Physiological tissue losses of feedlot cattle transported for slaughter in South Africa

by

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DECLARATION

I, Suné Bartman, hereby declare that this dissertation, submitted for the degree: MSc (Agric) Animal Science: Production Physiology and Product Quality, at the University of Pretoria, is my own work and has not been submitted by me for a degree at any other University.

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LIST OF ABBREVIATIONS

%	Percentage
%TL	percentage transportation loss
AA	amino acids
ACTH	adrenocorticotropic hormone
ACTH-RH	adrenocorticotropic hormone releasing hormone
ADH	antidiuretic hormone
ADP	adenosine diphosphate
AIC	Akaike Information Criterion
ATP	adenosine triphosphate
BAA	β-adrenergic agonist
BIC	Bayesian Information Criterion
BHB	β-hydroxy butyrate
cAMP	cyclic adenosine monophosphate
CCM	cold carcass mass
CDR%	cold dressing percentage
CK	creatine kinase
Cl^-	chloride
CNS	central nervous system
CPK	Creatine phosphokinase
CRF	corticotrophin-releasing factor
Cu	copper
DFD	dark, firm, dry
DNA	deoxyribonucleic acid
DR%	dressing percentage
ECF	extracellular fluid
ETC	electron transport chain
FCR	feed conversion ratio
Fe	iron
FFA	free fatty acids
FWD	food and water deprivation

GIT	gastrointestinal tract
GLDH	glutamate dehydrogenase
GLM	general linear model
GLMM	generalised linear mixed model
GOT	glutamic oxaloacetic transaminase
GPX	glutathione peroxidase
GPX1	glutathione peroxidase 1
GPX3	glutathione peroxidase 3
GTP	glutamic pyruvic transaminase
H	hydrogen
H2O	water
H_2O_2	hydrogen peroxide
HCO_3^-	bicarbonate
HPA	hypothalamic-pituitary-adrenal
HS	heat stress
Hsp	heat shock proteins
HSP 90	heat shock protein 90
HSP70	Heat Shock Protein 70
HSPA1A	heat shock protein family A member A1
ICF	intracellular fluid
K+	potassium
Kg	kilogram
LDH	lactate dehydrogenase
LDH	lactate dehydrogenase
LM	live mass
MANOVA	multiple analysis of variance
MDA	malondialdehyde
Mm	millimetre
Mn	manganese
mRNA	messenger RNA
N	nitrogen
NAD	nicotinamide adenine dinucleotide (oxidized)

NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate
NEFA	non-esterified fatty acids
O2•-	superoxide radicals
PCV	packed cell volume
PDK4	pyruvate dehydrogenase kinase 4
pH	percentage hydrogen
pHu	ultimate percentage hydrogen
RNA	ribonucleic acid
ROS	reactive oxygen species
SCF	subcutaneous fat
SOD	superoxide dismutase
SOD1	superoxide dismutase 1
SOD3	superoxide dismutase 3
TBW	total body water
TCA	tricarboxylic acid
THI	Temperature Humidity Index
UV	ultraviolet
VFA	volatile fatty acids
VIP	Vasoactive intestinal peptide
WCM	warm carcass mass
WDR%	warm dressing percentage

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ABSTRACT

Pre-slaughter loss of live mass due to transportation is a well-known occurrence in the beef production industry – predominantly regarding the economic sustainability thereof because it represents a potentially significant loss in monetary value. It is widely accepted that stress is a major contributor to the physiological tissue loss occurring during transport. However, the multi-factorial nature of pre-slaughter stress and the inevitability of some of these stressors, such as heat stress, fasting, handling and transport, has made it difficult in the past to predict the expected losses during a journey to the abattoir for slaughter. Individual variation among the cattle transported and slaughtered as a group has been presumed the main explanation for the variation seen in live mass losses, and the reason for reduced carcass mass. The purpose of this study was to determine if variation in certain fixed factors among transported feedlot cattle, including breed type, pre-slaughter mass, carcass classification, fat %, subcutaneous fatness (SCF), β-adrenergic agonist treatment, ADG, FCR, dressing % and CCM, significantly contribute to the variation in the percentage of transportation loss (%TL) of typical feedlot cattle transported for short (less than 100km) distances to the abattoir, as well as to determine the expected range of live mass loss due to transport based on these factors. Empty body mass prior to slaughter and post-transport masses were used to determine individual transport losses. Data on all the above factors were also recorded and used for the analysis. The results indicate that average losses between 1.51% and 2.01% can be expected for typical feedlot cattle in South Africa being transported for short distances (less than 100km) to the abattoir. The greatest contributing factor to the variation observed in transport losses was fat % (P = 0.007), which had a negative relationship with %TL. Higher fat % therefore correlated with lower transport losses. This also explains the significance of β-agonist treatment (P = 0.042), and the trends observed in breed type and carcass classification (for datasets 1 and 2), despite their insignificance (P = 0,957; P = 0.,160; P = 0,698). Live mass, cold carcass mass (CCM) and dressing % showed no trend and were all found to have insignificant effects on %TL (P = 0,519), (P = 0,300; P = 0.820), (P = 0,450; P = 0,230). Average daily gain, FCR and SCF also all had P >0.05 significance values, and therefore had no effect on %TL. The results of this study show that fatter animals have a tend to lose less physiological tissue as moisture, but also that the difference between losses of lean and fat animals are relatively small, consistently staying below 2.5%, regardless of fat %, β-adrenergic agonist treatment, breed or carcass classification. More research is needed to determine if longer transport distances have an effect on this value, and what the extent of that effect would be.

