

**EFFECT OF INDIGENOUS SLAUGHTER METHODS ON POST-MORTEM  
GLYCOLYTIC POTENTIAL AND SHELF-LIFE OF GOAT MEAT**

**MASTER OF SCIENCE IN AGRICULTURE  
(ANIMAL PRODUCTION)**

**C. M. SELOANE**

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GLYCOLYTIC POTENTIAL AND SHELF-LIFE OF GOAT MEAT**

by

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**SUPERVISOR: Dr ZM MDLETSHE**

**CO-SUPERVISOR: PROF O TADA**

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## **DECLARATION**

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Animal Production) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all materials contained herein has been duly acknowledged.

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**Seloane, CM (MR)**

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**Date**

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Everyone has a journey uniquely destined to travel on their own, and I quote “*if I have seen further [than others], it is by standing on the shoulders of the giants*” – Isaac Newtons. For the completion of this work, it would be remis of me to not take this opportunity and express my gratitude for all the support I have received to complete all the chapter contained herein my thesis. Postgraduate is a research journey full of headache, however, also comes with indescribable blessings that set one apart and I just have to acknowledge. With that being said, I would first like to acknowledge the commitment, unwavering support, and supervision bestowed by my supervisor to me Dr ZM Mdletshe, for making this work what it is. I will forever be indebted to you and thank you for ensuring a successfully completion of this dissertation.

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## **DEDICATION**

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## List of acronyms

<	less than
>	greater than
0d	same day of slaughter
14d	day fourteen of slaughter
1d	day one of slaughter
3d	day three of slaughter
7d	day seven of slaughter
ADP	adenosine diphosphate
ATP	adenosine triphosphate
CFP	chest floor piercing
CFU	colony forming unit, an estimate of variable number of bacteria
CIKS	centre for indigenous knowledge systems
CO <sub>2</sub>	carbon dioxide
DFD	dry, firm, dry or dark-cutting meat
<i>E. COLI</i>	<i>escherichia coli</i>
GP	glycolytic potential
H <sup>+</sup>	hydrogen ions
H <sub>2</sub> S	hydrogen sulphide
IK	indigenous knowledge
ISM	indigenous slaughter method (s)
<i>LS</i>	<i>longissimus dorsi</i>
<i>LTL</i>	<i>longissimus thoracis et lumborum</i>
MAP	modified atmosphere packaging
MMA	methylmalonic acid
NaCl	sodium chloride
a*	redness
b*	yellowness
L*	lightness
C*	chroma
H*	hue angle

NS	not significant
R	Pearson correlation
SAS	Statistical analysis systems
pHu	Ultimate pH
O <sub>2</sub>	oxygen
SNP	suprasternal notch piercing
TNI	transverse-neck insertion

## Abstract

The objectives of the study were to determine the effect of Transverse neck incision (TNI), Suprasternal notch piercing (SNP), and the under shoulder blade piercing at the chest floor point of the elbow (CFP) to the direction of the heart methods of slaughter treatments on post-mortem glycolytic metabolites and shelf-life properties of muscle tissue. A total of 18 non-descript male castrate goats which are a common type of goat found in villages across South Africa (averaging  $16.8 \pm 1.84$  kg) with live weights ranging between 18 and 21 kg, and sixteen months of age, were stratified based on live weight and randomly assigned into the TNI, SNP, and CFP slaughter treatments (6 goats/treatment). Goats were slaughtered with TNI, SNP, and CFP based on their treatment group and the *Musculus longissimus thoracis et lumborum* (LTL) was sampled at post-mortem for muscle tissue glycolytic potential, glycogen, lactate levels, ultimate pH, and colour measurements. Overall, the TNI, and CFP slaughter treatment had lower muscle tissue ultimate pH in addition to higher glycolytic potential, glycogen, lactate levels, redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ) values. The TNI and CFP slaughter treatments had higher glycolytic potential, glycogen, and lactate levels, consequently, improving the physicochemical properties of muscle tissue.

Overall, the slaughter technique did not influence ( $P \geq 0.05$ ) muscle tissue pH. Muscle tissue redness ( $a^*$ ) decreased over time, being highest ( $P \leq 0.05$ ) at day 0, followed by day 7 ( $P \leq 0.05$ ), and 14, respectively. Muscle tissue yellowness ( $b^*$ ) decreased ( $P \leq 0.05$ ) over time being highest for the CFP ( $P \leq 0.05$ ) on day 3, followed by the TNI and SNP ( $P \leq 0.05$ ) at day 7, and CFP ( $P \leq 0.05$ ) on day 11, respectively. Muscle tissue lightness ( $L^*$ ) decreased over time, being highest at day 0, followed by day 11, and day 3 being the lowest, respectively. The shelf-life of muscle tissue from goats slaughtered with TNI was longer compared to the SNP and CFP slaughter treatment.

**Keywords:** Glycolytic potential; lactate; Muscle tissue pH; indigenous slaughter methods; redness; yellowness.

## **CHAPTER 1: GENERAL INTRODUCTION**

### **1.1 Background**

In developing countries such as Africa and the Middle East, goat meat constitutes an important role as a high protein source for resource-limited households (Dossa *et al.*, 2015). The oxidative status of goat meat, causing them to become susceptible to lipid oxidation (Faustman *et al.*, 2010), results in high odour intensity and shorter keeping properties, triggering resource-limited households to be careful in the type of slaughter method to use when slaughtering a goat (Webb and O'Neill, 2008; Mdletshe *et al.*, 2021).

The demand and consumption of red meat in Southern Africa exceed the supply (Webb, 2014; RPO, 2018). Consumption of goat meat is generally low in developing countries (Webb, 2014). The major constraint is the age at slaughter, poor pre-slaughter care, and slaughtering of goats using indigenous techniques (Mdletshe *et al.*, 2020; 2021) which increases antemortem stress levels due to poor handling (Pophiwa *et al.*, 2020), thereby, exacerbating post-mortem oxidative status of muscle tissue. Successful progress has been made to explore alternative slaughter techniques that reduce antemortem stress, and post-mortem oxidative status of meat (Simela *et al.*, 2004; Sabow *et al.*, 2016, 2017). The comparison of slaughter techniques was based on stunning before slaughter and slaughter without stunning with little or no emphasis on respecting cultural standards. Although conventional slaughter methods improved the oxidative status of goat meat, therefore, physicochemical properties, glycolytic metabolites, and shelf-life properties, these techniques are conventional, which include making the animal unconscious before bleeding and, therefore, not culturally friendly. Some indigenous slaughter techniques can likely reduce the oxidative status of goat meat, improving the physicochemical, glycolytic metabolites, and shelf-life properties.

### **1.2 Problem statement**

Goats are important to resource-limited households in developing countries (Rumosa-Gwaze *et al.*, 2009). They provide meat, milk, and additional household income by selling them as live animals (Mdletshe *et al.*, 2018). However, indigenous methods are been utilised by the resource-limited households to slaughter goats without the practice of stunning (Mdletshe *et al.*, 2020). This is why goat meat normally has an

oxidative status of higher lipid oxidation rate, therefore, less glycolytic metabolites, poor physicochemical, and shelf-life properties (Simela *et al.*, 2004).

The possible lactate formed in the muscle at exsanguination represents the glycolytic potential as an expression of the energy level of the entire animal before slaughter (Maribo *et al.*, 1999; Roth *et al.*, 2006; Przybylski *et al.*, 2016). Lactate is also the result of the anaerobic digestion of glycogen into ATP, while glucose and glucose-6-phosphate are the intermediate products produced during the transformation process (Monin and Sellier, 2015; Pointon *et al.*, 2018). Glycolytic potential indicates the muscle glycogen concentration at pre-slaughter, which regulates the frequency of pH decline in meat post-mortem (Bakhsh *et al.*, 2019).

Efforts to understand the effect of indigenous slaughter techniques on post-mortem glycolytic metabolites, and physicochemical and shelf-life properties of goat meat, have focused on halal and kosher religious methods (Fuseini *et al.*, 2016; Sabow *et al.*, 2016), and paid little attention to indigenous slaughter methods practiced by resource-limited farmers in developing countries (Khalid *et al.*, 2015). The slaughter method promotes the rate at which glycogenolysis occurs in the muscle by depleting muscle glycogen content due to ante- and peri-mortem physical exhaustion (Grandin, 2017). Such evidence is not conclusive on indigenous slaughter methods and thus merits further investigation.

### **1.3 Justification**

The effect of TNI, SNP, and CFP is poorly understood. Such knowledge is crucial in making recommendations on an alternative slaughter method that is culture-friendly while improving glycolytic metabolites, physicochemical, and shelf-life properties of goat meat. The knowledge is crucial in paradigm shift and improving goat meat consumption, through the reduction of oxidative status. The effect of TNI, SNP, and CFP slaughter techniques on glycolytic metabolites, physicochemical, and shelf-life properties are likely to differ (Mdletshe *et al.*, 2021). Assessing the effect of TNI, SNP, and CFP slaughter techniques will contribute to the selection of a method that is culture-friendly while improving the oxidative status, physicochemical, and shelf-life properties of goat meat.



#### **1.4 Aim**

The study aimed to determine the relationship between TNI, SNP, and CFP on the post-mortem glycolytic metabolites and shelf-life of goat meat.

#### **1.5 Objectives**

The objectives of the study were to determine:

- i. The effect of TNI, SNP and CFP slaughter methods on post-mortem glycolytic potential, glycogen, lactate formation, ultimate meat pH (pHu), and colour.
- ii. The effect of TNI, SNP and CFP slaughter methods on post-mortem meat shelf-life.

#### **1.6 Hypothesis**

The hypotheses of the study were as follows:

- i. Slaughter methods do not affect post-mortem ultimate pH and colour, glycolytic potential, glycogen, and lactate formation of goat meat.
- ii. Slaughter methods will not affect post-mortem meat pH and colour in goat meat postmortem.

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## **CHAPTER 2: Literature review**

### **2.1 Introduction**

Generally, many control measures that have been exercised at slaughter are mainly to cease the contamination of meat as one of the reasons to improve its shelf-life. Even though food-borne infections are minimalised, the methods to slaughter animals particularly goats without prior stunning continue to be a global immense contributing factor to the meat shelf-life (Global Salm-Surv, 2003; Hendriksen *et al.*, 2009). In rural communities of Southern Africa, meat with a shortened shelf-life continues to be reported from indigenous slaughter-related practices (Sabow *et al.*, 2017; Mdletshe *et al.*, 2021). In this regard, this misery adds to the associated financial losses as a result of rapid meat spoilage and enormous food insecurity. Recognition of the type of slaughter before consumable meat can be derived from the live animal is, therefore, crucial in prolonging meat shelf-life (Qekwana *et al.*, 2016). Consumers demand is one which account for consumption of meat with high eating quality and safer for consumption with the progression of time (Bryhni *et al.*, 2002). The most basic high-quality meat associated traits are an attractive meat colour, meat juiciness and meat tenderness, and as well as flavour intensity (Simela *et al.*, 2004). In favour of the demands made by consumer on meat, goat meat role players in slaughter practices are persuaded to account for targeting and exerting methods of producing high-eating quality goat meat to consumers that can last longer (with prolonged shelf-life) at storage.

