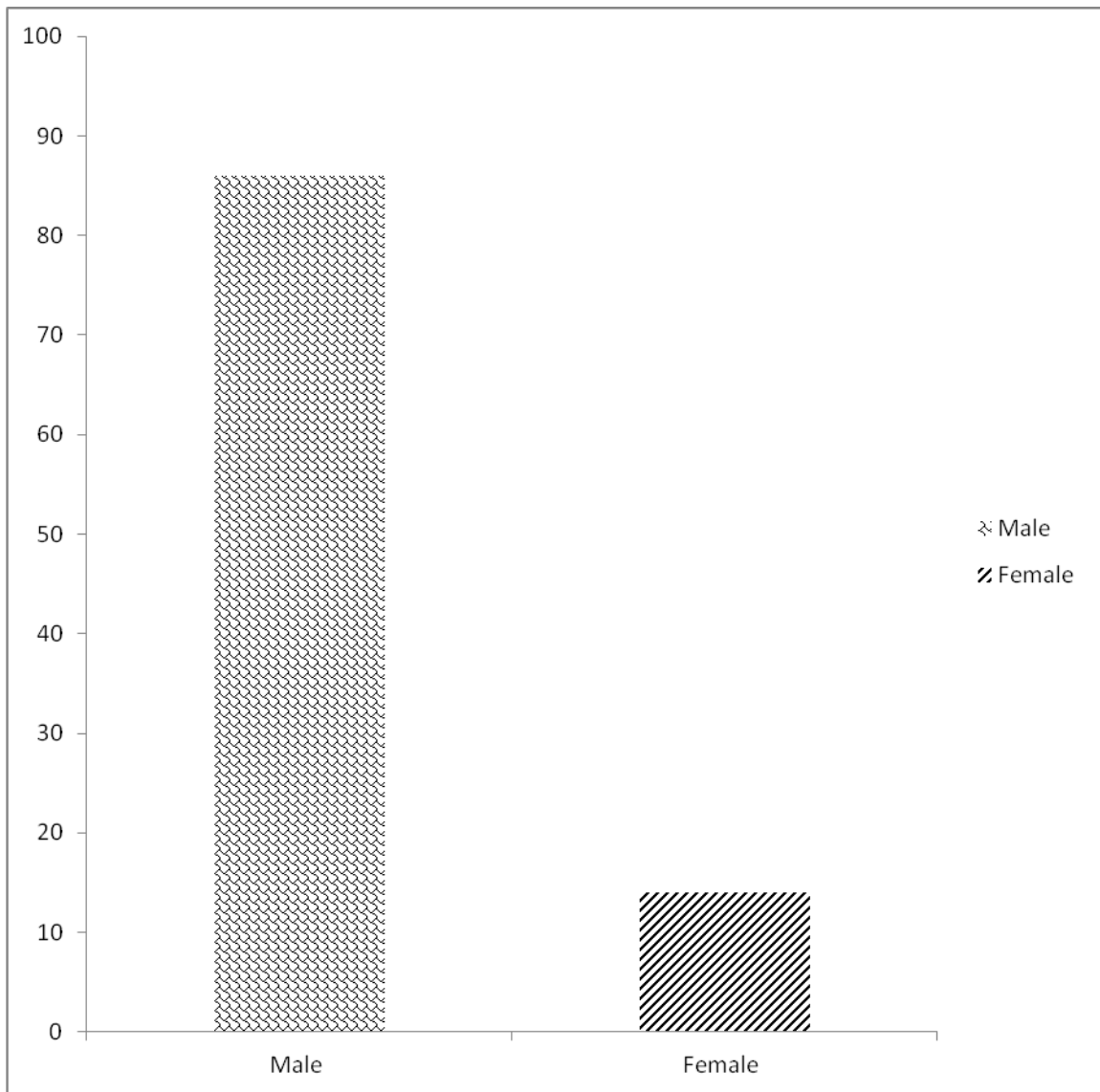
**Figure 3. 1 The gender of farmers involved in sheep husbandry in Eastern Cape Province of South Africa**
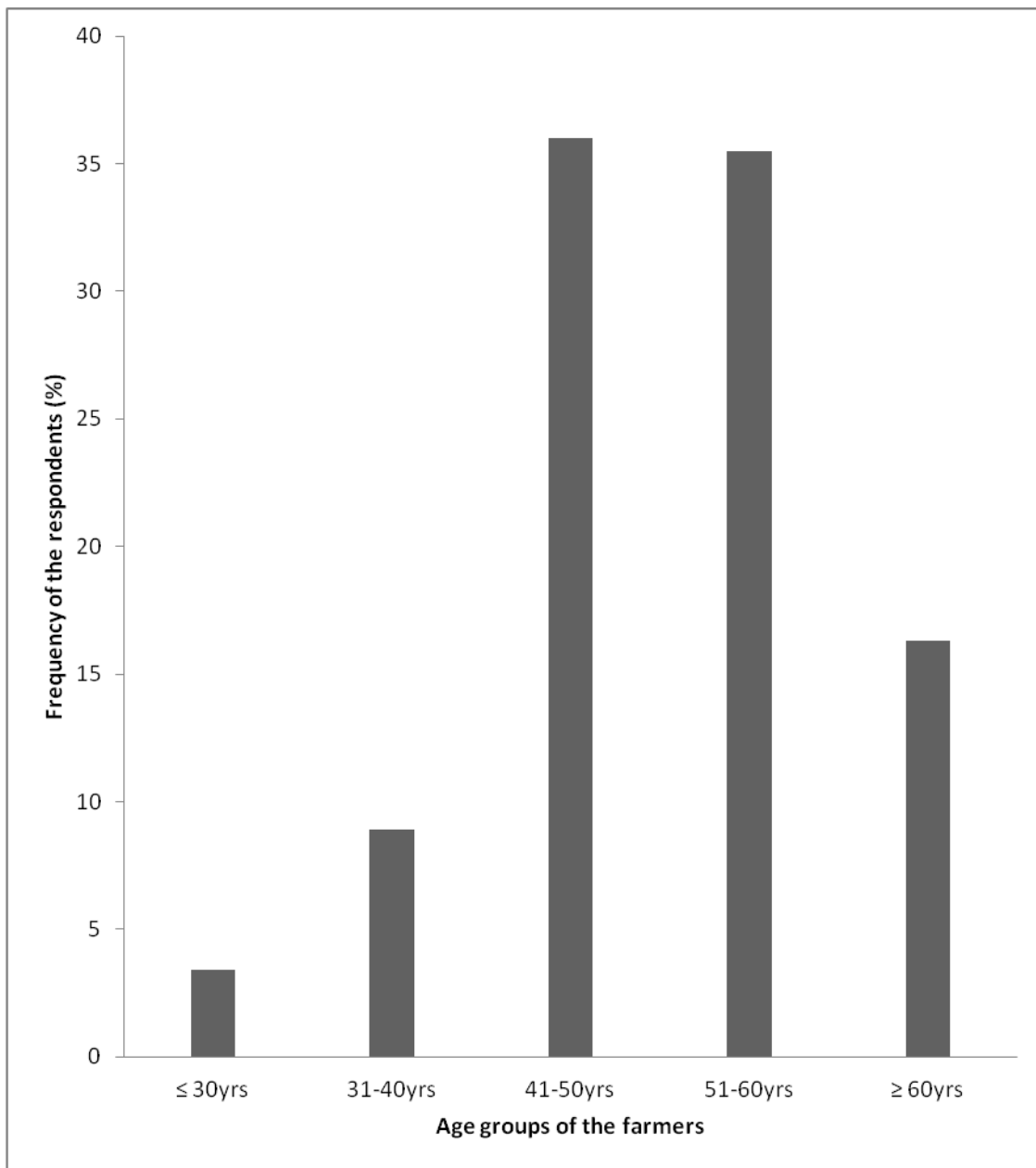
**Figure 3. 2 Age groups of farmers involved in sheep husbandry in Eastern Cape Province of South Africa**
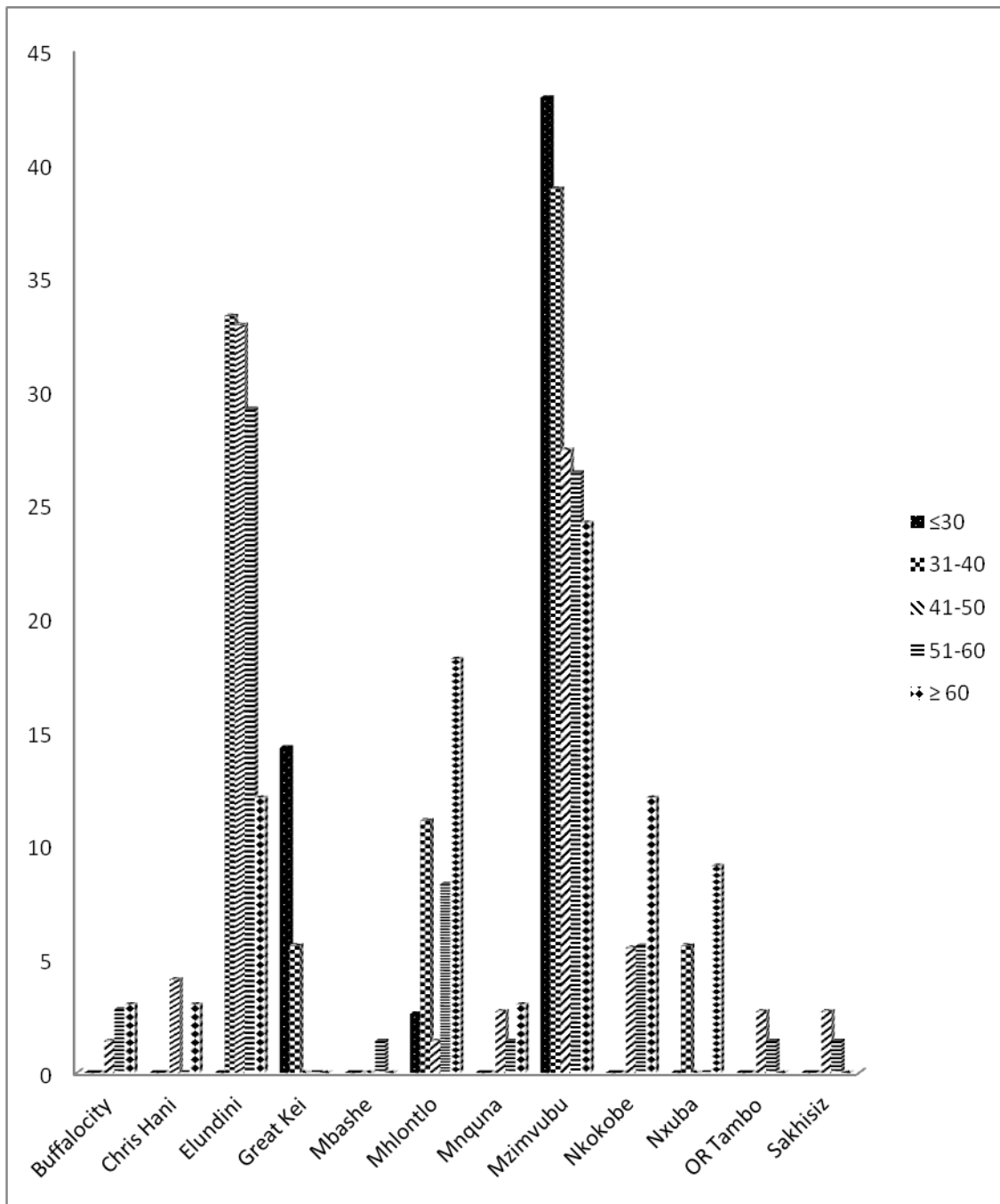
**Figure 3. 3 Sheep farmers' age-group distribution within Eastern Cape municipalities, South Africa**
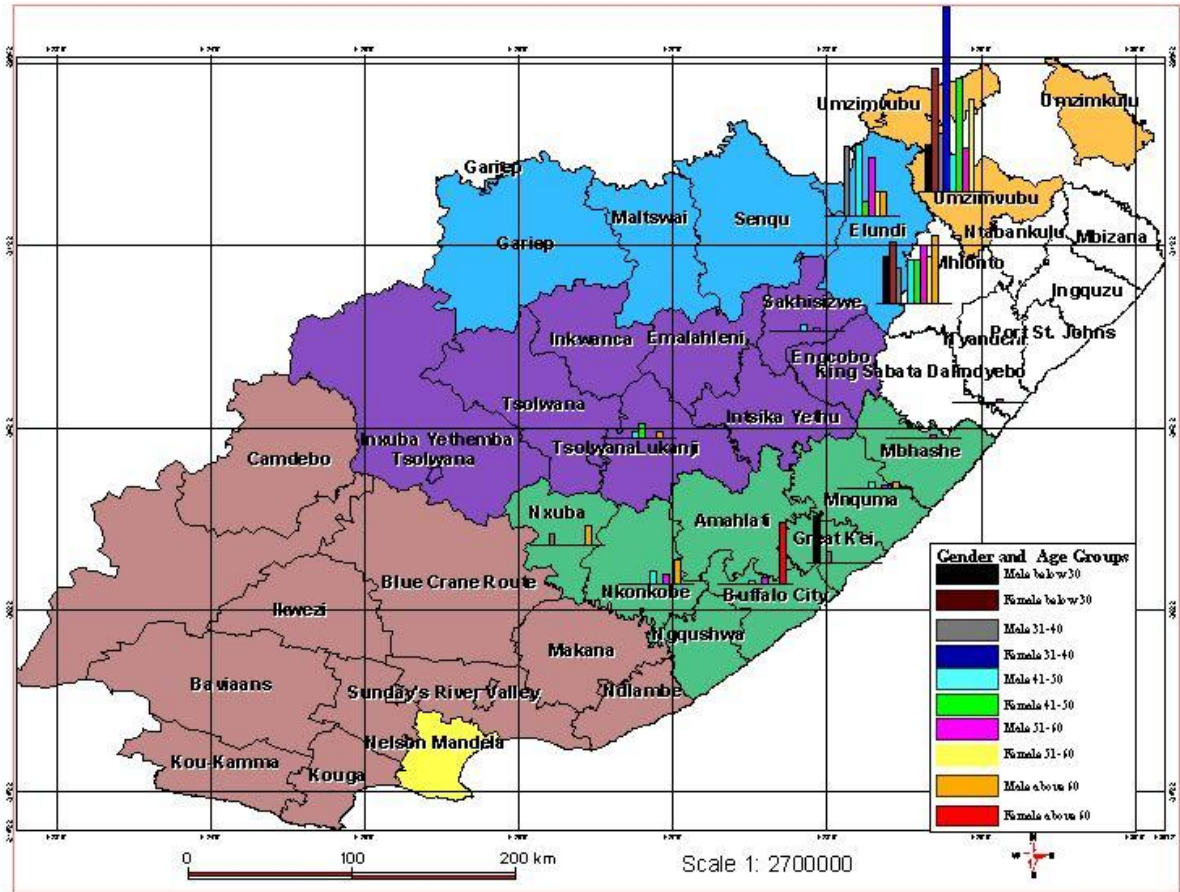
**Figure 3. 4 Pin-mapping showing the perceptions of farmers on the importance of pre-slaughter welfare of sheep in Eastern Cape municipalities, South Africa**

### 3.3.3 Perceptions on slaughter indicators for sheep breeds in Eastern Cape Province

Findings in this study further showed that all the respondents recognised season as a crucial slaughter indicator in ECP (Figure 3.5). Most of them (85.2%) were of the opinion that sheep should be consigned for slaughter during winter season. Spring was on the other hand, considered as a season when sheep culling should not be done. Divergent views were however recorded in respect of perceptions on live weight and age as slaughter indicators for sheep. As shown in Table 3.1, the opinions of most sheep farmers about live weight being a slaughter indicator were only different ($p \leq 0.05$) for Dormer but similar ($p \geq 0.05$) for Dohne Merino, South African mutton Merino, Dorper and other breeds. Differences in opinions could be attributed to phenotypic variations, which farmers might have noticed among the ovine species on their farms.

Contrasting opinions obtained in the current study may be due to farmers' knowledge about the impact of inbreeding depression or heterosis on growth performance of sheep. These key issues invariably influence the age at which the animal attains slaughter weight and could be responsible for the difference in views. Observations during data gathering revealed that younger farmers ($\leq 30$ years) were flexible about the live weight appropriate to offer Dorper for slaughter. Older male farmers ($\geq 60$ years), considered Dohne Merino and South African mutton Merino ripe for slaughter having attained 41 to 50kg live weight. It was noticed during data collection that female farmers (within 41 to 49 years) chose rather to offer other sheep genotypes for slaughter at $\leq 60$kg live weight. Majority of male farmers (66.7 to 100.0%) preferred sheep to be slaughtered for meat when they are $\leq 1$ year, as lambs but the female farmers indicated interest in slaughtering their sheep when older than, or equals 2 years.
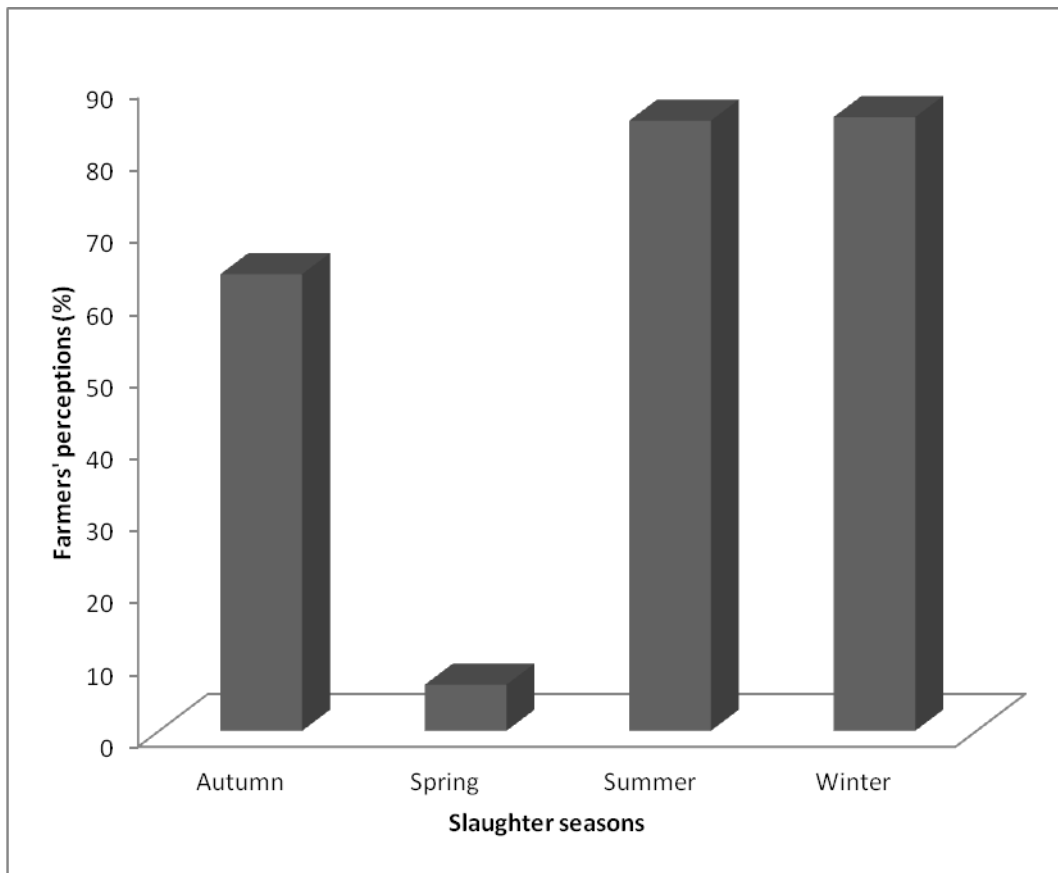
**Figure 3. 5 Season as a slaughter indicator for sheep in Eastern Cape municipalities, South Africa**

**Table 3. 1 Farmers' perception about age and liveweight of ovine species as slaughter indicators in Eastern Cape municipalities, South Africa**

| Breed | $X^2$-value | [1]Sig. |
|---|---|---|
| **Live weight of sheep at slaughter** | | |
| Dohne Merino | 12.479 | NS |
| South African mutton Merino | | |
| | 9.099 | NS |
| Dorper | 0.802 | NS |
| Dormer | 101.601 | *** |
| Others | 1.416 | NS |
| **Age of sheep at slaughter** | | |
| Dohne Merino | 26.478 | * |
| South African mutton Merino | | |
| | 38.743 | *** |
| Dorper | 21.093 | * |
| Dormer | 25.840 | ** |
| Others | 18.723 | NS |

[1]Significant at *p $\leq$ 0.05; **p $\leq$ 0.01; ***p $\leq$ 0.001 but NS not significant at p $\geq$ 0.05

## 3.5 Discussion

The results on unequal gender participation in sheep husbandry in the present study are consistent with previous reports (Jaim and Rehman, 1988; Banstola et al., 2004). The age at which people of different gender groups go into sheep farming also affect their perception about sheep welfare as recently reported (Paudel et al., 2007; Mapiliyao et al., 2012). Among several factors responsible for this, Yisehak (2008) and Ayoade et al. (2009) had implicated female pre-occupation with household chores, spouse dominance and inability to access adequate capital. The heavy domestic responsibilities on women are therefore responsible for their search for livelihood outside livestock husbandry and the reason for empowering men instead (Internal Strategic Perspective, 2005; Statistics South Africa, 2010). Although it is a common practice, in South Africa that women own livestock yet, the management of the herds is seen as the sole responsibility of men (Kleinbooi and Lahiff, 2007). This norm is connected with the colonial and apartheid history of the country where institutional support is either lacking or failing to implement redistributive land reforms in favour of women in livestock enterprise (Hoffman et al., 2007).

The initiative by Public-Private-Partnership (PPP) between the Alfred Nzo District and the Goldfields Foundation on Agricultural Development are responsible for higher concentration of sheep farmers around Mzimvubu and Elundini municipalities (Alfred Nzo District Municipality IDP, 2010). The provision of financial aid and technical supports for Small, Medium and Micro Livestock Enterprises (SMMLE'S) for about 2450 farmers in 330 villages around Umzimvubu and Matatiele justifies the result in the current study on land use for sheep husbandry within the region (Oledele and Monkhei, 2008).

Geographically, the higher mountain peaks in Elundini having 800mm to 1200mm rain per annum forms the catchment for Mzimvubu River where large volumes of water supports communal sheep grazing and livestock watering. The Elundini terrain (with slopes steeper than 1.8 in the Southern Drakensberg), also creates a scenic environment conducive for biking, hiking and skiing of livestock like sheep. Massive rearing of ruminants is possible in these municipalities due to availability of 56.40% grassland covering the entire total surface area. Farmers from these two municipalities (Elundini and Mzumvubo) have at their disposal basic resources to meet the welfare needs of the stock in the velds (Draft Integrated Development Plan Review, 2010).

In retrospect, during the winter season of the year 2010 when this study was conducted, most farmers within the study area (ECP) and in South Africa (at large) recorded massive sheep mortalities due to the outbreak of Rift Valley Fever (DREF Operation, 2010; GAR, 2010; Jasen van vuren et al., 2010; UNNC, 2010). The deteriorating effect of this zoonotic disease could be one of the major reasons why farmers' opinion favours slaughter of sheep in winter. Expectedly, consequential winter pasture contamination (in high altitude areas) because of the prevalence of trichostrongylic gastro-intestinal nematode (GIN) species which often affect the body conditions of sheep in the flock by raising the pathogenicity of this parasite has earlier been reported ( Perry et al., 2002;  Waller et al., 2004). Economic losses incurred by farmers in sheep and goats flocks due to severe pathogenic effects of Oestrosis are recognized in relation to livestock culling in winter (Cepeda-Palacios, 2001; Alcaide et al., 2003).

As opposed to health issues, seasonal demands for ovine meat during festive seasons (Christmas and Easter) have annually propelled farmers to consign their lambs or sheep for slaughter during winter (Panella et al., 1995). Mutton or lamb consumers in the Mediterranean countries would prefer to slaughter their sheep during winter because of their preference for lambs having pale colour, higher ultimate pH and low juiciness (San˜udo et al., 1996). From the foregoing, it can be deduced that the choices of season and slaughter weight are motivated by various factors. Farmers that are aiming at the possibility of having better wholesale prime cuts (Yardimei et al., 2008; Snowder and Duckett, 2011); choice quality meat grade and optimal meat yield with the expected carcass backfat (Santos-Silva et al., 2002) would therefore target the most suitable season and live weight for their decision. The interest of the male farmers for lambs is also consistent with the report on the slaughter of Apulian lambs between 45 to 90 days old in quest for lambs with fatty acid profiles that are suitable for consumers' health (Cifuni et al., 2000; Gallardo et al., 2011). A different opinion has been expressed where tougher meat from sheep met consumers taste (San˜udo et al., 1996).

**3.6 Conclusion**

Findings in this study have clearly shown that sheep farmers did not have adequate knowledge on the importance of *ante-mortem* welfare of sheep. Since the attainment of slaughter weight is not the same for all sheep genotypes, farmers were of the opinion that they could present their sheep for slaughter, as it seems appropriate for them. The slaughter weight of 60kg was considered the upper limit for culling different sheep genotypes by the middle-aged female farmers. In general, most of the respondents chose winter as the preferred season when sheep should be presented for slaughter. It could be concluded from this study that only two municipalities were knowledgeable about the importance of *ante-mortem* welfare of sheep in ECP. Although these farmers considered age at slaughter, live weight and season as crucial slaughter indicators for sheep, yet it is essential to determine if they are only producers or if they are both producers and consumers of meat from sheep raised on their farms.

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**CHAPTER 4: Preference of Xhosa speaking farmers for mutton and lamb from the natural velds in the Eastern Cape Province of South Africa**

**Abstract**

The aim of the study was to determine farmers' preference for meat types or anatomical parts from sheep raised on the natural velds. Data was generated by interviewing 203 sheep farmers within Eastern Cape municipalities in South Africa. The $X^2$ tests were computed in an attempt to determine farmers' attributes that might be pointers to their preference for meat types or anatomical parts from sheep. Results showed that farmers had more interest in mutton than other types of meat from sheep. Highest preference for 'mutton only' and combination of 'mutton and lamb' was shown by farmers within 51-60years of age. Liver, intestines and head were found to be the most preferred anatomical parts from sheep by the farmers. It was further revealed that most male farmers consume sheep meat because of personal interest (PI) and that traditional beliefs (TB) mainly influences the consumption of sheep meat by the female counterparts. It was concluded that sheep farmers are both producers and consumers of ovine meat from sheep raised on their farms (natural velds). Personal interest and traditional belief were found as the most important factors influencing their preference for various meat types and anatomical parts from sheep.

**Key words**: Anatomical parts, farmers' preference, meat types, neophobia, sheep farmers

**4.1 Introduction**

The preference for what to eat and what to abstain from by consumers, have received intensive advocacies in recent times (Cohen and Farley, 2008; Carlsson-Kanyama and Gonzalez, 2009; van't Riet et al., 2011). These advocacies are either congruent with the existing consumers' behaviour towards meat or are expressions of negative attitudes against the motives for its consumption (Holm and Møhl, 2000). Amidst a plethora of disciplines (as indicated in Chapter 3), meat production and its consumption have been under strong criticisms, rigorous debates and conflicting opinions (Richardson et al., 1994; Becker et al., 1998; Vartanian et al., 2007; Ruby and Heine, 2011). The pessimistic impression about meat has consequently produced a new orthodoxy supporting ambivalence towards meat and routine consumption of vegetarian or vegan diets (Povey et al., 2001; Te Velde et al., 2002; Delaney, 2008; Christina et al., 2011.).

In situations where a kind of meat is acceptable, consumers tend to question its origin in an effort to seek out healthier options for themselves (Hersleth et al., 2012). Knowledge of the source of meat has thus become a growing topic of interest, since buying or eating certified organic meat from the natural velds means that the animal has been treated ethically. Thus, the meat products from that source are considerd to be void of growth hormones, antibiotics, beta-agonists and other potentially harmful chemicals (Lind et al., 2009; Weissnar and du Rand, 2012). In South Africa, the natural velds form the basis of sheep farming where most ovine species are commonly raised for wool and meat products (Brand, 2000; Kösters et al., 2013). An estimate of 28.8 million sheep and flock size ranging between $\leq 50$ and $\geq 1800$ herds exist on these velds where over twenty sheep breeds are managed in various provinces (Agriculture, Forestry and Fisheries, 2011).

As a cutting-edge of diversity that encompasses all seven of South Africa's biomes, the Eastern Cape Province (ECP) offers unequalled range of climates and landscapes that support sheep production (Eastern Cape Development Corporation, 2011). Although the national herd size is unevenly distributed provincially, most of the herds are found in Eastern Cape (30%) followed by the Northern Cape (25%), Free State (20%) and the Western Cape (11%) respectively (Cloete and Olivier, 2010). Based on this information, the ECP which is situated on a land mass of 16958 km$^2$ forms 14% of South African's land area where majority of sheep breeds are raised for meat production (Agriculture, Forestry and Fisheries, 2011). Sheep meat that has formed an important component of the traditional food and a greater share in the meat market in South Africa include the lamb, mutton and offal (Bosman et al., 2002; Fayemi and Muchenje, 2012; Hoffman et al., 2013).

The term lamb has been used to designate carcass from ovine species under six or twelve months (weighing 32 kg live weight or more) and having not more than two permanent incisors. Meat from ovine species older than one year at slaughter but having more than two permanent incisors or have lost their third temporary incisor is referred to as mutton (Jeremiah, 1998; Schneller, 2009). Over the past ten years, the average mutton gross production in South Africa has increased continuously from 2001 until recent times. The amount of mutton produced in the country in the 2008 production period was 163 million kilogramme (Kg) and due to changing lifestyle of the consumers; the amount consumed reached its peak of 188 million Kg during the same period. Compared to 2001, an increase of 39% of mutton production and a slight increase of mutton consumption of 8.5% was recorded in 2010 (Agriculture, Forestry and Fisheries, 2011).

Going by massive production and consumption of sheep meat in South Africa, no evidence is available to show that the producers (farmers) are also consumers of meat types from various ovine species raised on natural velds.  Rather, the approach taken from available consumer-related studies are only focused on consumers who are non-farmers. Information is therefore available on consumers': perception for lamb from natural pasture (Weissnar and du Rand, 2012), preference for nutritional and sensory quality of lamb (Imami et al., 2011); acceptability of mutton (Bosman et al., 2002). The focus in recent times is even tilted to consumers' curiosity against mutton adulteration (Karabasanavar et al., 2011), their purchasing intention for lamb meat (Furnols et al., 2011) and factors influencing the demand for small ruminant meat (Juma et al., 2010).

It is however not clear why scientific literature is silent on farmers' preference for sheep meat considering their position as producers. This knowledge gap causes failure in understanding the consumer class they belong-either as 'meat eaters' or as 'non-meat eaters'. The dearth of information on this topical issue even makes it difficult to understand farmers' preference for lamb, mutton or anatomical parts. Based on this concern, it was hypothesized that male and female Xhosa speaking sheep farmers of different age groups had similar preference for mutton, lamb and their anatomical parts. The objective of the study therefore was to determine the preference of sheep farmers for lamb, mutton and anatomical parts from sheep raised on their farms (natural velds).

## 4.2 Materials and Methods

### 4.2.1 Study site

The study was conducted in Eastern Cape Province (ECP) of South Africa where the Xhosa speaking farmers are predominant. The municipalities used for this study comprised of Buffalo City, Chris Hani, Elundini, Great Kei, Mbashe, Mhlontlo, Mnquna, Mzimvubu, Nkokobe, OR Tambo, Nxuba and Sakhisizwe. The ECP is however located at latitude $32^{o}$ S and longitude $26^{o}$ E of the Republic of South Africa with a mean annual rainfall of 552mm. The vegetation is composed of several trees, shrubs and browse species such as: *Acacia karroo, Themeba triandra, Panicum maximum, Digitaria eriantha, Cynodon dactlylon, Pennisetum clandestinum* and *Eragrostis* species (National Department of Agriculture, 2010; Eastern Cape Development Corporation, 2011).

### 4.2.2 Survey description

A survey based on structured questionnaires was conducted among 203 sheep farmers in the ECP from May to October of 2010. This was done through questionnaire administration to farmers using a team of field-workers that were competent in communicating to them in vernacular (Xhosa) and English languages. These workers were screened to ascertain their suitability as interviewers. The screening was done to minimise challenges that might affect the reliability of the results. Training was also provided to these workers in order to ensure strict adherence to the goals of the study and to guarantee full participation of the target farmers. The survey questionnaires were administered to these sheep farmers through personal contacts in their respective farms. Both male and female farmers used were within ($n \leq 30$ to $n \geq 60$ years) age group and their selection was done on the basis of willingness to participate in the study.

Questions raised were intended to determine their preference for mutton and lamb harvested from the animals raised on their farms. These questions include: "What is your gender (Male or Female)? What is your age group ($\leq 30$ years; 31-40; 41-50; 51-60 & $\geq 60$ years)? Indicate the municipality where your farm is situated. Do you eat sheep meat (Yes, mutton only; Yes lamb only; both mutton and lamb; no interest in sheep meat)? If yes, why do you eat sheep meat (religious belief, traditional belief, personal interest, professional ethics and other reasons)? Which organ or anatomical part of sheep do you consider as meat (back, blood, brain, head, hide, intestines, kidney, leg, liver, loin, lung, neck, shoulder)?"

### 4.2.3 Description of statistical analysis

All data was analysed using SAS (version 9.1.3 of 2007). Chi square tests were computed to determine the relationship between farmers' attributes (age, gender and municipality) and their preference for meat types from sheep.