CHAPTER I: INTRODUCTION

For every abattoir-ready animal, the 24 – 48h preceding slaughter entails a certain degree of antemortem stress induced by the unavoidable pre-slaughter management events (Grumpelt *et al.,* 2015). These events elicit a stress response that results in the alteration of both behaviour and physiology, each having the potential to significantly affect the traits of economic importance as well as animal welfare in a negative manner (Grumpelt *et al.,* 2015; Njisane & Muchenje, 2014). Of particular interest in this study is the effect that the ante-mortem stress response has on tissue losses that then result in live- and carcass mass-losses, and which contributes to these losses.

Due to the multifactorial nature of the antemortem stressors (Chulayo *et al.,* 2016), defining the quantity, origin and rate of tissue loss in order to determine the exact effect thereof has not yet been accomplished under South African conditions. Considering the size of the beef industry in South Africa and its contribution to food security, job creation and economic growth (DAFF, 2019), ensuring maximum profitability to safeguard industry viability is of utmost importance. This is especially true due to growing threats such as climate change, increasing disease outbreaks, rising input-costs (DAFF, 2019) and the ever-intensifying attacks on the production procedures of the industry from an animal welfare perspective (Agbeniga, 2011). Identification and quantification of the multitude of pre-slaughter stressors could lead to solutions that may very well result in a significant increase in the overall profitability of beef production, along with the subsequent twofold benefit of lower per kg meat prices and increased animal welfare for the concerned consumer (González et al., 2015).

The purpose of this literature review is to evaluate the effects of the pre-slaughter events – grouped as fasting, transport and lairage – on the cumulative stress response of beef cattle and subsequently what physiological changes may occur that will cause tissue losses in animals. Physiologically, the loss of mass can be attributed to four primary stress induced insults, namely hypoglycaemia, dehydration, tissue catabolism and ion depletion (Grumpelt *et al.,* 2015). These stress responses allow an animal to actively respond to a perceived threat by increasing blood circulation, the availability of energy substrates and the oxygenation of muscles (Reichea *et al.,* 2019). Due to the stressful nature of the 24- to 48-h immediately prior to slaughter, these physiological responses occur and subsequently have a negative impact on the traits of economic importance, including live mass, carcass yield, intramuscular fat content and meat quality traits (Schaefer *et al.,* 2001)

Certain pre-slaughter stressors have an emotional origin, such as fear caused by the presence of humans or being in an unfamiliar environment with unfamiliar animals, while other stressors have a physical origin, such as the inability to find food or water, a pain stimulus or transport- induced fatigue (Reichea *et al.,* 2019). The quantity of LW lost is a function of the degree of emotional and physical stress experienced by an individual animal, and this in turn is greatly affected by environmental conditions, transport duration, the type of animal and the total time without food and water (Grumpelt *et al.,* 2015). The combined effect of the above-mentioned factors determines how much distress the animal experiences and thus how much the body's demand for energy and water increases, while also increasing the frequency of urination and defecation (González *et al.,* 2015). When no food and water are available to supply the increased energy demand for restoring homeostasis, body reserves are mobilized instead, ultimately depleting these reserves and causing dehydration (González et al., 2015).

The attempt to maintain or restore homeostasis upon experiencing a stress-stimuli occurs by activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in elevated levels of catecholamines and cortisol (Schaefer *et al.,* 2001). Cortisol leads to an increase of plasma glucose levels due to the release of glucose from the liver and also prevents the uptake of glucose by adipose tissue. In addition, cortisol is necessary for the activation of glycogen phosphorylase by adrenaline (Reichea *et al.,* 2019). Along with activation of the HPA axis is the activation of non-HPA axis events, both resulting in a number of biochemical changes that lead to the previously mentioned physiological insults (Schaefer *et al.,* 2001).