Mead (2004) highlighted how the slaughter procedure is practised and the glycogen levels as energy utilised in muscles during slaughter are all equated as a quality assurance on the shelf-life of meat for consumers, retailers, and producers. This meat shelf-life is conditional to the practices involved on the animal at slaughter (for instance, slaughter with stunning (SS) vs slaughter without stunning (SWS), processing which includes carcass chilling, condition of storage, and most importantly, the level of glycogen reserves in the body at post-slaughter (Huda *et al.*, 2012). Meat cuts for consumption are derived from live animals following either or both formal and informal slaughter sectors in South Africa and as well as other developing countries (Govender and Genis, 2010; Fayemi, 2013).

These slaughter sectors are both influencing the shelf-life of meat, which ultimately influences the acceptability of meat at purchase by consumers and in the long run, food security (Qekwana *et al.*, 2015). Informal slaughter sector of animals is by virtue

the opposite of formal slaughter whereby stunning practice is not implemented before slaughter (Van der Merwe, 2015).

## **2.2 Meat shelf-life**

Meat shelf-life components begin immediately after muscle-to-meat conversion is accomplished within the skeletal muscles (Lonergan *et al.*, 2010; Kim *et al.*, 2010), and are coupled with elements affecting meat discolouration such as meat colour and pH. This is because, at this stage, the animal slaughtered achieves its death, and the muscles are no longer supplied with sufficient glucose as energy (Sabow *et al.*, 2017). Meat spoilage is an inevitable problem during retail and storage, and therefore, pre-slaughter techniques which contribute to this challenge significantly merit attention. Common elements which are the most determinant factors for the shelf-life falls under two major categories: slaughter technique adopted and glycogen reserves (glycolytic potential) at post-mortem (Simela *et al.*, 2014; Ndaeva, 2018). The slaughtering technique is explicitly a pre-meat harvest approach, whereas glycogen reserves in muscles can be either post- or pre-harvest applications.

## **2.3 Determining the onset of meat shelf-life**

The onset of meat shelf-life is usually from post-slaughter abattoir operations, which have meat assessors who officially authorize meat which meets the standard for consumption (Gama, 2017). Consumers then purchase the meat through retailer distributions. The Meat Safety Act No. 40 of 2000 is one entity which governs meat product distribution (Rani and Pradhan, 2021; Rani *et al.*, 2023). The approval of meat for consumption is usually performed through visual inspection by meat inspectors within this sector (Njoga *et al.*, 2023). However, microbiological tests are not performed at the early stage of meat inspection, the reason being that meat is not subjected to microbial spoilage such as bacteria within fewer days of production at storage (Govender *et al.*, 2013).

The associated primary cause of shortened meat shelf-life is recently reported to be the glycogen in muscle tissue of goat meat, which caters for energy reserves at post-mortem (Webb *et al.*, 2018). This, consequently, subjects the meat's shelf-life analysis to provide an essential meat quality control role of food security. In South Africa, two governing entities primarily liable for dealing with meat production safety, processing

and the security of food are, “namely the Department of Agriculture, Forestry & Fisheries (DAFF) and the Department of Health (DoH) (Govender *et al.*, 2013).” However, these collective meat monitoring representatives still requires insightful data on the effect of informal slaughter on the role post-mortem glycolytic potential plays on meat shelf-life in place (Mdletshe *et al.*, 2020; 2021). For that reason, this data can benefit and improve the production of goat meat under resource-limited on indigenous slaughter methods adhered and utilised in rural based sectors. Therefore, a study that would provide conclusive information on the effect that energy reserves in the meat samples at post-mortem has for improvement production of meat shelf-life and would incorporate adaptable informal slaughter strategies, which results in meat production for consumption with prolonged shelf-life, thus as one strategy to contest food insecurity, is encouraged.

### **2.3.1 Management of goat meat and its shelf-life in resource-limited areas**

According to Adzitey and Nurul (2011), consumers purchase meat of poor-quality meat sold at retail outlets in developing countries such as in rural communities. This conforms to a result of less attention given to standard and handling methods of slaughter, which accommodates and safeguard the welfare of the animal. For instance, in South Africa, predominantly most rural areas, animals are not stunned before slaughter (Mdletshe *et al.*, 2021). This is mainly because cultural and religious beliefs sometimes dictate the slaughter methods (Mdletshe *et al.*, 2021). Consequently, these non-stunning slaughter practices become a considerable contributing factor to the misery caused by the meat shelf-life. However, heeding proper standards and slaughter procedures, as well as the use of households' by-products rich in glucose solution to treat meat cuts may aid in counteracting this challenge (Adzitey and Nurul, 2011).

### **2.3.2 Factors determining the shelf-life of goat meat**

Meat as a food product which is prone to rapid spoilage, it can impose a serious health risk if handled improperly (Bukachi *et al.*, 2021). Excess depletion of glycogen reserves in muscles due to pain experienced during slaughter may result in meat prone to bacterial growth pathogens in the early days of storage. This has been distinguished as a major hindrance to the prolonged shelf-life storage and safety of meat (Seeiso, 2009). Some of the serious complications associated with human health

arises from these bacteria which may be pathogenic micro-organisms, which spoil meat during its shelf-life duration and include spp. of *Diarrheagenic Escherichia coli* (Hobbs and Roberts, 1993; Yafetto *et al.*, 2019). The South African meat industry has recently reported from a government-commissioned database on the estimated economic costs in meat shortages, expenses, and absence from retailers as a result of the product lasting only for fewer days on the shelves (Meissner *et al.*, 2013; Rani *et al.*, 2017). This report endorses a review of management strategies to improve meat shelf-life and quality assurance slaughter practices (Nkwana, 2015; Drimie, 2016). Therefore, continuous efforts to derive and harvest meat cuts with improved shelf-life from live animals to be sold in retailers are greatly encouraged.

Further, the research focus should cover and credit all number of processes involved from the production to purchase and consumption stage of meat, including how the animals were raised, as feeding (type of feed) can to some extent, influence the wholesome meat appearance, transportation logistics, and slaughter practices. In all these stages, meat shelf-life can be influenced greatly and is paramount to carefully assess these stages for meat quality assurance (McDonald and Sun, 1999; Faria *et al.*, 2022), the reason being most consumers hold least knowledge or information on all parameters of meat quality. This results in consumers considering only the perceived quality and appearance of the product when making decisions during purchase (Becker *et al.*, 2000).

### **2.3.3 Effect of post-mortem storage duration on the shelf-life of goat meat**

Meat quality deterioration determined by the shelf-life begins at d0 (same day of animal slaughter) just after slaughter and continues through until when meat is purchased and cooked (Huda *et al.*, 2012). Many irreversible processes which drive the spoilage of meat, particularly regarding the microbiological process by bacterial growth during meat storage are a result of the lack of glycogen reserves in the carcasses of muscles (FAO, 1990). These micro-organisms utilize the less available energy which is insufficient in muscles for their growth. Meat with a shortened shelf-life, other than affecting food security, may also constrain producers to start selling it while unhygienic at lower prices in retailers (Qekwana *et al.*, 2012; Qekwana and Oguttu, 2014) to compensate for their production losses, thus endangering the health of consumers.

Meat undergoes several changes during which its quality deteriorates due to processes involving microorganisms, thus negatively affecting its storage and shelf-life placement days (Sabow *et al.*, 2016). These challenges, which influence the shelf-life of meat in most previously researched studies are not well outlined. For instance, changes in the glycolytic metabolites in muscle tissue post-mortem. Most studies have reported several pre-slaughter practices at the farm level which affect meat-eating quality (Ferguson and Warner, 2008; Guarnido-Lopez *et al.*, 2022), during the abattoir transportation of animals (Chulayo and Muchenje, 2013; Gajana *et al.*, 2013; Vimiso and Muchenje, 2013), at the periods of pre-slaughter (Miranda-de la Lama *et al.*, 2009, Muchenje *et al.*, 2009; Muchenje and Ndou, 2012) and the methods used during slaughter (for instance; stunning or non-stunning practice) (Muchenje *et al.*, 2009; Miranda-de la Lama *et al.*, 2012; Njisane and Muchenje, 2013)."

#### **2.3.4 Slaughter effect and the shelf-life of meat**

Processes that arise after animals have been informally slaughtered in rural setups, affecting the shelf-life of meat during its storage and retailers, are deserted (Qekwana *et al.*, 2015), yet the meat derived from these animals is considered the most important aspect in the communities (McMillin and Brock, 2005; Qekwana *et al.*, 2015). This is because meat contributes to the food security of people residing in rural communities. Even though focus of other studies was done on meat post-slaughter on its microbiological quality (Gill, 1996; Adzitey and Nurul, 2011; Huda *et al.*, 2012; Niyonzima *et al.*, 2013), post-mortem analysis and effect for glycogen in muscles of carcass on the meat shelf-life during informal animal slaughter if any, is not well outlined.

#### **2.4 Role of post-slaughter carcass energy reserves in muscles of goat meat**

Slaughter procedures are regulated by laws to ensure animals are not slaughtered inhumanely to prevent unnecessary pain in minimizing needless animal cruelty (Grandin, 2005). The post-mortem energy reserved in muscle tissue influenced by the method of slaughter used is therefore, of paramount importance to the shelf-life of meat products (Simela *et al.*, 2004). Since meat of high quality is a prerequisite demanded by consumers, various approaches have been studied to merge the demand. Nutritional and feeding strategies form part and parcel (Andersen *et al.*, 2005), and the welfare of the animal when transporting (Hughes *et al.*, 1996), and at



slaughter (Chulayo and Muchenje, 2013). Even so, many post-slaughter practices with post-mortem glycolytic potential analysis being one, contribute significantly to the shelf-life of meat Huda *et al.*, 2012). These practices comprise the way animals exhaust the glycogen levels in muscles to counteract the pain inflicted during slaughter, chilling, and other factors (Simela *et al.*, 2004). Although extensive research work on addressing some of these factors has been done, the scarcity of knowledge on aspects influencing meat shelf-life at the post-mortem stage remains multifaceted and is yet to be documented.