### 4.3 Results

### 4.3.1 Farmers interest and preference for sheep meat types

Majority of the respondents (92.1%) showed their interest in the consumption of various meat types from sheep (Figure 4. 1). Few of the farmers who did not indicate any interest in sheep meats were only 7.9% of the 203 respondents that were interviewed. The result implied that most of the farmers are both producers and consumers of meat types from ovine species raised on their farms (natural velds). It is clear that the interest shown in 'mutton only' by the male farmers was higher than their female counterparts but both of them ranked 'mutton only' as their most preferred meat (Figure 4. 2). Preference for 'lamb only' was however found to be the least among the categories of meat types chosen by the farmers.
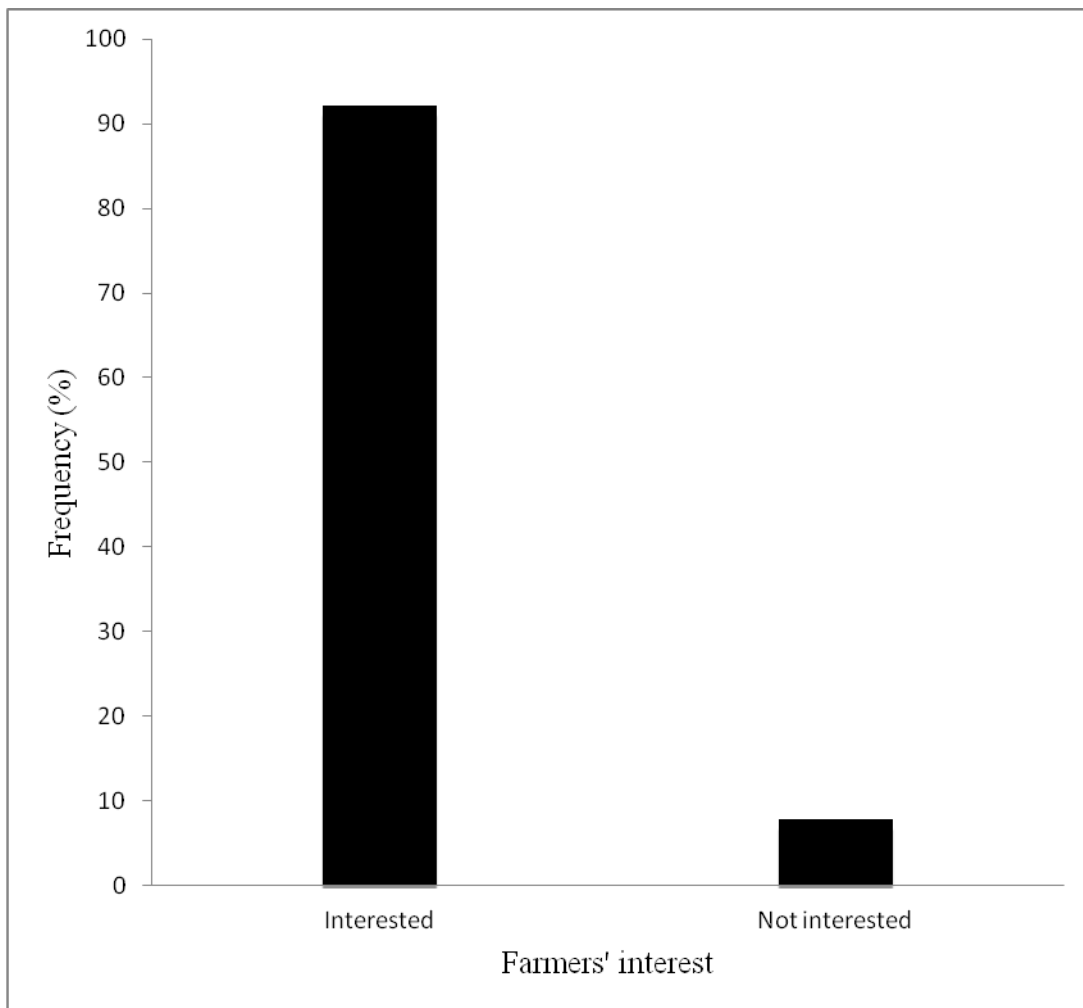
**Figure 4. 1 Interest in the consumption of sheep meat types by farmers from Eastern**

**Cape Province of South Africa**

Female sheep farmers

Male sheep farmers

100%

80%

60%

40%

20%

0%

Lamb   Mutton   Mutton &Lamb   Lamb   Mutton   Mutton &Lamb
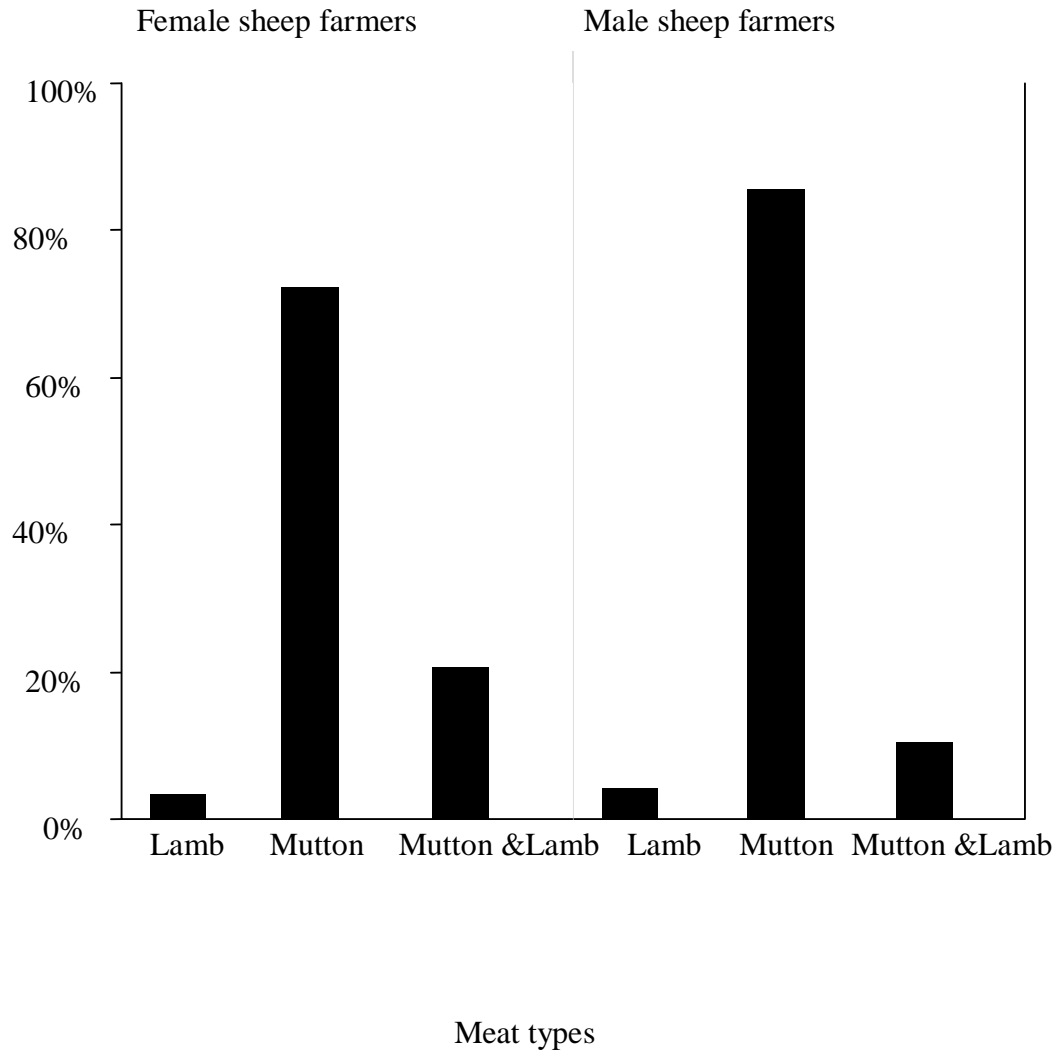
Meat types

**Figure 4. 2 Relationship between farmers' gender and their preference for sheep meat**

Further result revealed a significant relationship ($p < 0.001$) between farmers' gender and their preference for meat types (Table 4. 1). Although a significant relationship ($p < 0.001$) was also observed between farmers' municipality and meat types, absence of significant relationship ($p > 0.05$) was found between farmers' age group and preference for meat types. In Figure 4.3, farmers within 51-60years showed indication of having highest preference for 'mutton only' and also for combination of 'mutton and lamb'. On the contrary, younger sheep farmers ($\leq 30$ years) did not indicate any interest in 'mutton only'. The result presented in Figure 4.4 showed that sheep farmers demonstrated unequal preferences for various anatomical parts as meat from sheep. The most preferred anatomical parts by the sheep farmers in ECP were the liver, intestines and head. An indication portraying that most sheep farmers were biased against the blood and hide as meat from sheep was also observed. Preference for anatomical parts was not fund to significantly different ($p > 0.05$) among farmers across all the municipalities except for the back ($p < 0.01$), brain ($p < 0.05$) and loin ($p < 0.01$), respectively (Table 4. 2).

### 4.3.2 Factors influencing preference for sheep meat

However, the survey identified five major factors influencing farmers' preference for the consumption of sheep meat (Figure 4. 5). Majority of the male farmers (62.1%) were observed showing preference for sheep meats because of personal interest (PI) but traditional belief (TB) as the main factor influencing the consumption of sheep meat by the female sheep farmers. The farmers ranked religious belief and personal ethics as the least important factors influencing their preference for sheep meat.

**Table 4. 1: Farmers' preference for sheep meats from sheep raised on their farms**

| Parameters | $X^2$-value | [1]Significant |
|---|---|---|
| Gender*Meat types | 208.082 | *** |
| Municipality*Meat types | 233.834 | *** |
| Age group*Meat types | 8.329 | ns |

[1]Significant at ***$p < 0.001$; ns was not significant at $p > 0.05$

|  | Mutton only | Lamb Only | Mutton & Lamb |
|---|---|---|---|
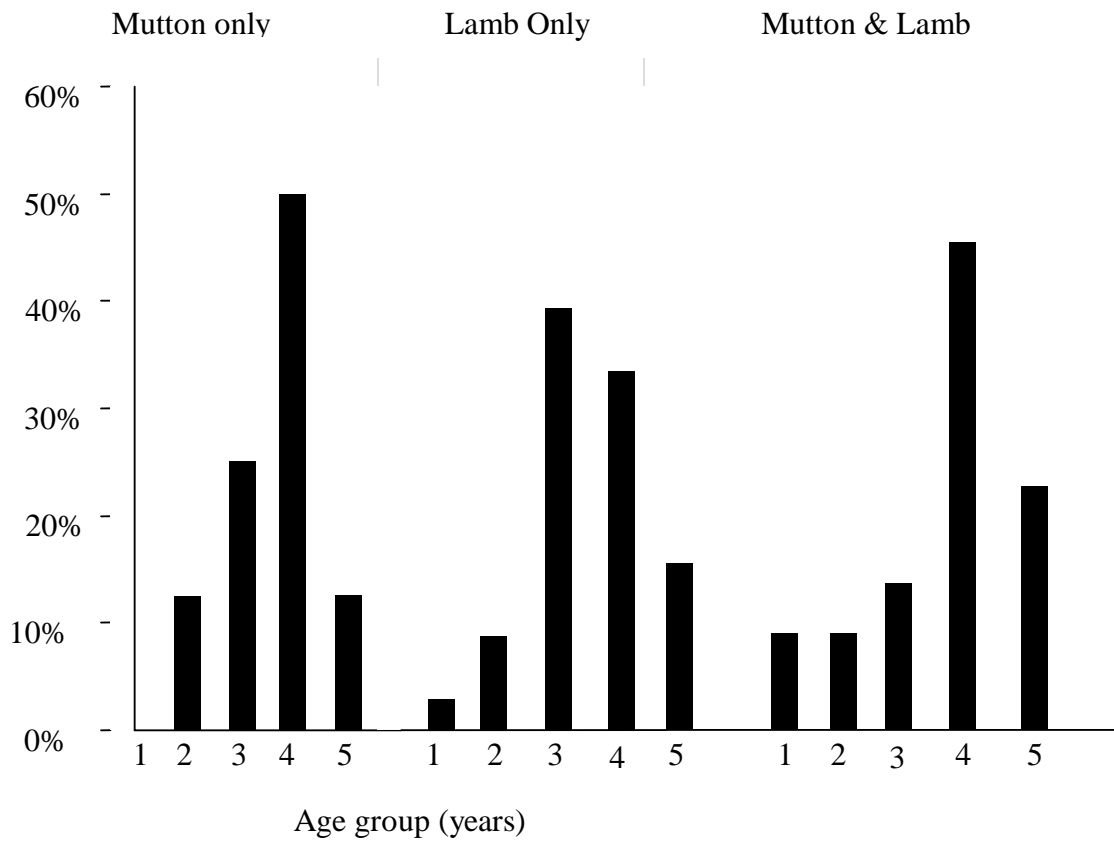
**Figure 4. 3 Relationship between farmers' age group and preference for sheep meat**

Key = 1: ≤ 30years; 2: 31-40years; 3: 41-50years; 4: 51-60years & 5: ≥ 61 years
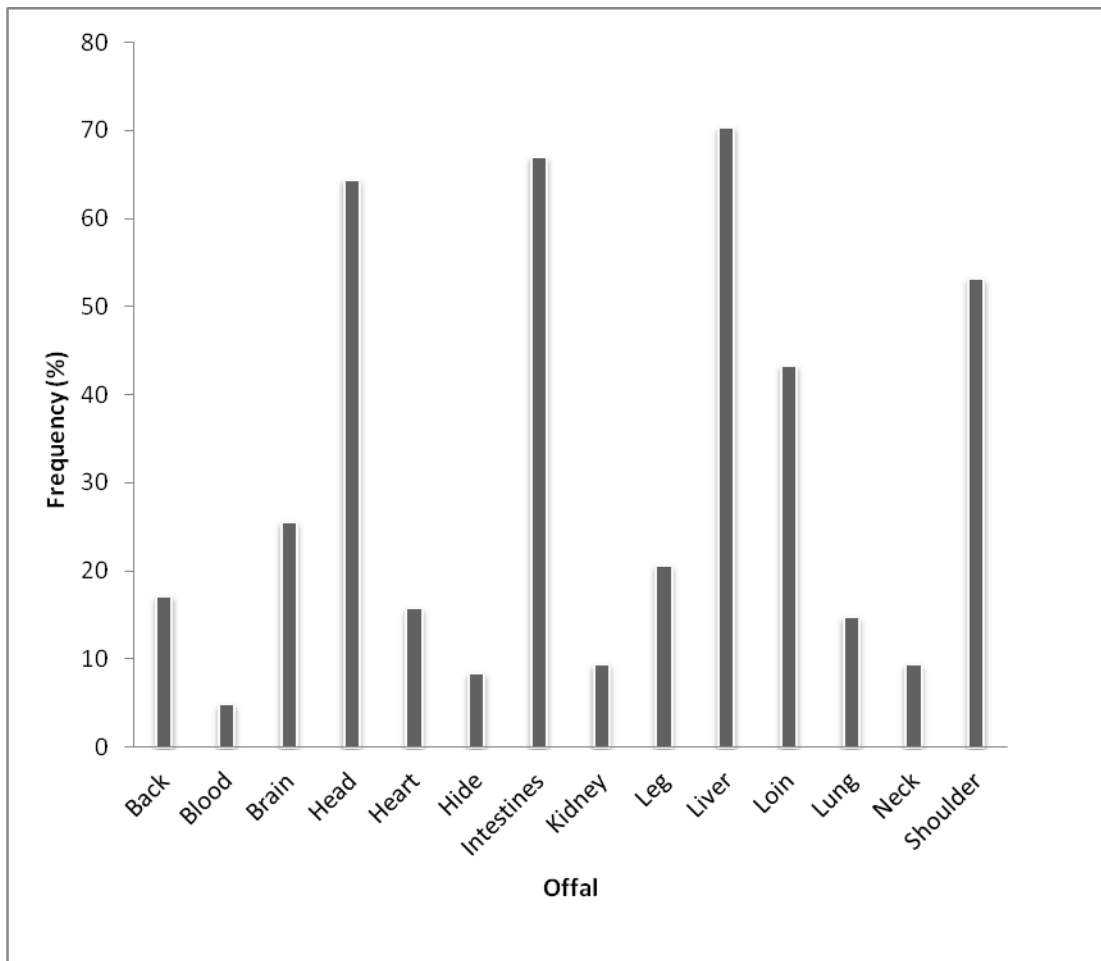
**Figure 4.4 Farmers' preference for different anatomical parts of sheep considered as Meat**

**Table 4. 2 Relationship between farmers' municipality and their preference for different anatomical parts from sheep**

| Anatomical parts | $X^2$-value | [1]Significance |
|---|---|---|
| Back | 60.556 | ** |
| Blood | 21.997 | ns |
| Brain | 34.546 | * |
| Head | 10.440 | ns |
| Heart | 21.321 | ns |
| Intestine | 16.677 | ns |
| Kidney | 11.333 | ns |
| Leg | 31.048 | ns |
| Liver | 36.379 | ns |
| Loin | 60.556 | ** |
| Lung | 13.883 | ns |
| Neck | 18.122 | ns |
| Shoulder | 24.047 | ns |

[1]Significant at *$p < 0.05$; **$p < 0.01$
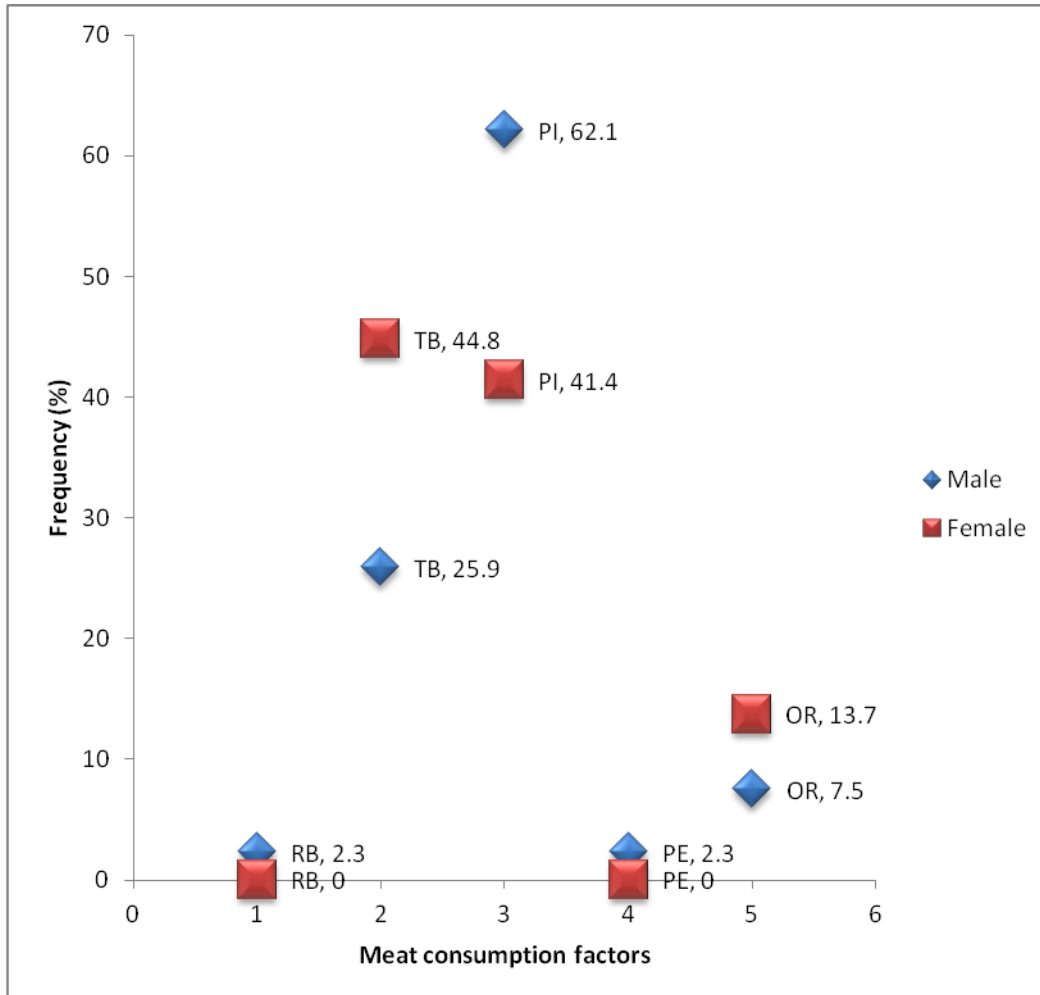
**Figure 4.5 Factors influencing consumption of lamb and mutton by sheep farmers**

Key = RB: Religious Belief; TB: Traditional Beliefs; PI: Personal Interest;

PE: Professional Ethics and OR: Other Reasons

**4.4 Discussion**

The survey-based approach used in the current study provided the evidence that majority of sheep farmers from the ECP are both producers and consumers of ovine meats from the veld grown sheep. The expression of positive attitude to sheep meats from the velds revealed that the farmers had trust in the origin of the meat being 'organic in nature.' Consistent with this impression, it was reported that raising sheep on natural pasture where no supplementary feeding, antibiotics or beta-antagonist was added produces meat with superior eating quality (Ẵdnǿy et al., 2005; Lind et al., 2009). Recent studies have also found that consumers manifest higher preference for such because they were perceived to be natural, authentic, safe and tasty (Sainsbury et al., 2011; Hersleth et al., 2012).

Inequality in farmers' choice of 'mutton only', 'lamb only' or 'mutton and lamb' suggested that inclination for each meat type was influenced by certain consumer motivating factors (Remco et al., 2009). In literature, these motivating factors were previously related to consumers' personality, status, sensory appeal, peer pressure, knowledge or learned predispositions to each meat type (Pollard et al., 2002, Lokuruka, 2006; Lever and Miele, 2012). In accordance with the theory of multiple preferences (Dovey et al., 2008; Aiking, 2011), low preference for lamb meat found among the farmers showed that their interests were not fixed on a specific kind of meat from sheep. This generally implied that heterogeneity of factors (including age, gender, ethics, personality traits et c.) influence consumers' disposition towards food and meat products (Kubberød et al., 2002; Martins and Pliner, 2005; Bruns-Weller, 2010; EFSA, 2011).

Based on personal interest, meat consumers may reject lamb meat or detest it because of its characteristic odour and flavour because of (Cramer, 1983; Erasmus et al., 2001).  It suffices therefore to allude that the choice of food partly depends on personal preference which in itself is determined by consumers' cognitive, affective and normative mechanisms (Rousset et al., 2005). Some other results from the current study have shown that male farmers had more interest in meat consumption than the females. This findings was consistent with the previous studies where higher preference for meat was considered a symbol of maleness or masculinity (Ruby and Heine, 2011; Rozin et al., 2012). These authors found that meat-eating was a masculine activity being perceived to bring sufficient satisfaction to men. As a form of dietary identity, Vartanian et al. (2007) reported that food (meat) is habitually chosen based on the likelihood that it would improve one's social standing, hegemonic masculinity or promote  gender roles.

This study found that female sheep farmers showed lower interest in sheep meats. Mooney and Lonz (1997) attributed this ambivalence from meat or preference for less of this kind of food (meat) than males as means to appear feminine. The authors further reported that female disposition to meat may sometimes be due to motivations for weight control by the females (de Silva and Rachman, 1987). In addaition, the perturbations that usually occur during menstrual period were also found to resort to disgust and nausea in menstruating females (Fessler, 2001). Consequently, imposition of dietary restraints, food neophobia tendencies (Elzerman et al., 2011; Korzen et al., 2011) or personal apathy for meat set in (Kaplan et al., 2000).  Few authors have therefore linked this apathy to gender-intuitive disgust sensitivity (Inbar et al., 2009; Olabi et al., 2009), emotivist perspectives (Fessler et al., 2003) and feminine psychographics or tendency to ascribe leanness to beauty (Asp, 1999).

A range of factors including cultural sentiment (Hoffman et al., 2005), personal interest (Erasmus et al., 2001) and religious belief (Vijoen and Gericke, 2001; Kruger et al., 2003), found to influence preference of sheep meat by consumers are also applicable to the sheep farmers.

**4.5 Conclusion**

This study has established the fact that sheep farmers are both producers and consumers of meat from ovine species that are raised on the natural velds. Although they exhibited a high preference for mutton, the liver was also found to be the most preferred anatomical part by the ECP sheep farmers. Low interest in 'lamb only' and neophilic tendency towards blood and hide were found among the farmers. Evidence has also been provided from the study that male farmer showed more interest in various meat types from sheep than the females and that those within 51-60years indicated greatest preference for sheep meat. However, most of the male farmers consume sheep meat mainly because of personal interest and the female farmers for traditional belief. In all the Eastern Cape municipalities, sheep farmers demonstrated similar preference for various anatomical parts except for the back, brain and loin muscle from sheep. Since the consumption of meat is usually preceeded by animal slaughter, it is necessary to determine if the method used during animal slaughter and stunning phases is appropriate to spare the slaughtered animal of avoidable pain or not.

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# CHAPTER 5: Quantitation of ovine Ubiquitin C-terminal hydroxylase (UCH L1) as a pain biomarker in electrically stunned sheep

**Abstract**

The objective of the current study was to quantitate the expressions of ovine Ubiquitin C-terminal hydroxylase (UCH L1) as a biomarker coding for pain in electrically stunned sheep. Blood samples were collected after exsanguination from Dohne Merino castrates (n = 30) and ewes (n = 30) and, were afterwards prepared for biochemical assays. Real-time PCR was performed using Maxima SYBR Green/ROX qPCR Master reaction mix Green 1 and a LightCycler® carousel-based system. Results revealed that the application of 110volts across the head did not induce immediate insensibility in 50% of the sheep. Higher expressions of ovine UCH L1mRNA caused by electric insults were indications that castrates sustained more traumatic brain injury than ewes during stunning. In addition, the age and sex of the sheep significantly ($p < 0.001$) influenced post-stunning expression of pain signals from castrates and ewes. This study has shown that brain-specific neuronal gene coding for pain in electrically stunned Dohne Merino were located within 9-42 (5'-...-3') and 231-208 (3'-...-5') exon-exon boundaries. In conclusion, it is evident that the "head-only" stunning method is not a zero pain-free approach and the quantitation of UCH L1mRNA is potentially viable as a biomarker for detecting pain in head-stunned sheep.

**Key words:** Brain injury, electrical stunning, pain biomarker, real time RT-PCR

## 5.1 Introduction

Meat occupies a special place in the human diet and this reality goes with the slaughter of livestock. Hitherto, in the history of meat consumption (Chapter 4), very little attention is given to quantitate the pain and suffering the animal may feel during the slaughter process (Zivotofsky and Strous, 2012). It is also not clear if the quality of pre-slaughter welfare enjoyed by these animals exonerates them from avoidable pain or not. Pleiter (2005) has shown that one of the humane ways to lessen animal's pain and suffering during slaughter is stunning. As a statutory requirement, all meat species are expected be stunned in order to ensure that they are unconscious during exsanguination (Gregory, 2007; Grandin, 2010). Compliance with this regulation is advantageous in that it supposedly minimises the pain experienced by the animal in a bid to meet consumers' expectation of getting meat of their choice (Prinz et al., 2010).

Apparently, several stunning methods have been introduced to the meat industry to achieve this goal. In bovine, avian, porcine or ovine abattoir, the use of captive bolt, gas, controlled atmosphere, water bath and electrical stunners are common (McKeegan et al., 2007; Hindle et al., 2010). Currently among these methods, electrical stunning is the most widely used for meat harvesting (Barbut, 2010), and particularly for sheep (Vergara and Gallego, 2000). The purpose of using the electrical stunning method is not the same everywhere but particularly in the African context, it depends on the aim for slaughtering the animal perhaps for traditional or for non-traditional use (Ndou et al., 2011). However, in the European Union, electrical stunning is used to induce unconsciousness during cutting and bleeding. In the United States, stunning is applied to immobilize the animal to make automatic cutting very easy (Lambooij et al., 2008).

The most parsimonious reason to justify the efficacy of electrical stunning is that it induces a grand mal epileptic-type seizure (Zivotofsky and Strous, 2012). Where this is done, animals are stunned by applying the stunner to the: "whole body", "head-and-chest", "head-to-brisket", "head-and-leg", "head-and-cloaca" or "head-only" respectively (Gregory, 2007; Weaver and Wotton, 2008; Prinz et al., 2010). As guided by religious institution, some Halal authorities accept "head-only" electrical stunning because it does not kill the animal instantly but causes a temporary loss of consciousness (Grandlin, 2007). Attendant pain that follow when stunning is not effective manifests in form of extended bleating, groaning, flight tendencies, corneal reflexes, hyperemia and rhythmic breathing indicating an active brain stem of conscious animals after-stunning (Grandlin, 2010; Zivotofsky and Strous, 2012).

The relationship between the electrical insults on the brain regions and affective-motivational components apparently pave way for neuronal excitability (Schreckenberger et al., 2005). This neuronal response however causes voltage spikes across the brain cells and abrupt perturbation from a quiescent state (Izhikevich, 2000). This scenario eventually triggers different nerve cell receptors causing neuronal signals or nervous disorder expressed as traumatic brain injury or pain in the spinal cord (Besson, 1997; Mondello et al., 2011). The unifying consequence is that the initial injury from this complex phenomenon causes cellular damage and disintegration leading to the release of cell type-specific proteins such as S-100B, glial fibrillary astrocytes, neuro-specific enolase and ubiquitin C-terminal hydroxylase (UCH L1) (Svelthana et al., 2012). Although in man, the elevated expression of a high brain specific neuronal protein (UCH L1) causes focal cerebral ischemia and spinal cord injury (Castegna et al., 2004; Papa et al., 2010) yet, all stunning methods potentially traumatize the animal too (Kilgour, 1978).

Thus, the use of pain biomarker such as UCH L1 becomes applicable for the quantitation of neuronal damage in form of sub-arachnoid haemmmorrhage and brain injury, (George et al., 2011; Yajun et al., 2012). To date, no empirical evidence is available where this neuronal-specific protein gene product (UCH-L1) was used as a biomarker to quantitate the traumatic pain experienced by sheep when subjected to a stunning procedure. In the current study, Dohne Merino sheep was used as a test animal being one of the leading composite breeds in South Africa. The hypothesis tested was that blood samples from Dohne Merino sheep would be a viable option to quantitate brain pain received during "head-only" stunning and that UCH L1mRNA would be an effective biomarker for detecting pain signals in the animal. The objective of the study therefore, was to quantitate the expressions of ovine UCH L1 mRNA as a biomarker coding for pain in electrically stunned Dohne Merino sheep.

## 5.2 Materials and Methods

### 5.2.1 Ethical clearance, data collection and sample preparation

The present study was performed with the consent of the Research Ethics Committee of the University of Fort Hare, South Africa (UFH/UREC, 7-REC-270710-028). Data was generated at a high-throughput Halal abattoir where "head-only" electrical stunning was used. The average ages of the stunned Dohne Merino were 11 and 36 months for castrates and ewes respectively. Prior to bleeding, the sheep arranged on a single line, were held in a restraining conveyor, and were eventually stunned in the head by applying 15.125kilo-Watts's power (or 110volts and 0.8 ohms). During exsanguination, blood samples (5 to 10 ml) were collected from the jugular vein of each ewe (n=30) and castrate (n=30) into heparinised vacutainer tubes to prevent coagulation. The samples were kept in a cooler box and the aliquots were stored frozen at-20°C until they were used for UCH-L1 assay.

### 5.2.2 Total RNA Extraction

Total mRNA was extracted rapidly from the previously collected Dohne Merino blood samples using the Zymo Whole-Blood RNA MiniPrep™ kit (Zymo Research). This kit was chosen for having the ability to extract high quality RNA ($A260/A280$ >1.8, $A260/A230$ >1.8) suitable for all downstream RNA-based manipulations. A buffer system combined with *Fast-Spin* column technology (according to manufacturer's instructions) was used for the extraction. A total of 600-µl blood RNA Buffer™ was added to 200-µl whole-blood sample following red blood cell lysis. The sample was mixed and was transferred to a Zymo-Spin IIIC™ Column in a Collection Tube and was centrifuged at ≥ 12, 000-x *g* for 2 minutes.

Thereafter, 400 µl of RNA Pre-Wash Buffer was added to the column and centrifuged at ≥ 12, 000-x *g* for 30 seconds. A total of 400-µl RNA wash buffer was added to the column and was centrifuged at ≥ 12,000-x *g* for 30 seconds. Then 100 µl RNA recovery buffer was then added to the Zymo-Spin™ IIIC column and the column was centrifuged at ≥ 12,000 x *g* for 30 seconds. Following this was the addition of 100-µl ethanol (95-100%) to the flow-through in the RNase-free tube from the above and mixed well by pipetting. The mixture was thereafter transferred to a Zymo-Spin™ IC column in a collection tube and centrifuged at ≥ 12,000 x *g* for 30 seconds. Before the flow through was discarded, 400 µl RNA prep buffer was added to the column and centrifuged at ≥ 12,000 x *g* for 1minute. Again, 800-µl RNA wash buffer was added to the column and centrifuged at ≥ 12,000-x *g* for 30 seconds and the flow-through was discarded.

The wash step was then repeated with 400 μl RNA wash buffer and the Zymo-Spin™ IC column was centrifuged in an emptied collection tube at ≥ 12,000 x *g* for 2 minutes. Carefully, the Zymo-Spin™ IC column was removed from the collection tube and transferred into an RNase-free tube. The DNase/RNase-free water ( ≥ 6 μl)  was added directly to the column matrix and centrifuged at 10, 000-x *g* for 30 seconds in order to finally elute the RNA.

**5.2.3 Reverse transcription for ovine Ubiquitin C-terminal hydroxylase, (UCH L1)**

Maxima SYBR Green/ROX qPCR Master Mix was optimized for its ability to produce sensitive and specific quantification of genomic, plasmid and cDNA templates (Table 5.1). Contained in the Master Mixes were buffer, dNTPs, thermostable hot-start DNA polymerase, SYBR® Green dye, KCl and $(NH4)_2SO4$ which were used to provide high specificity of primer annealing. The ROX Passive reference dye was included in the Master Mix to serve as an internal reference for normalisation of the SYBR Green 1 fluorescent signal. This is because the dye allows for correction of well-to-well variation that might occur due to pipetting inaccuracies and fluorescence fluctuations. In qPCR, DNA accumulates and fluorescent signal increases proportionally to the DNA concentration. The excitation and emission maxima of SYBR Green I were at 494 nm and 521 nm, and found being compatible with the use of any real-time cycler. The Master Mix was used with the real-time thermal cyclers [LightCycler® 480 SYBR Green I Master (LightCycler® 480 instrument) and LightCycler® FastStart DNA MasterPLUS SYBR Green I (LightCycler® carousel instrument)] that were chemically modified by the addition of heat-labile blocking groups to amino acid residues (Table 5.2).

**Table 5.1: Reverse transcription (RT-PCR) for Ovine Ubiquitin C-terminal hydroxylase (UCH L1 mRNA)**

| Reagent | Amount (µL) | Mastermix (x5) |
|---|---|---|
| 5x Reaction mix | 4 | 20 |
| Maxima Enzyme Mix | 2 | 10 |
| Distilled water | 9 | 45 |
| | **Amount of Mastermix** | |
| Template RNA | 5 | 5 |
| Total volume | 20 | 20 |

| Reagent | Concentration | Amount (µL) | Mastermix (x5) |
|---|---|---|---|
| Maxima SYBR Green/ROX qPCR Master Mix | - | 12.5 | 62.5 |
| Forward Primer | 0.3µM | 0.75 | 3.75 |
| Reverse Primer | 0.3µM | 0.75 | 3.75 |
| Distilled water | - | 6.0 | 30 |
| Master Mix amount | - | 20 | 20 |
| Template cDNA | < 500ng | **5** | **5** |
| Total volume | **-** | **25** | **25** |

**Table 5.2: Thermal cycling parameters for quantitative determination of Ovine Ubiquitin C-terminal hydroxylase (UCH L1 mRNA) in two steps RT-PCR**

| Steps/Stages | Time (seconds) | Temperature ($^{o}$C) | Cycle number | Cycling condition |
|---|---|---|---|---|
| Holding stage | 600 | 95 | 1 | - |
| Cycling (1$^{st}$) | 15 | 95 | 40 | - |
| Cycling (2$^{st}$) | 60 | 60 | | Pre-denaturation |
| Melt curve (1$^{st}$) | 15 | 95 | | Denaturation |
| Melt curve (2$^{nd}$) | 15 | 60 | | Annealing |
| Melt curve (3$^{rd}$) | 15 | 95 | | Elongation |

**5.2.4 Primers Design for ovine Ubiquitin C-terminal hydroxylase (UCH L1)**

The design process for ovine UCH L1 primers did not follow a conventional route (Reference Patent Number: PA156691/P). This is because the sheep genome is not fully sequenced and consequently, no reference sequence data is available for the requested genes. Instead, partial mRNA sequences from the GenBank accession (*Ovis aries* UCH L1-S27a protein mRNA, partial cds., ACCESSION AY566307, VERSION: AY566307.1 ) numbers were used to BLAST against the sheep EST database to extract long expressed sequence tags (EST) to represent nearly the full-length mRNA sequences. This approach was chosen to cater for exon-exon boundries within the EST sequence since the EST data was not fully curetted. This was necessary to prevent erroneous amplification that might result from contaminating genomic DNA in the cDNA samples. For this reason, the primers were designed to generate PCR products of approximately 200-350 base pairs (bp) long in order to maximize the potential of the primers to bind in different exons. Primers for quantitative PCR were used on the cDNA samples but were first tested to know if the primers bind to and amplify sheep genomic DNA. Caution was taken such that all the RNA samples were DNaseI to get rid of any residual genomic DNA and to purify the DNA-free RNA before it was synthesized into cDNA.