The first factor influencing the degree of live mass loss is the length of time the animal is deprived of food and water. The duration of food and water deprivation affects the extent of dehydration of body fluid components from muscle and non-muscle tissue, which further enhances the adrenocortical response to stress (Schaefer *et al.,* 2001). Secondly, the duration of transport and all transport-related events - such as handling, loading, and unloading –affects live mass loss (Chulayo *et al.,* 2016), as these stressors activate several biological pathways known to induce oxidative stress (Deters & Hansen, 2019) and also negatively affects the bodies energy reserves (Chulayo *et al.,* 2016). Thirdly, lairage may add to the stress of animals as they are introduced into unfamiliar surroundings and mixed with unfamiliar animals. This results in increased physical activity due to homosexual behaviour (e.g., bulling or riding behaviour), teasing and mounting (Mohan Raj *et al.,* 1992), as well as fighting. Animals may also spend extended

times in lairage prior to slaughter, increasing the duration deprivation (Liu *et al.,* 2012). Furthermore, environmental conditions throughout the pre-slaughter period also greatly influence an animals stress response and therefore the loss of live mass, particularly ambient temperatures and humidity.

The cost of rearing beef and the current emphasis on the importance of meat quality in the sale of beef products justifies taking the maximum amount of care of the live animal to prevent loss of potential yield, and to ensure the best possible lean meat quality. Good handling - which takes advantage of our knowledge of animal behaviour - is likely to promote animal welfare and prove to be desirable from an economic viewpoint (Warris,1990). Thus, procedures which reduce carcass shrink, eliminate bruising, and prevent the occurrence of dark cutting meat are generally also good for the animal's welfare. Only through concern for good meat quality and better animal welfare is a profitable industry that produces meat in an ethically acceptable and sustainable way a possibility (Warris,1990)

This study aimed to explore the possible reasons behind the variation seen in the % of physiological tissue loss of feedlot cattle when transported for short distances (less than 100 km) to the abattoir, as well as to determine the range within which these losses can be expected. The factors considered included cattle breed type, pre-slaughter mass, carcass classification, carcass fat %, subcutaneous fatness, β-adrenergic agonist treatment, average daily gains, feed conversion ratio, dressing % and cold carcass mass.

In order to achieve this aim, the following objectives were set:

1. Determine whether variation in certain factors among feedlot cattle significantly contributed to the variation seen in the % of physiological tissue loss of feedlot cattle transported for less than 100km to the abattoir.
2. Determine the expected range of live mass loss (%) resulting from transport based on the factors mentioned above.

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CHAPTER II: LITERATURE REVIEW

2.1 Describing the different types of live mass loss

Pre-slaughter live mass loss has long been known to impact the economic profitability of the meat production industry (Asplund *et al.,* 1982), representing a potentially significant loss in monetary value (Revell, 1968). This mass loss is frequently referred to as shrink and comprises of losses from both the live animal and carcass mass (Parish & Rhinehart, 2017). Loss of live mass in the pre-slaughter period occurs in two forms (Coffey *et al.,* 2001); the loss of gut fill and the loss of physiological tissue (Revell, 1968). Herbivore live mass is dynamic; daily feed consumption can comprise around 4% of their body mass, yet over a 24h period no significant change in live mass is observed (Hogan *et al.,* 2007). This owes to the fact that feed is fermented, metabolised, and then excreted in faeces and urine as well as bodily secretions and expired air (Hogan *et al.,* 2007). Loss of gut fill therefore refers to the loss in body mass that occurs when the stomach, digestive tract and bladder are emptied within the first few hours after fasting, when gut content cannot be replenished (Revell, 1968).

The rate at which the rumen residues are removed depends largely on the diet fed prior to fasting and the physiological stage of the animal (Hogan *et al.,* 2007). More digestible feeds would be excreted at a faster rate than less digestible feeds, resulting in faster rumen voiding (Hogan *et al.,* 2007). The type, quantity, and quality of feed that cattle consume determines the capacity of the gut and how much feed is contained within it, therefore it plays a role in the extent of shrink cattle experience. Feedlot cattle are fed high quality concentrate diets associated with less gut fill and therefore also less shrink or gut loss (González *et al.,* 2015). Losses from gut fill can, however, easily be recovered when animals regain access to feed and water (Revell, 1968). Tissue losses occur when the energy and water required to maintain homeostasis and continue essential bodily processes can no longer be extracted from external food sources (González *et al.,* 2015). The body compensates for this by drawing the required moisture and energy from various carcass tissue (Revell, 1968), comprising catabolism of both lipids and protein as well as depletion of muscle glycogen which then ultimately results in dehydration and an overall negative energy balance (Schaefer *et al.,* 2001).