Carefully considered methods which can, therefore, minimize pain during slaughter, may be crucial for the improved shelf-life of the final meat product. This is the reason that higher pre-slaughter pain and stress inflicted on animals, cause a more and faster depletion of glycogen reserves as energy in muscles, especially under informal (non-stunning) slaughter practices (NDA, 2007; Reilly, 2012; Mdletshe *et al.*, 2020; Nkosi *et al.*, 2021). Consequently, the lesser the glycogen reserves in meat during its shelf-life storage, the quicker meat deterioration can be achieved rapidly, particularly under high ambient temperatures (Lever *et al.*, 2010).

## **2.5 Practice of formal slaughter on the shelf-life of goat meat**

Animals are stunned prior when slaughtered formally to improve their quality meat shelf-life while minimizing pain (Sabow *et al.*, 2017). Stunned animals are then monitored for any signs such as duration of losing responsiveness, bleeding efficacy, and cardiac arrest through inspection. For this reason, this is to ensure that pain is minimal and less depletion of glycogen as energy reserves in the muscles are achieved (Mdletshe *et al.*, 2020). However, abattoirs based in South Africa considers the extent of carcass bruising as a factor used in approval of the carcasses for retailer purchase and consumption post-slaughter. Rendering animals insensible to pain by stunning would aid in depleting less glycogen to reduce the degree of meat spoilage during storage because glycogen as the energy reserves sustain the freshness of meat (Adzitey and Nurul, 2011). Furthermore, the rate of glycogen depletion in muscles after slaughter influences enzyme and microbial activities because they both are energy-dependent (Huda *et al.*, 2012).

## **2.6 Effect of slaughtering stressed animals on the glycolytic metabolites of meat**

Fresh meat and its shelf-life are greatly affected by preliminary meat energy reserves in muscles at storage (Lametsch *et al.*, 2003). The basic function of stunning the animals is to provide the main purpose of protecting and prevent them from being sensible to pain during slaughter until complete bleeding is achieved (Silayoi and Speece, 2007). Stress harms the carcass because it causes a considerable depletion of glycogen in muscle, causing the meat to be prone to microbial spoilage, and deteriorating the shelf-life of meat "(Yam *et al.*, 2005). According to Brody (1997)", stunning of animals is not only done to protect the welfare rights involving the human slaughter procedure, but also to ensure meat with sufficient energy reserves in muscle is harvested to delay spoilage, also to permit some enzymatic activity to improve its shelf-life (Silayoi and Speece, 2007). However, the stunning technique used has to be reliable because stunning can only be effective in controlling sensibility to pain, which is a factor that contributes to severe glycogen depletion in carcass muscle during slaughter (Yam *et al.*, 2005).

Glycogen available in the muscle of meat post-slaughter significantly used to determine the shelf-life storage of a meat product. The correct slaughter technique application maintains glycogen reserves in muscle and minimizes pain during slaughter (Calnan *et al.*, 2014; 2019; Abhijith *et al.*, 2021). Treating meat with household by-products rich in glucose, such as sugarcane when slaughter is done under communal setups can assist with improving the glucose available in muscles of carcass at storage, thus delaying or preventing rapid meat deterioration, and prolonging its shelf-life (Davies, 1995; Del Nobile *et al.*, 2009).

## **2.7 Post-mortem energy and microbiological assessment in muscle tissues of goat meat**

Post-mortem muscle energy reserves assessment is seldom performed; however, it can greatly influence the shelf-life of meat (Bekhit *et al.*, 2013). Furthermore, microbial assessment is not performed as part of a daily assessment by the communal farmers who practice informal animal slaughter, "and the Department of Veterinary Public Health" (DVPH) on the other hand does a monthly microbiological assessment of meat shelf-life only during audits (Singh *et al.*, 2011). The guidelines from European standards are put into place when performing microbiological assessments on their

meat shelf-life, and the retailer chains assess the level of bacterial spoilage on the shelf-life of meat using their standards (Sagoo *et al.*, 2007; Panseri *et al.*, 2018), for instance, Woolworths and Checkers. Laws exercised in South Africa for meat safety have no national microbial standards which they utilize in adhering their product to (Qekwana *et al.*, 2014).

The recommended contamination levels according to the European Union (EU) “by total aerobic bacteria and total coliforms do not exceed 5.0 and 2.5 log CFU/g, respectively” (Ukut *et al.*, 2010). These standards may differ from other regions in the world, as they are based on temperatures experienced in European environments. Thus, this review indorse for research on microbial analysis to be carried out in communal areas where animals are informally slaughtered. This could aid in setting up the own standards for the South African government, which when set mandatory, can be for usage in indigenous slaughter and by communal meat retailers.

## **2.8 Determination of goat meat with shortened shelf-life post-mortem**

Any carcass which lacks sufficient glycogen reserves at post-slaughter storage is correlated with having a shorter shelf-life, and prone to rapid spoilage (Huda *et al.*, 2012). Depending on the extent to which meat lacks sufficient energy reserves, and has been affected by microbial activities, meat spoilage may be partial or total (Wang *et al.*, 2013). This rapid carcass spoilage consequently results in considerable economic losses faced by the meat industry during its shelf-life storage duration (Rani *et al.*, 2017).

Adequate amount of glycogen reserves in the carcass is paramount during the shelf-life storage of meat to impair and prevent the rapid micro-organisms growth (Singh *et al.*, 2011). Additionally, previous research studies have focused on how chilling affects rigour mortis, and less if any, reported on glycogen reserves’ influence on rigour mortis. The severity of pain experienced by an animal during slaughter results in antemortem stress, which depletes the glycogen energy reserves in the muscles by decreasing muscle glycogen, hexokinase, lactate dehydrogenase activity, and lactic acid (Wang *et al.*, 2022; 2023), thus influencing meat shelf-life. Therefore, for this reason, the amount of glycogen turning into lactate during post-mortem metabolism affects lactic acid and meat colour (Davoli *et al.*, 2022).

## **2.9 Processes involved in goat meat from farm to consumer chain**

The animal feeding mechanism forms part of the animal growth which is regulated by its live weight gain from the feedlot to the time of abattoir slaughter of the final meat product at display under retail outlets (Aguayo-Ulloa *et al.*, 2014). In the supply chains for meat products, animals are transformed into meat products by processors (communal farms) shortly as they move out of feedlots and organise delivery to consumers for retail outlets. This meat chain comprises farmers, feedlot operators, slaughter operators, and to some extent food-service inspectors and retailers (Miranda-de la Lama *et al.*, 2014; Vargas-Bello-Pérez *et al.*, 2017).

The main aim of heeding all these processes involved in the meat supply chain is the preservation of the fresh appearance for the product, which the main attractive feature to lure consumers during purchase while display its extended shelf-life storage and retard the rapid bacterial growth. Admitting meat is food product highly prone to rapid spoilage (McDonald and Sun, 1999), therefore, some of the most key elements attributed to meat quality can be affected easily during the feedlot growth phase and pre-slaughter, which may also result in the occurrence of rapid growth of spoilage bacteria during storage. Consumers use the most important inherent meat quality cues involving colour, marbling, pH, tenderness, and juiciness as quality attributes coming into play when judging for meat quality at the point of purchase (Glitsch, 2000; Robbins *et al.*, 2003), with colour being possibly the most key factor (Faustman and Cassens, 1990; Kannan *et al.*, 2001; Webb, 2014; Davoli *et al.*, 2022).

## **2.10 Stages affecting the storage period and microbiological quality of goat meat**

Madoroba *et al.* (2021) stated that the slaughter phase which stretches to the final period of meat display in retail outlets is critical to meat shelf-life. Several factors affect meat shelf-life during its duration of storage, namely type of slaughter (stunning and non-stunning) method; bleeding efficiency (amount of blood absent in the carcass); duration of storage (in days); level of glycogen reserves depleted in muscle (due to stress and pain inflicted at peri-mortem); and bacterial growth. Meat shelf-life is therefore, significantly dependent on these critical factors throughout its storage period and retail display (Rosenvold and Andersen, 2003; Pearce *et al.*, 2011). In demand for meat of higher quality with improved shelf-life to reach the retail outlets for consumer purchase, meat processors, particularly during pre-slaughter conditions

must comprehend the aspects which influence this quality shelf-life (Young *et al.*, 2003; Kandeepan *et al.*, 2013).

### **2.11 Effect of slaughter practice on muscle tissue structure and energy reserves**

Meat colour post-slaughter depends on mitochondrial expansion in muscle apart from pigment, myoglobin, which when influenced by the stress imposed on the animal pre-slaughter permits the light to pass through (Lanari and Cassens, 1991; Priolo *et al.*, 2002; Luciano *et al.*, 2009). Therefore, during slaughter pain inflicted on animals must be avoided or minimized as this pain depletes glycogen reserves in the muscles of the carcass. Nychas *et al.* (2008) stated that the shelf-life goat meat produced under good slaughter practices that birth hygienic good slaughter routines, are regulated by the available energy in carcass muscle at post-slaughter during meat distribution in the supply series to retail outlets. This report was in line with the study of Doulgeraki *et al.* (2010) which reported on the role of bacteria in meat cuts during its shelf-life storage.

High levels of stress are caused by the antemortem poor handling, which increases the muscle glycogen depletion, which in turn decreases the activity of lactate dehydrogenase and hexokinase. This can prevent the pH of the muscle tissue from dropping (Wang *et al.*, 2022; Liu *et al.*, 2022). Such incidence leads to higher muscle fibre type I and lower IIB activity which worsens muscle glycogen depletion during pre-slaughter handling by increasing the stress hormones such as cortisol which increases the blood glucose and brain activity (Choe and Kim, 2014; Choe, 2018). This is also observed in poor bleed-out rate when the slaughter without prior stunning practices is utilized, causing a result of smaller wound size opening due to higher contractions in the muscle structure, restricting the higher volume of blood expelled from the carcass (Golebiewska and Poole, 2015).

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### **CHAPTER 3: Post-mortem glycolytic metabolites, meat pH and colour of muscle tissue for goats slaughtered with TNI, SNP, and CFP methods.**

#### **Abstract**

Slaughtering of goats by indigenous slaughter methods without the practice of stunning remains a constraint to the physicochemical properties of meat. The objective of the study was to determine the effect of transverse neck incision (TNI), suprasternal notch (SNP), and chest floor piercing (CFP) on post-mortem glycolytic potential (GP), meat pH, and colour. A total of eighteen (18) non-descript male castrated goats which are a common type of goat found in villages across Southern Africa, weighing (averaging  $16.8 \pm 1.84$  kg) and the body weight ranged between 18 and 21 kg live and sixteen months of age, were stratified based on live weight and randomly assigned into the TNI, SNP, and CFP slaughter treatments (6 goats/treatment). *Musculus longissimus thoracis et lumborum* (LTL) was sampled at post-mortem for muscle tissue glycolytic potential, glycogen, lactate levels ultimate pH, and colour measurements. The TNI slaughter technique had lower muscle tissue ultimate pH, and glycogen, and lactate levels, in addition to higher glycolytic potential, redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ) values. It was concluded that muscle tissue glycolytic potential and ultimate pH were maintained for the TNI and CFP slaughter technique when compared with the SNP slaughtering method. The use of TNI and CFP as an alternative to the SNP slaughter method should therefore be taken into consideration to improve the physicochemical characteristics of goat meat.