**5.2.5 Statistical analysis**

Chi square tests were computed to establish the relationship between slaughter age and sex, and UCH L1 mRNA expressions after stunning. Using the PROC FREQ procedure of SAS (version 1.9.3, 2007), the significance was tested at $p < 0.05$.

## 5.3 Results

Result has shown that within the same nucleotide region of 24bp, the sequence for the forward primer (5'-TCCGGGTCTCATCTGTCTCCTCCT-3') and the reverse primer (3'-CGTCCATCTTCCAGTTGCTTGCCA-5') were found to be 9-42 and 231-208 respectively (Figure 5.1). The GC-content of 58.33% for the forward primer and 54.17% for the reverse primer were within the normal GC range of 45-60% portraying high annealing strength (Table 5. 3). The result of using SYBR Green Master mix in the temperature-dependent dissociation between DNA-strands gave a typical primer-dimer formation (Figure 5.2). The derivative Tm was higher (86.94°C) and that of the primer-dimer had characteristically lower Tm of 65°C. It is clear from the foregoing that at a higher inflection point (Tm), 50% of the primer was annealed. This result therefore reflected the stability of interaction between the primer-target gene and indicating a rise in absorbance intensity (hyperchromicity) that produced the desired single stranded UCH L1 mRNA amplicon.

In Figure 5. 3, the negative melting curve illustrating the absence of UCH L1 mRNA in about 50% of the sheep (before and after stunning) is presented. At a high Tm (76.1°C), the negative UCH L1 mRNA expression generated a form of dissociation that was characteristic of a reaction with a baseline fluorescence signal. This baseline region showed that the animal did not experience any traumatic pain after stunning and that the post-stunning effect caused instant insensibility (Figure 5.4). In contrast, the melting curve indicating positive UCH L1 mRNA expression (after stunning) was attained at 82.13°C Tm (Figure 5.5).

```
1   TCTCGCCCTT CCTGTGTCTC CGGGTCTCAT  CTGTCTCCTC CTTTTCCTCA CCCTCAGGTG

61  GAACCGCCGC CAGCATGCAG ATTTTCGTGA AGACCCTGAC GGGGAAGACC ATCACTCTTG

121 AGGTCGAGCC CTCGGATACA ATAGAAAATG TGAAGGCCAA GATCCAGGAT AAGGAAGGAA

181 TTCCTCCTGA CCAGCAAAGA CTGATCTTTG CTGGCAAGCA ACTGGAAGAT GGACGTACTT

241 TGTCTGACTA CAACATTCAA AAGGAGTCCA CTCTTCATCT AGTGTTGAGA CTTCGTGGTG

301 GCGCTAAGAA AAGGAAGAAG AAGTCTTACA CCACTCCCAA GAAGAACAAG CATAAGAGAA

361 AGAAGGTTAA ATTGGCTGTT CTGAAATACT ATAAGGTGGA TGAGAATGGC AAAATCAGTC

421 GCCTTCGCCG GGAGTGTCCC TCAGATGAAT GTGGTGCTGG AGTTTTTATG GCCAGTCACT

481 TTGACAGACA TTATTGTGGC AAATGTTGTC TGACCTATTG TTTCAACAAA CCAGAAGACA

541 AGTAATTGTA CATTGGTTAA TAAACATATG AGCTAACATT TAAAAAAAAA AAAAAAAAAA

601 AAAAAAAAA GCTCGCTCAG CCAGCTTGCC CTGCTTTCTG AGACATATGA CCTCTGGCCC

661 CAGCCGCTAG ACCTCTCCCG ACCTCACCTC TGACTTCAGC AGCCAAGTGT GAATGCAGAG

721 AGCAAAGCCC CAAGGAGGAA GCTCGGGCCT GAGCATAGCA GAGGGCTCCT TGCTGGGTTA

781 GGATGGAGCT CCCCAAGTTT TCCCAGCAGA AGGGATGACC TTTCATTCTG TTTTC
```

**Figure 5. 1 Primer location (>*Ovis aries* UCH L1-S27a EST: > TC65096 TC34746) for**

**Ovine Ubiquitin C-terminal hydroxylase (UCH L1)**

**Key**: Red and Bright green colours above denote forward and backward primers.

**Table 5. 1 Primer parametres used for Ovine Ubiquitin C-terminal hydroxylase (UCH**

**L1) quantification**

| Primer characteristics | Template strand | Length (bp) | Start | Stop | [a]Tm | [b]GC (%) |
|---|---|---|---|---|---|---|
| Forward primer | Plus | 24 | 19 | 42 | 59.69 | 58.33 |
| Reverse primer | Minus | 24 | 231 | 208 | 59.71 | 54.17 |
| Product length | 217 | | | | | |

| | |
|---|---|
| Two primers complementarily | Max complementarity in continuous: 3 bp, free energy= 1.50 Kcal/mol |
| | 5'-TCCGGGTCTCATCTGTCTCCTCCT-3' |
| | ||| |
| | 3'-ACCGTTCGTTGACCTTCTACCTGC-5' |
| Two primers complementarily | Max complementarity in discontinuous: 8 bp |
| | 5'-TCCGGGTCTCATCTGTCTCCTCCT-3' |
| | || || ||| | |
| | 3'-ACCGTTCGTTGACCTTCTACCTGC-5' |

[a]Tm: Melting temperature; [b]GC: Guanine-Cytosine

The fluorescence signal produced was clearly typical of a curve consisting of the baseline region, exponential growth and linear phases. The positive amplification for this result occurred at $11.25\Delta R$ after successive thermal cycling (Figure 5. 6). The presence of the single curve at the peak of amplification showed the absence of contaminating products such as contaminating DNA or primer-dimer that could have appeared as additional peaks different from the desired amplicon. The expression of UCH L1 mRNA therefore indicated that the sheep experienced traumatic pain in the brain during stunning and that they were still conscious before exsanguination.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| mRNA Quantity | 0.0002 | 0.0002 | 7.00E-05 | 7.00E-05 | 1.40E-05 | 1.40E-05 | 2.90E-06 | 2.90E-06 |
| Cт Mean | 29.51 | 29.51 | 31.21 | 31.21 | 34.87 | 34.87 | 34.87 | 36.78 |
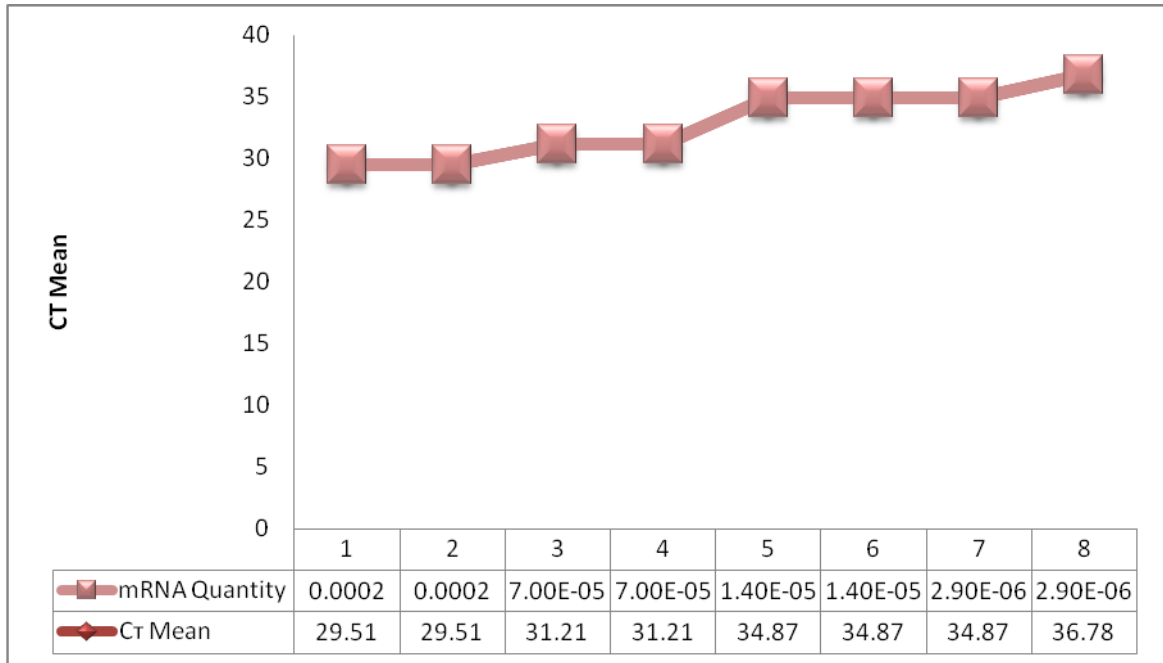
**Figure 5. 2 Standard Reverse Transcription-Polymerase Chain Reactions (RT-PCR) curve for Ovine Ubiquitin C-terminal hydroxylase (UCH L1 mRNA)**
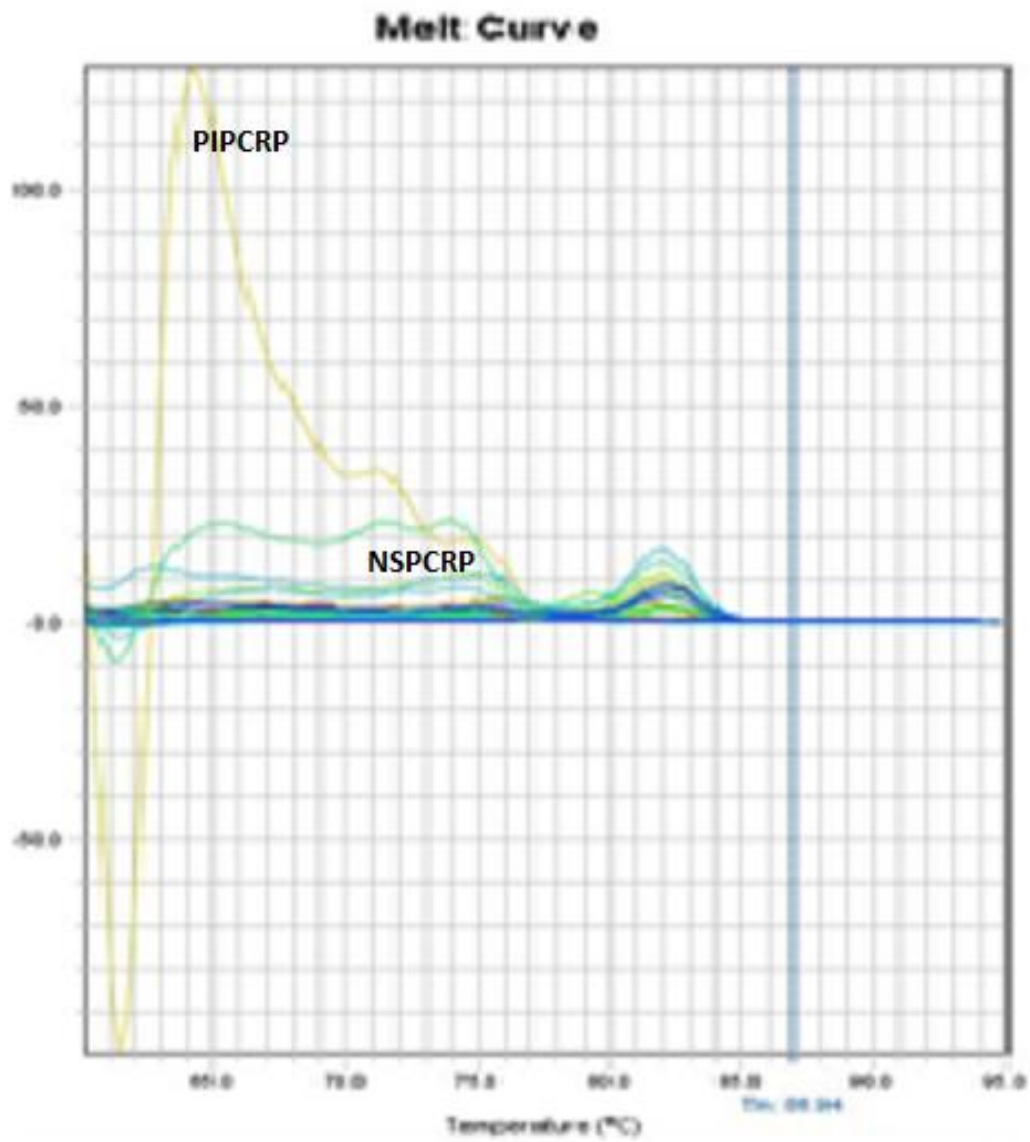
**Figure 5. 3 Derivative melting curve for standard samples for ovine UCH L1 expression**

(NSPCRP: Non-Specific PCR Products & PIPCRP: Peak of interest)
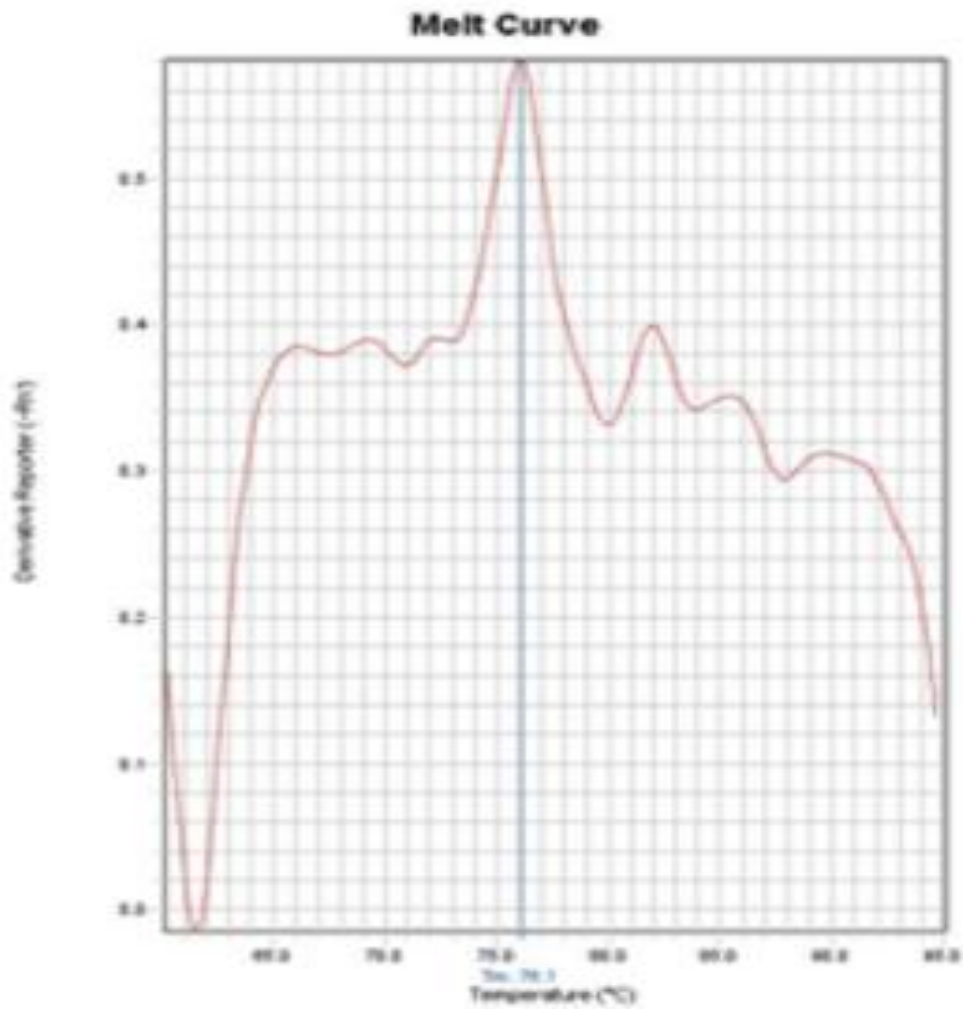
**Figure 5. 4 Negative reaction showing absence of UCH L1 mRNA expression by Dohne Merino sheep before and after stunning**
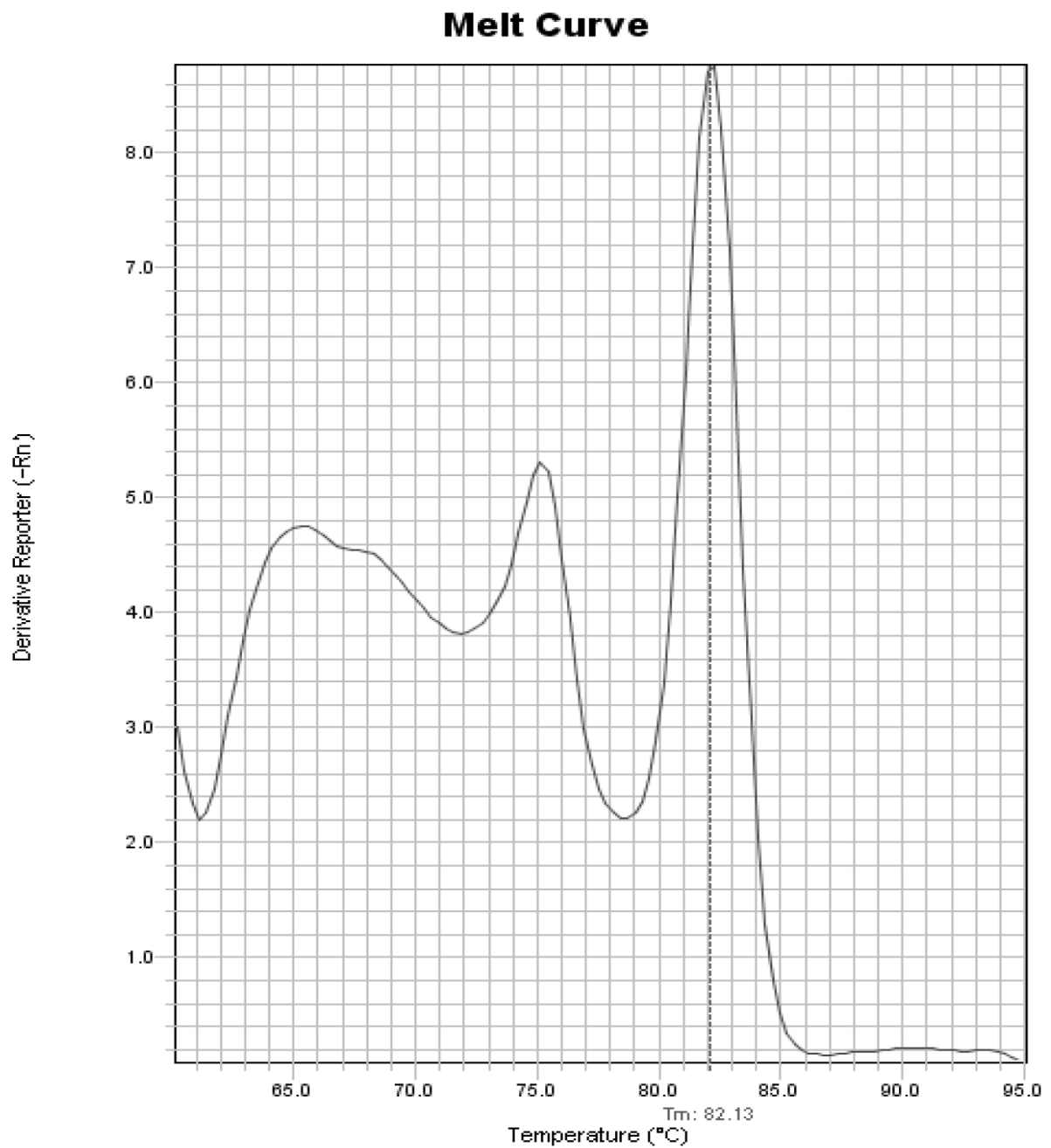
**Figure 5. 5 Melt curve for positive post-stunning expression of UCH L1 mRNA by Dohne Merino sheep**
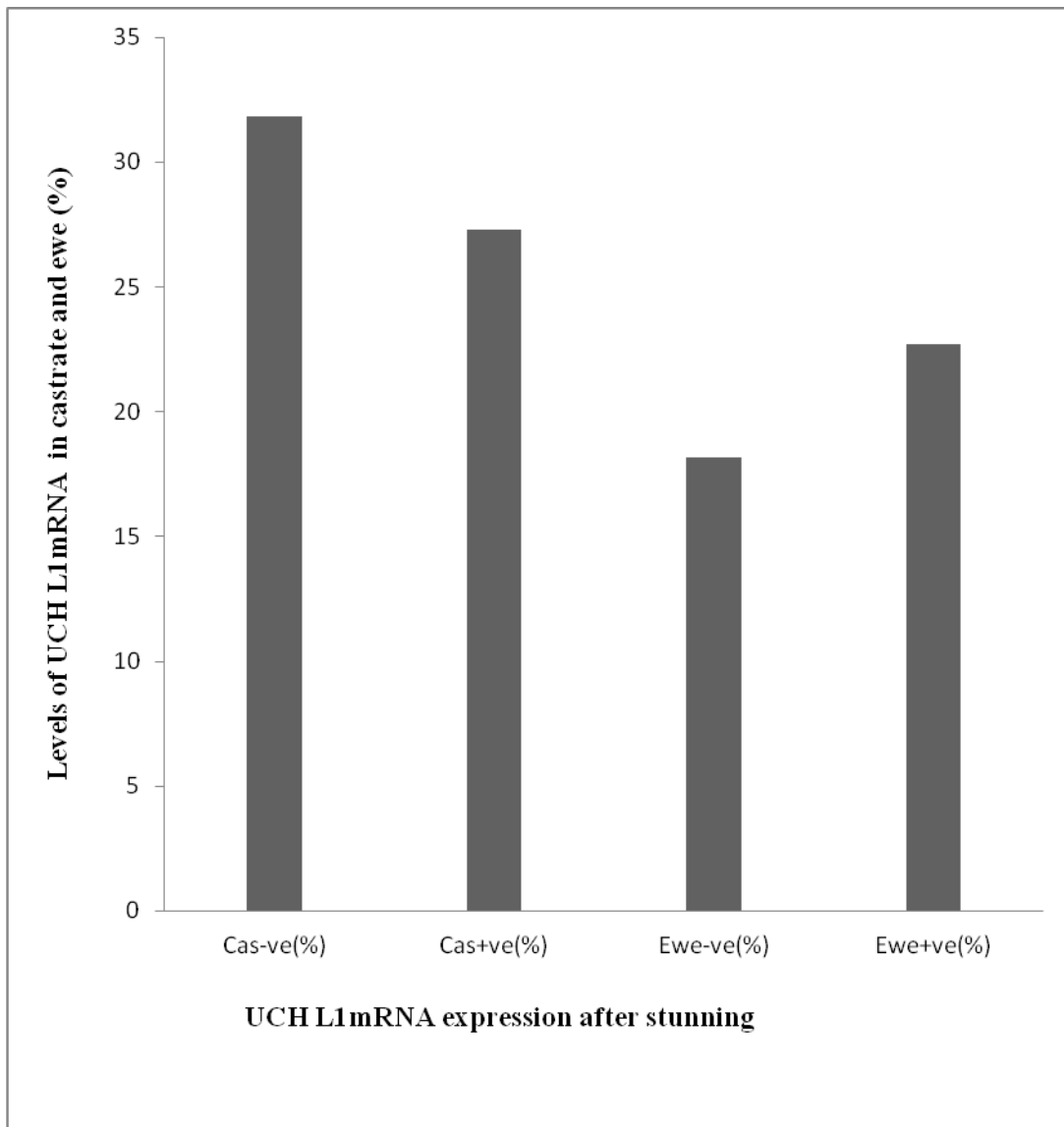
**Figure 5. 6 Expressions of UCH-L1 by Dohne Merino sheep castrate and ewe after**

      **electrical stunning**

*(Cas-ve(%) and Ewe-ve (%) were castrates and ewes that recorded no UCH L1 after stunning but Cas+ve (%) and Ewe+ve(%) were castrates and ewes that expressed CH L1 after stunning)

**Table 5. 2 Effects of age and sex on the expressions of UCH-L1 mRNA in electrically stunned Dohne Merino sheep**

| Parametres | [1]Sig. |
|---|---|
| Sex | NS |
| Age | NS |
| Sex*Age | *** |

[1]Significant at $p < 0.001$; NS: Not significant at $p > 0.05$.

**Table 5. 3 Physical activities observed for presence or absence of UCH L1 mRNA**

**expressions in Dhone Merino ewes and castrates**

| Body parts | Sheep with negative UCH L1 expressions | Sheep with positive UCH L1 expressions |
|---|---|---|
| Head | Loose and floppy spasm from 3 to 6 seconds. | Some twisting of the head and neck regions |
| Legs | Uncoordinated paddling movements; extended and flexed hindlegs. | No movement from the fore and hind legs |
| Neck | Neck flexing from 3 to 6 seconds. | Absence of neck flexing |
| Eyes | Nystamus (vibrating eye), blinking. | Single feeble eye (corneal) reflex |
| Tongue | Hanging out, straight and limp. | Stiff curled, going in and out |
| Mouth | No groaning before exsanguination | Groaning observed before exsanguination. |

## 5.4 Discussion

The quantitation of UCH L1 mRNA in the Dohne Merino blood has revealed that pain experienced by castrates and ewes was influenced by their physiological differences. As earlier reported (Wilhelm and Pingoud, 2003), the amount of UCH L1 mRNA product detected was directly linked with the melt peak resolution and that the area under the curve of a melt peak. The generation of a single product (UCH L1mRNA) during a melt run therefore suggested an association between a time-temperature binding pattern of the SYBR green 1 and the growth of the peak (Aniko and Delano, 2006). As previously reported, it could be affirmed that the use of intercalating dyes such as SYBR Green 1 for melting curve analysis is a reliable technique that is ideal for amplicon detection and differentiation (Papp et al., 2003).

The findings in this study were consistent with the previous reports where the expressions of UCH L1 mRNA portrayed traumatic brain injury in man and vertebrates (Tisherman et al., 1990; Day and Thompson, 2010; George et al., 2011). Some authors have also associated the elevated serum UCH L1 mRNA with abnormal blood-brain barrier function (Blyth et al., 2011) while attributing neuronal vulnerability to traumatic brain injury caused by electrical or mechanical insults (Arnaoutakis et al., 2011; George et al., 2011). A profound sympathetic discharge resulting in artery hypertension, high pulmonary lymph flow and elevated catecholamines were found in dogs experiencing pains in the brain (Millen et al., 1985). It could be argued therefore, that the release of electrical impulses in response to affective-motivational mechanism at the tonic phase triggered some nociceptions in the animal (Schreckenberger et al., 2012).

Thus, the transmission of these emotional signals initiated sensory transduction by the receptor leading to "action potential or graded potential" that manifested as pain in the sheep and expressed as UCH L1 mRNA in the blood samples (Yajun et al., 2012). Without making clarity on sex or age of the animal, a recent study has reported some differences in severity of pain experienced when some Merino sheep were stunned using the captive bolt (Finnie et al., 2012). The varying adaptive response to painful stimuli otherwise known as hyperalgesia and heritability differences could play some roles in the obtained result. Since neuronal excitability is affected by dehydration, the degree of dehydration of the castrates and ewes prior to stunning might be responsible for the varying nociceptive manifestations (Sneddon, 2004; Fitzpatrick et al., 2006; Grandlin, 2010). Moreover, the present study has revealed a major weakness of "head-only" stunning with positive expressions of UCH L1 mRNA in about 50% of the Dohne Merino castrates and ewes. This revealed that the passage of current through the head did not produce a stunned state in the sheep (Vogel et al., 2011), but rather exposed about half of the sheep to avoidable brain trauma.

Prior to exsanguination, physical activities that symbolize ineffective stunning were also found responsible for positive expressions of UCH L1 in the sheep. In sheep, groaning, curled and moving tongue, flight attempts and tendencies to return to normal rhythmic breathing were observed as a sign of consciousness before the sheep were re-stunned to induce insensibility (Maria et al., 2000; Velarde et al., 2002). Generally, all the behavioural responses accountable for ineffective stunning, poor head-stunner contact (McKeegan et al., 2006; Grandlin, 2010) were collectively found in sheep having positive UCH L1 mRNA expressions. The body resistance by the sheep was comparatively lower compared to the dissipated electrical power from the stunner.

**5.5 Conclusion**

This study has given evidence that UCH L1 mRNA is a reliable biomarker to quantitate pain from head-stunned sheep. The quantitation of UCH L1 mRNA has demonstrated that half of the stunned sheep experienced traumatic brain pain before exsanguination. Castrates were more susceptible to the pain than ewes. Although the current study (Chapter 5) has shown how male (castrate) and female (ewe) sheep respond to traumatic brain injury (TBI) during electrical stunning yet, no information was provided about the pregnancy status of these slaughtered ewes. The next experimental phase (Chapter 6) however used reliable pregnancy biomarkers to ascertain the pregnancy status of these ewes at the point of slaughter.

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# CHAPTER 6: Biomarkers coding for ovPAG-1 mRNA expression and pregnancy status in Dohne Merino ewes

*(Published by the Tropical Animal Health and Production, see Appendix 11.4)*

**Abstract**

The objective of the present study was to determine the pregnancy status of Dohne Merino ewes at slaughter. This was done by collecting blood samples from the ewes (n = 60) during exsanguination to assay for pregnancy biomarkers using radio-immunological and TaqMan Reverse Transcription-Polymerase Chain Reaction (RT-PCR) techniques. The expressions of ovine pregnancy-associated glycoprotein (ovPAG-1) within a range of $1.068E^{-09}$ to $8.977E^{-07}$ indicated 43.33% ewes with pregnancy ($\Delta$mRNA) signals at the point of slaughter. The assay validation further showed that half of these ewes were truly pregnant and most of the ewes that exhibited "true positives" were within 56-60kg live weight and had an average age of 30 months. The biomarkers used in this study have shown that 21.66% Dohne Merino ewes were truly pregnant at the point of slaughter.


**Key words:** Abattoir; Dohne Merino sheep; pregnancy; RNA extraction; Reverse-Transcription PCR

**6.1 Introduction**

Typically, the slaughter of animals of both sexes occurs everywhere and conventionally stunning is done first before exsanguination. This is generally achieved by asphyxiating the animals with gas, shooting with a captive bolt pistol or shocking them with electric current (Lawrie and Ledward, 2006). When electric stunning is used for sheep, traumatic pains accompany the practice and the animal experience pain when they are stunned before conversion to meat (Chapter 5). In South Africa, Dohne Merino is one of the commonest composite sheep breeds that are mostly presented for slaughter at the registered abattoirs. This breed was developed in 1930s as a cross breed between the local Merino and the German Mutton Merino at the Dohne Research Farm in the Eastern Cape Province (Kotzé, 1951; McMaster and Kotze vader van die, 1991; Van Wyk et al., 2008).

The Merinos have received a topmost rating among the registered breeds by the South African Studbook Association due to their immense contributions to the subsistence economy and to the meat-wool industries (Swanepoel, 2006; Cloete and Olivier, 2010). A decline in the number of sheep in South Africa (from approximately 30 to 22 million) was reported between the early 1980s and 2007 (Cloete and Olivier, 2010) due to the outbreak of rift fever valley disease, predation, theft, slaughter and other factors (Chapter 1). The conversion of these animals to meat products has been found to be one of the key reasons for the decline in sheep population nationwide and in the sub-Saharan of Africa too (Ngbede et al., 2012). This is because not only the conventional non-breeding, infertile, old or accidentally injured ewes are slaughtered for meat but even the productive pregnant ones (Abdulkadir et al., 2008; Muhammad et al., 2009; Addass et al., 2010).

The aftermath therefore, is the wastage of pregnancies with singleton, twins, triplets or multiple foetuses at different gestational stages (Mailafia and Ramalan, 2010; Bokko, 2011). The danger of slaughtering pregnant ewes lies in the possibility of converting the most productive animals to meat and of eroding useful genetic resources. The need for accurate diagnosis that could provide useful information whether in culling of non-pregnant ewes or ascertaining their pregnancy status becomes critical for the sustainability of this meat species. In an effort to identify the cause of pregnancy wastages, primary attention is mostly directed to infections while the non-infectious causes amounting to almost 70% are perhaps ignored (Christianson, 1992; Grazul-Bilska et al., 2010). Conventionally, the simplest method of observing return to oestrus may often prove unreliable since records on dates of natural service are sometimes unobserved or unrecorded by the farmers (Gordon, 2005).

With the advent of sensitive radioimmunoassay (RIA) technique, non-pregnant livestock can be routinely screened with almost 100% accuracy through progesterone assay (Calamari, 2001; Gordon, 2005). Being pregnancy-stage dependent, the estimation of progesterone (P4) from the serum through RIA has generated diagnostic information with high precision (Liu et al., 2004). In terms of cumulative duration of its effects, this ovarian steroid hormone (P4) is central in preparing the endometrium for implantation of fertilized ovum and thus very important in pregnancy establishment and maintenance (Skinner et al., 2000, Clemente et al., 2009). Hence, during early pregnancy stage, P4 stimulates proliferation and expansion of epithelial cells and alveolar morphogenesis (Buser et al. (2011). In mid-to-late pregnancy, the impact of its inhibitory effects prevents the reflux of any accumulated milk into the interstitial space (Marton et al., 2009).

However, the expression of the genes for pregnancy-associated glycoprotein (ovPAG-1) during implantation time and persistence as pregnancy advances provides a link between P4 and ovPAG-1 as highly sensitive pregnancy biomarkers (Garbayo et al., 2000; Jerome, 2012).

These proteins are detectable in the maternal blood around the time of attachment of the foetal placenta when the trophoblast binucleate cells start to migrate and fuse with the endometrial cells forming the fetomaternal syncytium (Gordon, 2005). Based on its rapidity, high throughput and ease of standardisation, amplification of DNA by real-time PCR has become one of the best methods in diagnostic laboratories (Valdazo-Gonza´ lez, 2007).

In this study, the use of two diagnostic tools was necessary because the ovPAG-1mRNA only produced pregnancy signals (or $\Delta$mRNA signals coding for pregnancy) which reflects the status of the animal at follicular phase but bridges the limitations of return to oestrus reported by Gordon (2005). Hence, the use of RIA served the purpose of validating the actual pregnancy status of the ews at slaughter by comparing their ovP4 concentration with the existing standards (or threshold values). The resolution to collect blood samples for diagnostics became the only alternative since logistics at the abattoir did not permit the examination of the reproductive tracts for the status of *corpus lutea,* oviduct, uterus and ovaries of the slaughtered ewes. Therefore, the rationale for this study was to assay the blood samples to determine the pregnancy status of Dohne Merino ewes at slaughter.