Due to the greater water holding capacity of protein compared to fat, greater levels of tissue moisture loss are typically encountered in animals with higher lean-to-fat ratios, implying that physiological tissue loss is a function of internal factors such as animal age, sex, breed, and body condition (Parish & Rhinehart, 2017). The quantity of tissue loss

increases when animals are subjected to external sources of stress, which is an unavoidable part of pre-slaughter management. Therefore, live mass loss is also a function of the external stress-inducing factor such as the total time without feed and water, transport duration, environmental conditions, and handling procedures (Schaefer *et al.,* 2001). Animals ready for slaughter suffer from hunger and fatigue before, during and after transport and the strong social structure among cattle elicits an immediate nervous reaction when miTong Xing with unfamiliar animals during lairage, all contributing to the cumulative stress response. Just like stress, shrink is a cumulative phenomenon with high variability among species, breed, and specific pre-slaughter conditions (Coffey *et al.,* 2001). The greater the degree of stress an animal is subjected to, the greater the mass it loses and the bigger the loss of monetary value (Revell, 1968).

In a study done on long-haul (longer than 400km) transport of North American cattle, the mean shrink experienced was 5.3± 1.79% of live mass, increasing between 7:00 AM and 18:00 PM, and decreasing thereafter. The rate of loss was shown to be greater when temperatures were higher and for longer times travelled. For every hour spent on the road, shrink was shown to increase at a decreasing rate, reaching a maximum value at 40h travel time. The research done under conditions in North America shows that the average shrink values for commercial long-haul travel of cattle ranges between 2 and 14% of their LW, the actual value being dependant on several factors unique to each situation (González *et al.,* 2015).

2.2 Physico-chemical aspects of stress

Any situation causing fear, anxiety or restlessness in an animal is perceived as a threat to their survival, eliciting a physiological adaptive response known as a distress response (Hernández-Hernández-Cruz *et al.,* 2016). The amygdala is the part of the brain in charge of responding to and controlling such fears or anxieties by means of activating the physiological stress responses of the body (Agbeniga, 2011). Upon detecting a fear-eliciting stimulus, the amygdala activates the two central integrated processes, namely the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA-axis), to initiate a reaction appropriate for the type of stressor (O'Neill, 2019). The sympatho-adrenal component of the autonomic response is usually the first and fastest to act in response to a fight or flight situation and is mediated by the catecholamines epinephrine and norepinephrine (Agbeniga, 2011; O'Neill, 2010). Acute stressors like handling, loading, transport and unloading activate an autonomic response that initiates a rapid release of catecholamines (O'Neill, 2019). This alters carbohydrate metabolism to ensure that glucose is purposed towards essential bodily functions (Lacourt & Tarrant, 1985) and to

help the animal cope with adverse situations (O'Neill, 2019). The catecholamine-induced effect on glucose homeostasis is associated with greater adenosine triphosphate (ATP) production, higher rates of aerobic glycolysis and an increased glucose availability from glycogenolysis and gluconeogenesis (O'Neill, 2019). The precursor to norepinephrine and epinephrine, dopamine (O'Neill, 2010), is also secreted during stressful conditions and is responsible for guiding the animals away from dangerous environments and towards those that provide food and safety (O'Neill, 2019), as well as playing a role in gluconeogenesis (Matsumura *et al.,* 1980). Synthesis of these three catecholamine neurotransmitters is all dependent on the availability of their amino acid precursor tyrosine, a non-essential large neutral amino acid (O'Neill, 2010). Ante-mortem stress provokes a fight or flight response that increases the synthesis and utilisation of dopamine, norepinephrine, and epinephrine to such an extent that tyrosine is often the rate-limiting factor, compromising further synthesis of the catecholamines (O'Neill, 2010). When catecholamine synthesis is compromised, numerous behavioural and physiological changes occur that make animals less resistant to stress and unable to function normally (O'Neill, 2010). Some of these physiological changes include increased heart and respiration rates, elevated body temperatures and redistribution of visceral blood volume towards the skeletal muscle and brain (Agbeniga, 2011; O'Neill, 2010). Figure 2.1 shows the physiological pathway of a response to a fear stimulus.