**Keywords:** *longissimus muscle*, glycolytic potential, indigenous slaughter methods; meat pH, colour.

### 3.1 Introduction

For many centuries, goats have been an integral part of resource-limited households. They provide red meat, skin, fibre, additional household income by selling live animals, and social functions such as a measure of social status, and the performance of cultural beliefs (Mdletshe *et al.*, 2018). They are adapted to arid and semi-arid ecotypes characterized by low annual rainfall, and low quantity and quality vegetation during the cool-dry seasons. Consumption of goat meat is generally low in developing countries (Webb, 2014). The major constraint is age at slaughter, poor pre-slaughter care, which increases antemortem stress levels due to poor handling (Pophiwa *et al.*, 2020), association of goat meat with cultural beliefs, high odour intensity in meat, and slaughtering of goats using indigenous techniques (Mdletshe *et al.*, 2020; 2021).

Goat slaughter without stunning, which results in unconsciousness and death through bleeding, is an ongoing problem that has long been seen as the primary constraint on the physicochemical characteristics (Mdletshe *et al.*, 2021), and reduced consumption of goat meat (Sabow *et al.*, 2015; 2017). In Southern Africa, the TNI, SNP, and CFP are the common indigenous slaughter methods without stunning. Although animal welfare activists regard them inhumane, they are permitted by the South African Meat Safety Act of 2004, therefore, prompting scholars to explore alternative methods that are culturally friendly while improving the visual appearance of goat meat (Mdletshe *et al.*, 2021). They are linked with enhance the antemortem stress (Mdletshe *et al.*, 2020), high post-mortem muscle tissue pH, water-holding capacity, redness, and chroma values (Mdletshe *et al.*, 2021).

The amount of glycogen that can be converted into lactate during post-mortem metabolism is measured by glycolytic potential, which influences the production of lactic acid and meat colour (Davoli *et al.*, 2022). Increased antemortem stress causes a substantial decline in muscle glycogen, which in turn causes a decrease in hexokinase and lactate dehydrogenase activity in addition to lactic acid (Wang *et al.*, 2022; 2023). The average threshold value for glycolytic potential in goats reared in pasture, electrical stunned and bled should range from 105 to 115 ( $\mu\text{moles/g}$ ) (Simela *et al.*, 2004). The effect of TNI, SNP, and CFP slaughter methods on post-mortem muscle tissue glycogen and lactate levels, pH, and colour, of meat, however, remains unknown. However, understanding their effects can help resource-limited households choose a culturally-friendly and low-stress alternative, leading to better quality and longer shelf-life. The objective of the study was to determine the effect of TNI, SNP,



and CFP slaughter techniques on post-mortem muscle tissue glycolytic potential, glycogen and lactate levels, pH, and colour. It was hypothesized that post-mortem glycolytic potential, glycogen and lactate levels, pH, and colour are not influenced by TNI, SNP, and CFP slaughter techniques.

### **3.2. Methods and materials**

#### **3.2.1 Compliance with ethical aspects of the study**

The University of Limpopo Animal Research Ethics Committee approved the care and use of goats, ensuring that they complied with national and international standards for animal welfare and ethics (Reference number: AREC/11/2022: PG).

#### **3.2.2 Description of the study site and goat management**

The study was conducted at the Senwabarwana rural village of Bochum (23°53'24" S, 29°45'25" E) in Blouberg using random sampling, under Capricorn district municipality, Limpopo Province, South Africa. A total of eighteen non-descript male castrate goats with an average of 16 months old and a body weight (averaging  $16.8 \pm 1.84$  kg) with a live body weight range of between 18 and 21 kg were purchased from the local rural farmers. Resource-limited farmers managed goats in free-grazing communal rangelands. Goats were held in lairage for an estimated 16h before slaughter with full access to water but without feed.

#### **3.2.3 Experimental design and treatments**

At slaughter, goats were weighed using a multi-purpose hanging scale for goats, stratified based on live weight, and randomly assigned to the three (TNI, SNP, and CFP) slaughter treatments (6 goats/treatment). The South African Meat Safety Act of 2004, which permits the informal slaughter of food animals outside of an abattoir for personal use, was followed by the local rural farmers in the slaughtering of goats. In short, the TNI, SNP, and CFP procedures were used to slaughter goats following Mdletshe *et al.* (2020; 2021) (See Figure 3.1 A, B, and C).



figure 3. 1: Images of a spear (A), suprasternal notch piercing (B), and chest-floor point-of-elbow (C).

Used with permission of MDPI, adapted from Mdletshe *et al.* (2020).

All of the goats were slaughtered in a nearby village where they were purchased, following guidelines modelled following the 2004 South African Meat Safety Act, which allowed for the slaughter of goats without stunning for personal use. Goats in the three slaughter treatments underwent transverse neck incision (TNI), piercing at the chest floor point of the elbow (Figure 3.1C; CFP), and suprasternal notch piercing with a short spear (Figure 3.1B; SNP). The goats that were to be slaughtered were chosen at random the day before, weighed, and kept in a different enclosure with unlimited access to water but no food as explained according to Mdletshe *et al.* (2020; 2021). In short, the objects that were used were a short spear (figure 3.1A) and a sharp knife, which were specifically made for slaughtering goats. Using a sharp knife, the TNI slaughtering procedure was carried out by Sabow *et al.* (2016).

The main superficial and deep nerves of the cervical region, as well as the trachea, oesophagus, carotid arteries, jugular veins, and muscles (brachiocephalic, sternocephalic, sternohyoid, and sternothyroid) were cut by the knife. The direction in which the knife moved changed with each cut. The SNP slaughter process was performed by two experienced slaughtermen using a short spear (Figure 3.1) with a goat allowed to stand upright with hind legs. While the second slaughterman held the right front leg and the spear, piercing the suprasternal notch in the direction of the heart, the first slaughterman held the left front leg and the head (using horns). Three slaughtermen used a short spear to perform the CFP method (Figure 1A). Two of the slaughtermen held the goat's front and hind legs while the third person carrying the spear pierced the goat next to the floor near the heart and pointed the elbow in the direction of the heart. Spears and knives used in the dressing and slaughtering of carcasses were sanitised for one minute in boiling water. Throughout the bleeding procedure, blood was collected in buckets.

### **3.2.4 Meat sampling and storage**

The weight of the dressed carcass was  $6.94 \pm 1.54$  kg on average. Forty-five minutes after dressing the carcass, the muscle's pH (CRISON pH25, CRISON instrument SA, Spain) was measured in triplicate between the eighth and thirteenth ribs. After weighing and measuring the meat pH, the left and right *longissimus thoracis et lumborum* (LTL) were extracted immediately, vacuum-packed, and put in polystyrene cooler boxes (less than 4°C) before being taken to the laboratory to be tested for muscle tissue glycolytic metabolites, pH, and colour.

### 3.2.5 Determination of glycolytic potential

The *M. Longissimus thoracis* (LT) was frozen at  $-70^{\circ}\text{C}$  for 24 hours after slaughter before being subjected to testing for glycolytic metabolites. The glycolytic metabolites were determined using the enzymatic method given by Dalrymple & Hamm (1973) for the glycogen content and by Gutmann & Wahlefeld (1974) for the amount of lactate in the meat sample. In summary, 0.85 M  $\text{HClO}_4$  was used to dissolve 0.5g of frozen muscle tissue sample and homogenised it for 1 minute in 4.5 mL of ice-cold perchloric acid solution. For ten minutes, the homogenates were centrifuged at  $2,700 \times g$  and  $4^{\circ}\text{C}$ . After neutralising the supernatant fraction with 10 M KOH, it was kept in separate tubes at  $-80^{\circ}\text{C}$  for further lactate and glycogen analyses. A lactic acid kit from Nanjing Jiancheng Bioengineering Institute (China) was used to perform a spectrophotometric analysis of the lactate present in the supernatant fraction. After two hours of incubation at  $55^{\circ}\text{C}$  in acetate buffer (pH 4.8) with amyloglucosidase (A7420, Sigma-Aldrich Inc., St. Louis, MO), the glycogen in the supernatant fraction was hydrolyzed to glucose by an enzyme. A concentration of 10 M KOH was used to neutralise the supernatant portion following incubation. With the aid of a commercial glucose oxidase kit (Shanghai RongSheng Biotech Co. Ltd.), glucose and lactate were measured spectrophotometrically.

The Monin & Sellier (1985) formula was used to compute the glycolytic potential (GP) of muscle tissue, which is expressed as a measured ( $\mu\text{moles/g}$ ) lactate per 1g of muscle tissue. The following formulae were used:

$$\text{GP} = (2 \times [\text{glycogen}]) + [\text{lactate}].$$

### 3.2.6 Determination of Meat pH and Colour

Using a portable pH metre probe (CRISON pH25, CRISON instrument SA, Barcelona, Spain), the pH of the meat was determined 45 minutes after dressing the carcass and 24 hours (pHu) post-mortem. A colour metre (HunterLab, ColorFlex EZ Spectrophotometer, Reston, VA, USA) was used to measure the colour of the meat three times within 24 hours post-mortem.

### 3.2.7 Statistical Analysis

Statistical Analysis Software (SAS, 2010) was used for analysing all of the data. A general linear model was used to test the effect TNI, SNP, and CFP slaughter method on post-mortem muscle tissue pH, colour ( $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ), glycolytic potential, glycogen, and lactate levels. The least-square means were compared pairwise using the PDIFF option.

The model used is shown below:

$$Y_{ij} = \mu + S_i + \varepsilon_{ij}$$

Where;

$Y_{ijk}$  = Response variables (Glycolytic potential, glycogen, lactate, meat pH,  $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ );

$\mu$  = population mean;

$S_i$  = effect of indigenous slaughter method;

$\varepsilon_j$  = residual error.

The muscle tissue pH and colour ( $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ), glycolytic potential, lactate, and glycogen were correlated using the correlation procedure (PROC CORR) to determine the Pearson correlation coefficients. For differences between least-square means,  $p < 0.05$  was used as the significance threshold.