## 6.2. Materials and Methods

### 6. 2.1 Experimental procedures and data collection

All experimental procedures were reviewed and approved by the Research Ethics Committee of the University ofFort Hare, South Africa (UFH/UREC, 7 - REC-270710-028). The data for this study was generated over a period of one year at a high-throughput Halal abattoir. The live weights of 60 clinically healthy Dohne Merino ewes were determined at the lairage using an electronic weighing scale. The mean age of the ewes (21 months or four-tooth; 30 months or six-tooth and $\geq$ 48 months or full mouth) were estimated through a general chronological guide using cementum (or dentition) method (AGFACTS, 2003). Whole blood was from each ewe (5 to 10 ml) were collected from the jugular vein into heparinised vacutainer tubes during exsanguination at the abattoir. Samples were immediately placed in a cooler box until centrifugation. Centrifugation was done three hours post-slaughter because of the distance between the abattoir and the laboratory. The Eppendorf centrifuge 5403® (Clements, Sydney, NSW, Australia) machine was used for harvesting the serum at 3000 rpm for 15 minutes. The harvested serum was stored frozen (at-20°C) until used for ovP4 assay.

### 6.2.2 Assay optimization and Radioimmunoassay (RIA) for ovine progesterone (ovP4)

A heterologous double-antibody from the bovine PAG 67 kDa subunit and the rabbit antiserum raised against a mixture of caprine 55 and 59 kDa PAG subunits were used as the first antibody. This step was taken as the ovPAG-1 has not been purified to homogeneity and because PAGs from different ruminants are identical in their sequences and immunoreactivity (Xie et al., 1991; 1998; Garbayo et al., 2008). Since radioactive iodine ^{125}I has the potential to increase the sensitivity of the assay by conjugating the iodinated compound to the steroid molecule, it was adopted for radio-iodination of the antigen (Lindberg et al., 1974).

Hence, the reason for using it in this study to compete with $^{125}$I labelled progesterone tracer, as binding sites on tubes coated with polypropylene rabbit progesterone antibodies (stable at 2-8$^o$C).

At room temperature (25$^o$C), samples and reagents were mixed thoroughly by gentle inversion before use and then assayed in duplicates. One-hour incubation at 37$^o$C in a water bath was adopted to give higher binding without compromising the accuracy and quality control. The quality of the tracer was monitored for radiochemical purity using 12x 75mm polypropylene tubes T (total counts) and non-specific binding (NSB). Radioactivity associated with the tubes was quantified using a Packard Auto-Gamma 5780$^{®}$ (United Technologies, Packard, Australia) counter which had been programmed to calculate the standard and the tested samples due to its compatibility with the standard (12 x 75 mm tubes, Vortex mixer) for counting $^{125}$I progesterone bounded to the polyclonal rabbit progesterone antibodies in the test tube.

### 6.2.3 Assay validation of the ovP4 using Radioimmunoassay (RIA)

Calculations on sensitivity and specificity were done to determine the validity of the diagnostic tests on the sera as follows:

1. **True positive**: the ewe was pregnant at slaughter and the test was positive.

2. **False positive**: the ewe was not pregnant at slaughter but the test was positive.

3. **True negative**: the ewe was not pregnant at slaughter and the test was negative.

4. **False negative**: the ewe was pregnant at slaughter but the test was negative.

5. **Sensitivity:** The sensitivity of the method was determined as the minimum detectable concentration at two standard deviations from the zero binding value. This was mathematically expressed with the probability that pregnant ewes were screened positive using the equation: [true positives/(true positives + false negatives)].

6. **Specificity:** The specificity is the probability that non-pregnant ewes were screened negative using the equation: [true negative / (true negative + false positive).

### 6.2.4 Total RNA Extraction

Total mRNA was rapidly extracted from the previously collected Dohne Merino blood samples (n = 60) using the Zymo Whole-Blood RNA MiniPrep™ kit. This kit was chosen for having the ability to extract high quality RNA ($A260/A280$ >1.8, $A260/A230$ >1.8) suitable for all downstream RNA-based manipulations. A buffer system combined with *Fast-Spin* column technology (according to manufacturer's instructions), was used for the extraction. A total of 600-µl blood RNA Buffer™ was added to 200-µl whole-blood sample following red blood cell lysis. The sample was mixed and was transferred to a Zymo-Spin IIIC™ Column in a Collection Tube and was centrifuged at ≥ 12,000-x *g* for 2 minutes.

Thereafter, 400 µl of RNA Pre-wash buffer was added to the column and centrifuged at ≥ 12, 000-x $g$ for 30 seconds. A total of 400-µl RNA wash buffer was added to the column and was centrifuged at ≥ 12,000-x $g$ for 30 seconds. Then 100 µl RNA recovery buffer was then added to the Zymo-Spin™ IIIC column and the column was centrifuged at ≥ 12,000 x $g$ for 30 seconds. Following this, was the addition of 100-µl ethanol (95-100%) to the flow-through in the RNase-free tube from the above and mixed well by pipetting.

The mixture was thereafter transferred to a Zymo-Spin™ IC column in a collection tube and centrifuged at ≥ 12,000 x $g$ for 30 seconds. Before the flow through was discarded, 400 µl RNA prep buffer was added to the column and centrifuged at ≥ 12,000 x $g$ for 1minute. Again, 800-µl RNA wash buffer was added to the column and centrifuged at ≥ 12,000-x $g$ for 30 seconds and the flow-through was discarded. The wash step was then repeated with 400 µl RNA wash buffer and the Zymo-Spin™ IC column was centrifuged in an emptied collection tube at ≥ 12,000 x $g$ for 2 minutes. Carefully, the Zymo-Spin™ IC column was removed from the collection tube and transferred into an RNase-free tube. The RNA, ≥ 6 µl of DNase/RNase-free water was added directly to the column matrix and centrifuged at 10,000-x $g$ for 30 seconds to finally elute the solution.

**6.2.5 Primer and Probe Design**

Probes and primers were designed using the primer express software version 1.0 following manufacturer's instructions (Applied Biosystems, Foster City, CA). The amplicons for the ovPAG-1 were generated as indicated in the GenBank graphics (Figure 6. 1) with the pregnancy primers amplified within 332-358 (for forward) and 359-419 (for reverse) at 24 bp regions. The sequence of primer and probe for PAG-1 was chosen from the regions of nucleotide sequence that provided the most variability from the other members of bPAG and bPRP families (GenBank:M73961.1). The oligo analyserPrimer and probe were designed with Oligo Analyzer, from Integrated DNA technologies as shown in Table 6.1. The GC-content of 40-60% was used within the primer length of 18-30bp at 52-58$^o$C melting temperature (Table 6. 2).

```
   1 cttggagcca ggaaagaagc atgaagtggc ttgtgctcct tgggctggtg gccttctcag
  61 aatgcatagt gaaaatacct ctaaggagag tgaagaccat gagaaacacc tcagtggaa
 121 aaaagatgct gaacagtttc ctgaaggaac atgcttacag actgtctcag tttcttttc
 181 gtgcctcaaa tctgactatt caccctctga gaaacattat ggatatgctc acgtgggta
 241 acatcaccat tggaacaccc cctcaggaat tccaggttgt ctttgacaca gctcatctg
 301 acttgttggt gccctccatc aattgcctca gcccaaccaa gagaccctgt gtaaacaag
 361 ataagttcaa acatcaccag tcttccacct tccggtttac caatgacacc tcagaatct
 421 actttggttc tggaacaatg agaggatttg ttgctcatga cacagttcgg ttggggacc
 481 ttgtaagtac tgaccagccg tttggtctaa tcttttttgga atcctggctt atatcccatt
 541 ttgatggcat cttgggcttg aactatccca aaatatcctt ctctggagcc tccccatct
 601 ttgacaagct gaagaatgaa ggtgcctttt ctgagcctgt ttttgccttc acttgaaca
 661 aagacaagca ggagggcagt gtggtgatgt ttggtggggt agaccaccgc actacaagg
 721 gagagctcaa ctgggtacca ttgatccacc cgggcgagtg gagtataccc tggaccgca
 781 tctccatgag aagaaaggtt attgcttgtt ctggtggctg tgaggccctt tgggcaccg
 841 ggacatcact gatccttggc ccaagaacag tggttgaaaa catacagaag acatcggtg
 901 ccacacaaca gtgtttcgag tactttgttt catgttctgc ggtctatgcc tgccctcta
 961 ttgtcttcac catcaacggc atcaactacc cagtgccacc tcaagcctac tcgtcaagg
1021 attctagagg ccagtgctat tccccctttc aagtgaacag agcgaatcca ctgcagaga
1081 actggatcct gggtgacgtc ttcctgaggc ggtatttctc agtctttgat gaggaaatg
1141 acaggattgg cctggcacgg gcagtgtaaa tgctgggagt ggttcaggaa cagtaaggc
1201 ctatcctaac acacactcac tcacccttttg ggcactcctg cccatgatgc ggtgaactg
1261 tatttg
```

**Figure 6. 1 Complete coding sequence for ovine pregnancy-associated glycoprotein-1 mRNA (GenBank: M73961.1)**

**Key**: Red text indicates the **forward primer**, yellow text indicates **the probe** and blue text indicates **the reverse primer**.

**Table 6. 1** Primer parametres used for quantification of whole blood samples from Dohne Merino ewes

| Parameter | Particulars |
|---|---|
| Primer length | 18 – 30 bp |
| Melting temperature ($T_m$) | 52°C – 58°C |
| | The Tm difference between forward and reverse primers must not exceed 5°C |
| GC content | 40 – 60% |
| 3' –end sequence | G's or C's at the 3' –end of the primers sequence promotes specificity of the primer. More than 3 G's or C's should be avoided |
| Hairpin | Hairpin formation at the 3' –end higher than $\Delta G$ -2 kcal/mol should be avoided |
| | Internal hairpin formation higher than $\Delta G$ -3 kcal/mol should be avoided |
| Self dimer | Self-dimer formation at the 3' –end higher than $\Delta G$ -5 kcal/mol should be avoided |
| | Internal self dimer formation higher than $\Delta G$ -6 kcal/mol should be avoided |
| Cross dimer | Cross dimer formation at the 3' –end higher than $\Delta G$ -5 kcal/mol should be avoided |
| | Internal cross dimer formation higher than $\Delta G$ -6 kcal/mol should be avoided |

$\Delta G$ = Gibbs free

**Table 6.2 Primers and probes for Real-Time (RT-PCR), including data on the sequence, length, melting temperature, molecular weight and Guanine-Cytosine (GC) content**

| Name | Accession Number | Sequence | | Position |
|---|---|---|---|---|
| OvPAG-1 | M73962 | Forward: CAACCAAGAGACCCTGTAGTAAAC-3´ | 5´- | 332-358 |
| | | Reverse:5´- AAGTAGATTCTGAAGGTGTCATTG-3´ | | 359-419 |
| Probe[a] | | FAM- AAACATCACCAGTCTTCCACCTTCCGGTTT- MGBNFQ | | |

Forward primer (OligoAnalyzer 3.1)

| Length (bp) | Melting temperature ($^{o}$C) | Guanine-Cytosine (GC) content (%) | Molecular weight (g/mole) |
|---|---|---|---|
| 24 | 55.1 | 45.8 | 7323.8 |

Reserve primer (OligoAnalyzer 3.1)

| Length (bp) | Melting temperature ($^{o}$C) | Guanine-Cytosine content (%) | Molecular weight (g/mole) |
|---|---|---|---|
| 24 | 52.7 | 37.5 | 7446.9 |

TaqMan Probe (OligoAnalyzer 3.1)

| Length (bp) | Melting temperature ($^{o}$C) | Guanine-Cytosine content (%) | Molecular weight (g/mole) |
|---|---|---|---|
| 30 | 63.2 | 46.7 | 9036.9 |

[a] = FAM refers to the reporter and MGBNFQ refers to the quencher; bp=base pairs

### 6.2.6 Real-Time (RT-PCR) Analysis

Taqman quantitative real-time reverse-transcriptase polymerised chain reaction (RT-PCR) was used to measure the gene expression levels. Fifty nanograms of total RNA was reverse-transcribed into complementary DNA (cDNA) and used for this real-time RT-PCR analysis. As previously described (Bustin, 2000; Patel et al., 2003), a quantitative detection method, TaqMan real-time RT-PCR was used for analysing mRNA levels in the blood samples. Real-Time RT-PCR detection was done using ABI PRISM 7700 sequence detector and the software version 1.7 (Applied Biosystems). The reaction mixtures contained 1μM forward, reverse primers, 0.25 μM TaqMan probe, 0.2 mM deoxynucleotide triphosphates, 5.5 mM Magnesium chloride, and TaqMan buffer, and were collectively dispensed into a 96-well plate. The thermal cycling proceeded with 40 cycles of $95^{\circ}C$ for 15 seconds and $60^{\circ}C$ for 1 minute. The standard curve was generated by serial dilution of plasmid containing ovPAG-1 mRNA to quantify concentrations of samples.

### 6.2.7 Statistical Analysis

Data for ovP4 were analysed by the General Linear Model (GLM) procedures of SAS (version 9.1.3 of 2007) considering the relationship between slaughter weight and progesterone concentration. Significance differences were compared at 5%.

## 6.3. Results

Details of ovine progesterone (ovP4) concentrations in different reference groups of Dohne Merino sheep are summarised in Table 6. 3. Four reference groups were indentified based on the threshold of $\geq$ 3.18nmol/L used in this study. The reference group having 7.01 to 21.48nmol/L range of ovP4 did show that 21.66% of Dohne Merino ewes were truly pregnant at slaughter. The correlation coefficient ($R^2$) of 0.991 showed that the amplification efficiency for the standard (TaqMan RT-PCR) kit used was as high as 82.5% (Figure 6. 2). The slope of the standard curve therefore demonstrated good optimisation and reproducibility from run to run. The direction of the standard curve showed that the pregnancy signal of the sheep at the later stage of gestation, in the third trimester was recorded from 0.01 and those at the earliest pregnancy phase or at the follicular phase is detected after several mRNA cyclying (e.g $9E^{-07}$). No mRNA amplification (Table 6. 3) was observed for the non-pregnant ewes as indicated with zero fluorescence ($\Delta$mRNA). The expression of ovPAG-1 mRNA within a range of $1.068E^{-09}$ to $8.977E^{-07}$ revealed that 43.33% of the ewes had pregnancy signals at the point of slaughter. The threshold values ($C_T$) between the tested samples and the standard, were similar and thus indicating the pregnancy status of the ewes.
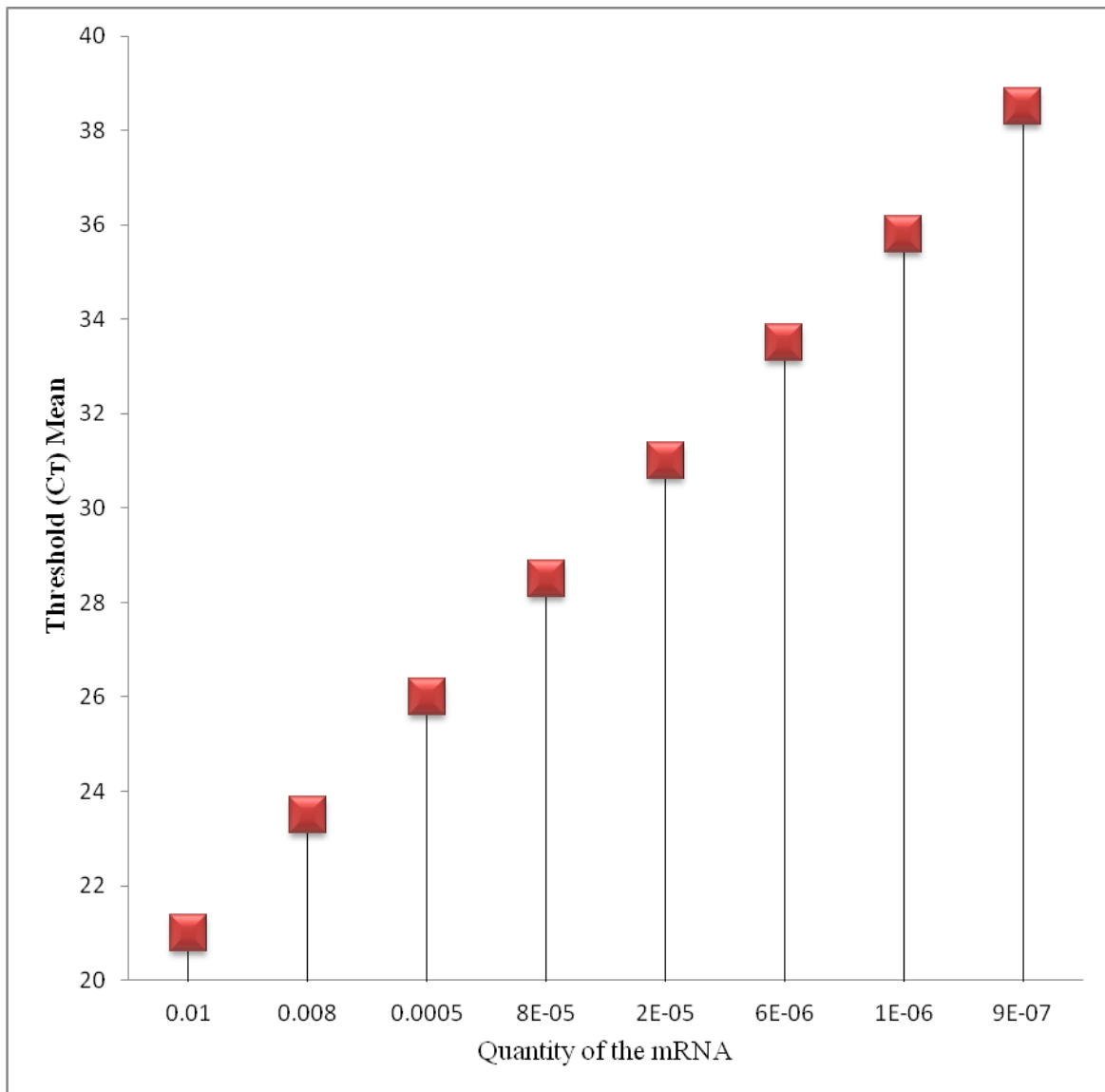
**Figure 6. 2 Standard TaqMan Reverse Transcription-Polymerase Chain Reactions (RT-PCR) curve for ovine pregnancy-associated glycoprotein (ovPAG-1mRNA)**

**Table 6. 3 Assay validation of ovine pregnancy-associated glycoprotein (ovPAG-1mRNA) from the TaqMan Reverse Transcription-Polymerase Chain Reaction (RT-PCR) technique**

| Reference group | Positive (pregnant) | Negative (not pregnant) |
|---|---|---|
| Range of threshold value ($C_T$) | 33.7495-39.7792 | Nil |
| Quantity of mRNA | $1.068E^{-09}$ to $8.977E^{-07}$ | Nil |
| Ewe with fluorescence Signals (%) | 43.33 | 0.00 |
| Ewes with no fluorescence Signals (%) | 0.00 | 56.67 |

The result in Figure 6.3 showed that most of the Dohne Merino ewes (51.7%) having ≥ 3.18nmol/L ovP4 threshold values were slaughtered at a mean age of 30 months. This implies that six-tooth ewes with elevated ovP4 levels formed the majority class among the sheep presented for slaughter at the abattoir. This result suggested that age plays significant role in the concentration of ovP4 in ewes and a crucial factor considered before sheep are presented for slaughter. In addition, the result in Figure 6.4 showed the relationship between the live weight of the ewes at slaughter and the concentration of ovP4 in them. From the result, ewes with live weights within 56-60kg recorded a highest ovP4 concentration that corresponds to 8.6nmol/L. Both results (in Figure 6.3 and 6.4) however revealed that six-tooth ewes within 56-60kg had the highest ovP4 values at the point of slaughter.
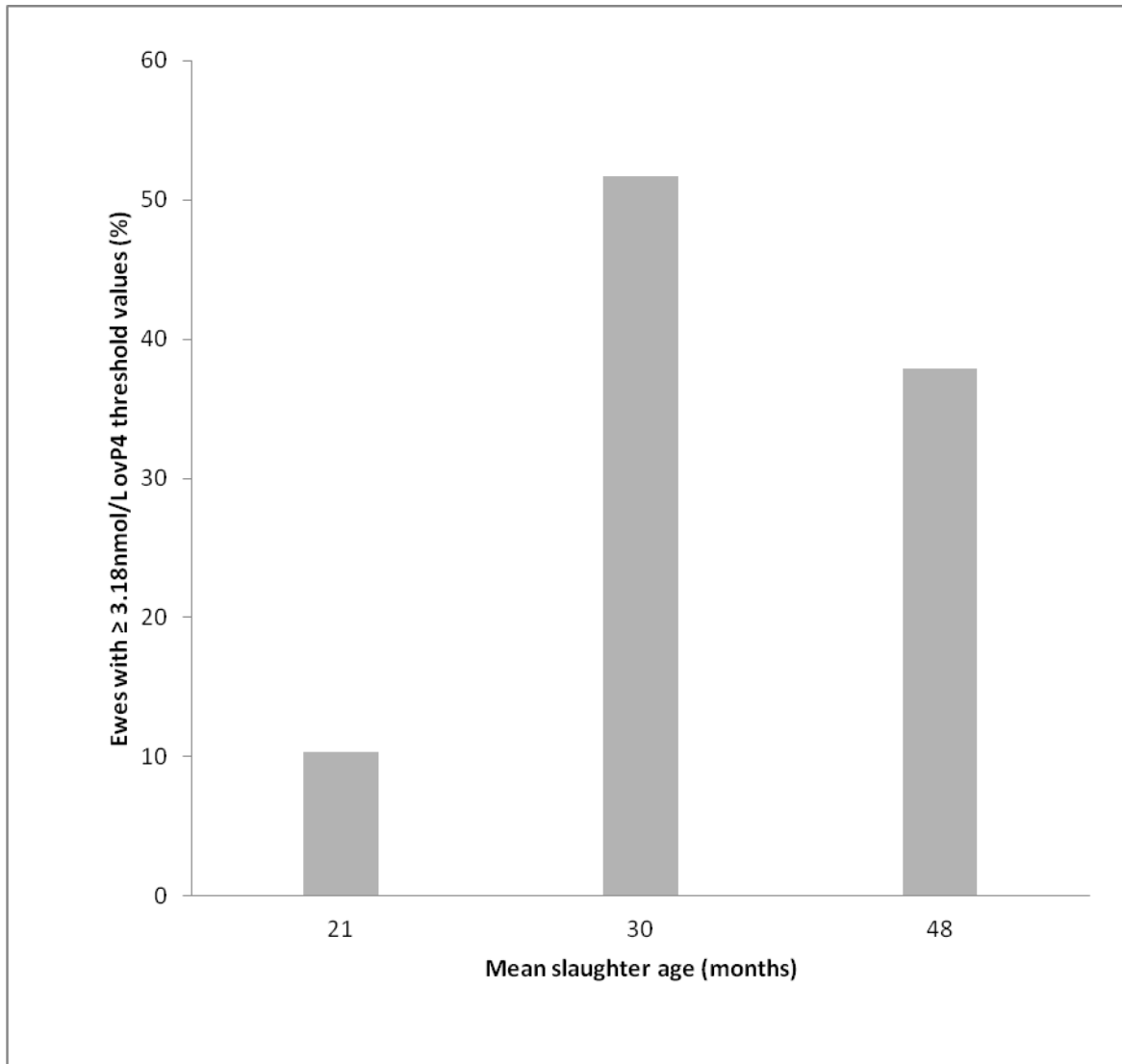
**Figure 6. 3 Relationship between mean slaughter age and proportion of Dohne Merino ewes with the threshold of ≥ 3.18nmol/L ovine   progesterone (ovP4) concentrations**
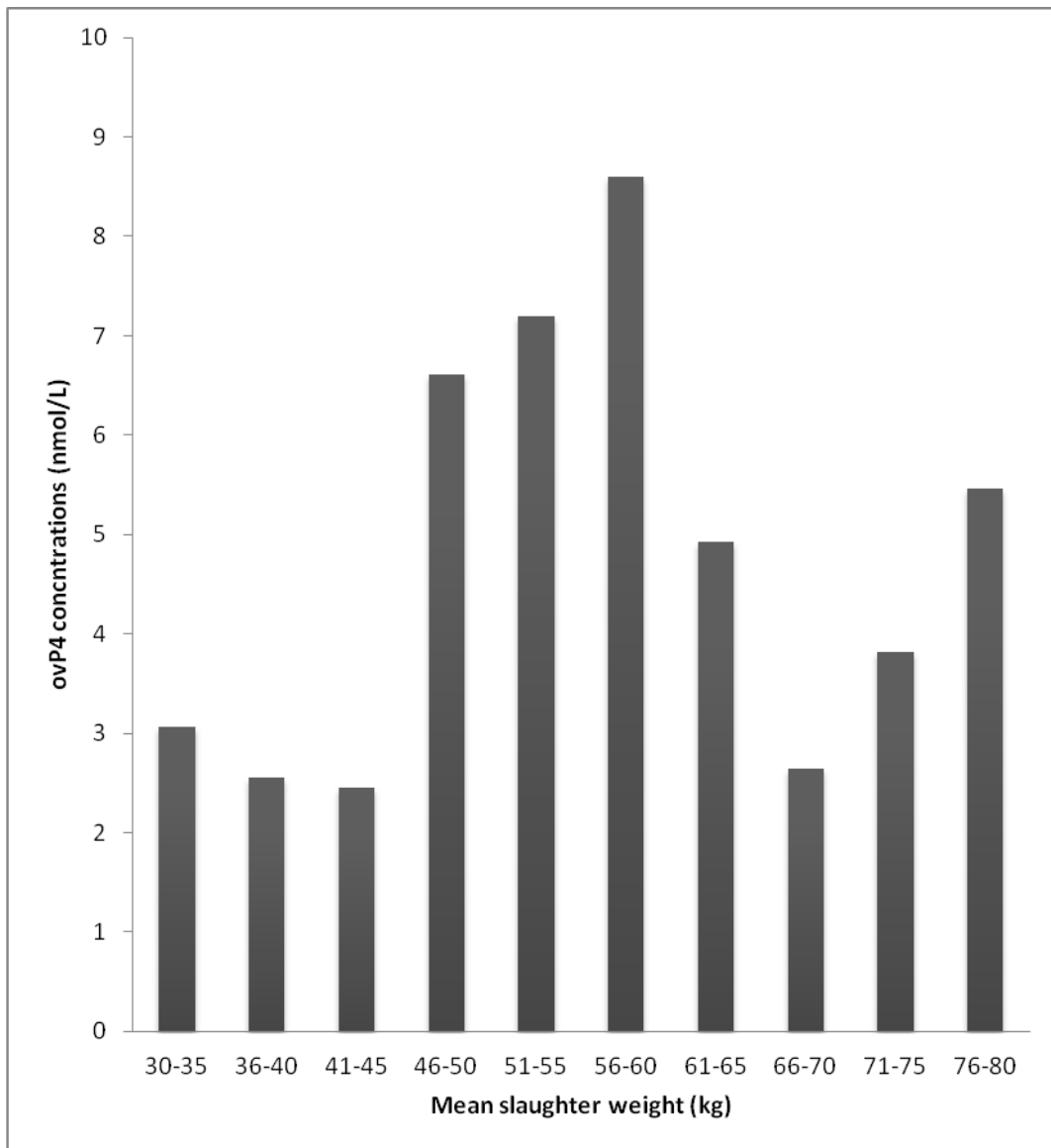
**Figure 6. 4 Relationship between mean slaughter weight and ovine progesterone (ovP4) concentrations in Dohne Merino ewes**

**Table 6. 4 Assay validation of the ovine progesterone (ovP4) from Radioimmunoassay**

| Reference group | True Positive | False positive | True negative | False negative | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|
| Proportion of ewe (%) | 21.66 | 35.00 | 43.34 | 0.00 | 100 | 55.30 |
| Range of ovP4 values (nmol/L) | 7.01-21.48 | 1.01-6.00 | 0.00-0.99 | *NA | *NA | `*NA |

*NA: Not Available and threshold value for ovine pregnancy is $\geq$ 3.18 nmol/L

**6.4 Discussion**

High sensitivity and specificity recorded in this study were typical of Coat-A-Count RIA and confirmed previous claims that RIA gives highly predictive and accurate values (Willard et al., 1995; Karen et al., 2003). It therefore suggests that the RIA used in the present study was most sensitive for giving a high level of accuracy similar to the result obtained when a monoclonal antibody was used to assay bovine PAG-1 (Whitlock and Maxwell, 2008). In comparison with the works of Gordon (2005) and (Karen et al., 2003), the use of transrectal ultrasonography gave lower sensitivities of 82.6% and 62% respectively. The absence of false negative cases still describes the uniqueness of this study in that the diagnostic tests did not show negative results for ewes that were actually pregnant. Although 35% of false positive cases were observed, the result only implied that the affected ewes were possibly in their follicular phase and were not actually pregnant. Cases of false positive found in this study were similar to the findings by Ganaie et al. (2009).

Going by these findings, sheep with ovP4 concentration $\leq 0.99$nmol/L and those within 1.01-6.00 nmol/L were not pregnant but might be in their follicular or early luteal phase at the point of slaughter. The slaughter of sheep at this physiological stage concurred with previous studies where ovine offsprings from conception to the end of organogenesis fall victim of wastages at the abattoir (Inskip and Dailey, 2005; Menchaca, 2002). In their studies, Gray et al. (2001) and Spencer et al. (2004) found variations in ovP4 levels in ewes at slaughter. The variations observed in their findings were comparable to 10.56-15.66nmol/L ovP4 levels reported on Awassi and Merino ewes between 0-18days of pregnancy (Karen et al., 2003) but lower than 35.30nmol/L ovP4 levels in Trakia Merino ewes carrying single foetus at 20th day of pregnancy (Yotov, 2007).

Generally, the combination of these findings showed that sheep at different stages of preganancies having varying levels of ovP4 are converted to meat at the abattoir. In response to estrogen secretion, the growth of endometrial glands during hyperplasia has been found to contribute to hormonal fluctuations in ewes at different stages of pregnancy (Gray et al., 2001). The extra-ovarian syntheses that follow due to hypertrophy were ascribed to the advancement in conceptus elongation and higher pregnancy rates in sheep (Spencer et al., 2004; Satterfield et al., 2006). It could be thought from this study that the weight gain observed due to hormonal changes at different stages of pregnancy and age of the ewes plays some roles in the decision to present the sheep for slaughter. In this regard, Santos-Silva et al. (2002) reported the benefit of getting sheep meat with low fat content when priority was placed on the age and live weight of Branco Merino at the point of slaughter. In another study, consumers' preference for heavy ewes that could produce mutton with dissectible fat and less bone-muscle ratio was observed by Cloete et al. (2004); Daniel et al. (2011) and Bulent et al. 2012).

In a work reported by Nephew et al. (1993), the expressions of fluorescence (ΔmRNA) signals were not the same in all the sheep. The reason for this could be ascribed to variation in their placenta gene at various stages of pregnancy. Furthermore, Joa˜o et al. (2008) found that the stimulating effect of interferon genes and oligoadenylate snythetase could also account for the dissimilarity in the mRNA expressions in the pregnant ewes. Although from the ovPAG 1mRNA tests, the number of ewes that manifested pregnancy signals doubled those that actually exhibited 'true positives' from the ovP4 diagnostics. In their study, Ruder et al. (1988) claimed that the impact of incomplete cross-reactions with antibodies in pregnancy specific proteins due to the inability of RIA to measure ovine antigen might be responsible for the result obtained in this study.

Several authors have shown that having high ovP4 in serum (> 3.18-6.36 nmol/L) from spontaneously ovulating species (sheep, cow, guinea pigs, dogs and horses) was an indication of cyclic ovarian activity (active *Corpus lutea)* or pregnancy (Nagy et al., 1998; Nagy et al., 2005; Forsyth, 2010; Nagy and Juhasz, 2012). In contrast, Hellgren et al. (1990); Silva et al. (2007) and Barbato (2009) indicated that ewes, cows or bears with ovP4 level lower than 3.18nmol/L threshold values were not pregnant or found in their anovular phase. In respect of this in a similar study, Evans and Walsh (2012) associated the low level of ovP4 concentration (< 3.18nmol/L) in ewes with implantation failure and increased intrauterine mortality. Silva et al. (2007); Marton et al., (2009) however presented a different result that ewes with exactly 3.18nmol/L (or 1.0ng/mL) or within 1.27 to 3.18nmol/L ovP4 levels might be cycling but were in their luteal phase.

Considering the proportion of the pregnant ewes from the sample size (n = 60), 51.7% obtained in this study was higher than 42.4% reported from the slaughtered pregnant Merino ewes in the Southern Australia flocks (Kleemann and Walker, 2005). The pattern of foetal wastage observed in this study was similar to 52% earlier reported at the Bamenda municipal abattoir, Cameroon where visual assessment or palpitation was used for determining the pregnancy status of slaughtered cows (Ndi et al., 1994).

**6.5 Conclusion**

The outcome of both diagnostics techniques [(TaqMan Reverse Transcription-Polymerase Chain Reaction (RT-PCR) & radio immuno assay (RIA)] used in this study has provided evidence that some pregnant Dohne Merino ewes were slaughtered at the abattoir. The affected ewes had their pregnancy terminated at 30 months old within 56-60kg live weights. Judging from the RIA diagnostic test, 21.66% of these ewes were truly pregnant at slaughter and this proportion was half of the pregnancy signals detected from the ovPAG1mRNA analysis. In every abattoir, the major goal of slaughtering either pregnant or non-pregnant sheep is to convert them to animal protein source such as the offal. The evaluation of their nutritive constituents and general eating quality is therefore worth evaluation.

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# CHAPTER 7: Characterisation of muscle fibre orientation, nutrient constituents and tenderness of offal from Dohne Merino sheep

*(Submitted to Animal Journal)*

**Abstract**

The current study characterised the muscle fibre orientation of offal from Dohne Merino castrates (n = 69) and ewes (n = 69) in relation to nutrient constituents and tenderness. Scanning electron microscopy, energy dispersive spectroscopy and the universal Instron apparatus were respectively used for characterising the fibre orientation, nutrient constituents and tenderness of the offal. The electron micrographs showed uniformity in fibre orientation (isotropy) for the lung, mouth muscle and fillet. Significant differences ($p < 0.05$) were observed in the fibre length of the mouth muscle and the fibre thickness of the lung from the castrates. Maximum levels of crude protein (CP) and digestible crude protein (DCP) were found in the fillet of Dohne Merino castrates. Compared to other offal, the tracheas from the ewes had the highest ($p < 0.05$) Warner-Bratzler Shear Force (WBSF) values. The energy dispersive spectroscopy revealed uneven levels of nutrients in all the offal. The type of offal, age and sex of the animal significantly influenced the muscle fibre orientation, the nutrient compositions and tenderness of Dohne Merino offal.

**Key words**: Anisotropy; isotropy; scanning electron micrographs; micronutrients; Warner-Bratzler Shear Force

## 7.1 Introduction

The term muscle refers to multiple bundles of fibres held together by connective tissues. The muscle tissue consists of highly specialized fibres and each muscle fibre is formed by fusion of several undifferentiated cells known as myoblasts into long, cylindrical or multi-nucleated cells (Scott et al., 2001). On the basis of structural and functional characteristics, the muscle tissue is classified into three types: cardiac, smooth and skeletal (Schoenfeld, 2010). Cardiac muscle tissue is striated and forms the bulk of the wall of the heart. Like the cardiac muscle, smooth muscle fibres are usually involuntary, non-striated and found within the walls of organs such as oesophagus, intestines, bronchi, blood vessels and the arrector pili in the skin. Unlike smooth muscle, skeletal muscle tissue is striated but attached to bones and aligned perpendicular to the long axes of the fibres (Saladin, 2010). Cardiac and skeletal muscles are striated because they contain sarcomeres and are packed into highly regular arrangements of bundles known as isotropy.

The smooth muscle fibres have neither and thus exhibit anisotropic orientation. The orientation of the fibres have been found to affect meat quality traits with respect to the cut made parallel or perpendicular to the muscle (Xia et al., 2007). As reported on beef round, careful characterization of muscle fibres helps in evaluating value-added strategies for the meat cuts (Von Seggern et al., 2005). Therefore understanding muscle fibre orientation, intramuscular texture and nutrient constituents is useful during meat fabrication as it makes identifying meat cuts and its carving easier (Senaratne et al., 2009; 2010). In their work, Mohan et al. (2009) noted that the dynamics of offal depend largely on several physical and chemical properties of muscle including gender of the livestock species, age and weight at slaughter, *ante-mortem* conditions and nutritional compositions.

As previously discussed, offal from the slaughtered sheep whether pregnant or not constitutes part of the balanced diets in different ethnic groups as variety meats or edible non-carcass by-products (Magoro, 2007; Sun et al., 2011). Thus, awareness on muscle foods has recently increased with emphasis on the need to assess the nutritional significance and the quality of offal from various meat species (D'arco et al., 2012; Hoffman et al., 2013). Commonly, offal is excised from the adipose, epithelial, connective and nervous tissues of red meat species like the cattle, goat and sheep (Nollet, 2011; Fayemi and Muchenje, 2012b). Guided by Meat Council Regulation (EC No 700/2007), offal has become widely acceptable for its value addition in militating against food crisis, combating malnutrition and for improving household protein intakes (SKAPS, 2010; Florek et al., 2012). Based on these uses, it is not viable discarding edible offal such as the lung, liver, heart, kidneys, oesophagus, trachea, rumen, reticulum, omasum, abomasum, spleen, brain, spleen, pancreas, trotters, tongue, tail, and intestines as waste (Fernandes et al., 2010; Toldrá et al., 2012).

Hence, one of the motivations for studies on the dietary shift towards the consumption of offal in recent times (Fayemi and Muchenje, 2012a; Hoffman et al., 2013). As a means of characterising fibres, the use of a multi-purpose device like the scanning electron microscope (SEM) is known for characterising animate and non-animate materials (Clarke, 2002). Most SEM's have user-friendly intuitive interfaces to generate high-resolution of sample surfaces or images of shapes of objects revealing details less than 1nm (Suzuki, 2002). In science, the energy dispersive spectroscopy (EDS) from SEM has been reliably used to examine microfabric and crystallographic orientation in many materials and to indentify compositional maps based on differences in trace elements (Goldstein, 2003; Egerton, 2005). Since, SEM analysis is considered to be non-destructive; the x-rays generated by electron interactions do not lead to loss of volume from the sample.

It rather makes it possible to analyse the same sample materials repeatedly (Suzuki, 2002; Anderhalt, 2007; Gaige, 2007). Its sensitivity in providing valid information about the surface morphology and elemental composition of living tissues formed the basis for using it in the current study. Despite the rise in the consumption of offal globally (Fernandes et al., 2010; Florek et al., 2012; Hoffman et al., 2013), information is unavailable on the connection between the muscular fibre orientation, nutrient constituents and tenderness of offal. As indicated previously, it was thus decided in the current investigation to use offal from Dohne Merino being one of the leading registered sheep in South Africa (Chapter 2). Therefore, the objective of this study was to characterise the muscle fibres of the offal from Dohne Merino sheep in relation to their nutrient constituents and tenderness.

## 7.2 Materials and Methods

### 7.2.1 Sample preparation

A total of 138 Dohne Merino sheep sourced from communal and commercial farms were used for this study. At slaughter, the average age of the castrates (n = 69) was 11 months and the ewes (n = 69) were 36 months old. Complete sets of offal comprising the liver, lung, rumen, omasum, abomasum, reticulum, small intestine, large intestine, kidney, spleen, trachea, mouth muscle, tongue, heart and fillet were collected from each sheep after exsanguination. Subsequently, these were processed using scanning electron microscopy (SEM). In order to be able to view the samples under scanning electron microscope, ≤ 5g offal from each one was excised and fixed (stabilised) in 10% formalin solution in a way that the ultrastructure of the animal tissues remained as close to the living material as possible.

The samples were re-fixed in 2.5% sodium cacodylate-buffered gluteraldehyde (pH 7.2) at $4^{o}C$ for 2 hours and post-fixed again with 1% sodium cacodylate-buffered uranyl acetate for 120 minutes. Afterwards, distilled water was used to rinse the sample as a transitional solvent to prevent shrinkage of the sample in the process of dehydration in ethanol.

**7.2.2 Sample dehydration, sputter coating and critical point drying**

Sequentially, each sample was then held for 20 minutes in ethanol in ascending grades of: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% to 100% respectively during the period of dehydration. In order to improve the electrical conductivity of the sample surface in the SEM, a thin film of gold palladium was used for sputter coating to enhance x-ray microanalysis. Critical Point Drying (CPD) followed thereafter using the Hitachi critical point dryer HCP-2 (Hitachi Koki Co Ltd, Tokyo Japan) to prevent the samples from distortion and to boost good structural preservation. This was done by mounting the samples on aluminium stubs having double-sided carbon tape followed by sputter coating with gold-palladium (Au-Pb) using the Eiko IB.3 Ion Coater (EIKO Engineering Co TD, Japan). For high-resolution images of offal with sufficient contrast, a conductive coating is indispensable in order to suppress surface charging, minimize radiation damage and increase electron emission from the surface. The samples were finally observed under the JEOL JSM-6390LV scanning electron microscope (SEM) for the determination of surface micrographs and energy dispersive spectroscopes (EDS) of the offal.

**7.2.3 Determination of crude protein (CP) and digestible crude protein (DCP)**

The elemental constituents from offal were auto-generated from the energy dispersive x-ray spectroscopy (EDS) of SEM. The respective crude protein (CP) and digestible crude protein percentage (DCP) values from each sample were obtained as derivatives from N-values using the equations below:

i. Crude protein (CP, %): [N% x 6.25]

ii. Digestible crude protein percentage (DCP, %): This calculated value was generated using 70 percentage of the crude protein value (CP, %) as reported by Schroeder (1994) and the Western Beef Resource Committee (2010), respectively.

**7.2.4 Determination of Warner-Bratzler Shear Force (WBSF) values of the offal**

Warner-Bratzler Shear Force (WBSF) values were determined as indicators of tenderness of the offal using the Universal Instron apparatus. Prior to shearing, sample were cooked at an average of 88$^{o}$C for 45 minutes using Sous vide apparatus. After cooking, the offal was cooled for an average of 10 minutes. Coring (of fillet and liver) was done parallel to the grain of the offal and sheared perpendicular to the fibre direction through the WBSF device at cross head speed of 400mm/minute for one shear in the centre of each core. The remaining offal that could not be cored (due to their orientation) was cut with a sharp scalpel into an approximate size of 10mm before shearing.

### 7.2.5 Statistical analysis

Data collected on WBSF, fibre length (micrometer, μm); fibre thickness (μm), nitrogen-sulphur ratio, crude protein (CP) and percentage digestible crude protein (DCP) were analysed using the PROC GLM procedures of SAS (version 9.1.3 of 2007). Post-hoc tests were done with Tukey's Studentised Range (HSD) for multiple comparisons of means.

### 7.3 Results

### 7.3.1 Fibre orientation and tenderness of offal from Dohne Merino sheep

The results of the energy dispersive x-ray spectroscopy (EDX) revealed significant ($p < 0.05$) variations in fibre orientations and tenderness of Dohne Merino offal (Table 7.1). These variations implied that different offal in each sheep were not aligned the same way regarding the orientation of their fibres and that each required unequal shearing force (WBSF) to cut through the fibres. The scanning electron micrographs illustrating how the orientations of the offal vary with respect to the direction of the fibres also confirmed the same observation (Figures 7.1a & 7.1b). As opposed to anisotropy, the fibre orientation found in the small intestine, fillet, lung and mouth muscle indicated isotropy. Typical micrographs presented in Figure 7.2a & 7.2b implied that fibres with isotropic orientation can be seen in different shapes.

In Table 7. 2, the results on fibre length and fibre thickness of offal from the castrates and ewes are presented. Comparing the length of all the intestinal offal, the results showed that the ewe from small intestine had the longest fibre length of $112.3 \pm 0.31$ μm. Among the non-intestinal offal, the mouth muscle had the longest fibre length of $201.2 \pm 0.54$ μm.

No significant difference ($p > 0.05$) was found in the fibre lengths of the reticulum, spleen and mouth muscle from the castrates and ewes. The lung from the castrate however had maximum ($p < 0.05$) fibre thickness of $73.0 \pm 0.90\mu m$ in comparison with other offal. Gender effects did not produce any significant difference ($p > 0.05$) between the fibre thickness of the spleen and kidney from castrates and ewes, respectively. The characteristic lengths and thickness of the fibre in the present study cannot be compared with any previous report due to paucity of information.

**Table 7. 1 Muscular fibre orientation and Warner-Bratzler shear force (N) of Dohne Merino of offal**

| Offal | Fibre orientation | Ewe ( n = 69) | Castrate (n = 69) |
|---|---|---|---|
| **Intestinal offal** | | | |
| Abomasum | Anisotropy | $57.9 \pm 2.19^a$ | $40.9 \pm 1.55^b$ |
| Large intestine | Anisotropy | $49.8 \pm 1.85^a$ | $30.5 \pm 1.31^b$ |
| Omasum | Anisotropy | $59.1 \pm 4.32$ | $56.2 \pm 3.06$ |
| Reticulum | Isotropy | $49.7 \pm 3.68^a$ | $44.2 \pm 3.68^b$ |
| Rumen | Anisotropy | $62.8 \pm 2.55^a$ | $57.6 \pm 1.81^b$ |
| Small intestine | Isotropy | $45.3 \pm 2.26^a$ | $15.8 \pm 1.59^b$ |
| **Non-intestinal offal** | | | |
| Fillet | Isotropy | $34.2 \pm 1.87^a$ | $23.4 \pm 1.32^b$ |
| Heart | Anisotropy | $33.2 \pm 1.80^a$ | $18.7 \pm 1.27^b$ |
| Kidney | Anisotropy | $18.2 \pm 0.96$ | $18.7 \pm 0.68$ |
| Liver | Anisotropy | $18.5 \pm 1.33^a$ | $13.4 \pm 0.94^b$ |
| Lung | Isotropy | $25.5 \pm 1.23^a$ | $15.6 \pm 0.87^b$ |
| Mouth muscle | Isotropy | $52.0 \pm 5.78^a$ | $44.7 \pm 4.09^b$ |
| Spleen | Anisotropy | $27.4 \pm 1.59^a$ | $22.5 \pm 1.12^b$ |
| Tongue | Anisotropy | $35.0 \pm 3.05^a$ | $46.8 \pm 2.16^b$ |
| Trachea | Anisotropy | $111.0 \pm 5.78^a$ | $73.7 \pm 4.09^b$ |
| Oesophagus | Anisotropy | $104.7 \pm 4.63^a$ | $69.3 \pm 3.28^b$ |

[a, b,] Values within the same row having different superscripts were significant ($p < 0.05$)

**Table 7. 2 Fibre length and fibre thickness of offal from Dohne Merino sheep**

| Offal | Fibre length (µm) | | Fibre thickness (µm) | |
|---|---|---|---|---|
| | Castrate (n=69) | Ewes (n=69) | Castrate (n=69) | Ewes (n=69) |
| **Intestinal offal** | | | | |
| Abomasum | $63.9 \pm 0.12^a$ | $54.2 \pm 0.17^b$ | $4.6 \pm 0.09^b$ | $5.8 \pm 0.48^a$ |
| Large intestine | $80.3 \pm 0.11^a$ | $74.1 \pm 0.08^b$ | $8.8 \pm 0.72^a$ | $6.7 \pm 0.54^b$ |
| Omasum | $88.0 \pm 0.17^b$ | $99.5 \pm 0.19^a$ | $8.9 \pm 0.51^b$ | $21.1 \pm 0.36^a$ |
| Reticulum | $42.1 \pm 0.16$ | $40.4 \pm 0.18$ | $17.7 \pm 0.60^b$ | $22.4 \pm 0.84^a$ |
| Rumen | $76.4 \pm 0.26^a$ | $67.2 \pm 0.22^b$ | $24.2 \pm 0.78^a$ | $18.6 \pm 0.60^b$ |
| Small intestine | $54.2 \pm 0.12^b$ | $112.3 \pm 0.31^a$ | $23.0 \pm 0.36^b$ | $40.2 \pm 0.84^a$ |
| **Non-intestinal offal** | | | | |
| Lung | $134.7 \pm 0.25^a$ | $59.6 \pm 0.16^b$ | $73.0 \pm 0.90^a$ | $46.3 \pm 0.24^b$ |
| Spleen | $46.0 \pm 0.14$ | $44.2 \pm 0.16$ | $13.5 \pm 0.48$ | $13.4 \pm 0.48$ |
| Heart | $48.0 \pm 0.28^b$ | $55.4 \pm 0.64^a$ | $11.4 \pm 0.36^b$ | $22.4 \pm 0.84^a$ |
| Liver | $43.9 \pm 0.12^a$ | $38.8 \pm 0.12^b$ | $9.1 \pm 0.72^b$ | $21.0 \pm 1.08^a$ |
| Trachea | $16.2 \pm 0.14^a$ | $13.4 \pm 0.17^b$ | $5.1 \pm 0.36^a$ | $3.8 \pm 0.24^b$ |
| Mouth muscle | $201.2 \pm 0.54$ | $198.0 \pm 0.39$ | $23.5 \pm 1.08^a$ | $18.9 \pm 0.84^b$ |
| Tongue | $91.1 \pm 0.28^b$ | $111.7 \pm 0.81^a$ | $10.4 \pm 0.41^b$ | $14.7 \pm 0.58^a$ |
| Fillet | $86.8 \pm 0.55^a$ | $81.7 \pm 0.20^b$ | $24.2 \pm 0.89^a$ | $18.7 \pm 0.77^b$ |
| Kidney | $38.2 \pm 0.33^a$ | $26.2 \pm 0.22^b$ | $26.0 \pm 0.64$ | $26.2 \pm 0.64$ |
| Oesophagus | $49.2 \pm 0.12^b$ | $82.3 \pm 0.31^a$ | $23.0 \pm 0.36^b$ | $34.2 \pm 0.84^a$ |

[a, b,] Values within the same row having different superscripts were significant ($p < 0.05$)
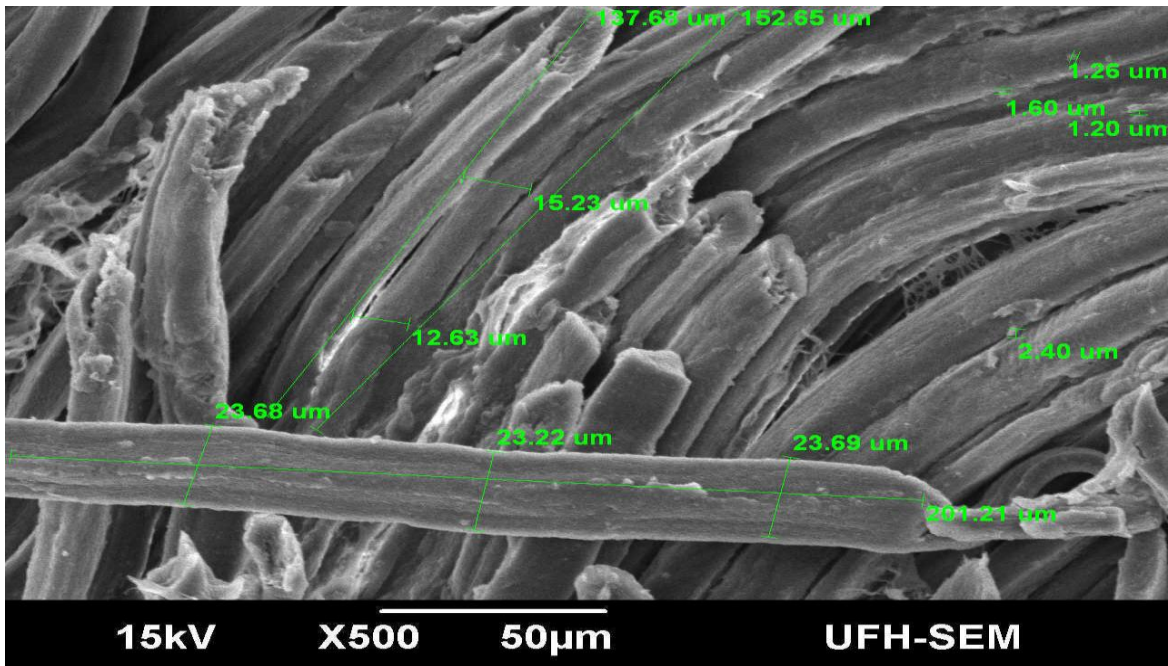
**Figure 7. 1a Longitudinal isotropic micrograph of the mouth muscle from Dohne Merino castrate**
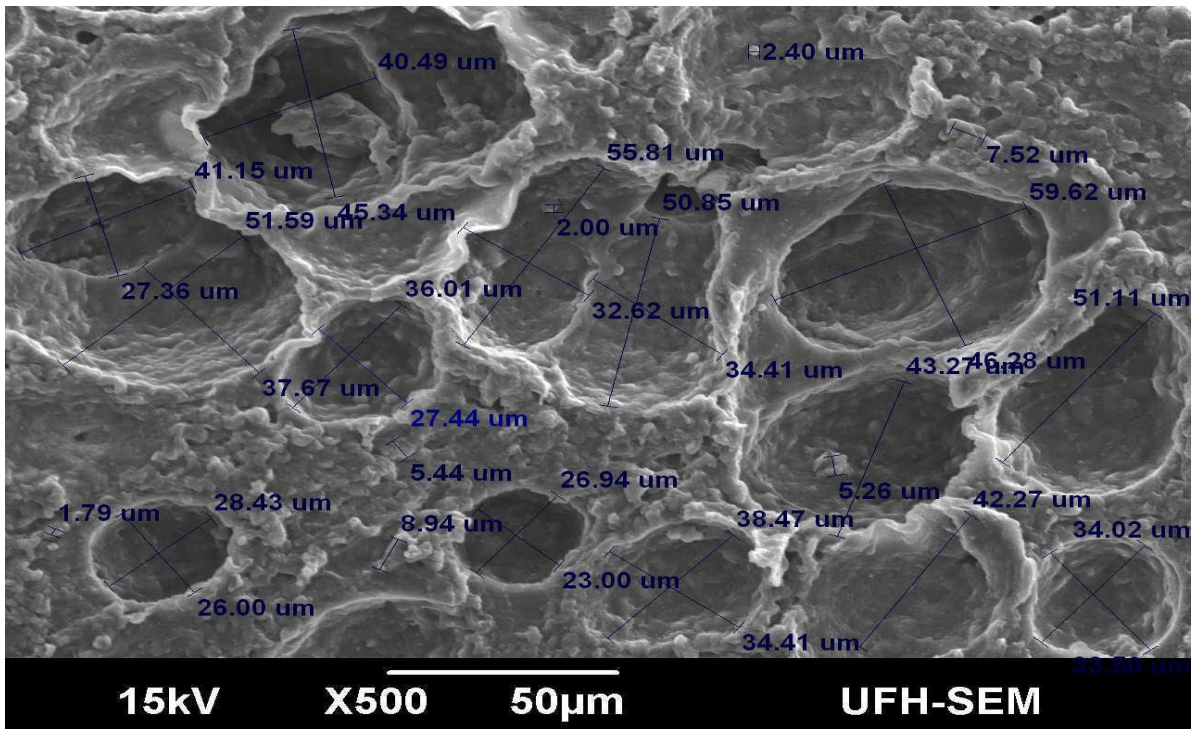


**Figure 7.1b: Circular isotropic micrograph of the lung from Dohne Merino ewe**
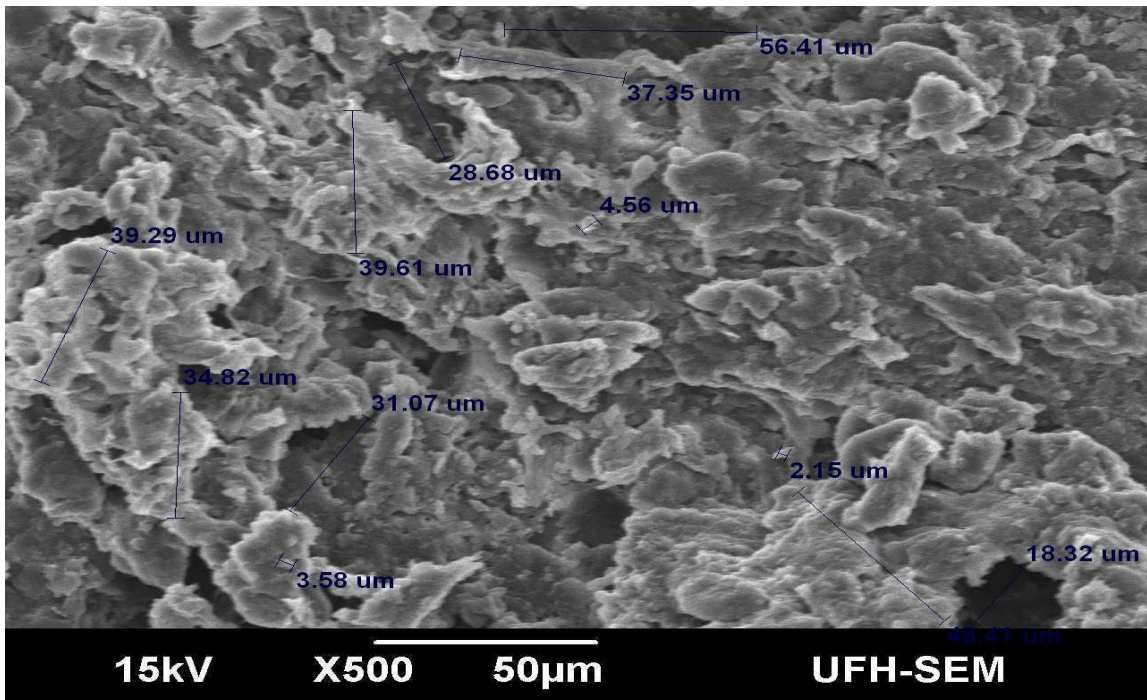
**Figure 7. 2a Anisotropic micrograph of liver from Dohne Merino castrate**
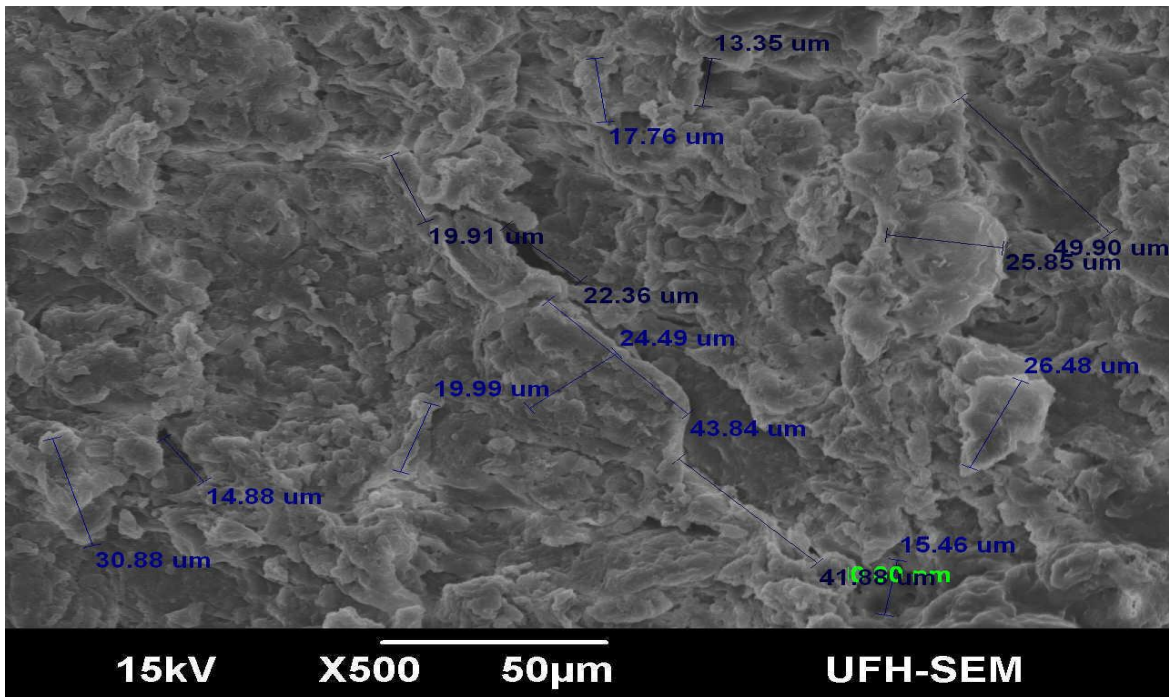


**Figure 7. 2b Anisotropic micrograph of kidney from Dohne Merino ewe**

**7.3.2 X-ray microanalysis of protein and mineral contents in Dohne Merino offal**

The nutrient composition in the offal from Dohne Merino revealed that the nitrogen (N) contents in the castrates were higher ($p < 0.05$) than the ewes except in the large intestine (Table 7. 3). Among all the intestinal offal, highest amount of N was found in the rumen from the castrates. This amount was also responsible for the highest CP ($p < 0.05$) and (DCP) levels from the rumen. Conversely, the abomasum from ewes had the least N-levels which also translated to the lowest amounts of CP ($27.6 \pm 0.09$) and of DCP of $19.3 \pm 1.03\%$, respectively. No significant difference ($p > 0.05$) was noticed in the sulphur (S) levels between the castrates and ewes from omasum and rumen. While the reticulum from the ewes contained the highest S levels ($p < 0.05$), the least ($p < 0.05$) quantity of S, was found in the large intestine of the sheep. Except for rumen and small intestine, the N/S ratios were significantly higher ($p < 0.05$) in the castrates than in the ewes. In Table 7. 4, the results on N levels in most of the non-intestinal offal from the castrates were found to be higher than those from the ewes. Among all the non-intestinal offal, the N-contents in the fillet were found to be the highest ($P < 0.05$). This value was invariably accountable for the highest CP and DCP presented in Figures 7.3 & 7.4, respectively.

The nutritional composition revealed that the nitrogen (N) contents in the Dohne Merino castrates were higher ($p < 0.05$) than the ewes for all the intestinal offal except the large intestine (Table 7.3). The higher N levels in rumen particularly, could be related to maximal crude protein (CP) ($72.5 \pm 3.78\%$) and digestible crude protein (DCP) ($50.8 \pm 5.01$) values. On the contary, the least N-values in the abomasum of the ewes also translated to the lowest amounts of CP ($27.6 \pm 0.09$) and of digestible crude protein (DCP) of $19.3 \pm 1.03\%$, respectively. The sulphur (S) levels in the omasum and rumen of Dohne castrates and ewes were not different ($p > 0.05$) from each other.

The highest (p < 0.05) N/S ratio found in the large intestine from the castrates suggested an imbalance in nitrogen-sulphur content with the sulphur content being the lowest in the large intestine. Non-intestinal offal (particularly the fillet from the castrates) was found having the highest nitrogen content ($p \leq 0.05$; 11.2 ± 0.54) (Table 7.4). This value was invariably accountable for the highest crude protein (CP, $p \leq 0.05$) and digestible crude protein (DCP, $p \leq 0.05$) contents in the fillet as well. Incidentally, the results on the CP and the DCP contents in the non-intestinal offal followed similar pattern (Figures 7.3 & 7.4). Among all the non-intestinal offal, the liver from the ewes had the lowest CP and DCP levels respectively (Figure 7.3). Generally, the results showed that the protein contents in the intestinal offal were significantly higher (p < 0.05) than those in the non-intestinal ones. In addition, most of the offal from the castrate Dohne Merino was richer in protens than those from the ewes. Although lung from the castrates recorded highest CP and DCP levels, yet its Cd levels was also high (15.55 ± 0.61%) possibly due to the grazing environment where the animals were managed before slaughter. It is worth noting that SEM uses the percentage (%) to show the quantity of the nutritional constituents of each offal relative to the size of the sample analysed.

**Table 7. 3 Nitrogen-Sulphur ratio and protein contents from intestinal Dohne Merino offal**

| Offal | Meat Source | Nitrogen (%) | Sulphur (%) | N/S Ratio | Crude protein (%) | Digestible Crude Protein (%) |
|---|---|---|---|---|---|---|
| Abomasum | Castrate | $6.5 \pm 0.49^a$ | $1.0 \pm 0.31^a$ | $6.8 \pm 0.56^a$ | $40.3 \pm 0.11^a$ | $28.2 \pm 1.92^a$ |
|  | Ewe | $4.4 \pm 0.28^b$ | $0.7 \pm 0.28^b$ | $6.2 \pm 0.52^b$ | $27.6 \pm 0.09^b$ | $19.3 \pm 1.03^b$ |
| Large intestine | Castrate | $5.8 \pm 0.36^b$ | $0.2 \pm 0.01^b$ | $27.7 \pm 1.22^a$ | $36.4 \pm 1.51^b$ | $25.5 \pm 0.65^b$ |
|  | Ewe | $8.6 \pm 0.54^a$ | $0.8 \pm 0.03^a$ | $10.4 \pm 0.98^b$ | $54.0 \pm 1.63^a$ | $37.8 \pm 0.93^a$ |
| Omasum | Castrate | $7.4 \pm 0.51^a$ | $0.9 \pm 0.31$ | $8.0 \pm 0.91^a$ | $46.0 \pm 1.23^a$ | $32.2 \pm 1.46^a$ |
|  | Ewe | $5.4 \pm 0.40^b$ | $0.8 \pm 0.40$ | $6.8 \pm 0.57^b$ | $33.8 \pm 1.01^b$ | $23.7 \pm 1.32^b$ |
| Reticulum | Castrate | $9.2 \pm 0.72^b$ | $1.1 \pm 0.05^b$ | $8.7 \pm 0.32^a$ | $57.8 \pm 4.35^a$ | $40.5 \pm 2.13^a$ |
|  | Ewe | $8.4 \pm 0.54^a$ | $1.8 \pm 0.03^a$ | $3.6 \pm 0.21^b$ | $52.5 \pm 2.10^b$ | $34.8 \pm 1.06^b$ |
| Rumen | Castrate | $11.6 \pm 0.22^a$ | $1.0 \pm 0.08$ | $6.4 \pm 0.19$ | $72.5 \pm 3.78^a$ | $50.8 \pm 5.01^a$ |
|  | Ewe | $8.3 \pm 0.54^b$ | $1.2 \pm 0.24$ | $6.8 \pm 0.87$ | $51.9 \pm 3.33^b$ | $36.3 \pm 5.02^b$ |
| Small intestine | Castrate | $6.8 \pm 0.12$ | $1.0 \pm 0.09^a$ | $7.0 \pm 0.05^b$ | $42.5 \pm 0.56^a$ | $29.8 \pm 0.71^a$ |
|  | Ewe | $5.6 \pm 0.18$ | $0.6 \pm 0.07^b$ | $8.9 \pm 0.12^a$ | $35.0 \pm 0.43^b$ | $24.5 \pm 0.76^b$ |

[a, b,] Means within the same column having different superscripts were significantly different

$(p < 0.05)$

**Table 7. 4 Nitrogen and sulphur contents from non-intestinal Dohne Merino offal**

| Offal | Meat Source | Nitrogen (%) | Sulphur (%) | N/S Ratio |
|---|---|---|---|---|
| Fillet | Castrate | $11.2 \pm 0.54^a$ | $1.3 \pm 0.07$ | $8.5 \pm 0.99^a$ |
| | Ewe | $8.9 \pm 0.44^b$ | $1.0 \pm 0.08$ | $5.7 \pm 0.77^b$ |
| Heart | Castrate | $6.6 \pm 0.22^a$ | $0.9 \pm 0.01$ | $7.4 \pm 1.01^a$ |
| | Ewe | $4.9 \pm 0.53^b$ | $0.7 \pm 0.03$ | $6.6 \pm .97^b$ |
| Kidney | Castrate | $8.9 \pm 1.64$ | $1.7 \pm 0.13^a$ | $5.3 \pm 0.65$ |
| | Ewe | $9.4 \pm 1.64$ | $1.1 \pm 0.03^b$ | $4.8 \pm 0.63$ |
| Liver | Castrate | $9.7 \pm 2.05^a$ | $1.2 \pm 0.31^a$ | $4.5 \pm 0.54^b$ |
| | Ewe | $3.9 \pm 1.11^b$ | $0.3 \pm 0.01^b$ | $11.4 \pm 1.73^a$ |
| Lung | Castrate | $7.0 \pm 0.41^b$ | $1.4 \pm 0.07^b$ | $10.0 \pm 1.02^a$ |
| | Ewe | $9.9 \pm 0.93^a$ | $2.3 \pm 0.16^a$ | $8.2 \pm 0.94^b$ |
| Mouth muscle | Castrate | $7.1 \pm 0.75^a$ | $0.8 \pm 0.64^a$ | $4.0 \pm 0.07^b$ |
| | Ewe | $5.0 \pm 0.52^b$ | $0.5\pm0.21^b$ | $11.1 \pm 1.71^a$ |
| Tongue | Castrate | $10.3 \pm 0.19^a$ | $1.3 \pm 0.05^a$ | $6.3 \pm 0.45^a$ |
| | Ewe | $7.7 \pm 0.10^b$ | $0.8 \pm 0.03^b$ | $3.4 \pm 0.36^b$ |
| Trachea | Castrate | $7.5 \pm 0.77^a$ | $0.9 \pm 0.16^a$ | $2.5 \pm 0.92^b$ |
| | Ewe | $5.2 \pm 0.38^b$ | $0.5 \pm 0.07^b$ | $11.5 \pm 1.07^a$ |
| Oesophagus | Castrate | $6.8 \pm 0.12$ | $1.0 \pm 0.09^a$ | $7.0 \pm 0.05^b$ |
| | Ewe | $5.6 \pm 0.18$ | $0.6 \pm 0.07^b$ | $8.9 \pm 0.12^a$ |

[a, b,] Means within the same column having different superscripts were significantly different