## 3.3. Results

### 3.3.1 Determination of muscle tissue pH at 24 h post-mortem

Goat meat pH and colour are shown in Table 3.1. The slaughter method did not affect ( $P \geq 0.05$ ) meat pH at 45 minutes post-mortem. The slaughter method had an effect ( $P \leq 0.05$ ) on the ultimate meat pH (pHu). The ultimate pH for TNI (6.30 vs 6.72) and CFP (6.32 vs 6.72) were lower ( $P \leq 0.05$ ) when compared with the SNP slaughter treatment. The correlation between muscle tissue ultimate pH, colour ( $a^*$ ,  $b^*$ ,  $C^*$ ,  $L^*$ , and  $H^*$ ), and glycolytic metabolites (glycolytic potential, glycogen, and lactate levels) are given in Table 3.2. Correlation between the muscle tissue ultimate pH, colour ( $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ), and glycolytic metabolites (glycolytic potential, glycogen, and lactate levels) are given in Table 3.2. The ultimate pH was positively correlated ( $r = 0.67^{***}$ ;  $P \leq 0.001$ ) for muscle tissue glycogen in addition to negative correlation ( $r = -0.61$ ;  $P \leq 0.001$ ) for lactate levels, respectively.

### **3.3.2 Determination of muscle tissue colour at 24 h post-mortem**

The slaughter method had an effect ( $P \leq 0.05$ ) on muscle tissue redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ). Muscle tissue redness was higher ( $P \leq 0.05$ ) for TNI (15.3 vs 12.6) and CFP (15.2 vs 12.6) when compared with the SNP slaughter treatment. Muscle tissue yellowness ( $b^*$ ) was higher SNP when compared with TNI (12.4 vs 10.4) and CFP (12.1 vs 10.4) slaughter treatments. Muscle tissue chroma values were higher ( $P \leq 0.05$ ) for the SNP when compared with the TNI (20.1 vs 17.3) and CFP (19.9 vs 17.3) slaughter treatments.

The correlation between muscle tissue redness ( $a^*$ ), yellowness ( $b^*$ ), lightness ( $L^*$ ), chroma ( $C^*$ ), and hue ( $H^*$ ) angle values are given in Table 3.2. Muscle tissue redness ( $a^*$ ) had a strong positive correlation with yellowness ( $r = 0.86^{***}$ ;  $p \leq 0.001$ ), chroma ( $r = 0.97^{***}$ ;  $p \leq 0.001$ ), and hue angle values ( $r = 0.80^{***}$ ;  $p \leq 0.001$ ) respectively.

Muscle tissue yellowness had a positive correlation with lightness ( $r=0.44^{**}$ ;  $P \leq 0.01$ ), chroma ( $r = 0.96^{***}$ ;  $***P \leq 0.001$ ), and hue angle values ( $r = 0.47^{**}$ ;  $P \leq 0.01$ ) respectively. Lightness and Chroma values were positively correlated ( $r= 0.56^{***}$ ;  $p \leq 0.001$ ). The muscle tissue chroma values were positively correlated with ( $r = 0.68^{***}$ ,  $p \leq 0.001$ ) with the hue angle.

### **3.3.3. Determination of glycogen, lactate, and glycolytic potential for goat muscle tissue at 24h post-mortem**

There was an association ( $P \leq 0.05$ ) between the TNI, SNP, and CFP slaughter methods on muscle tissue glycogen, lactate levels and glycolytic potential (GP). The muscle tissue glycogen levels were higher for the TNI (12.9 vs 11.2) and SNP (12.5 vs 11.2) when compared with the CFP slaughter treatment. Muscle tissue lactate levels were higher for TNI (20.9 vs 19.6) and SNP (21.9 vs 19.6) when compared with the CFP slaughter treatment. The TNI had a muscle tissue higher in glycolytic potential (22.4 vs 20.9) and SNP (23.2 vs 20.9) when compared with the CFP slaughter treatment.

The correlation between muscle tissue glycolytic potential, glycogen, and lactate levels is given in Table 3.2. Glycolytic potential had a positive correlation with muscle tissue glycogen ( $r= 0.65^{***}$ ;  $p \leq 0.001$ ) and lactate levels ( $r = 0.39^*$ ;  $p \leq 0.05$ ), respectively.

**Table 3. 1:** Effect of TNI, SNP, and CFP slaughter methods on the meat pH, glycolytic metabolites ( $\mu\text{moles/g}$ ), and meat colour of longissimus et thoracis musculus from Nguni goats

Variable	Slaughter method			Significance
	TNI	CFP	SNP	
<b>Meat pH</b>				
pH <sub>45 min</sub>	6.69 $\pm$ 0.11	6.48 $\pm$ 0.11	6.59 $\pm$ 0.11	NS
pH <sub>24h</sub>	6.30 $\pm$ 0.12 <sup>a</sup>	6.32 $\pm$ 0.12 <sup>a</sup>	6.72 $\pm$ 0.12 <sup>b</sup>	*
<b>Glycolytic metabolites</b>				
Glycolytic potential ( $\mu\text{moles/g}$ )	17.4 $\pm$ 1.38 <sup>a</sup>	16.2 $\pm$ 1.38 <sup>a</sup>	13.9 $\pm$ 1.38 <sup>b</sup>	*
Glycogen ( $\mu\text{moles/g}$ )	12.9 $\pm$ 6.19 <sup>a</sup>	11.2 $\pm$ 6.67 <sup>b</sup>	12.5 $\pm$ 5.65 <sup>a</sup>	*
Lactate ( $\mu\text{moles/g}$ )	20.9 $\pm$ 5.63 <sup>a</sup>	19.6 $\pm$ 5.99 <sup>b</sup>	21.9 $\pm$ 4.94 <sup>a</sup>	*
<b>Meat colour</b>				
a*	15.3 $\pm$ 0.49 <sup>a</sup>	15.2 $\pm$ 0.49 <sup>a</sup>	12.6 $\pm$ 0.49 <sup>b</sup>	*
b*	12.4 $\pm$ 0.58 <sup>a</sup>	12.1 $\pm$ 0.58 <sup>a</sup>	10.4 $\pm$ 0.58 <sup>b</sup>	*
L*	28.6 $\pm$ 1.01	29.7 $\pm$ 1.01	27.2 $\pm$ 1.01	NS
C*	20.1 $\pm$ 0.83 <sup>a</sup>	19.9 $\pm$ 0.83 <sup>a</sup>	17.3 $\pm$ 0.83 <sup>b</sup>	*
H*	0.67 $\pm$ 0.02	0.66 $\pm$ 0.02	0.64 $\pm$ 0.02	NS

<sup>a,b,c</sup> Means in the same row are significantly different at \* $p < 0.05$ ; NS: Not significant; TNI: transverse neck incision; SNP: suprasternal notch piercing; CFP: chest floor piercing; pH<sub>45min</sub>: pH 45 min post-mortem; pH<sub>24h</sub>: ultimate pH; a\*: redness; b\*: yellowness; L\*: lightness; C\*: chroma; H\*: Hue

**Table 3. 2:** Pearson Correlation between pHu, the colour parameters of a\*, b\* L\*, C\*, H\*; and the glycolytic metabolites of GP, Glycogen, and Lactate

Variables	pHu	a*	b*	L*	C*	H*	GP	Glycogen	Lactate
pHu		0.12	0.22	0.15	-0.17	0.05	0.67***	-0.73***	-0.61***
a*			0.86***	0.02	0.97***	0.80***	-0.05	0.21	0.28
b*				0.44**	0.96***	0.47**	0.12	0.21	0.21
L*					0.22	0.56***	0.10	0.03	0.08
C*						0.68***	0.03	0.22	0.26
H*							-0.07	0.22	0.30
GP								0.65***	0.39*
Glycogen									0.95***
Lactate									

Correlated at \*P ≤ 0.05; \*\*P ≤ 0.01; \*\*\*P ≤ 0.001. pHu: ultimate pH<sub>24h</sub>; a\*: Redness; b\*: Yellowness; L\*: Lightness; C\*: Chroma; H\*: Hue; GP: Glycolytic potential



There was a strong positive correlation between glycogen and lactate values ( $r = 0.95^{***}$ ;  $p \leq 0.001$ ).

### **3.4 Discussion**

The objective of the study was to determine the effect of TNI, SNP, and CFP slaughter techniques on post-mortem glycolytic potential, meat pH, and colour. The findings that the ultimate pH of the muscle tissue was higher for the SNP compared to TNI and CFP values could be explained by the high levels of stress caused by the antemortem handling, which increased the muscle glycogen depletion which in turn decreased the activity of lactate dehydrogenase and hexokinase. This prevented the pH of the muscle tissue from dropping (Wang *et al.*, 2022; Liu *et al.*, 2022). These observations could be explained by the differences in the goats' unaccounted-for dietary intake, which may have had a confusing effect on the muscle tissue that stores glycogen. The observations could also be explained by higher muscle fibre type I and lower IIB activity which worsens muscle glycogen depletion during pre-slaughter handling by increasing the stress hormones such as cortisol which increase the blood glucose and brain activity (Choe and Kim, 2014; Choe, 2018). More research on the impact of nutritional status on the integrity of goats' glycolytic potential should be done to assess the blood metabolites creatinine, urea, albumin, glucose, cholesterol, and total protein on muscle fibre type of muscle fibres in goat meat with low glucose in muscle tissue. The current study's findings, which showed that TNI and CFP had higher glycolytic potential than SNP slaughter treatment, can be explained by low muscle tissue glycogen resulting from high antemortem stress (discussed previously), which causes pyruvate to bond with hydrogen electrons to form lactate, which functions as a buffer to stop  $pH_u$  from falling any further. To understand the stress biochemical enzymes influencing post-mortem glycolysis, further research is necessary.

Findings that the meat redness ( $a^*$ ) was higher for both TNI and CFP slaughter treatments may perhaps have been caused by stress prolonged on the goats before slaughter, which is linked to the production of stress hormones (as discussed earlier). Additionally, poor bleeding techniques and wound size may have contributed, as they are connected to the failure to sever the neck carotid arteries and vertebrae arteries, which supply blood to the brain and cause a poor bleed out rate (Anil *et al.*, 2000;

2006). Therefore, blood heme (haemoglobin and myoglobin) was retained in the meat. Furthermore, the presence of myoglobin in meat influences aerobic metabolic capacity by improving type IIB muscle fibre, which increases ATPase activity and accelerates the depletion of glycogen in muscle tissue.