$(p < 0.05)$
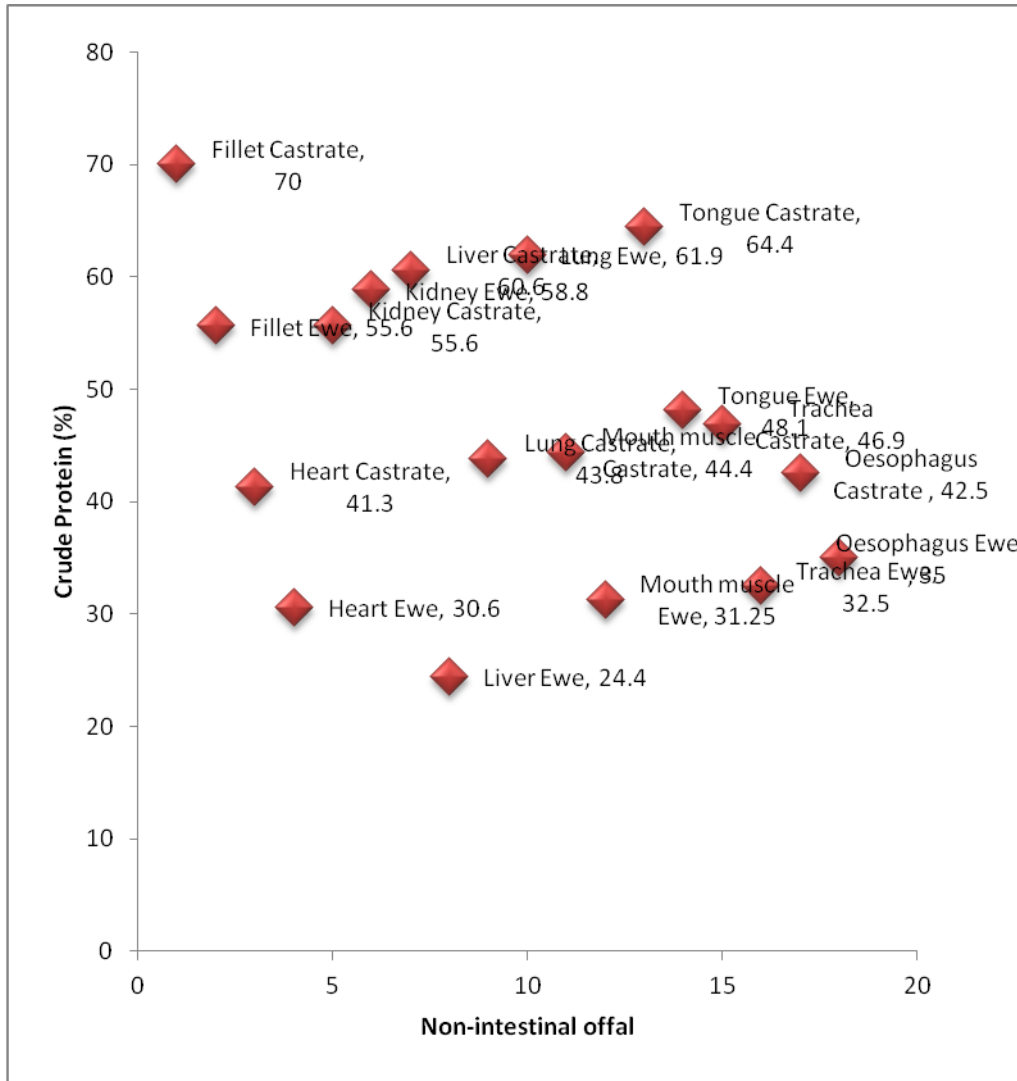
**Figure 7. 3 Crude protein contents from non-intestinal offal of Dohne Merino castrates and ewes**

185
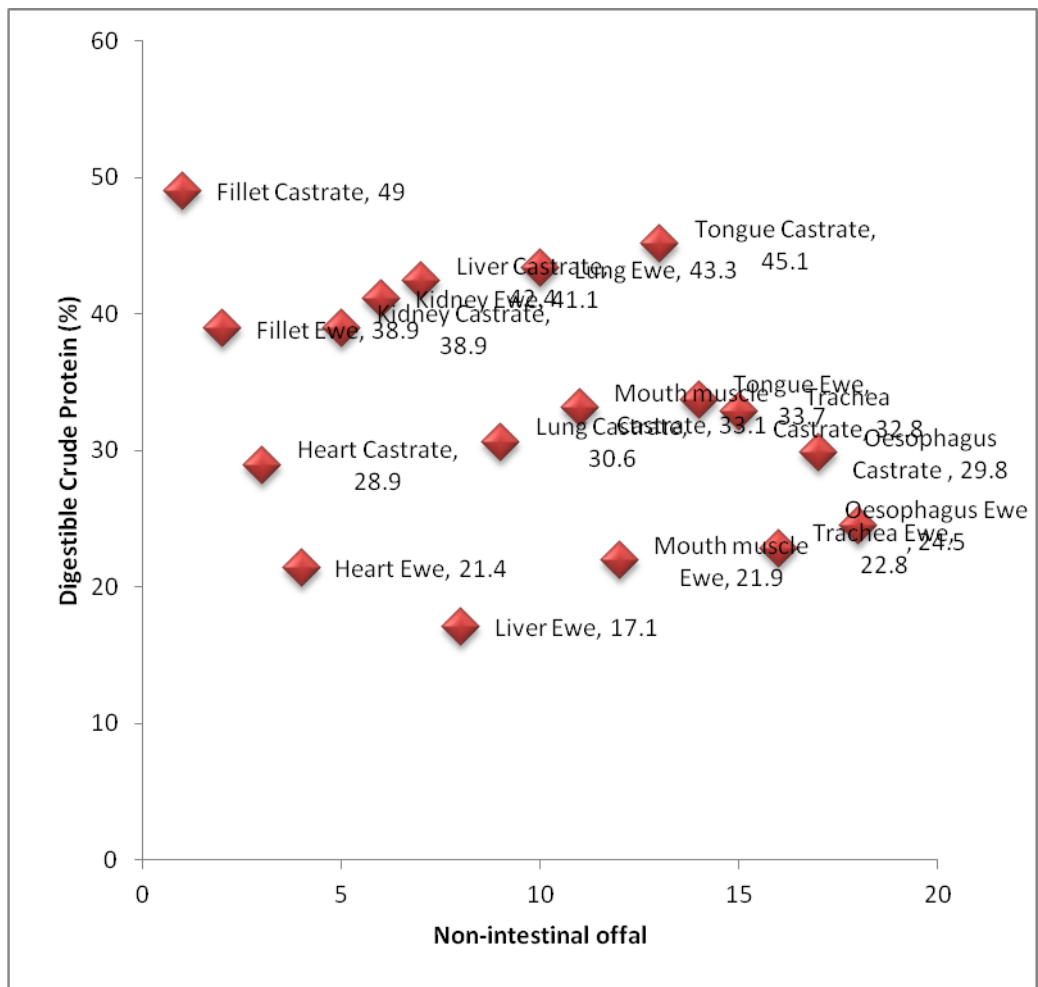
**Figure 7.4 Digestible crude protein contents from non-intestinal offal of Dohne Merino castrates and ewes**

### 7.3.3 Detectable contents Sodium and some heavy metals in the Dohne Merino offal

The result of the x-ray emission that produced signals with the information on the elemental constituents of the lung is presented in Figure 7. 5. The image displayed by SEM generated varying amounts of few elements found in the lung. Apart from the main elements that are fundamental building blocks of the muscle, highest amount of Cadmium (Cd, $15.55 \pm 0.61\%$) was detected in the lung from the castrates. The level of Cd in this specimen could be attributed to high deposition of this trace element (in the lung) from the grazing environment where the animals were managed before slaughter. As shown in Figure 7.6, a typical x-ray is presented showing a number of trace elements that were detected in the heart of the castrates. Aluminium (Al), arsenic (As) and sodium (Na) were also found at different levels in some intestinal and non-intestinal offal of the ewes (Figure 7.7). The proportions of A's in the large intestines and the omasums were higher than in other organs. In ascending order, the silicon levels in the offal from the castrates were: spleen > small intestine > omasum > large intestine > heart (Figure 7.8). Copper (Cu) was only detected in four offal from Dohne Merino ewes and presented in descending order as follows: tongue < lung < rumen < omasum (Figure 7.9). This order showed that omasum contained the highest level of Cu but the least level was found in the tongue.
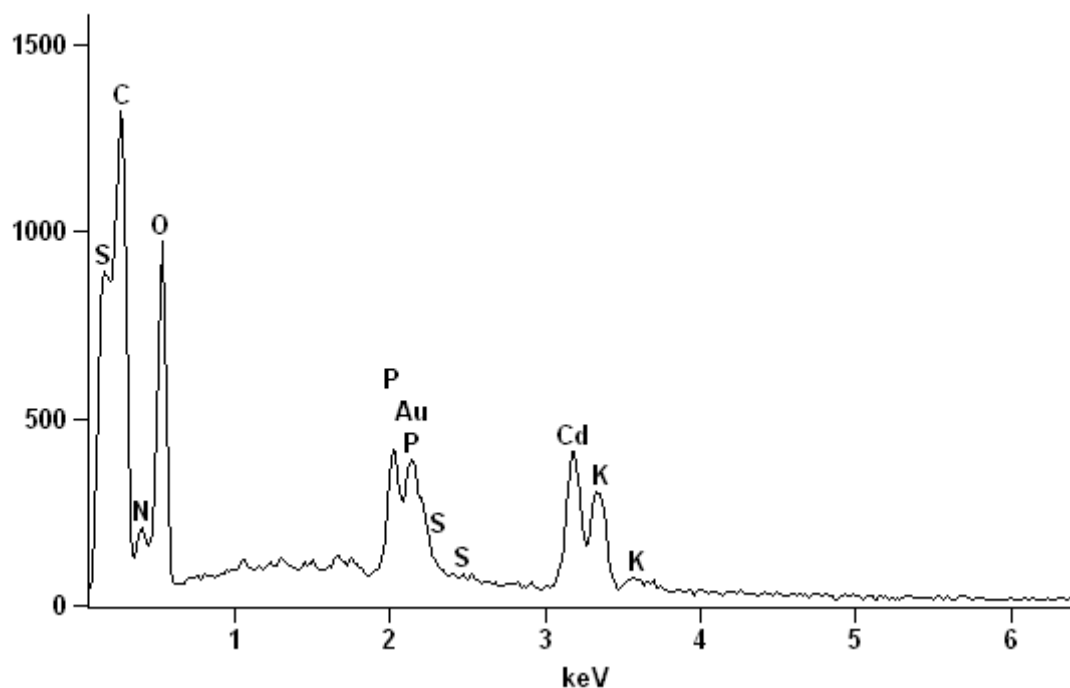
Weight %

| | C-K | N-K | O-K | P-K | S-K | K-K | Cd-L | Au-L |
|---|---|---|---|---|---|---|---|---|
| *Lung castrate (2)_pt1* | 18.33 | 6.93 | 28.41 | 2.97 | 0.36 | 2.89 | 15.55 | 24.55 |

Weight % Error (+/- 1 Sigma)

| | C-K | N-K | O-K | P-K | S-K | K-K | Cd-L | Au-L |
|---|---|---|---|---|---|---|---|---|
| *Lung castrate (2)_pt1* | +/- 0.39 | +/- 0.87 | +/- 0.70 | +/- 0.09 | +/- 0.10 | +/- 0.13 | +/- 0.61 | +/- 4.18 |

Compound %

| | C | N | O | P | S | K | Cd | Au |
|---|---|---|---|---|---|---|---|---|
| *Lung castrate (2)_pt1* | 18.33 | 6.93 | 28.41 | 2.97 | 0.36 | 2.89 | 15.55 | 24.55 |

**Figure 7. 5 X-ray microanalysis showing maximum levels of cadmium, phosphorus and potassium in the lung of Dohne Merino castrate**

Full scale counts: 1875          Heart castrate (2)_pt1
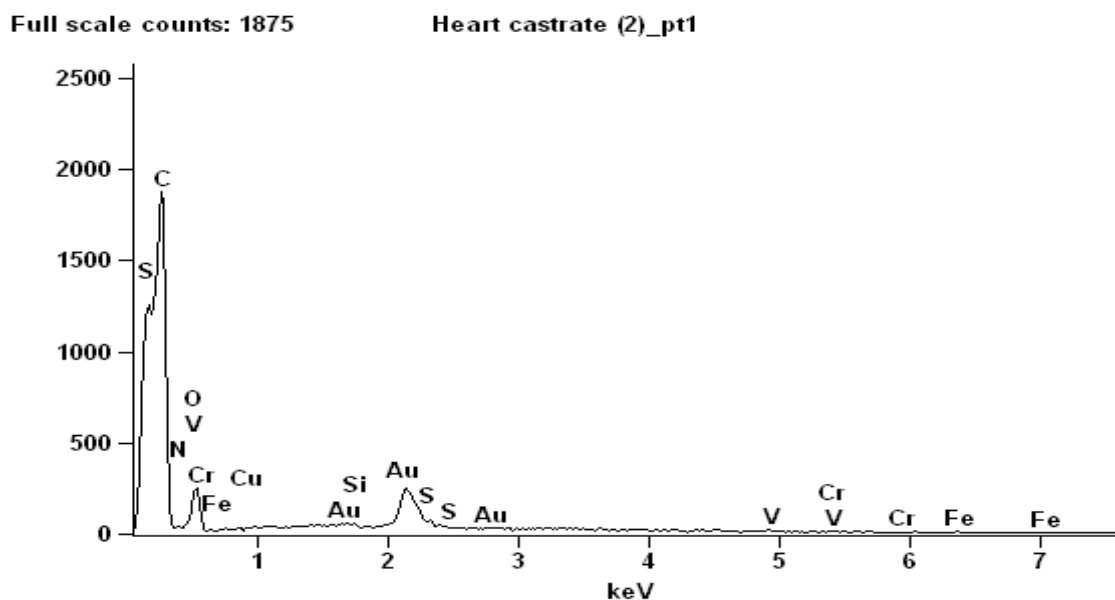
Atom % Error (+/- 1 Sigma)

| | C-K | N-K | O-K | Si-K | S-K | V-K | Cr-K | Fe-K | Cu-K | Au-L |
|---|---|---|---|---|---|---|---|---|---|---|
| *Heart castrate ( 2)_pt1* | +/- 0.86 | +/- 2.86 | +/- 1.08 | +/- 0.04 | +/- 0.08 | +/- 0.07 | +/- 0.08 | +/- 0.11 | +/- 0.21 | +/- 0.52 |

Compound %

| | C | N | O | Si | S | V | Cr | Fe | Cu | Au |
|---|---|---|---|---|---|---|---|---|---|---|
| *Heart castrate (2) _pt1* | 49.89 | 11.86 | 23.84 | 0.14 | 0.68 | 0.47 | 0.36 | 0.66 | 1.03 | 11.07 |

**Figure 7. 6 X-ray microanalysis of the heart showing the presence of vanadium and chromium in Dohne Merino castrate**
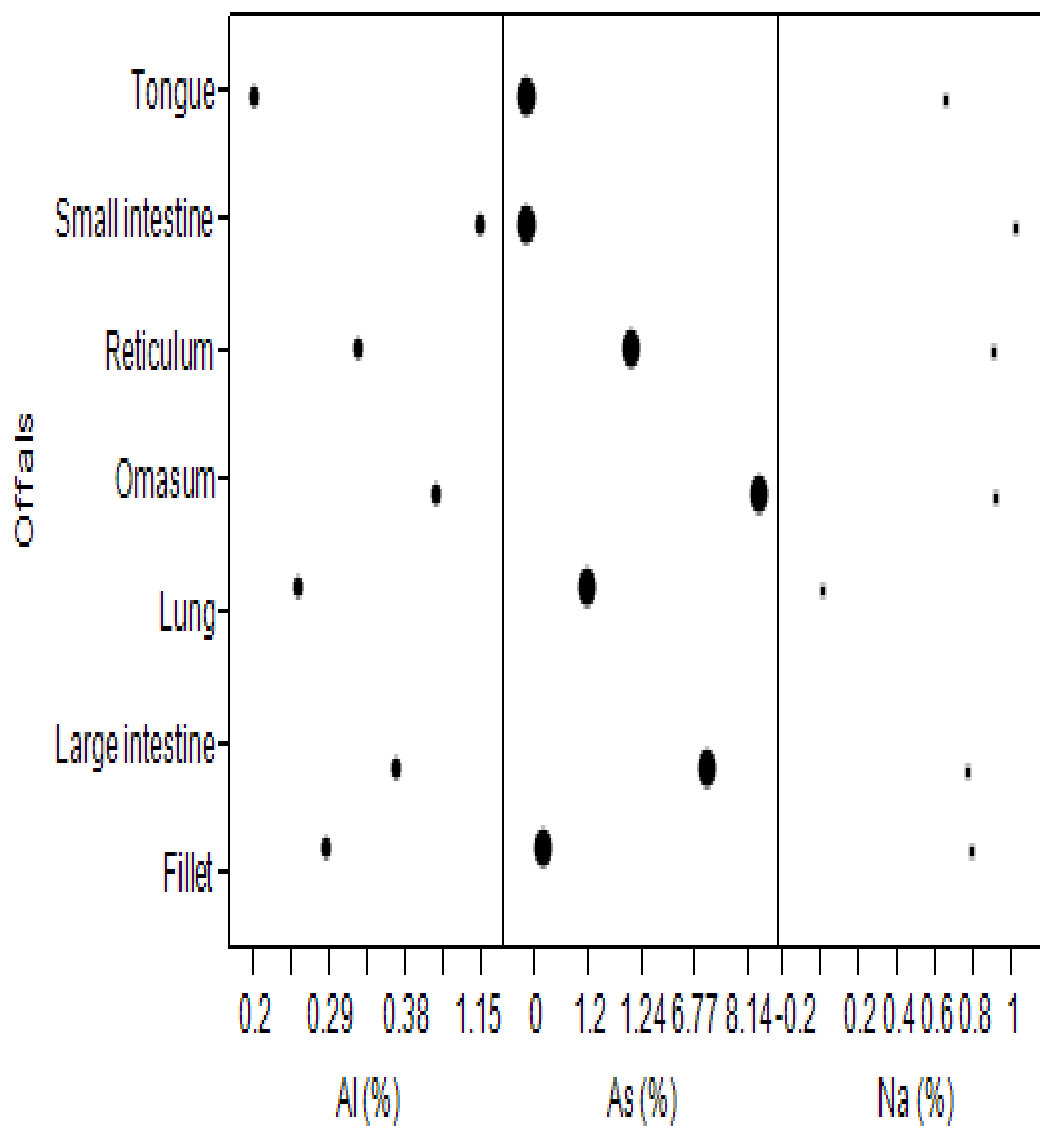
**Figure 7. 4 Aluminium, arsenic and sodium concentrations in some intestinal and non-intestinal offal of Dohne Merino ewes**
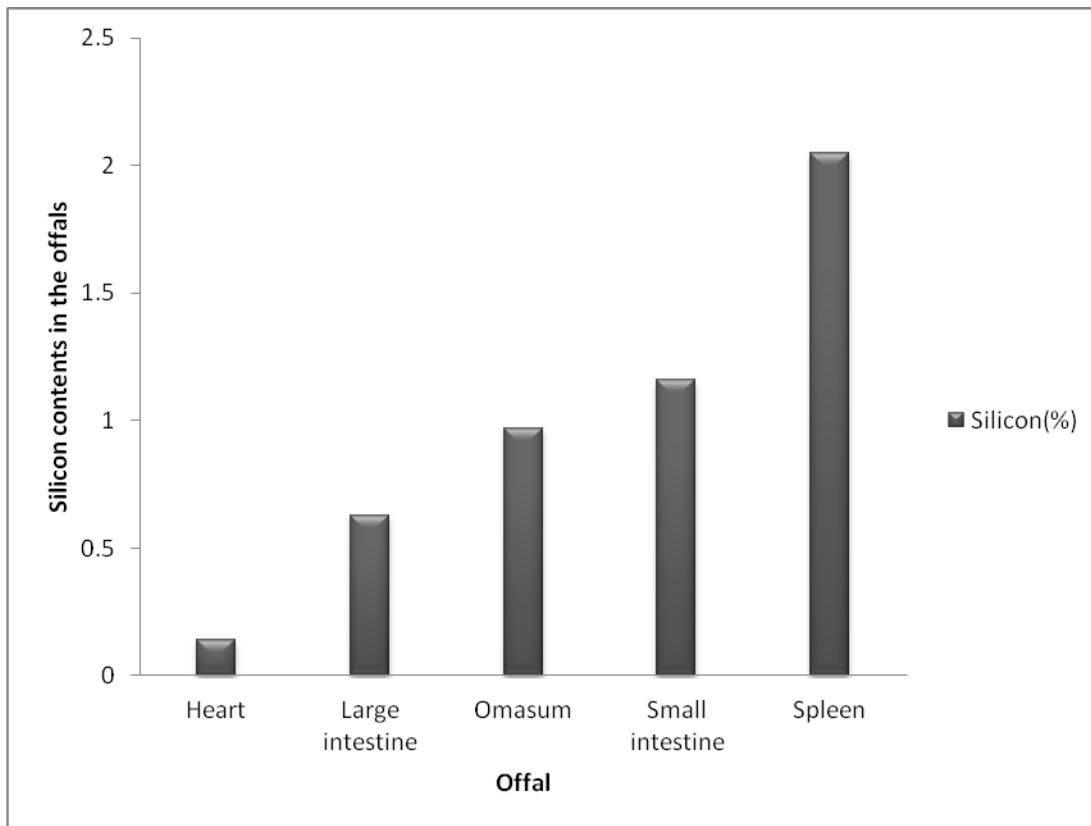
**Figure 7. 5 Silicon levels in some intestinal and non-intestinal offal of Dohne ewes**
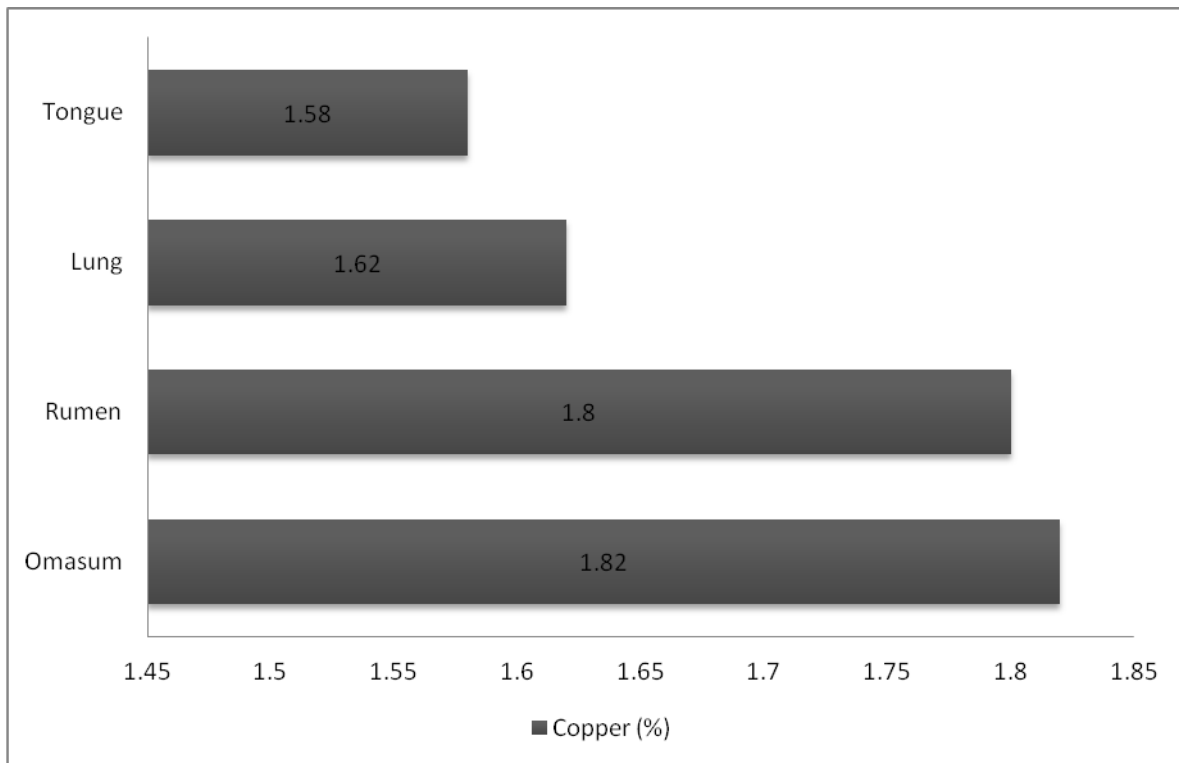
**Figure 7. 6 Levels of copper in the tongue, lung, rumen and omasum of Dohne Merino ewes**

**7.4 Discussion**

In their views, Alegre *et al.* (2005); Anderhalt (2007) and Gaige (2007) linked the basis for this type of orientation in most muscle fibres to varying fibre arrangement, lengths and the peculiarities of the fusiform muscles. While imaging high-contrast fluorescent protein, Rizzo and Piston (2005); Owicki (2000) found rotational mobility (size) and the fluorescence lifetime of the protein being responsible for anisotropy. In anisotropic materials, Kocks (2000) ascribed occurrence of different fibre orientations to the effect of excitation on molecular weight, binding state, viscosity and alignment between excitation and emission dipoles of the materials. Although these factors were not investigated in this study yet, the dearth of literature on fibre orientation suggest the possibility that the factors accountable for non-meat materials could be responsible for our findings too.

It is obvious from these results that anisotropy was predominant among the examined offal. A confirmation of these findings was reported by Sharafi and Blemker (2010) where most (bundles) of the skeletal muscle fibres from the fascicle cross-sections were found to be highly anisotropic. Judging from the dissimilarity in the shapes from the lung and mouth muscle, Martini *et al.* (2008) reported a scenario where skeletal isotropic muscles could appear with a number of shapes such as flat bands, spindle shaped and even large protrusions. Intrinsically, offal that manifested isotropic orientation gave indication that they have characteristics that are similar to the rectus (or straight) muscles. This assertion was established on rectus muscles where fibres that were longitudinally aligned parallel to one another were seen to display isotropic orientation (Martini *et al.,* 2008). These authors also drew similar allusion to fibres with concentric shape (for instance lung) and thus portraying a different way by which muscles with isotropic fibres are arranged around an opening or recess.

On different note, Gaige (2007) and Cao et al. (2010) associated the oblique pattern of short fibres from the lung to the circular shape of the visceral layer due to inner pleura linings, outer parietal layer and clusters of microscopic air sacs (alveoli). From the foregoing, our findings seem to challenge the assumption of transverse isotropy being implicit in most finite element model of muscle microstructure (Blemker et al., 2005; Lemos et al., 2004). Going by reports from Scho¨nfisch (1997) and Kocks (2000), various factors including density, heat capacity, conductivity and textural patterns of the material could be attributed to isotropic alignment of the fibres in the examined offal. As expected, the trachea of the ewes had the highest ($p < 0.05$) WBSF value and by implication, offal with low WBSF values were tender than those with higher WBSF values.

In this regard, the report of Muchenje et al. (2008) on skeletal muscle where more shearing force was required to cut through tougher bovine loin muscle seem similar and relevant. Since more WBSF is required to shear tougher meat, the presence of cartilaginous rings in the trachea could be ascribed to the relative toughness of this offal (trachea) compared to other offal. In addition, the results showing that the tenderness of omasums was not significantly different ($P > 0.05$) showed certain degree of similarity in the thickness of the omasal leaves from both the ewes and castrates. The tenderness of the lung following a similar pattern as the omasums suggested that almost the same amount of shearing force was required to cut through the lung from both sexes. Generally, the WBSF values of most offal from ewes indicated a pattern depicting huge effects of age and gender on the tenderness of different offal. Higher WBSF values from most offal from Dohne Merino ewes agreed to higher force values previously recorded for shearing ovine skeletal muscle (Hopkins *et al.,* 2007). Since the castrates used in this study were younger in age than the ewes, Harper (1999) found low structural linkage due to collagen content and cellular matrix as underlying factors.

Similar to reports on the tenderness of M. *longissimus* muscle by Warner et al. (2006, 2007), the adhesive force in the connective tissue of ewes underscored the rationale why most offal from Dohne Merino ewes' had higher WBSF values than those from the castrates. In essence, a biological effort which begins with the fusion of satellite cells to the muscles fiber leads to the formation of new myofibrils and consequently increases the cross-sectional area (i.e length*thickness) of muscle fibre. It could be presumed therefore that the characteristics of the fibres depend on the: myofibrils, fibre types, oxidative capacity and the glycogen content of the muscle fibres (Choe *et al.,* 2009). Charge and Rudnicki (2004) rather reported that the pattern of protein expression in the myofibrils and the activities of the satellite cells might affect the length and other features of the muscle fibres.

The general observation from the current study revealed overlapping nutrient levels by the offal having the same or almost the same amount of protein contents. Incidentally, the protein contents in the intestinal offal were found to be significantly higher ($p < 0.05$) than those in the non-intestinal ones. Generally, highest accumulation of CP in the rumen is possible due to the synthesis of synthesis of microbial protein in the muscular sacs of the rumen which may account for the retention of 75-85% total N and nucleic acids within its sacs (Dijkstra et al., 1998; Fujihara and Shem, 2011). It is worth mentioning that the result (in Figure7. 5) was not meant to imply that gold (Au) was present in the lung but rather to show that Au was used for sputter coating at some stage in electron scanning. This was done to prevent the accumulation of static electric fields on the specimen (during imaging) which could cause scanning faults or image artifacts (Rudenberg and Rudenberg, 2010).

In agreement with previous studies, few authors found that heavy metals accumulate more in the offal than in the muscle and in most other types of food (Miranda *et al.,* 2005; Hernandez

and Benedito, 2005). Ferraresi and Corticelli (2002); Forte and Bocca (2008) therefore ascribed the augmented levels of these heavy metals to the effect to long-term exposure of the animals to the environmental pollution from the soil, waste water or deposition of fumes from industrial areas. The extremely long biologic half-life (30-35 years) makes Cd a cumulative toxicant, with liver and kidney as the main organs of accumulation (Madeddu *et al.,* 2011). Since the Cd levels in the current study was more than the recommended 10-25µg/day average daily intake, Hermida *et al.* (2006) stated the possibility of health risks if such offal is consumed. Considering public health therefore, it is suggested that raising ovine species in a zero-cadmium environment or tolerable cadmium environment might not pose any health risks to consumers. Although, the potassium (K) level of 2.9 ± 0.13% (11.55µg/day) was lower than the Recommended Daily Allowances (RDA) for adults, the value of K obtained in the present study was below the RDA for matured male and females reported by the Joint FAO/WHO (2006) and Ngassapa *et al*. (2010); Othman (2011). The need to fortify the sheep diet with adequate dietary K is therefore recommended. Generally, the composition of fibres in each muscle is genetically and/or environmentally determined and individual differences are significant (Sedki et al., 2003; Sun et al., 2011).


**7.5 Conclusion**

By combining muscle fibre characterisation with mechanical measurements of tenderness of the offal, good predictions of muscle fibre orientation and nutrient constituents was possible. The outcome of the energy dispersive x-ray spectroscopy in this study produced variations in fibre orientations, tenderness and nutritional compositions of the examined offal. The study further showed that most of the offal with anisotropic orientation had higher WBSF values than those with isotropic orientation, which suggested that isotropic offal, are comparatively tender. It was observed in this study that the fibre length and fibre thickness of omasum and

the mouth muscle from Dohne Merino ewes were respectively longer and wider than observed from others. It was found that rumen from castrates with the longest fibre length and the thickest fibre also had the highest CP and DCP levels. The fillets from the castrates had the highest crude protein and digestible crude protein contents. The levels of the trace nutrients in the offal also revealed the condition of the environment where these animals managed before slaughter. It is of utmost importance therefore to investigate how the ambient environmental conditions affect other eating qualities of offal such as pH and colour development.

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**CHAPTER 8: Effect of *post-mortem* ambient conditions on pH and the development of colour by Dohne Merino offal**

*(Submitted to Meat Science)*

**Abstract**

The aim of this study was to determine the effect of *post-mortem* ambient conditions on pH and the development of colour by Dohne Merino offal. A data logger was used along with colour-guide $45^0/0^0$ colorimeter and pH metre to generate data on ambient conditions, colour, pH and temperature of the offal from the castrates (n = 69) and ewes (n = 69) from 1 to 30hour post-slaughter. Data collected were analysed using PROC GLM and PROC CANCORR procedures of Statistical Analysis System (SAS). Results revealed that ambient conditions were negatively correlated (p > 0.05) with the pH and temperature of most offal except the trachea (r = 0.5060, p < 0.05) and the heart (r = 0.5272, p < 0.05). The saturation index (SI) of few non-intestinal offal showed moderate relationship with the ambient temperature (AT). Dew point indicated a negative correlation (r = -0.5955) with the total colour difference ($\Delta E^*$) for the fillet. The canonical relationship between the relative humidity and the whiteness index (WI) of both intestinal and non-intestinal offal followed a similar pattern. In conclusion, the *post-mortem* ambient conditions in the cold room affected the pH and influenced the development of colour by the offal.

**Key words**: canonical correlation analysis, offal, saturation index (SI), total colour difference ($\Delta E^*$), whiteness index (WI)

**8.1 Introduction**

Colour is one of the most significant organoleptic properties of meat (Girolami et al., 2003). This is because colour gives the first visual impression to consumers concerning meat product at the point of purchase (Mancini and Hunt, 2005). Consumers therefore use colour as an indicator of freshness and wholesomeness that consequently influences their acceptability or rejection of meat (Jacob and Thomson, 2012). Mostly, the colour of meat is predominantly influenced by the concentration, chemical condition and lipid oxidative status of myglobin in the sarcoplasm of muscular fibers (Girolami et al., 2003; Ramos et al., 2009). In a broad sense, other factors affecting the colour of meat include muscle structure (which depends on pH and marbling) (Brewer et al., 2001), microbial growth (Stivarius, Pohlman & McElyea-Waldroup, 2002), oxygen consumption rate (Wulf and Wise, 1999) and drip losses (Muchenje et al., 2009).

As discussed previously (Chapter 7), numerous factors including *ante-mortem* handling, *post-mortem* conditions, age of animal at slaughter, weight at slaughter, genetics, diet, influence the colour and other qualities of meat or offal (Mancini and Hunt, 2005; Hoffman et al., 2013). In the meat industry however, practical experience has shown that ambient conditions play notable roles on the colour and pH of meat or offal (Puremist, 2012). Thus, a combination of ambient conditions in the cold room such as temperature, relative humidity, dew point, air movement and thermal radiation interrelate to create an environment that detracts or enhances meat quality (Sahin et al., 2003). Occurrence of negative traits is therefore possible when the ambient temperature (AT) is above the evaporative critical temperature (Marai and El-Kelawy, 1999). Besides, the temperatures at which muscles enter rigour when they are converted to meat have an effect on its colour (Hood, 1980; Farouk and Swan, 1998).

Hence, the collective effects of high temperature and low pH in the muscles result in colour development (Hood, 1980). Therefore, the control of temperature in a meat plant or sausage kitchen is important since temperature is critical to understand relative humidity. In view of this, appropriate temperature control is required to maintain high humidity without condensation during meat storage (Food and Agricultural Organisation, 1991). When the temperature is within the normal USDA range of 60% for beef, 42% for pork, 65% for chicken, 58% for turkey and 70% for fish, humidification reduces shrinkage, extends shelf life, preserves visual appeal of meat products and hence boosts marketability or profits (Puremist, 2012).

In relation to beef colour during storage, a relative humidity of nearly 90%, air velocity of about 0.5m/s and temperatures closer to the freezing point are suitable for maintaining the colour and freshness of beef in a stable condition (Arnau et al., 2012). Consequently, the development of brownish metmyoglobin concentrations due to discoloration caused by oxygen partial pressure, temperature and pH particularly affect meat quality (Mancini and Hunt, 2005; Saito et al., 2007; Suman et al., 2009). Meat in the foregoing contexts referred to the *Musculus longissimus* muscles for example the *M. longisimuss thoracis*, *M. longisimuss dorsi*, *M. longisimuss cervicis*, *M. longisimuss capitis*, *M. semimembranosus* and *M. semitendinous* (Jacob and Thomson, 2012) and not the offal. On record, dearth of information still exists on the effects of ambient conditions on the colour, muscle pH and muscle temperature of offal. Thus, the objective of the study was to determine the effects of *post-mortem* ambient conditions on pH and colour development by offal from Dohne Merino sheep.

## 8.2. Materials and Methods

### 8.2.1 Data collection and sample preparation

In this study, offal from Dohne Merino castrates (n = 69) and ewes (n = 69) sourced from a high-throughput abattoir were used. The mean ages of the sheep were 11 and 36 months for the castrates and ewes respectively. From each, sample size weighing 20-40g were excised from the liver, lung, heart, rumen, kidney, abomasum, rumen, omasum, reticulum, oesophagus, tongue, fillet, spleen, small intestine, large intestine using a sharp scalpel.

### 8. 2. 2 pH measurement of the offal

The pH and temperature of the Dohne Merino offal were measured using a digital pH metre (Crison pH25 instruments S.A., Alella, Spain) equipped with a penetrating electrode. The pH metre was calibrated using pH4, pH7 and pH9 standard solutions (CRISON Instruments, SA, and Spain) before each measurement were taken. After calibration, the respective measurements were taken at 1h, 6h, 24h and 30h post-slaughter.

### 8.2.3 Determination of ambient conditions

A data acquisition system was used for logging ambient temperature, relative humidity and dew points of the environment surrounding the offal at 1h, 6h, 24h and 30h post-slaughter. This was done by connecting a data logger (MT668 Major Tech Pvt Ltd, South Africa) with an in-built device for saving data to a laptop computer equipped with Microsoft Excel and programmed to take readings every 60 minutes from 1h to 30h post-slaughter.

## 8.2.4 Colour measurement of the offal

Colour measurements were taken on the surface of fresh offal from Dohne Merino castrates and ewes from 1h, 6h, 24h and 30h post-slaughter under average ambient temperature of $18.65^{\circ}C$, relative humidity of 44.25% and dew point of 4.18. A Minolta colour-guide $45^{0}/0^{0}$ colorimeter (BYK-Gardener GmbH, USA) having illuminant $D_{65}$ at $10^{\circ}$ observation angle and 20mm aperture size was calibrated using the green, black and white colour samples to determine L*, a* and b* colour coordinates (Commission International de l' Eclairage, 1976). The colour coordinates L*, a* and b* indicating lightness, redness and yellowness were respectively determined on each offal from 1, 6, 24 and 30 hours post-slaughter. Other colour parameters determined in this study were as described by Saricoban and Yilmaz (2010) for:

i. Whiteness index (WI) $= 100 - \sqrt{(100-L^*)^2 + a^{*2} + b^{*2}}$

ii. Saturation index (SI) $= \sqrt{a^{*2} + b^{*2}}$

iii. Total colour difference $(\Delta E^*) = \sqrt{(L_o-L^*)^2 + (a_o-a^*)^2 + (b_o-b^*)^2}$ where subscript 'o' refers to the colour reading of control offal' samples used as the reference and a larger $\Delta E^*$ indicates greater colour change from the reference offal' sample.

## 8.2.5 Statistical analyses

The Statistical Analysis System (SAS version 9.1.3 of 2007) was used for all the statistical analyses. The PROC GLM procedure of SAS was used considering the effects of post-slaughter timing on redness (CIEa*) and cooking regimes on the WBSF values of the offal. Significant differences between the least square means were performed using the PDIFF test of SAS, with significance level of $p < 0.05$.

The PROC CANCORR command of SAS was used to determine the potential multivariate (canonical) relationships among the ambient conditions and colour coordinates of the offal.

## 8. 3 Results

### 8.3.1 Post-slaughter timing and redness (CIEa*) of intestinal offal from Dohne Merino

The relationships between post-slaughter timing and CIEa* indicated considerable variations among the intestinal offal (Table 8.1). The effect of gender on CIEa* values for abomasum and rumen were not significant ($p > 0.05$). The redness value of reticulum from castrates was significantly higher ($p < 0.05$) than the one obtained from the ewes. The redness of the large intestines, small intestines and omasum followed a similar pattern with the castrates having significantly lower CIEa*values ($p < 0.05$) than the ewes. The CIEa* values of the omasum and rumen were not different ($p > 0.05$) from one-hour (1) to thirty (30) hour post-slaughter (hPS). This implies that there were no changes in the rate of discolouration, consumption or diffusion of oxygen in the offal from 1-30hPS.

### 8.3.2 Post-slaughter timing and redness (CIEa*) of non-intestinal offal from Dohne Merino

The age of the sheep did not have any significant effect ($p > 0.05$) in the *post-mortem* colour (CIEa*) of the kidney, lung and oesophagus of Dohne Merino castrates and ewes (Table 8.2). However, the redness values of reticulum, fillet and liver were significantly higher ($p < 0.05$) than those obtained from the ewes. There was no colour difference ($p > 0.05$) from 1hPS to 30 hPS by fillet, heart and lung. Only the kidney, liver and spleen attained their ultimate blooming at 30hPS among all the non-intestinal offal.

**Table 8. 1 Least squares means and standard error of means (± SEM) for the effects of gender and post-slaughter**

**timing on redness (CIE a\*) of intestinal Dohne Merino offal**

| Offal | Gender | | Post-slaughter timing for castrate & ewe | | | |
|---|---|---|---|---|---|---|
| | Castrate (n=69) aged 11 months) | Ewe (n=69) 36months) | 1h | 6h | 24h | 30h |
| Abomasum | $5.5 \pm 0.86$ | $7.0 \pm 0.87$ | $3.5 \pm 1.21^b$ | $6.5 \pm 1.23^{ba}$ | $8.4 \pm 1.34^a$ | $6.7 \pm 1.23^{ba}$ |
| Large intestine | $1.1 \pm 0.87^b$ | $5.7 \pm 0.88^a$ | $0.7 \pm 0.21^c$ | $5.3 \pm 1.24^a$ | $5.0 \pm 1.24^a$ | $2.6 \pm 0.81^b$ |
| Omasum | $2.1 \pm 0.39^b$ | $3.5 \pm 0.40^a$ | $1.7 \pm 0.56$ | $3.3 \pm 0.57$ | $3.0 \pm 0.57$ | $3.3 \pm 0.57$ |
| Reticulum | $14.0 \pm 0.96^a$ | $2.8 \pm 0.97^b$ | $2.3 \pm 1.34^b$ | $3.9 \pm 1.37^{ba}$ | $7.4 \pm 1.37^a$ | $4.7 \pm 1.37^{ba}$ |
| Rumen | $3.0 \pm 0.63$ | $4.7 \pm 0.64$ | $4.2 \pm 0.89$ | $2.9 \pm 0.91$ | $3.2 \pm 0.91$ | $5.2 \pm 0.91$ |
| Small intestine | $4.9 \pm 1.62^b$ | $9.7 \pm 1.64^a$ | $12.0 \pm 2.26^a$ | $6.9 \pm 2.31^b$ | $4.9 \pm 2.31^b$ | $5.3 \pm 2.31^b$ |

[a,b,c] Means in the same row with different superscripts are significantly different at $p < 0.05$.

**Table 8. 2 Least squares means and standard error of means (± SEM) for the effects of gender and post-slaughter timing on the redness (CIE a\*) of non-intestinal offal from Dohne Merino**

| Offal | Gender | | Post-slaughter timing for castrate & ewe | | | |
|---|---|---|---|---|---|---|
| | Castrate (n = 69) | Ewe (n = 69) | 1h | 6h | 24h | 30h |
| Fillet | $13.3 \pm 0.49^a$ | $10.9 \pm 0.49^b$ | $10.1 \pm 0.68$ | $11.9 \pm 0.70$ | $12.0 \pm 0.70$ | $12.0 \pm 0.70$ |
| Heart | $11.4 \pm 0.74^b$ | $15.4 \pm 0.75^a$ | $12.8 \pm 1.04$ | $14.1 \pm 1.06$ | $12.6 \pm 1.06$ | $14.1 \pm 1.10$ |
| Kidney | $14.0 \pm 0.82$ | $13.4 \pm 0.83$ | $13.8 \pm 1.15^{ba}$ | $13.3 \pm 1.17^{ba}$ | $11.8 \pm 1.70^b$ | $15.9 \pm 1.2^a$ |
| Liver | $12.6 \pm 0.61^a$ | $10.5 \pm 0.61^b$ | $12.7 \pm 0.85^{ba}$ | $10.4 \pm 0.87^{bc}$ | $2.4 \pm 0.87^c$ | $13.7 \pm 0.9^a$ |
| Lung | $18.7 \pm 1.53$ | $22.6 \pm 1.55$ | $19.5 \pm 2.14$ | $22.6 \pm 2.19$ | $21.4 \pm 2.19$ | $19.1 \pm 2.19$ |
| Oesophagus | $7.9 \pm 1.34$ | $11.2 \pm 1.36$ | $6.7 \pm 1.88^b$ | $13.4 \pm 1.92^a$ | $8.7 \pm 1.92^{ba}$ | $9.5 \pm 1.92^{ba}$ |
| Spleen | $10.4 \pm 0.65$ | $9.1 \pm 0.66$ | $8.9 \pm 0.91^b$ | $7.6 \pm 0.93^c$ | $8.2 \pm 0.93^b$ | $14.2 \pm 0.93^a$ |
| Trachea | $10.7 \pm 1.10^b$ | $13.5 \pm 1.10^a$ | $14.3 \pm 1.50^a$ | $10.3 \pm 1.5^b$ | $11.9 \pm 1.50^b$ | $11.8 \pm 1.50^b$ |
| Tongue | $4.9 \pm 1.60^b$ | $9.7 \pm 1.60^a$ | $12.0 \pm 2.30^a$ | $6.9 \pm 2.31^b$ | $4.9 \pm 2.31^b$ | $5.3 \pm 2.30^b$ |

[a,b,c]Means in the same row with different superscripts are significant at $p<0.05$.

### 8.3.3 Ambient conditions, pH and colour parameters of Dohne Merino offal

In Table 8. 3, no significant relationship ($p > 0.05$) was observed between the pH of the intestinal offals and all the ambient factors. The dew point and AT were moderately correlated ($p < 0.05$) with the muscle temperature (MT) of the omasum and for reticulum, MT and AT ($r = 0.5617*$) were related ($p < 0.05$). Concerning the Whitish Index (WI) for all intestinal offals, AT and dew points showed strong canonical correlation ($r \geq 0.8574*$). Among all the ambient factors, RH only showed significant relationship ($p < 0.05$) between the total colour difference ($\Delta E*$) and omasum ($r = 0.5150*$) and this could be linked to the retention of moisture in the omasal leaves of the sheep. Similarity of the AT, at 1h and 24hPS (Figure 8. 1) however, was accountable for the canonical relationship presented on colour parameters in Table 8.2. The effects of dew points on the colour parameters of offal on the other hand, followed similar pattern between 12hPS and 24hPS (Figure 8.2) but RH influenced the colour parameters differently at 6hPS and 30hPS (Figure 8.3).

The results of canonical correlations in Table 8.4 revealed that ambient conditions were negatively correlated ($p > 0.05$) with the pH and muscle temperature (MT) of most offal except ($p < 0.05$) the trachea ($r = 0.5060*$) and the heart ($r = 0.5272*$). The extent to which the colour of the offal deviated from an ideal white portrayed an inverse relationship between relative humidity (RH) and the Whiteness index (WI) of all the non-intestinal offal. The result on non-intestinal offal showed that AT moderately correlated ($p < 0.05$) with the saturation index (SI) of the trachea ($r = 0.5087*$) and heart ($r = 0.5315*$) as evident by the vividness of their colours.

213

**Table 8. 3 Canonical correlations between ambient conditions, pH and muscle**

**temperature of non-intestinal offal from Dohne Merino**

| Offal | Ambient Conditions | pH | MT |
|---|---|---|---|
| Liver | AT | -0.2548 | 0.2041 |
| | RH | -0.1852 | 0.1017 |
| | Dew point | -0.1912 | 0.1597 |
| Spleen | AT | -0.3104 | 0.4891 |
| | RH | -0.0636 | 0.0193 |
| | Dew point | -0.2703 | 0.4084 |
| Lung | AT | 0.0529 | 0.3423 |
| | RH | -0.2477 | -0.0348 |
| | Dew point | 0.1011 | 0.2927 |
| Trachea | AT | 0.0729 | 0.5060* |
| | RH | -0.0968 | -0.1444 |
| | Dew point | 0.0760 | 0.4674 |
| Kidney | AT | -0.2079 | 0.3677 |
| | RH | -0.3024 | -0.1425 |
| | Dew point | -0.1425 | 0.3371 |
| Oesophagus | AT | -0.2409 | 0.0904 |
| | RH | -0.2611 | -0.1104 |
| | Dew point | -0.1714 | 0.1041 |
| Heart | AT | -0.1619 | 0.5272* |
| | RH | -0.4987 | -0.1118 |
| | Dew point | -0.0255 | 0.4660 |
| Fillet | AT | -0.0721 | 0.2518 |
| | RH | -0.1204 | -0.0417 |
| | Dew point | -0.0186 | 0.2028 |

AT: Ambient temperature; RH: Relative Humidity; MT: Muscle Temperature, significantly correlated at $*p < 0.05$.

**Table 8. 4 Canonical correlations between ambient conditions and colour parametres of non-intestinal offal from Dohne Merino**

| Offal | Ambient Conditions | L* | a* | b* | WI | SI | ΔE* |
|---|---|---|---|---|---|---|---|
| Liver | AT | 0.254 | 0.1926 | 0.1169 | **-0.639*** | 0.1988 | **0.6067*** |
| | RH | 0.0669 | -0.2786 | -0.037 | -0.4346 | 0.0918 | 0.2394 |
| | Dew point | 0.1985 | 0.1951 | 0.0604 | -0.48 | 0.1565 | 0.4806 |
| Spleen | AT | 0.2274 | -0.0473 | 0.0074 | **0.6367*** | 0.4012 | 0.2185 |
| | RH | -0.0673 | -0.4033 | -0.1692 | **-0.5286*** | -0.0213 | 0.0588 |
| | Dew point | 0.2417 | 0.0093 | 0.0148 | **0.6990*** | 0.3530 | 0.2000 |
| Lung | AT | 0.1528 | -0.0672 | -0.0739 | **0.8909*** | 0.4168 | 0.0474 |
| | RH | 0.0352 | 0.1180 | 0.2954 | **-0.5840*** | -0.0438 | 0.1442 |
| | Dew point | 0.1247 | -0.0820 | -0.1114 | **0.9366*** | 0.3669 | 0.0098 |
| Trachea | AT | 0.3657 | 0.1814 | -0.0062 | **0.6610*** | 0.5087* | 0.2398 |
| | RH | -0.1015 | -0.1178 | -0.0495 | -0.4691 | -0.1489 | -0.0037 |
| | Dew point | 0.3575 | 0.1970 | -0.0027 | **0.7061*** | 0.4717 | 0.2254 |
| Kidney | AT | 0.1868 | -0.1666 | -0.0323 | **0.8785*** | 0.4139 | -0.4269 |
| | RH | 0.1114 | 0.0770 | -0.2644 | **-0.5645*** | -0.1603 | 0.0297 |
| | Dew point | 0.1508 | -0.1994 | 0.0096 | **0.9214*** | 0.3816 | -0.3992 |
| Oesophagus | AT | 0.3234 | -0.1927 | 0.2802 | **0.9508*** | 0.0879 | 0.1808 |
| | RH | -0.0026 | 0.2297 | 0.0328 | **-0.5383*** | -0.1232 | 0.0526 |
| | Dew point | 0.2966 | -0.2301 | 0.2389 | **0.9745*** | 0.1037 | 0.1574 |
| Heart | AT | 0.2180 | -0.0713 | -0.2168 | **0.9169*** | 0.5315 | 0.1919 |
| | RH | -0.2023 | 0.0562 | 0.0804 | **-0.5881*** | -0.1306 | -0.0676 |
| | Dew point | 0.2255 | -0.0864 | -0.2262 | **0.9627*** | 0.4753 | 0.1730 |
| Fillet | AT | -0.3721 | 0.0599 | 0.0035 | **0.9359*** | 0.2717 | **-0.5955*** |
| | RH | 0.1102 | -0.1066 | -0.0997 | **-0.5578*** | -0.0635 | 0.2758 |
| | Dew point | -0.3543 | 0.0812 | -0.0088 | **0.9714*** | 0.2276 | **-0.5889*** |

AT: Ambient temperature; RH: Relative Humidity; L*: Lightness; a*: redness; b*: Yellowness; WI: Whiteness Index;

SI: Saturation Index; ΔE*:Total Colour Difference; significantly correlated at *p < 0.05.

**Figure 8. 1 Canonical dimension for relationships between ambient temperature and colour parameters of Dohne Merino offal at different post-slaughter timing**

**Key:** 1 (in black), 2 (in red), 3(in green) & 4 (in blue) represents 1h, 6h, 24h & 30h post-slaughter timing

216
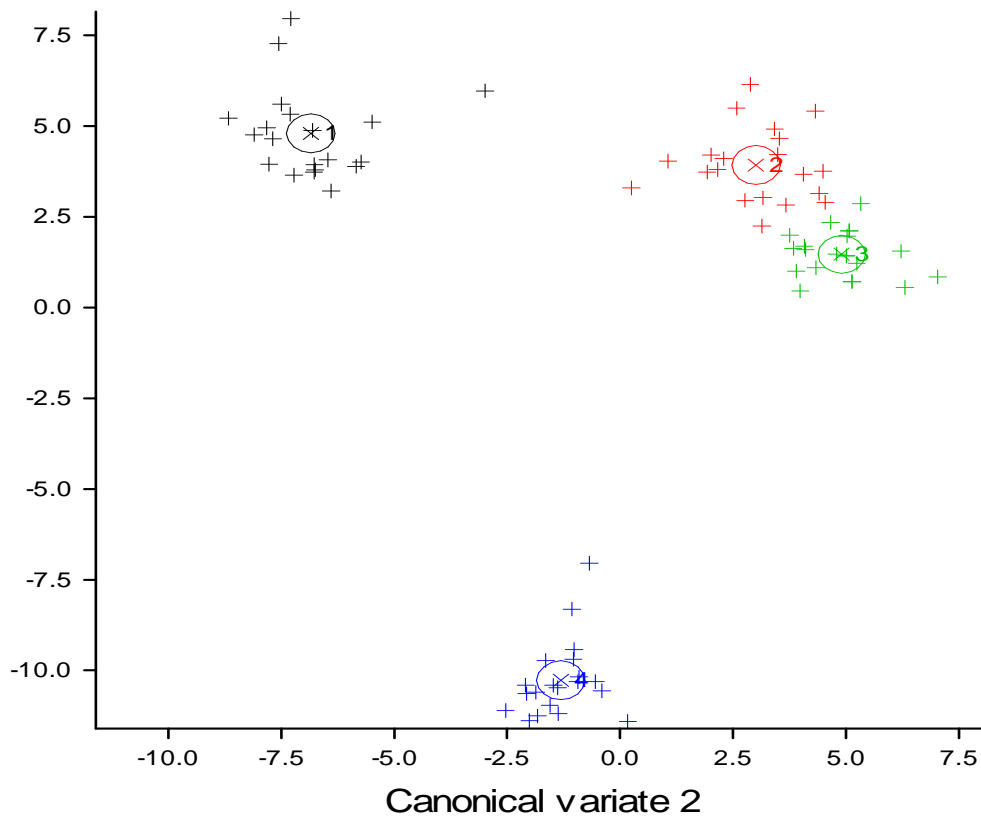
**Figure 8. 2 Canonical dimension for relationships between dew points and colour parameters of Dohne Merino offal at different post-slaughter timing**

**Key:** 1 (in black), 2 (in red), 3(in green) & 4 (in blue) represents 1h, 6h, 24h & 30h post-slaughter timing

217
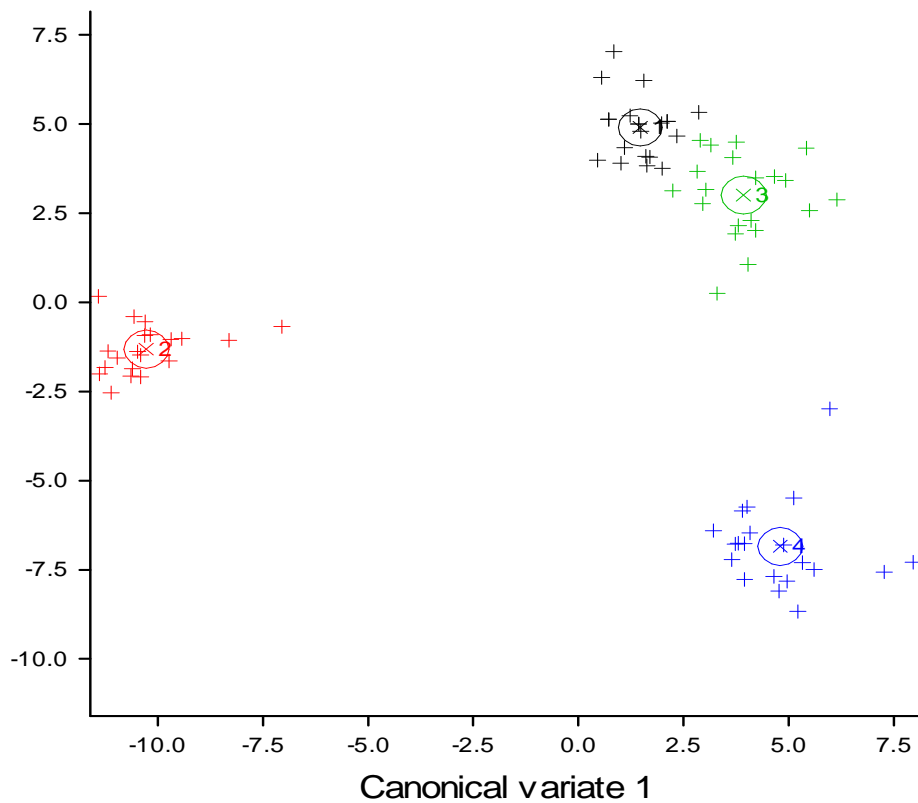
**Figure 8. 3 Canonical dimension for relationships between relative humidity and colour parameters of Dohne Merino offal at different post-slaughter timing**

**Key:** 1 (in black), 2 (in red), 3(in green) & 4 (in blue) represents 1h, 6h, 24h & 30h post-slaughter timing

### 8.3.4 Relationships among ambient conditions, colour and pH of Dohne Merino offal

The results in Table 8.5 showed inverse relationships between the ambient temperature (AT) and the lighteness (L*) of the rumen and the omasum. The dew point was found exerting similar effects on the L* of these organs as well. The results therefore implied that as the AT and the dew ponts were falling, the rumen and the omasum were becoming lighter. Both AT and the dew points were found having positive correlations ($p \leq 0.05$) on the redness (a*) and the yellowness (b*) of the rumen but negative effects on the abomasum and the omasum. The relationships between AT and the dew point further demonstrated positive effects ($p \leq 0.05$) on whiteness index (WI) of all the intestinal offal. On the contrary, the relative humidity (RH) of the environment had negative effects ($p \leq 0.05$) on the WI of the intestinal offal. A consistency in the relationships between the AT and the dew point had strong effects on the saturation index (SI) of the omasum and reticulum. The RH only was noticed with a significant effect on the total colour difference ($\Delta E*$) of the omasum. The combined effects of the ambient conditions on the pH values were found to produce weak correlations among the intestinal offal (Table 8.6). Significant variations were only observed for the internal temperature (MT) in the reticulum and within the omasomal leaves as presented in Table 8.6.

**Table 8. 5 Canonical correlations between ambient conditions and colour coordinates of intestinal offal from Dohne Merino**

| Offal | Ambient Conditions | L* | a* | b* | WI | SI | ΔE* |
|---|---|---|---|---|---|---|---|
| **Rumen** | AT | -0.1379 | 0.0573 | 0.2762 | 0.9433* | 0.1320 | -0.3761 |
| | RH | 0.1908 | -0.1690 | -0.1838 | -0.6036* | 0.2832 | 0.3028 |
| | Dew point | -0.1376 | 0.0696 | 0.2862 | 0.9855* | 0.0291 | -0.3807 |
| **Abomasum** | AT | 0.1005 | -0.2680 | -0.0810 | 0.9239* | 0.3669 | -0.1388 |
| | RH | 0.2853 | -0.2680 | -0.2803 | -0.6035* | 0.0535 | 0.4141 |
| | Dew point | 0.0291 | -0.2419 | -0.0441 | 0.9720* | 0.2816 | -0.2151 |
| **Omasum** | AT | -0.0963 | -0.2429 | -0.2071 | 0.9261* | 0.6116* | -0.4386 |
| | RH | 0.3395 | 0.0935 | -0.0469 | -0.6134* | -0.1000 | **0.5150*** |
| | Dew point | -0.1394 | -0.2443 | -0.1754 | 0.9739* | 0.5455* | -0.4844 |
| **Reticulum** | AT | 0.1908 | -0.2063 | 0.3433 | 0.9278* | 0.5672* | -0.0966 |
| | RH | -0.1119 | 0.0090 | -0.3399 | -0.6067* | -0.0626 | 0.0380 |
| | Dew point | 0.1772 | -0.1631 | 0.3452 | 0.9732* | 0.5042* | -0.1010 |
| **Large intestine** | AT | 0.2680 | -0.2008 | 0.0459 | 0.8574* | 0.4798 | 0.0912 |
| | RH | 0.1610 | 0.1627 | 0.1068 | -0.6527* | 0.1168 | 0.2811 |
| | Dew point | 0.2239 | -0.2004 | -0.0094 | 0.9197* | 0.3943 | 0.0376 |
| **Small intestine** | AT | 0.1174 | 0.2712 | -0.1844 | 0.9083* | 0.1971 | -0.0143 |
| | RH | -0.0762 | -0.0439 | 0.4193 | -0.5433* | -0.0657 | 0.2365 |
| | Dew point | 0.1547 | 0.2499 | -0.2681 | **0.9457*** | 0.1584 | -0.0339 |

AT: Ambient temperature; RH: Relative Humidity; MT: Muscle Temperature; L*: Lightness; a*: redness; b*: Yellowness; WI: Whiteness Index; SI: Saturation Index; ΔE*: Total Colour Difference. Significantly correlated at *p < 0.05.
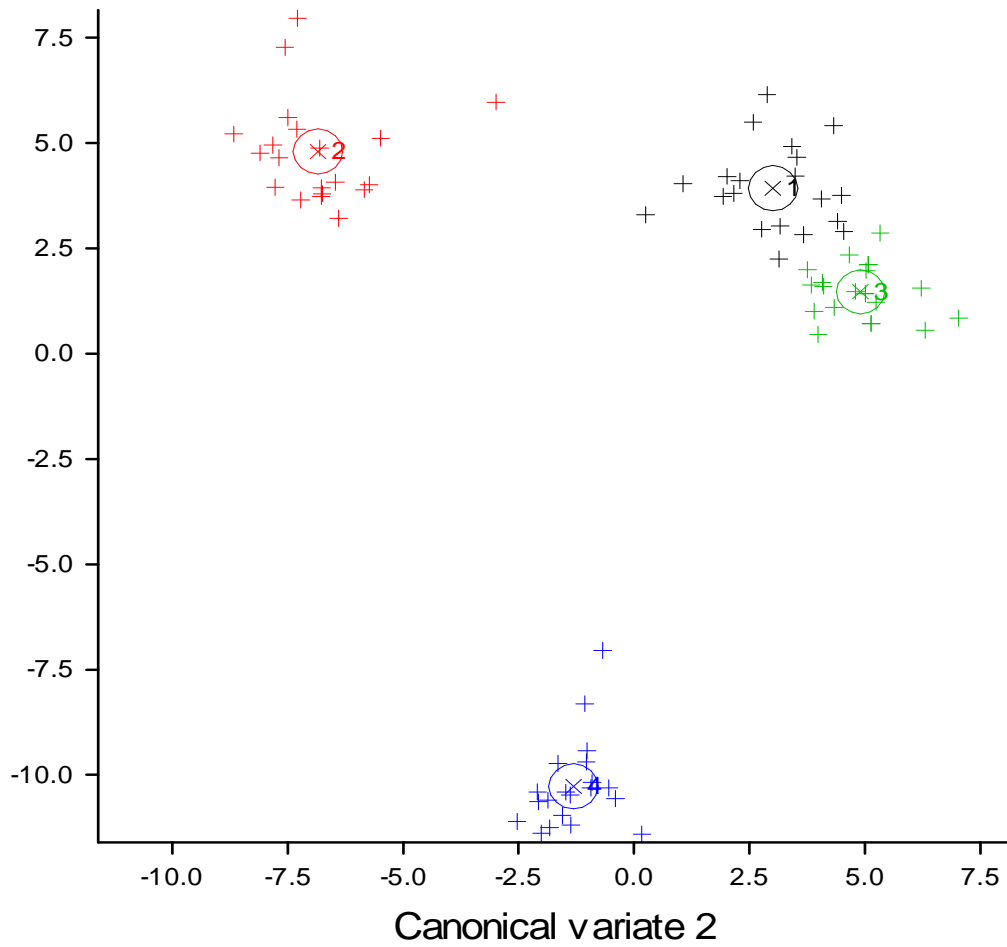
**Table 8. 6 Canonical correlations between ambient conditions, muscle pH and muscle temperature of intestinal offal from Dohne Merino sheep**

| Offal | Ambient Conditions | pH | MT |
|---|---|---|---|
| Rumen | AT | -0.2442 | 0.1432 |
| | RH | 0.1521 | 0.2745 |
| | Dew point | -0.2501 | 0.0398 |
| Abomasum | AT | -0.1066 | 0.3199 |
| | RH | -0.0216 | 0.0574 |
| | Dew point | -0.0701 | 0.2370 |
| Omasum | AT | -0.0206 | **0.6142*** |
| | RH | -0.0628 | -0.0913 |
| | Dew point | -0.0483 | **0.5475*** |
| Reticulum | AT | -0.3183 | **0.5617*** |
| | RH | 0.0834 | -0.0644 |
| | Dew point | -0.3018 | 0.4995 |
| Large | AT | -0.2183 | 0.4749 |
| intestine | RH | -0.2656 | 0.1289 |
| | Dew point | -0.1269 | 0.3869 |
| Small | AT | 0.2481 | 0.1731 |
| intestine | RH | -0.4293 | -0.0461 |
| | Dew point | 0.3061 | 0.1321 |

AT: Ambient temperature; RH: Relative Humidity; MT: Muscle Temperature. Significantly correlated at $*p < 0.05$.

**8.4 Discussion**

The result on the redness of the offal is similar to a previous study on beef where ultimate blooming of the meat was reached at 30-hour post-slaughter due to the levels of cytochrome and myoglobin pigments in the muscle (Young et al., 1999). By implication, most of the offal in this study attained ultimate redness longer than 4 hPS contrary to earlier report by Farouk and Lovatt (2000) but lower than 36hour obtained from chilled *M. longisimuss dorsi*, *M. semimembranosus* and *M. semitendinous* muscles of Merino crosses (Jacob and Thomson, 2012). The differences in our findings could be ascribed to the type of muscle, muscle region, *ante-mortem* conditions of the animals. The reason for having offal from older animals having a darker and redder colour could be attributed to higher myoglobin content in the ewes (Nishida and Nishida, 1985). It could be deduced from these results that meat colour varies according to the concentration of pigments, the chemical state of the pigments, muscle region and the way light is reflected on the meat (Abudullah and Matarneh, 2010).

Moreover, the average RH of 44.25% recorded in the present study is within the recommended range of 45-60% for meat packaging, boning and cutting rooms but lower than 80-95% range for meat chilling and ripening (FAO, 1991; Puremist, 2012). Since RH affects water activity on the surface of meat product, Arnau and Gou (2001) therefore reported that the storage of hams at 50-55% RH after salting produced whiteness on some parts of the rind due to precipitation of salt. It may be inferred consequently that the relationship between the equilibrium RH (at 44.25%) and moisture sorption isotherms of the offal will give a better idea on the stability of WI and extension of shelf life in connection with the activities of psychrophillic bacteria during autolysis.

In agreement with McNeil et al. (1987), at lower temperatures, oxygen was observed penetrating deeper into the meat while the oxymyoglobin layer thickens. This could mean that the change in oxymyoglobin colour of the fillet to metmyoglobin was accelerated at a higher AT of $18.65^{\circ}C$ obtained in this study.

**8.5 Conclusion**

The present study has shown that only the spleen, kidney and liver attained their ultimate redness (CIEa*) at 30hPS. It was observed that ambient conditions had higher influence on the whiteness index of most intestinal offal and also had a positive correlation with the pH and muscle temperature of trachea and heart. An inverse relationship was found between the relative humidity and the whiteness index of all the offal. Dew point indicated a negative correlation with the total colour difference (ΔE*) for the fillet. Saturation index (SI) of few non-intestinal offal showed moderate relationship with the ambient temperature (AT). In conclusion, the *post-mortem* ambient conditions in the cold room affected the muscle pH and had different effects on the development of various colour attributes of the offal.

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# CHAPTER 9: Cooking regimes and its effect on tenderness of Dohne Merino offal

**Abstract**

The effect of varying cooking regimes on the tenderness of Dohne Merino offal was determined in the current study. The offal used were collected from the castrates ($n = 69$) and ewes ($n = 69$) and were cooked under three regimes as rare, medium rare and welldone. The offal for the rare group were held in the Sous vide water bath at $75^{\circ}$C for 60 minutes; those in the medium rare, at $88^{\circ}$C for 45 minutes and the welldone group, at $100^{\circ}$C for 30 minutes respectively. Among all the intestinal offal, the tenderness of the tongue was negatively correlated ($r = -0.4$, $p < 0.05$) with the age of sheep at slaughter. The sex of the sheep did not have any significant effect ($p > 0.05$) on the Warner-Bratzler Shear Force (WBSF) value of the omasum from the castrates and ewes. It was found that offal cooked at $100^{\circ}$C for 30 minutes, recorded the least ($p < 0.01$) WBSF values except the heart. In conclusion, the study revealed that cooking Dohne Merino offal at $100^{\circ}$C for 30 minutes in an enclosed temperature-controlled water bath produced the most tender offal.

**Key words**: Canonical correlation analyses, Sous vide cooking, sheep offal, tenderness.

**9.1 Introduction**

In an attempt to maintain homeostasis, the *post-mortem* ambient conditions cause several metabolic and structural changes while muscle is being converted to meat (Chapter 8). In the process, tenderisation of the muscle becomes one of the notable observations due to changes in the properties of the muscle fibres and connective tissues (Taylor et al., 1995). Characteristically, cooking influences the force (kg) required for shearing and serves as a means of improving the textural properties of meat products (Sofos, 2008; Ayadi et al., 2009), developing sensory qualities (Muchenje et al., 2008) and enhancing aroma intensity (Walsh et al., 2010; Mora et al., 2010). Given the potential negative effects of eating uncooked meat, fresh meat or offal is usually thermal processed before consumption. This thermal processing is done mostly by cooking to improve wholesomeness and general qualities of meat products (Wrangham and Conklin-Brittain, 2003; Geesink et al., 2011).