Muscle tissue yellowness ( $b^*$ ) values for the TNI and CFP were found to be higher than those for the SNP slaughter treatment. This finding could be explained by assuming that muscle tissue glycogen was depleted faster, which led to higher lactate production (as was previously discussed). This prevented further muscle tissue pH decrease and created an environment that was favourable for the *Micrococcus* and *Flavobacterium* that cause meat yellowness. When contrasting the TNI and CFP chroma ( $C^*$ ) values to the SNP slaughter treatments, the higher values could be attributed to inadequate bleeding efficiency, which leads to blood retention in the muscle tissue. This blood retention results in the formation of metmyoglobin, which is responsible for met discoloration. These observations could have been influenced by the presence of oxygen in haemoglobin as a result of poor bleeding efficiency, accelerating lipid oxidation, and causing yellow muscle tissue discoloration (Alvarado *et al.*, 2007). The effects of the TNI, SNP, or CFP treatments on the rancidity and shelf-life of goat meat require further investigation.

### **3.5 Conclusion**

The TNI and CFP treatments were related to lower lactate levels, higher ultimate pH, glycolytic potential, and values of chroma ( $C^*$ ), yellowness ( $b^*$ ), and redness ( $a^*$ ) in the muscle tissue as compared to the SNP slaughter treatment. These findings suggest that TNI and CFP might be a good substitute for the SNP slaughtering technique. Measurements of shelf-life, serum cortisol levels and the composition of muscle fibre types in goats slaughtered with TNI and CFP should be performed for comparison to goats slaughtered using the SNP method.

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## **CHAPTER 4 Shelf-life properties of *longissimus musculus* from goats slaughtered with the TNI, SNP, and CFP methods**

### **Abstract**

The objective of the current study was to determine the effect of TNI, SNP, and CFP slaughter techniques on shelf-life properties of muscle tissue from 18 non-descript male castrate goats (6 goats/treatment), averaging  $16.8 \pm 1.84$  kg, with live weights ranging between 18 and 21 kg randomly allocated three slaughter methods, the TNI, SNP, and CFP. Muscle tissue pH and colour were measured for 14 days using *Musculus longissimus thoracis at lumborum* (LTL). Overall, the slaughter technique did not influence muscle tissue pH. Muscle tissue redness ( $a^*$ ) decreased over time, being highest ( $P \leq 0.05$ ) at day 0, followed by day 7 ( $P \leq 0.05$ ), and 14, respectively. Muscle tissue yellowness ( $b^*$ ) decreased ( $P \leq 0.05$ ) over time being highest for the CFP ( $P \leq 0.05$ ) on day 3, followed by the TNI and SNP ( $P \leq 0.05$ ) at day 7, and CFP ( $P \leq 0.05$ ) on day 11, respectively. Muscle tissue lightness ( $L^*$ ) decreased over time, being highest at day 0, followed by day 11, and day 3 being the lowest, respectively. The shelf-life of muscle tissue from goats slaughtered with TNI was longer compared to the SNP and CFP slaughter treatment.

**Keywords:** chroma, indigenous slaughter, redness, pH, yellowness, Hue, stunning, shelf-life.

## 4.1 Introduction

Indigenous and traditional slaughter techniques, such as the transverse neck incision (TNI), suprasternal notch (SNP), and chest flow point of elbow (CFP) piercing, have been used in successful attempts to reduce antemortem stress at slaughter for goats while maintaining the cultural standards of resource-limited households (Mdletshe *et al.*, 2020). Goat meat's physicochemical and glycolytic metabolite characteristics are significantly influenced by the method of slaughter (see Chapter 3; Mdletshe *et al.*, 2021). While pH is an indicator of meat quality, meat colour influences consumer purchasing choices and shelf life (Faustman and Cassens, 1990; Bekhit *et al.*, 2013).

Indigenous slaughter methods remain a solitary main limiting factors to goat meat quality (Mdletshe *et al.*, 2021). The metabolic pathway of goat meat varies with breed, pre-slaughter management, and slaughter technique (Sabow *et al.*, 2017). In Southern Africa, TNI, SNP, and CFP are common indigenous slaughter techniques used for slaughtering goats (Mdletshe *et al.*, 2020). These methods of indigenously slaughtering animals without stunning are legalised by the Meat Act of 2004, where the slaughtering of animals is performed for personal use (Qekwana *et al.*, 2016; 2017). The slaughter methods without stunning have been associated with higher muscle glycogen, and lower lactate and glycolytic potential values (see chapter 3). The indigenous slaughter methods cause high antemortem and post-mortem stress (Mdletshe *et al.*, 2020; 2021), resulting in high post-mortem meat pH, redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ) values (see Chapter 3).

The TNI, SNP, and CFP are traditional and indigenous slaughter techniques used by resource-limited households when performing traditional ceremonies and they have cultural meaning (Qekwana *et al.*, 2014). To reduce antemortem stress for goats and enhance the physicochemical characteristics and glycolytic metabolites of goat meat, there is currently a growing interest in the acceptance of traditional and indigenous slaughtering methods (see Chapter 3). Limited research has been done, if any is available, on how the shelf-life characteristics of goat meat is affected by the slaughter methods of TNI, SNP, and CFP. Therefore, the objective of the current study was to determine the effect of TNI, SNP, and CFP slaughter methods on the shelf-life properties of goat meat. It was hypothesized that slaughtering goats with the TNI, SNP, and CFP techniques would not affect the shelf-life properties.

## **4.2. Materials and methods**

### **4.2.1 Compliance with Ethical Aspects**

The compliant with ethical standards has been described in section 3.2.1.

### **4.2.2 Description of the study site and goats' management**

The study site and goat management have been described in section 3.2.2.

### **4.2.3 Experimental design and treatments**

The experimental design and allocation of treatments have been described in section 3.2.3.

### **4.2.4 Data collection**

Data was collected for 14 days using post-mortem *musculus longissimus thoracis et lumborum* (LTL) for the determination of muscle tissue pH and colour.

#### **4.2.4.1 Meat Sampling and Storage**

The collection and storage of *Longissimus thoracis et lumborum* (LTL) was described in section 3.2.4.

#### **4.2.4.2 Meat Colour and pH**

Determination of muscle tissue pH and colour was described in section 3.2.6.

### **4.2.5 Statistical analysis**

All data was analysed using Statistical Analysis Software (SAS, 2010). A repeated measure procedure was computed to test the effect of TNI, SNP, and CFP slaughter methods on goat meat shelf-life properties (muscle tissue pH;  $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ). The PDIFF option was used to create pairwise comparisons of the least square means.

The model used is shown below:

$$Y_{ij} = \mu + S_i + T_j + (S \times T)_{ij} + \epsilon_{ij}$$

Where;

$Y_{ijk}$  = Response variables (muscle tissue pH,  $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ );

$\mu$  = population mean;

$S_i$  = effect of either TNI, SNP or CFP slaughter method;



$T_j$  = the effect of time in days

$(S \times T)_{ij}$  = the interaction between slaughter treatment (TNI, SNP, and CFP) and time in days

$\varepsilon_j$  = residual error.

The significance threshold for differences between least square means was set at  $p \leq 0.05$ .

### **4.3. Results**

#### **4.3.1 Muscle tissue pH**

The effect of slaughter treatment and time on muscle tissue pH and colour are given in Table 4.1. Slaughter treatment, time, and the interaction between slaughter treatment and time had no effect ( $P \geq 0.05$ ) on the muscle tissue pH.

#### **4.3.2 Muscle tissue colour**

Slaughter treatment and the interaction between slaughter treatment and time had no effect ( $P \geq 0.05$ ) on muscle tissue redness ( $a^*$ ). Muscle tissue redness generally decreased over time (see Figure 4.1). Muscle tissue redness for day 0 was higher ( $P \leq 0.001$ ) compared to day 3 (15.8 vs 13.0), day 7 (15.8 vs 13.5), day 11 (15.8 vs 12.2), and day 14 (15.8 vs 11.7). Muscle tissue redness was also higher ( $P \leq 0.05$ ) for day 3 when compared to day 14 (13.0 vs 11.7). Furthermore, muscle tissue redness was higher ( $P \leq 0.05$ ) for day 7 compared to day 11 (13.0 vs 12.2), and day 14 (13.0 vs 11.7).

Time and the interaction between slaughter treatment and time had an effect ( $P \leq 0.05$ ) on muscle tissue yellowness ( $b^*$ ) (see Figure 4.2). Muscle tissue yellowness was lower for day 0 compared to day 3 (12.7 vs 15.2), and day 7 (12.7 vs 15.8). Muscle tissue yellowness ( $b^*$ ) was higher ( $P \leq 0.05$ ) for day 3 compared to day 11 (15.2 vs 12.9), and day 14 (12.7 vs 12.2). Furthermore, muscle tissue yellowness ( $b^*$ ) was higher for day 7 compared to day 11 (15.8 vs 12.9), and day 14 (15.8 vs 12.2).