Cooking therefore involves thermal application to food material in order to make it microbiologically safe, palatable and digestible (Combes et al., 2005). Characteristically, cooking enhances aroma intensity, juiciness (Walsh et al., 2010; Mora et al., 2010), shelf life and improves the textural properties of meat products (Sofos, 2008; Ayadi et al., 2009). Temperature and duration of cooking have been found to have a large effect on physical properties of meat and particularly on meat texture or the force (kg) required for shearing (Combes et al., 2003; Muchenje et al., 2008). As postulated by Kilcast (2004), the inextricable link between the food structure and textural responses generates either somesthesis (tactile or surface response from skin) or kinesthesis (proprioception or a deep response from muscles and tendons).

This link necessitates the need for the use of a mechanical device to evaluate mechanical tenderness of meat products. Warner-Bratzler shear device is however one of such widely used equipment to measure the toughness or tenderness of meat after cooking (Harris and Shorthose, 1988; Lepetit and Culioli, 1994). In the process of cooking, certain changes take place in the muscle and tenderisation is one of such due to changes in the properties of the muscle fibres and connective tissues (Taylor et al., 1995). This occurs since both the adenosine triphosphate (ATP) and calcium ions ($Ca^{2+}$) are involved in the contraction-relaxation process of the muscle. The *post-mortem* reduction in ATP levels and a rise in $Ca^{2+}$ lead to formation of irreversible cross-bridges between myosin heads and actin leading to the occurrence of rigour mortis in the tissue. The formation of rigour bonds therefore results in the toughening of the muscle (Maltin et al., 2003).

One way of tenderising toughened muscle is the application of heat as thermal treatment modifies the toughness of meat and other sensory traits during cooking process (Chiavaro et al., 2009). The complexity of the connective tissues in the offal affect its doneness when conventional cooking method is used (Garcı́a-Linares et al., 2004). The need for a method that will bring about even doneness during cooking of offal therefore calls for a heat-stable device that can influence the cooking properties of offal in a consistent way. The use of Sous vide apparatus then become relevant in this study as it does the cooking under controlled conditions of temperature-and- time inside a heat-stable vacuumized pouches or containers at programmable alarm of $1^{o}$ increments (Schellekens 1996; Douglas, 2012; Pulger et al., 2012).

Vacuum sealing is beneficial in Sous vide cooking in that it allows heat to be efficiently transferred from the water bath to the offal and thus minimising water, flavour and nutrient losses (Badwin, 2011).

By cooking at temperature higher than 50$^o$C, Sous vide gives more choice over doneness and tenderness than traditional cooking methods (Schellekens, 1996). This method has the potential to increase the shelf life, inhibits off-flavours that might be caused due to oxidation, retains moisture and loss of volatile compounds due to evaporation (Church and Parsons, 2000; Garcı´a-Linares et al., 2004; Stea et al., 2006). Through this cooking method, tough meat cuts are tenderised and made tender within medium or a medium-rare doneness and the breakdown of myosin in the process makes the material more palatable (Baldwin, 2010; Myhrvold et al., 2011). Going by the benefits of using Sous vide device, it was decided in this study to determine the effects of varying cooking regimes on the tenderness of offal from Dohne Merino sheep, which is one of the leading ovine species in South Africa.

## 9.2 Materials and Methods

### 9.2.1 Data collection and sample preparation

A total of 138 offal from Dohne Merino castrates (n = 69) and ewes (n = 69) sourced from a high-throughput abattoir were used. The mean ages of the sheep were 11 and 36 months for the castrates and ewes respectively. From each, samples weighing 20-40g were excised from the liver, lung, heart, rumen, kidney, abomasums, rumen, omasum, reticulum, oesophagus, tongue, fillet, spleen, small intestine, large intestine using a sharp scalpel.

### 9.2.2 Cooking regimes of the offal

Sous vide cooking method of sealing offal in vacuum plastic bags and submerging them in a temperature controlled water bath was used. Offal from Dohne Merino castrates and ewes were sub-divided into three cooking groups as rare, medium- rare and welldone.

The offal for the rare group were held in the water bath at 75$^{o}$C for 60 minutes; those in the medium rare, at 88$^{o}$C for 45 minutes and the welldone group, at 100$^{o}$C for 30 minutes respectively. The minimum cooking regime adopted in the present study was selected based on the recommended 75$^{o}$C thermal centre for doneness of meat (American Meat Science Association, 1995).

### 9.2.3 Determination of tenderness of Dohne Merino offal

After cooking in each group, the offal was cooled within 5-10 minutes for the determination of their tenderness. After cooling,  sub-samples measuring 10mm core diameter were cored from the fillet and liver due to the nature of their muscle but, an approximate size of 10-15mm of the offal that could not be cored were cut with a sharp scalpel. The coring was parallel to the grain of the offal and shared perpendicular to the fiber direction using a Warner-Bratzler shear force (WBSF) device mounted on a Universal Instron machine (cross head speed = 400mm/minute, one shear in the centre of each core). Three replicate measurements were taken from each streak for WBSF test and the mean maximum load for the cores, which represented the average of the peak force (N) of each sample, was recorded.

### 9.2.4 Statistical analyses

The PROC GLM and PROC CANCORR procedures of the Statistical Analysis System (SAS version 9.1.3 of 2007) were used considering the effects of varying cooking regimes on the WBSF values of the offal from Dohne Merino castrates and ewes. Significant differences between the least square means were performed using the PDIFF test of SAS, with significance level of $p < 0.05$.

**9.3 Results**

**9.3.1 Effect of age on tenderness of Dohne Merino offal**

The result showing the effect of age on the tenderness of intestinal offal from Dohne Merino sheep is presented in Figure 9.1. A negative correlation ($r = -0.2$, $p < 0.05$) found between age and tenderness of reticulum implied that age had little effect on the variability of Warner-Bratzler Shear Force (WBSF) value recorded on reticulum. A positive relationship observed between age and tenderness of abomasum gave an indication that age of Dohne Merino at slaughter contributed significantly ($r = 0.4$; $p < 0.05$) to the tenderness of the ovine abomasum. It could be deduced therefore that abomasum of young Dohne Merino sheep or castrates would require lower shearing force and vice versa. Except for kidney and tongue, tenderness of most non-intestinal offal showed positive but weak relationships with the age of the sheep (Figure 9. 2). The strength of association found for tenderness of the tongue ($r = -0.4$, $p < 0.05$) revealed that the force required to shear the tongue is inversely associated with the age of the animal. The implication therefore is that tongues from ewes would be less tender and thus require high shearing force.

**9.3.2 Effect of cooking regimes on tenderness of Dohne Merino offal**

In general, based on gender effect and variations in the connective tissues, the WBSF values of the ewes were higher than the values obtained from the castrates. The results indicating the effects of gender and varying cooking regimes on the tenderness of intestinal Dohne Merino offal showed  that intestinal offal from the castrates were tender than those from the ewes (Table 9.1). However, the force required to shear the omasum did not produce any significant difference ($p > 0.05$) between the castrates and ewes.

Except the reticulum, all the intestinal offal cooked at 100$^{\circ}$C for 30 minutes had the least WBSF values suggesting that offal belonging to "welldone group" were tenderest.

As presented in Table 9.2, the effects of cooking regimes were statistically significant ($p < 0.05$) for most of the non-intestinal offal from castrates and ewes except the kidney ($p > 0.05$). Non-intestinal offal from the castrates were found having lower WBSF values as compared to those from the ewes. The effects of cooking regimes on the non-intestinal offal followed a similar pattern previously found in the intestinal ones with the welldone group having the least WBSF values. Intrinsically, the trachea from the ewes had highest WBSF values ($p < 0.05$) than others due to the presence of tough cartilaginous rings. Offal under the rare group had higher ($p < 0.05$) WBSF values and those in the welldone group ($p < 0.05$) produced lower WBSF values. These results therefore suggested that cooking offal at higher temperature within a shorter duration contributed significantly to its tenderness and vice versa.

**Figure 9. 1 Canonical correlation for the effects of age at slaughter on the tenderness of the intestinal offals**
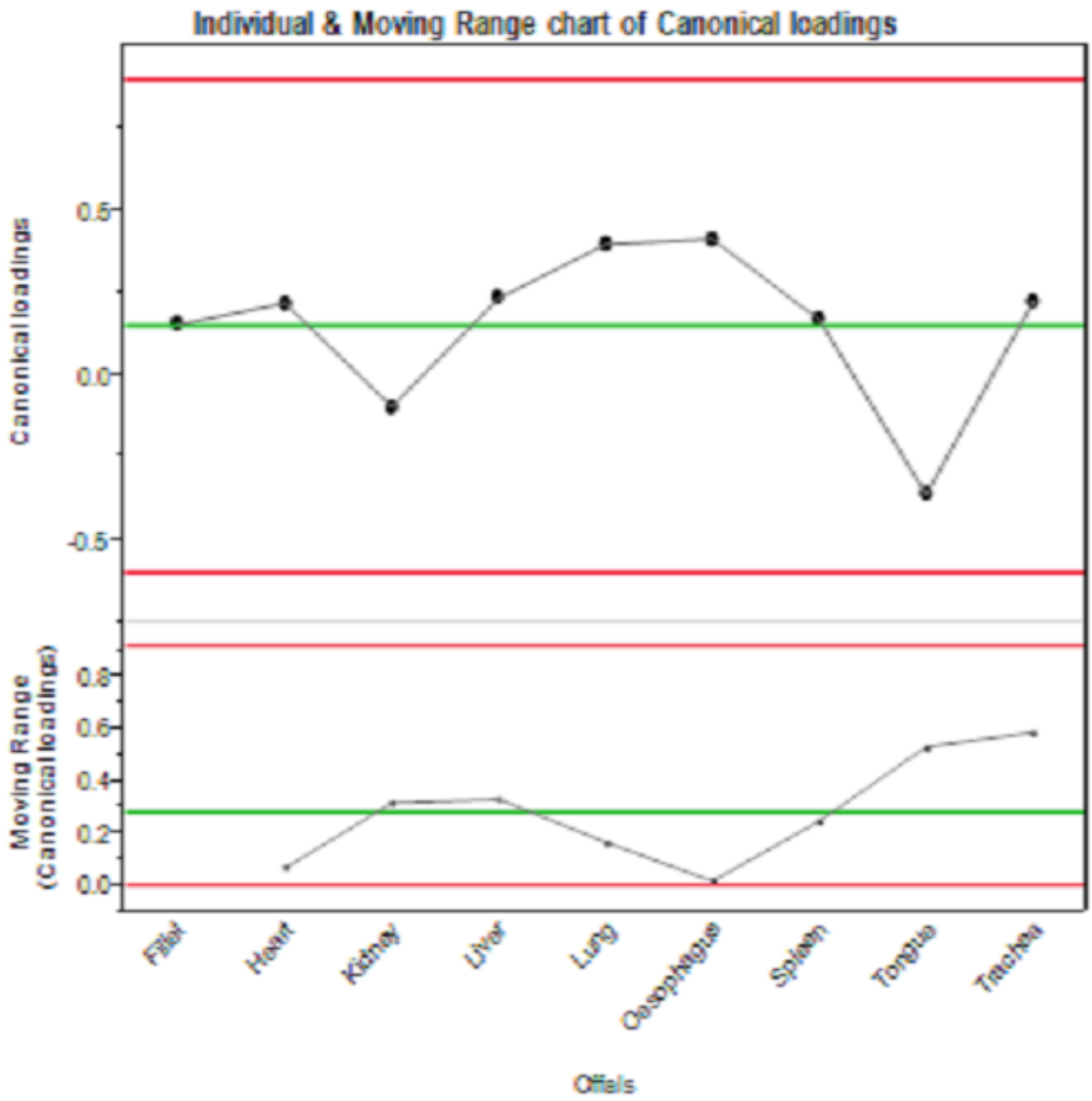
**Figure 9. 2 Canonical correlation for the effects of age at slaughter on the tenderness of non-intestinal offal**

**9.3.2 Relationships between cooking regimes and tenderness of Dohne Merino offal**

In general, based on gender effect and variations in the connective tissues, the WBSF values of the ewes were higher than the values obtained from the castrates. The results indicating the effects of gender and varying cooking regimes on the tenderness of intestinal Dohne Merino offal showed that intestinal offal from the castrates were tender than those from the ewes (Table 9.1). However, the force required to shear the omasum did not produce any significant difference ($p > 0.05$) between the castrates and ewes. Except the reticulum, all the intestinal offal cooked at $100^{o}C$ for 30 minutes had the least WBSF values suggesting that offal belonging to "welldone group" were tenderest.

As presented in Table 9. 2, the effects of cooking regimes were statistically significant ($p < 0.05$) for most of the non-intestinal offal from castrates and ewes except the kidney ($p > 0.05$). Non-intestinal offal from the castrates were found having lower WBSF values as compared to those from the ewes. The effects of cooking regimes on the non-intestinal offal followed a similar pattern previously found in the intestinal ones with the welldone group having the least WBSF values. Intrinsically, the trachea from the ewes had highest WBSF values ($p < 0.05$) than others due to the presence of tough cartilaginous rings. Offal under the rare group had higher ($p < 0.05$) WBSF values and those in the welldone group ($p < 0.05$) produced lower WBSF values. These results therefore suggested that cooking offal at higher temperature within a shorter duration contributed significantly to its tenderness and vice versa.
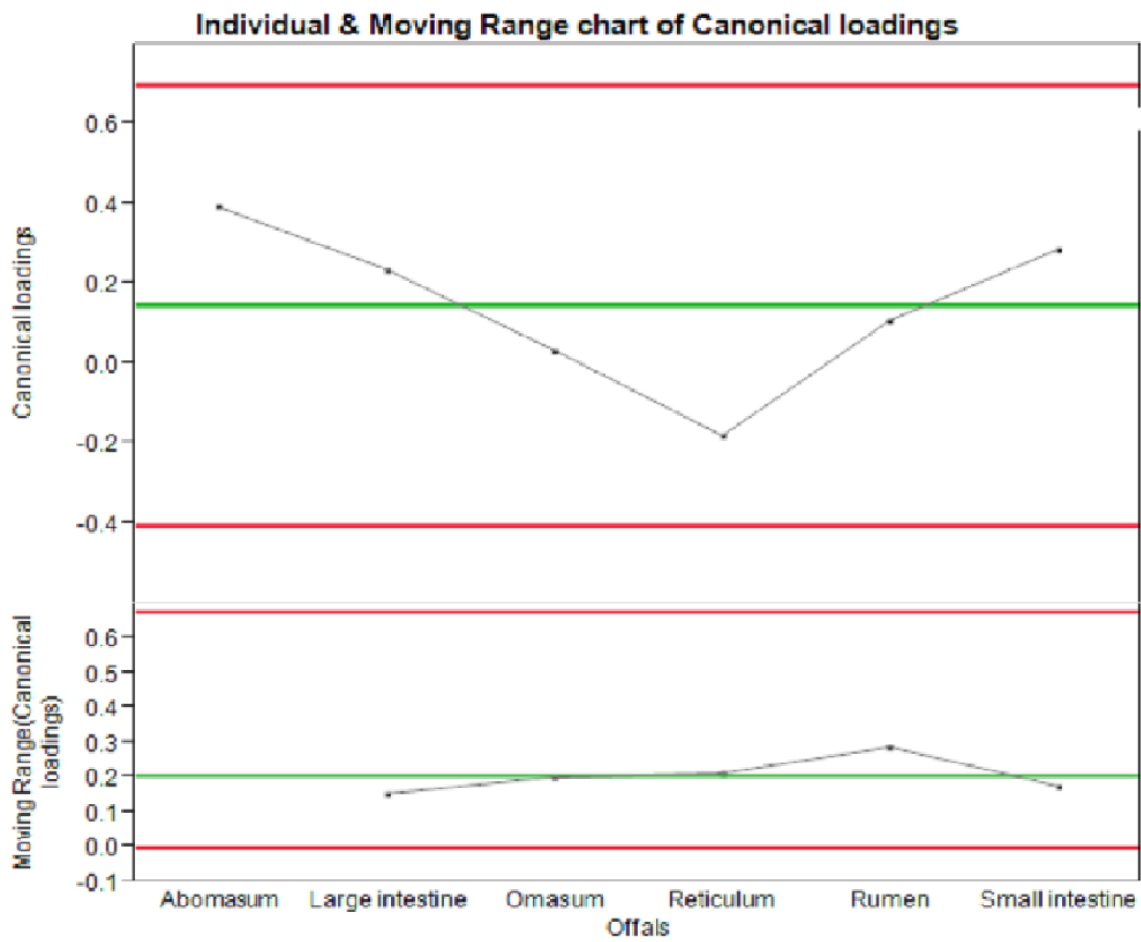
**Table 9. 1 Effects of gender and varying cooking regimes on the Warner-Bratzler Shear Force (WBSF) of intestinal Dohne merino offal**

| Offal | Gender | | Cooking regimes | | |
|---|---|---|---|---|---|
| | Ewe (n=69) | Castrate (n=69) | Rare[1] (n =69) | Medium rare[2] (n=69) | Welldone[3] (n=69) |
| Abomasum | $57.9 \pm 2.19^a$ | $40.9 \pm 1.55^b$ | $62.1 \pm 1.81^a$ | $48.6 \pm 2.01^b$ | $33.9 \pm 2.04^c$ |
| Large intestine | $49.8 \pm 1.85^a$ | $30.5 \pm 1.31^b$ | $66.2 \pm 2.02^a$ | $43.8 \pm 2.25^b$ | $30.2 \pm 2.29^c$ |
| Omasum | $59.1 \pm 4.32$ | $56.2 \pm 3.06$ | $86.1 \pm 2.62^a$ | $54.1 \pm 2.91^b$ | $28.4 \pm 2.96^c$ |
| Reticulum | $49.7 \pm 3.68^a$ | $44.2 \pm 3.68^b$ | $64.0 \pm 1.82^b$ | $83.2 \pm 1.30^a$ | $48.1 \pm 1.94^c$ |
| Rumen | $62.8 \pm 2.55^a$ | $57.6 \pm 1.81^b$ | $81.0 \pm 1.28^a$ | $61.2 \pm 1.42^b$ | $40.1 \pm 1.44^c$ |
| Small intestine | $45.3 \pm 2.26^a$ | $15.8 \pm 1.59^b$ | $63.7 \pm 2.86^a$ | $40.2 \pm 3.18^b$ | $16.0 \pm 3.23^c$ |

[a, b, c] Means within the same row having different superscripts were significantly different (p< 0.05); [1]Cooking at 75°C for 60 minutes; [2]Cooking at 88 °C for 45 minutes and [3]Cooking at 100 °C for 30 minutes

**Table 9. 2 Effects of gender and varying cooking regimes on the Warner-Bratzler Shear Force (WBSF) of non-intestinal Dohne merino offal**

| Offal | Gender | | Cooking regimes | | |
|---|---|---|---|---|---|
| | Ewe (n = 69) | Castrate (n = 69) | Rare[1] (n = 69) | Medium rare[2] (n = 69) | Welldone[3] (n = 69) |
| Fillet | $34.2 \pm 1.87^a$ | $23.4 \pm 1.32^b$ | $46.9 \pm 1.61^a$ | $25.6 \pm 1.79^b$ | $17.9 \pm 1.82^c$ |
| Heart | $33.2 \pm 1.80^a$ | $18.7 \pm 1.27^b$ | $31.9 \pm 2.13^a$ | $29.7 \pm 2.36^a$ | $14.7 \pm 2.40^b$ |
| Kidney | $18.1 \pm 0.96$ | $18.7 \pm 0.68$ | $24.9 \pm 0.56^a$ | $16.9 \pm 0.63^b$ | $12.5 \pm 0.64^c$ |
| Liver | $18.5 \pm 1.33^a$ | $13.4 \pm 0.94^b$ | $29.7 \pm 0.86^a$ | $12.9 \pm 0.96^b$ | $6.7 \pm 0.98^c$ |
| Lung | $25.5 \pm 1.23^a$ | $15.6 \pm 0.87^b$ | $31.5 \pm 1.07^a$ | $19.8 \pm 1.19^b$ | $10.8 \pm 1.21^c$ |
| Oesophagus | $104.0 \pm .78^a$ | $73.7 \pm 4.09^b$ | $132.0 \pm 4.31^a$ | $61.1 \pm 1.42^b$ | $40.1 \pm 1.44^c$ |
| Spleen | $27.4 \pm 1.59^a$ | $22.5 \pm 1.12^b$ | $36.2 \pm 1.16^a$ | $23.8 \pm 1.29^b$ | $15.2 \pm 1.32^c$ |
| Tongue | $35.0 \pm 3.05^a$ | $46.8 \pm 2.16^b$ | $59.8 \pm 2.15^a$ | $48.5 \pm 2.29^b$ | $21.7 \pm 2.32^c$ |
| Trachea | $114.7 \pm 4.63^a$ | $69.3 \pm 3.28^b$ | $135.2 \pm .55^a$ | $108.1 \pm 3.77^b$ | $53.9 \pm 3.82^c$ |

[a, b, c] Means within the same row having different superscripts were significantly different ($p < 0.05$); [1]Cooking at 75$^o$C for 60 minutes; [2]Cooking at 88 $^o$C for 45 minutes and [3]Cooking at 100 $^o$C for 30 minutes

**9.4 Discussion**

The current investigation addressed the effects of varying cooking regimes on the tenderness of ovine offal from Dohne Merino sheep. General findings from the study provided some insights on how various anatomical parts of the sheep responded to thermal treatment and different cooking durations. The characteristic thickness of the reticulum formed by mucosal layers and folds of *tunica muscularis* in the reticulum was responsible for the result obtained on the relationship between age and tenderness of reticulum from Dohne Merino sheep. Since no information is available on the relationship between age of sheep and the tenderness of its intestinal offal, it could be thought from the foregoing result that the three separate layers (*tunica muscularis)* of abomasum do not develop to maturity at the same time. As found in this study, the age-dependency of the maturation of its inner oblique, middle circular and outer longitudinal of the abomasum has a well defined thickness that commensurate with the advances in the animal age.

Similar to earlier report by Jeremiah et al. (1999) on lambs, the tenderness of offal from castrates could be attributed to the fact that muscles from lambs are younger than those from ewes were.  As stated by Purslow (2005), in view of the fact that ewes (used in this study) were older than the castrates were, it was possible that those ewes had stronger connective bonds and capacity to impose higher resistant on fiber dissolution during cooking. This invariably means that cooking meat at higher temperature for shorter period produces tender meat (Combes et al., 2003; Chiavaro et al., 2009). Conversely, Walsh et al. (2010) presented a contrary opinion indicating that slow cooking at 72$^{\circ}$C in a water bath had no significant effect on the tenderness of beef muscles. The disparity between the two results could be due to the type of muscle cooked, age of the sheep at slaughter and the condition of the meat being cooked (Tateo et al., 2008).

**9.5 Conclusion**

The present study has shown that various anatomical parts of Dohne Merino sheep responded differently to varying cooking regimes. Generally, because of age and gender differences, most offal from the ewes was tougher than those from the castrates were. The shearing force for the omasum and kidney from Dohne Merino castrates and ewes was not significantly different from each other. Among all the offal, trachea from Dohne Merino ewe had the highest Warner-Bratzler Shear Force (WBSF) value and kidney from the castrates, the least WBSF value. Sous vide cooking at $100^{o}C$ for 30 minutes produced the most tender offal from Dohne Merino sheep.

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**CHAPTER 10: General Discussion, Conclusion and Recommendation**

**10.1 General discussion**

The Eastern Cape Province has the largest agricultural sub-sector in the Republic of South Africa with significant numbers of sheep and other livestock (Eastern Cape Development Corporation, 2011; National Department of Agriculture, 2010). The concentration of the farmer has been found to be highest in the ECP bacuase of the agro-ecological suitability of the province for sheep husbandry. Although it is assumed that the perception of farmers on the *ante-mortem* welfare of the available sheep genotypes has the potential to boost quality of ovine products such as offal. On record, the pre-slaughter perceptions of farmers who raise these sheep for meat and meat products is not certain. As one of the key objectives of the current study, a survey was conducted and their opinions about the *ante-mortem* welfare and slaughter indicators for Dohne Merino sheep were also investigated. The null hypohesis ($H_o$) tested in Chapter 3 was that the perception of the farmers on the significnce of *ante-mortem* welfare of sheep and their slaughter indicators were not different.

On the contrary, the result showed significant differences which led to the rejection of the null hyphotesis. It was observed in Elundini that male farmers were more enlightened about the importance of pre-slaughter welfare of sheep than the female farmers. This still confirms an established norm on gender inequality or male dominance in livestock husbandry. Partly due to efforts from the Internal Strategic Perspective (Alfred Nzo District Municipality, 2010; Draft Integrated Development Plan Review, 2010) and indigenous knowledge, majority of the farmers considered age of the animal, live weight and winter season as crucial slaughter indicators for sheep. The outcomes of the study suggested that farmers in most Eastern Cape Province Municipalities do not have adequate knowledge on *ante-mortem* welfare of sheep and their slaughter indicators.

Similar to the suggestion by Dwyer (2009), it is recommended that emerging and established farmers are motivated to adopt international welfare quality codes to improve on their perceptions on pre-slaughter welfare of their stock and slaughter indicators. In line with this, another hypothesis ($H_o$) was tested with the aim of investigating the preference of Xhosa speaking farmers for mutton, lamb and various anatomical parts from the natural velds (Chapter 4). Rejection of the $H_o$ hypothesis was also found since majority of the respondents indicated highest preference for "mutton only". Evidence was as well provided on multiple preferences for different anatomical parts by all the farmers. The male farmers and those within 51-60years nevertheless indicated more interest in various meat types from sheep than the females and younger farmers. These were majorly found to be caused personal interest and the traditional beliefs of the farmers.

It is also worth noting that the pain inflicted on the sheep during stunning did not give substantive evidence that "head-only" stunning method is pain-free (Chapter 5). The hypothesis tested ($H_o$) in this regard assumed no differences in the real-time quantitation of UCH L1 in the castrates and the ewes of Dohne Merino sheep. The choice of a brain specific biomarker (UCH L1) has provided evidence that about 50% of the head-stunned sheep experienced some degrees of avoidable pain before exsanguination. Differential expressions of the ovine UCH L1mRNA therefore gave an indication that bio-impedance against electric insults is higher in ewes than in castrates. These findings also led to the rejection of the tested hypothesis and the acceptance of the alternative hypotheis. Based on the outcome of this quantitation (ovine UCH L1mRNA), the stakeholders in the meat industry would need some orientations that can boost the technical efficiency of their stunning standards at the slaughterhouses.

As a follow up, a monitoring study was then undertaken to assess the pregnancy status of the ewes offered for slaughter  under the stunning condition above. The null hypothesis tested was that none of the Dohne Merino ewes offered for slaughter was pregnant or that the expressions of ovP4 & ov mRNA by the sheep was not different (Chapter 6). The aftermath of the diagnostic test with the use of highly sensitive pregnancy biomarkers (ovP4 and ovPAG 1mRNA) disproved the tested hypothesis. Thus, the Radio-immunology assay (RIA) for ovP4 showed that about 22% of  the ewes were "truly pregnant" at slaughter and the TaqMan RT-PCR method produced more pregnancy ($\Delta$mRNA) signals of 44% than RIA. This implies that pregnant ewes are being converted into meat in various registered abattoirs and consumers eat this meat without knowing the status of the animal before slaughter.

In Chapter 7, the muscular fibre and the nutrient constituents were characterised using energy dispersive x-ray spectroscopy but dissimilarities were found among the offal. The hypothesis that the fibre orientations and nutrient constituents of the offal Dohne Merino castrates are similar to those of the ewes was tested. The need to reject this hypothesis was found from the study because the energy dispersive x-ray spectroscopy showed uniformity in fibre orientation (isotropy) for the lung, mouth muscle and fillet. In addition, the fibre length of the mouth muscle and the fibre thickness of the lung from the castrates also differed significantly. Higher crude protein and digestible crude protein contents were observed in the lung of the castrates as compared to other offal. High aluminium and sodium contents were detected in the small intestines of ewes. The type of offal, gender and status of the sheep significantly influenced the muscular fibre characteristics, the nutrient compositions and tenderness of Dohne Merino offal.

However, the relationships between ambient conditions, pH and colour parameters of of Dohne Merino offal were compared (Chapter 8). The comparism was done on the basis of the Ho that the ambient conditions had similar effects on the pH and colour development of the offal. Results showed a moderate relationship between the ambient temperature (AT) and the saturation index (SI) for two non-intestinal offal namely, the trachea and heart. An inverse relationship was obtained between the dew point and the total colour difference ($\Delta E^*$) for the fillet. The canonical relationship between the relative humidity and the whiteness index (WI) of both intestinal and non-intestinal offal followed a similar pattern. Only the spleen, kidney and liver attained their ultimate redness (CIEa*) at 30hours post-slaughter. These results invariably implied the rejection of the null hypothesis. Further tests on the impacts of cooking regimes on the tenderness of the offal from the castrates and ewes were also conducted (Chapter 9). Thus, it was therefore hypothesised that cooking regimes had similar effects on the tenderness of the offal from castrates and ewes of different age groups. Varying responses of these anatomical parts (or offal) to thermal treatment consequently provided evidence to reject the null hypothesis. It was observed that various anatomical parts of Dohne Merino sheep responded differently to varying cooking regimes .The offal that were cooked at the boiling point for the shortest duration of 30 minutes, were found to be more tender than those in other cooking groups.

**10.2 Conclusions**

Poor response received from sheep farmers on pre-slaughter welfare has established the need for urgent awareness on *ante-mortem* welfare of their ovine species in ECP of South Africa. High relationship between liver and lung, intestine and heart, head and intestine implied Xhosa speaking farmers place high premium on these organs as their choicest meat from sheep. It could be inferred that personal interest is the most important factor influencing the consumption and preference for mutton and lamb by Xhosa speaking farmers. It is obvious in the study that slaughtering an approximate of 43%, six-tooth ewes Dohne Merino portends the risk on the extinction of genetic resources Dohne Merino if the trend goes unchecked. As recently reported for beef (Sepehr et al., 2012), the practice of slaughtering breeding pregnant ewes for meat or offal production could be a constraint to security of products from sheep. Hence, threatening the sustainability of mutton-lamb production from such a synthetic genotype.

However, the use of UCH L1 as a brain-specific neuronal biomarker for assessing pain in sheep has shown that electrical stunning does not guarantee zero-pain for the head stunned sheep. The impact of this practice might be severe on the pregnant ewes and their offspring when stunned electrically before exsanguination. The variations in fibre characteristics, tenderness and nutritional compositions in the examined offal were largely influenced by their anatomical differences, physiological status and gender of the sheep. Impact of ambient conditions on the eating quality of the offal has shown that colour coordinates of the meat could be compromised if the immediate environment is not congenial to sustain the desirable traits in the meat. Cooking Dohne Merino offal at $100^{\circ}$C for 30 minutes in an enclosed temperature-controlled water bath would produce tender meat for consumers.

**10.3 Recommendations for further research**

More emphasis should be placed on pre-slaughter welfare of ovine species in Eastern Cape Province. Workshops, farmers' summits, seminars and other relevant forums should be organised for sheep farmers to address factors that could affect the *ante-mortem* welfare and quality of its meat or offal. Other aspects that require further research include:

1. Balancing gender inequality in sheep farming in ECP and for achieving a balance in pre-slaughter welfare of sheep in the entire Province

2. Use of more pain-biomarkers to assess the technical efficiency of stunning methods on bio-impedance of many meat species

3. Non-invasive diagnostics ewes at lairage, determination of pregnancy stage, type of pregnancy (normal or ectopic), foetal sex, foetal number (single, twins, triplets, multiples). *Post-mortem* examination of the *corpus lutea*, ovaries will be needed to further confirm the ovPAG mRNA signals from the blood samples.

4. Food enrichment studies on the amelioration of nutrient imbalance in sheep offal.

5. Standardising ambient conditions that give optimal eating quality of offal from sheep

**10.4 References**

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**11: Appendices**

**Appendix 11.1: Perception of farmers on *ante-mortem* welfare of sheep in Eastern Cape Province**

**A. Sheep farmers' demographic information**

1. What is your gender?   Male  ☐  Female  ☐

2. What is your age group? < 30 years ☐   31-40 ☐   41-50 ☐

   51-60 ☐   > 60 years ☐

3. Indicate your municipality where your sheep farming is based.....................................

4. Which breed of sheep do you rear on your farm? You are allowed to tick more than one options in the table below:

| S/No | Breeds of sheep | Yes | No |
|------|-----------------|-----|-----|
| i | South African mutton Merino | | |
| ii | Dohne Merino | | |
| iii | Dorper | | |
| iv | Merino crosses | | |
| v | Others | | |

**B. Pre-slaughter welfare of sheep**

5. Do you agree that issues on pre-slaughter welfare of sheep are important?

Yes ☐ No ☐ Not Sure ☐

1.  At what **age** do you consider proper to dispose your sheep breeds for slaughter without compromising their welfare? You are allowed to tick more than one option (s) please.

| S/N | Sheep breeds | ≤ 1 yr | 1-2yrs | 2-3yrs | >3yrs |
|---|---|---|---|---|---|
| i | South African mutton Merino | | | | |
| ii | Dohne Merino | | | | |
| iii | Dorper | | | | |
| iv | Merino crosses | | | | |
| v | Others | | | | |

2.  At what **live weight** do you consider proper to dispose your sheep breeds for slaughter without compromising their welfare? You are allowed to tick more than one option (s) please.

| S/N | Sheep breeds | ≤ 40kg | 41-49kg | 51-59kg | >60kg |
|---|---|---|---|---|---|
| i | South African mutton Merino | | | | |
| ii | Dohne Merino | | | | |
| iii | Dorper | | | | |
| iv | Merino crosses | | | | |
| v | Others | | | | |

3. At what **<u>season</u>** do you consider proper to dispose your sheep breeds for slaughter without compromising their welfare? You are allowed to tick more than one option (s) please.

| S/N | Sheep breeds | Autumn | Spring | Spring | Winter |
|-----|-------------|--------|--------|--------|--------|
| i | South African mutton Merino | | | | |
| ii | Dohne Merino | | | | |
| iii | Dorper | | | | |
| iv | Merino crosses | | | | |
| v | Others | | | | |

C. **Perceptions of sheep farmers on sheep meat**

10. Do you eat sheep meat?

Yes, mutton only [ ]    Yes, both mutton and lamb    [ ]

Yes, lamb only [ ]    No interest    [ ]

4.  What do you consider as meat in sheep? Please tick either Yes or No in the table

    below:

| Sheep Part | Yes | No | Sheep part | Yes | No |
|---|---|---|---|---|---|
| Loin |  |  | Liver |  |  |
| Shoulder |  |  | Lung |  |  |
| Neck |  |  | Intestines |  |  |
| Back |  |  | head |  |  |
| Brain |  |  | Kidney |  |  |
| Blood |  |  | leg |  |  |

5.  Tick the reason (s) why you think that your choice of either yes or no in the question

    above is right.

    Religion [ ] Tradition [ ] Personal interest [ ]

    Professional ethics [ ]    Others [ ]

**Appendix 11.2:** Meat in African context-from history to science by *African Journal of Biotechnology* Vol. 11(6), pp. 1298-1306.

**Appendix 11.3:** Maternal slaughter at abattoirs: history, causes, cases and the meat industry by *SpringerPlus* 2013, 2:125 (DOI: 10.1186/2193-1801-2-125).

**Appendix 11.4:** Biomarkers coding for ov PAG-1 mRNA expression and pregnancy status in Dohne Merino ewes at an abattoir by *Tropical Animal Health and Production* (DOI 10.1007/s11250-013-0404-5).