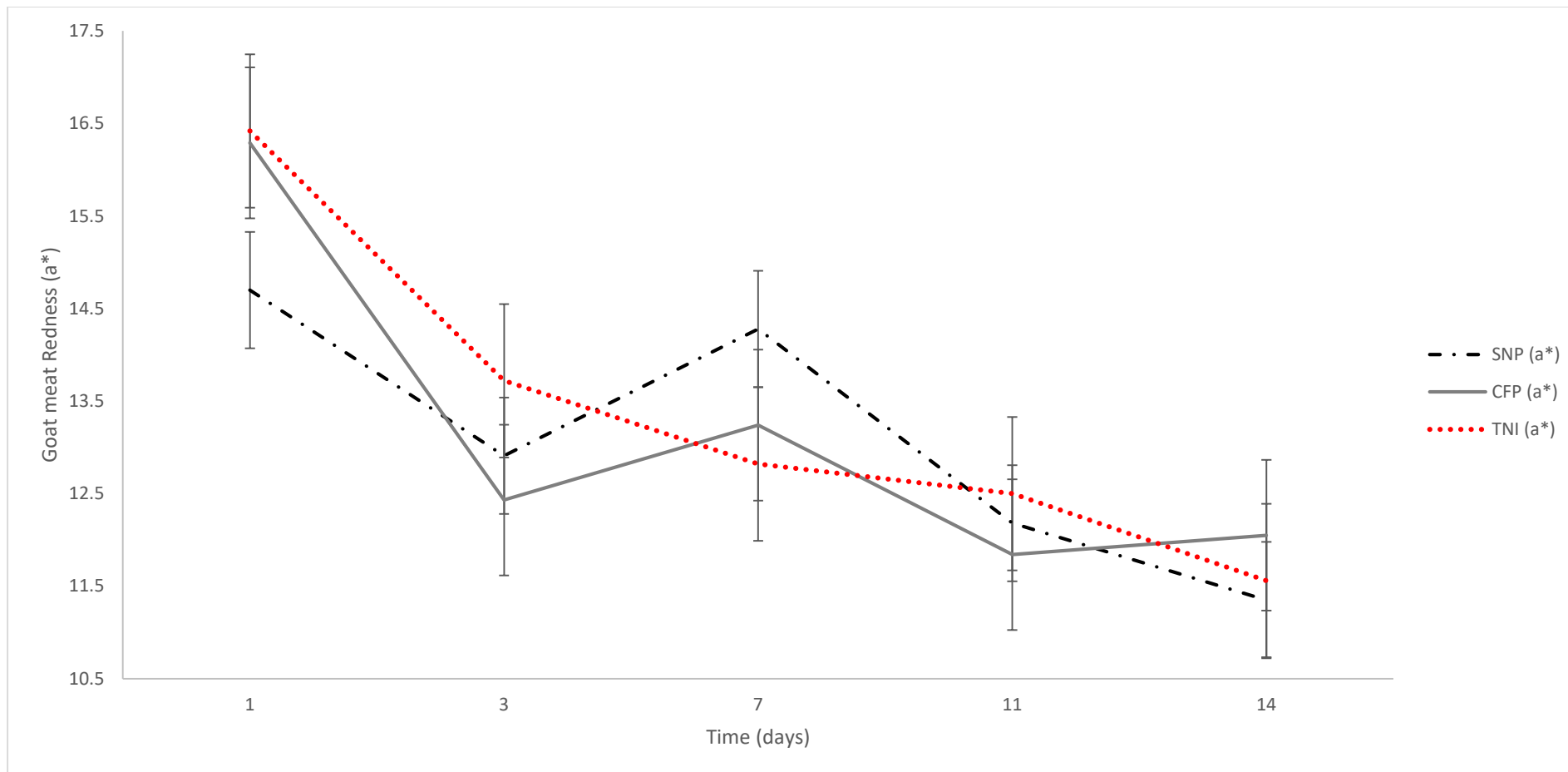
Time affected muscle tissue lightness ( $P \leq 0.05$ ). Muscle tissue lightness generally decreased over time (see Figure 4.3). Muscle tissue lightness ( $L^*$ ) was higher ( $P \leq 0.05$ ) at day 0 compared to day 3 (29.5 vs 22.4), day 7 (29.5 vs 25.2), day 11 (29.5 vs 26.1), and day 14 (29.5 vs 25.8). Furthermore, muscle tissue lightness was lower ( $P$

**Table 4. 1:** Effects of SNP, CFP, and TNI slaughter methods on colour parameters ( $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$  and  $h^*$ ), and pH of post-mortem musculus longissimus thoracis et lumborum (LTL) of goat meat.

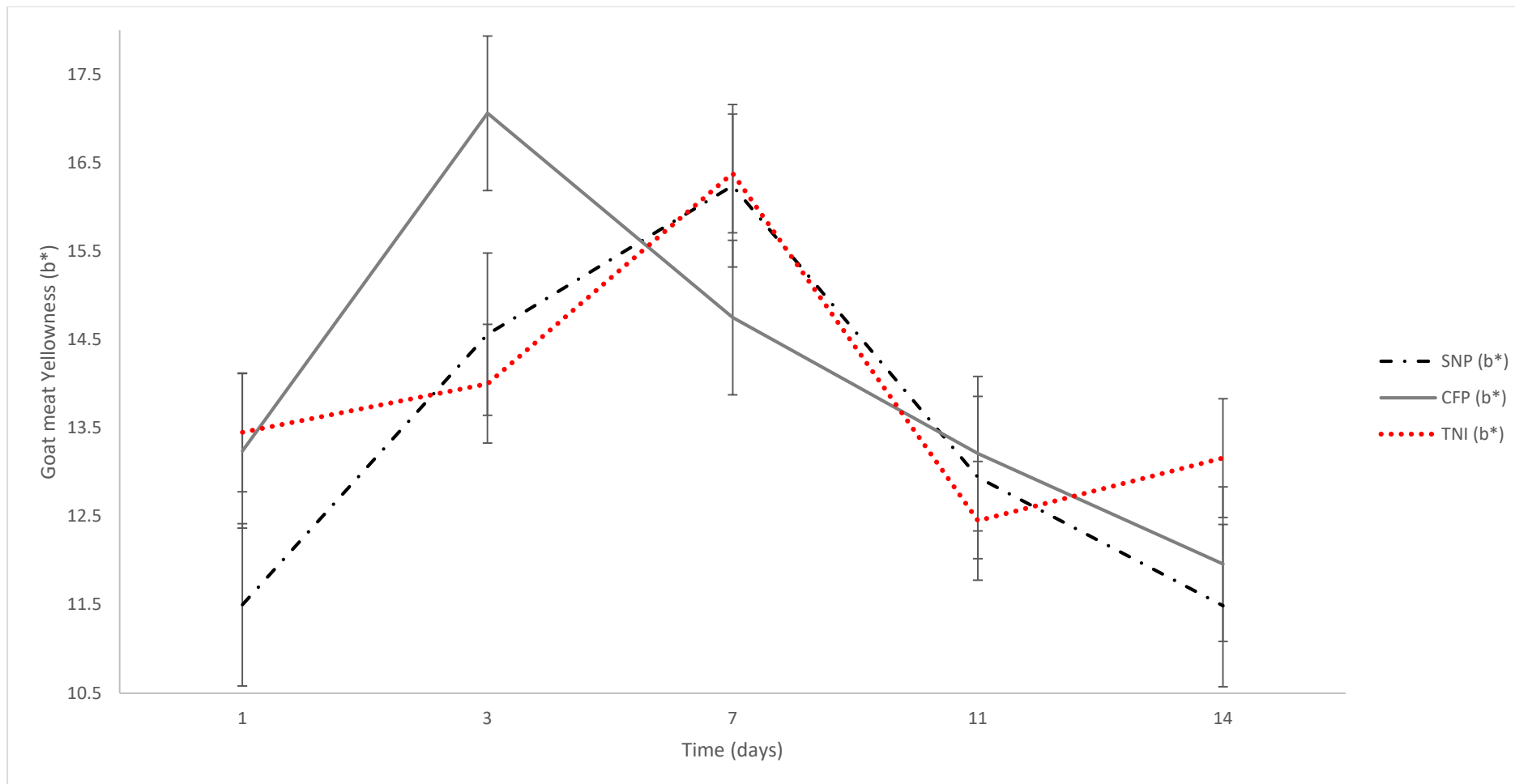
Variable	Slaughter method			Significance		
	TNI	SNP	CFP	Treatment	Time	Treatment × Time
<b>pH</b>	6.58 ± 0.04	6.50 ± 0.04	6.56 ± 0.04	NS	NS	NS
<b>Colour</b>						
<b><math>a^*</math></b>	13.40 ± 0.32	13.09 ± 0.32	13.17 ± 0.32	NS	***	NS
<b><math>b^*</math></b>	13.89 ± 0.35 <sup>b</sup>	13.35 ± 0.35 <sup>b</sup>	14.05 ± 0.35 <sup>a</sup>	NS	***	*
<b><math>L^*</math></b>	25.96 ± 0.62 <sup>b</sup>	25.27 ± 0.62 <sup>b</sup>	26.14 ± 0.62 <sup>a</sup>	NS	***	NS
<b><math>C^*</math></b>	26.25 ± 1.97	22.24 ± 1.97	22.51 ± 1.97	NS	NS	NS
<b><math>H^*</math></b>	0.53 ± 0.06	0.61 ± 0.06	0.58 ± 0.06	NS	NS	NS

Table of Significance for GLM for the least square means values of chicken breast meat and their standard errors.

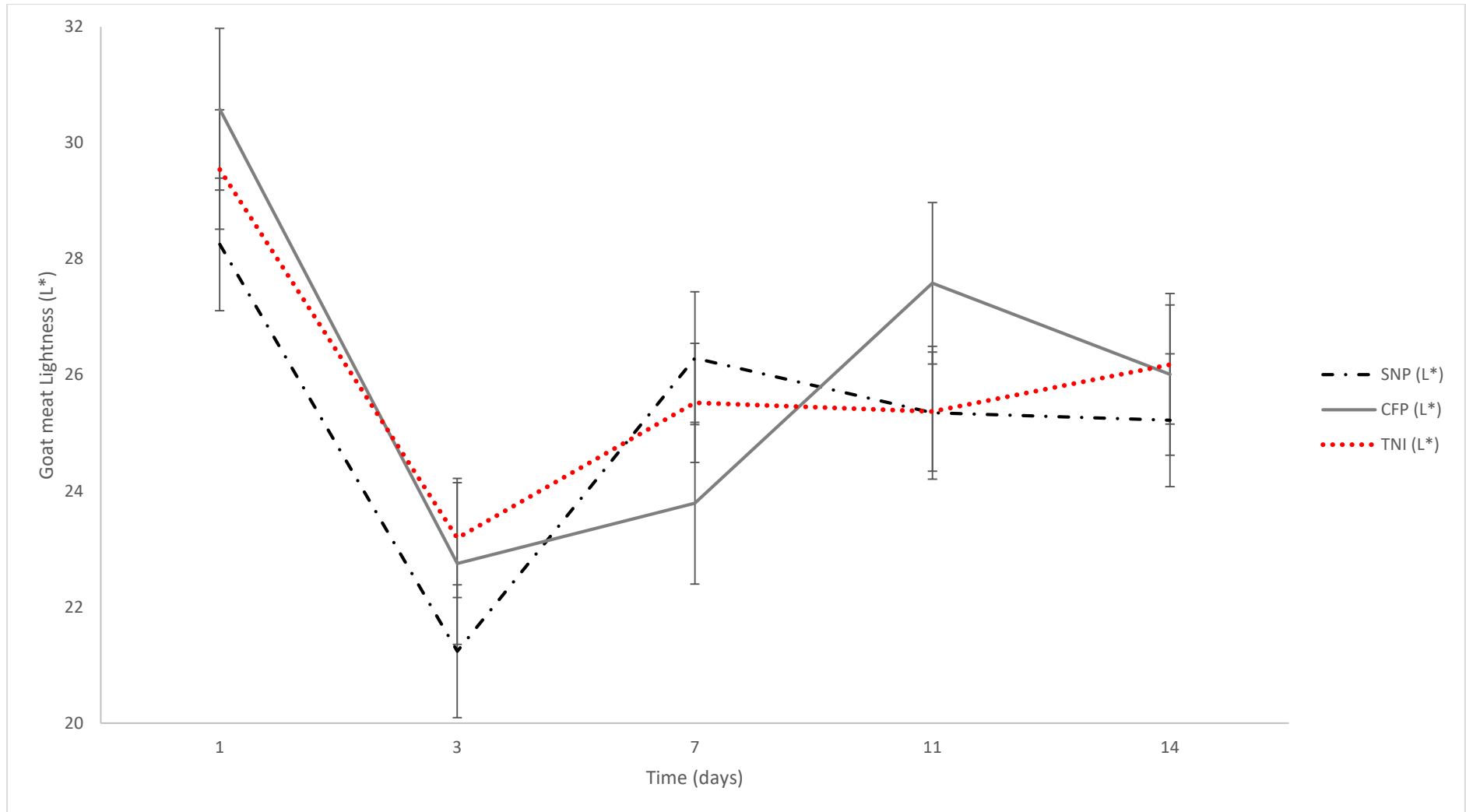
<sup>a,b</sup> Means in the same row with different superscripts are significantly different at \*  $p \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; NS: not significant; TNI: transverse neck incision; SNP: suprasternal notch piercing; CFP: chest floor piercing; pH<sub>45min</sub>: pH 45 min post-mortem; pH: ultimate pH;  $L^*$ : lightness;  $a^*$ : redness;  $b^*$ : yellowness;  $H^*$ : Hue;  $C^*$ : chroma.



**Figure 4. 1:** An illustration of muscle tissue redness ( $a^*$ ) for goats slaughtered with TNI, SNP, and CFP during the shelf-life period (days).



**Figure 4. 2:** Observed muscle tissue yellowness (b\*) for goats slaughtered with TNI, SNP, and CFP with the shelf-life period (days).



**Figure 4. 3:** Observed muscle tissue lightness (L\*) for goats slaughtered with TNI, SNP, and CFP during the shelf-life period (days).

$\leq 0.05$ ) for day 3 compared to day 7 (22.4 vs 25.2), day 11 (22.4 vs 26.1), and day 14 (22.4 vs 25.8).

#### **4.4 Discussion**

The objective of the study was to determine the effect of TNI, SNP and CFP slaughter treatments on the pH and colour ( $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ) for 14 days. Slaughter treatment did not affect muscle tissue pH. These observations could be explained by high ultimate pH above 5.7 at post-mortem which could be influenced by high ante- and peri-mortem stress exhibited by the animal during slaughter (Hoffman and Fisher, 2001; Kim *et al.*, 2014; Lee *et al.*, 2022). This could also be further explained by poor bleed-out rate for the SNP, and CFP slaughter practices as a result of smaller wound size opening, restricting higher volume of blood expelled from the carcass (Golebiewska and Poole, 2015). Poor bleed-out rate for the TNI slaughter treatment could be explained by not severing the internal carotid arteries which maintain the supply of blood to the brain. To reduce blood retention in the carcass during bleeding, resource-limited farmers should be educated about major blood vessels to sever/ cut to allow for high total blood volume expelled during bleeding apart from wound size and patency.

The finding that muscle tissue redness ( $a^*$ ) values were higher for the TNI and CFP compared with SNP slaughter treatment during early post-mortem is an indication that the meat might have retained more blood haems (haemoglobin and myoglobin) in the muscle as a result of poor bleed out rate. The blood retained in the muscle tissue could best be explained by the insufficient bleeding achieved from the slaughter of goats using the TNI method (Golebiewska and Poole, 2015). These results concur with the findings reported by both Rosen (2004) and Alat (2011), who observed that the blood in the body of an animal is less drained in greatest amounts when an animal is more stressed during the perimortem phase. These observations caused muscle tissue discolouration for the TNI to have a darker red appearance compared to SNP and CFP methods. The observation could best be explained by high ultimate pH influenced by lactate production, as a result of high glycogen depletion caused by acute stress levels at antemortem (see Chapter 3), limiting the production of lactic acid which has antimicrobial properties, preventing the spoilage of meat. The presence of oxygen in the retained blood in the form of oxymyoglobin promotes lipid oxidation, a substrate for myoglobin oxidation causing muscle tissue discolouration (Everse and Hsia, 1997;

Alvarado *et al.*, 2007), accelerating spoilage through surface microbial growth, causing rapid meat spoilage due to multiplication of microorganisms.

The lower values for muscle tissue yellowness ( $b^*$ ) for the SNP compared to the TNI and CFP slaughter treatment could be explained by the effect of a higher bled-out rate and lower muscle pH achieved in the early post-mortem (as discussed earlier). These observations could also be influenced by the absence of blood myoglobin and the oxygenation of available haemoglobin in the muscle tissue for the SNP slaughter treatment (Lindahl *et al.*, 2001; Karamucki *et al.*, 2013; Fernández *et al.*, 2021).

The finding that muscle tissue lightness ( $L^*$ ) was higher for the CPF and TNI slaughter treatment could be explained by prolonged stress during slaughter, resulting in the high muscle twitching (relaxation and contraction) action, causing the muscle to become less firm and intact, thus allowing light to penetrate the muscle when meat is displayed on shelves. Such gives rise to meat muscle with a lighter colour which is despised by the consumers in retail outlets (Hughes *et al.*, 2017; Purslow *et al.*, 2020). This implies that the fibre shape and type might have also affected the bulk optical properties of muscles (Swatland, 2002; 2004) as a result of the contraction and relaxation of the muscle fibres by the animal when coping with pain and stress during slaughter.

#### **4.5 Conclusion**

The difference in meat redness ( $a^*$ ), yellowness ( $b^*$ ), and Lightness ( $L^*$ ) was significantly associated with slaughter treatment and time. Higher muscle tissue redness ( $a^*$ ), yellowness ( $b^*$ ), and lightness ( $L^*$ ) were observed for the TNI, and CFP, resulting in delayed colour change compared to the SNP slaughter treatment. The TNI and CFP are the recommended alternative to SNP slaughter treatment.

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## **CHAPTER 5: General discussion, recommendation, and Conclusion**

### **5.1 General discussion**

The broad objective of the study was to assess the relationship between TNI, SNP, and CFP slaughter techniques on post-mortem glycolytic metabolites and shelf-life properties of goat meat. The main hypothesis tested was that TNI, SNP, and CFP slaughter methods would not affect post-mortem glycolytic metabolites (glycolytic potential, glycogen, and lactate formation) and shelf-life properties (meat pH, and colour parameters of  $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $H^*$ ) of goat meat. Eighteen (18) non-descript male castrates which are a common type of goat found in villages across Southern Africa, averaging weight of  $16.8 \pm 1.84$  kg with the live weight ranging between 18 and 21 kg, and sixteen months of age, were stratified based on live weight and randomly assigned into the TNI, SNP, and CFP slaughter treatments (6 goats/treatment). Goats were slaughtered with TNI, SNP, and CFP based on their treatment group and the *Musculus longissimus thoracis et lumborum* (LTL) was sampled at post-mortem for muscle tissue glycolytic potential, glycogen, lactate levels, ultimate pH, and colour measurements.

The hypothesis tested in Chapter 3 was that there would be a similar effect on the post-mortem glycolytic metabolites and the physicochemical properties of goat meat slaughtered with TNI, SNP, and CFP slaughter treatment. Although goat meat's physicochemical properties varied with the type of indigenous slaughter, TNI and CFP methods were associated with goat meat with higher glycolytic potential as energy reserves in muscle tissue post-mortem when compared to SNP slaughter treatment. Therefore, the hypothesis that there will be similar post-mortem glycolytic metabolites of muscle tissue slaughtered by TNI, SNP, and CFP slaughter methods was rejected. The lower muscle tissue ultimate pH in addition to higher glycolytic potential, glycogen, lactate levels, redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ) values for the TNI, and CFP slaughter treatment was attributed to lower antemortem stress, wound patency, and bled-out rate. The study has shown that the TNI and CFP slaughter treatments had higher glycolytic potential, glycogen, and lactate levels, consequently, improving the physicochemical properties of muscle tissue.

Chapter 4 was documented to test the hypothesis that TNI, SNP, and CFP slaughter treatments will not influence the post-mortem shelf-life of goat meat. The muscle tissue yellowness ( $b^*$ ) reduced with time, reaching its highest on day 3 for the CFP, day 7 for the TNI and SNP, and day 11 for the CFP ( $P < 0.05$ ). The observed difference in the

decrease of muscle tissue yellowness ( $b^*$ ) could be influenced by blood myoglobin enhancing lipid oxidation, consequently reducing the shelf-life of meat from the SNP slaughter treatment. The study showed that TNI had a longer shelf-life compared to SNP and CFP slaughter treatments.

## **5.2 Conclusions**

Results of the current study revealed that TNI and CFP slaughter treatment improved post-mortem muscle tissue ultimate pH, glycolytic potential, glycogen, lactate levels, redness, ( $a^*$ ), yellowness, and chroma ( $C^*$ ) values, and shelf-life. It can be concluded that TNI and CFP can be used as an alternative to SNP treatment to improve the visual appearance and shelf-life of goat meat.

## **5.3 Recommendations**

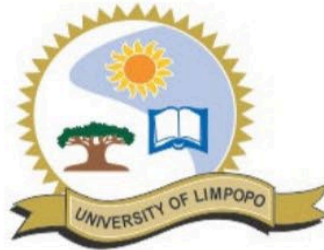
It can be recommended that TNI and CFP be used as an alternative to the SNP slaughter treatment to improve the visual appearance and shelf-life of goat meat.

Areas that require further research include the following:

1. Determining the effect of TNI, SNP, and CFP slaughter treatments on blood metabolites;
2. Determining the effect of TNI, SNP, and CFP slaughter treatments on post-mortem muscle fibre types;
3. Assessment of the measurements of shelf-life by integrating microbial, colour, and lipid oxidation parameters.

## 6. Appendices

### Appendix 1: Ethical approval letter for the goats trial



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**ANIMAL RESEARCH ETHICS  
COMMITTEE CLEARANCE CERTIFICATE**

**MEETING:** 27 September 2022

**PROJECT NUMBER:** AREC/11/2022: PG

**PROJECT:**

**Title:** Effect of Indigenous Slaughter Methods on Postmortem Glycolytic Potential and Shelf-Life of Goat Meat.  
**Researcher:** CM Seloane  
**Supervisor:** Dr ZM Mdletshe  
**Co-Supervisor/s:** Dr O Tada  
**School:** Agricultural and Environmental Sciences  
**Degree:** Master of Science in Agriculture (Animal Production)

  
**PROF JW NGAMBI**  
**CHAIRPERSON: ANIMAL RESEARCH ETHICS COMMITTEE**

The Animal Research Ethics Committee (AREC) is registered with the National Health Research Ethics Council, Registration Number: **AREC-290914-017**

**Note:**

- i) i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
- ii) ii) The budget for the research will be considered separately from the protocol.
- iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.