The biological effects of catecholamines are facilitated by two different classes of transmembrane receptors - α-adrenergic receptors and β-adrenergic receptors - located on a variety of mammalian tissue (O'Neill, 2019). Upon stimulation of these receptors with agonists epinephrine and norepinephrine, different responses take place of which the effects are directed towards a similar goal; mobilisation of energy reserves and redirection thereof to locations where immediate reaction is required (O'Neill, 2019). Both agonists and antagonists have the ability to bind to hormone receptors, the difference being that agonists elicit a hormonal response and antagonists do not – they serve as blockers against agonist activity (O'Neill, 2019). When an α-agonists stimulates an α-adrenergic receptor, inhibition of adenylate cyclase leads to contraction of the smooth muscle in blood vessels supplying peripheral organs, relaxation of smooth muscle in the gastrointestinal tract (GIT) and blood platelet aggregation. Activation of adenylate cyclase takes place when a β-agonist binds to a β-adrenergic receptor, leading to increased heart rate, higher rates of gluconeogenesis and relaxation of the smooth muscle in the bronchi and blood vessels supplying the skeletal muscle (Voet & Voet, 1990). Activation of the catecholamine system when animals experience a stress-

stimuli results in increased blood flow to the brain, and this in turn allows then to better manage the stress (O'Neill, 2019).

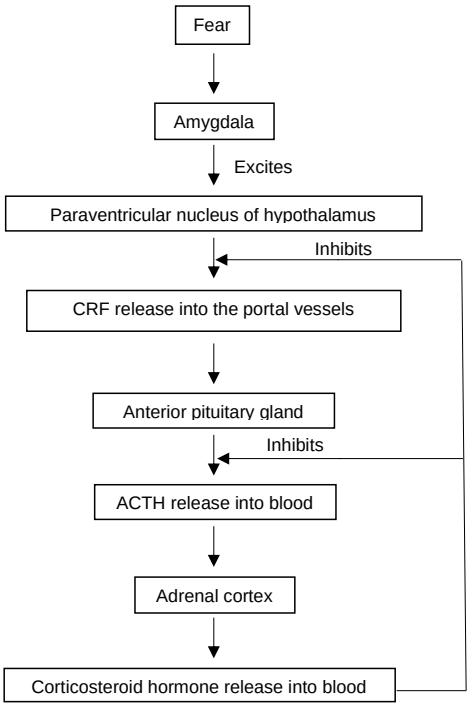

Figure 2.1 Physiological pathway of the response to a fear stimulus

Norepinephrine regulates the release of corticotrophin-releasing factor (CRF) from the brain via a α1-adrenergic receptor: the paraventricular nucleus of the hypothalamus responds to norepinephrine by releasing CRF, which passes through the hypophyseal-portal vessel to the pituitary gland where it induces the release of adrenocorticotropic hormone (ACTH) into the general circulation, thereby activating the HPA axis (Agbeniga, 2011; O'Neill, 2019). The HPA axis is responsible for maintaining a long-term stress response, which is characterized by the presence of glucocorticoids (Deters & Hansen, 2019). The releases of CRF from the hypothalamus - the site in the brain where the endocrine and nervous systems meet (Dukes, 2015a) - and subsequent release of ACTH from the pituitary gland stimulates the zona fasciculata of the adrenal cortex to secrete cortisol, the primary glucocorticoid in mammals. On the surface of the adrenal

fasciculata cells, receptors for ACTH are found that stimulates adenylyl cyclase activity upon ACTH binding. This increases the activity of intracellular cyclic adenosine monophosphate (cAMP), which in turn stimulates the synthesis of cortisol. ACTH therefore can be considered a regulator of cortisol secretions. ACTH secretion itself is controlled by adrenocorticotropic hormone releasing hormone (ACTH-RH) secretions from neurons in the hypothalamus into the Hypothalamo-hypophyseal portal system. Normally, ACTH and cortisol secretions display a circadian rhythm, however under stressful conditions the secretion of cortisol is more continuous and could lead to hypertrophy of the zona fasciculata in the case of chronic stress (Dukes, 2015a). Elevated plasma cortisol levels cause an increase in glucose levels by stimulating the synthesis of enzymes involved in gluconeogenesis, for which the main substrates are muscle derived amino acids. Cortisol acts on adipose tissue by decreasing its sensitivity to insulin to make more blood glucose available for utilization by the brain and muscle and simulates lipolysis of adipose tissue to increase the fatty acid levels in the blood. Cortisol also acts on muscle and other tissue by stimulating protein degradation to increase blood levels of amino acids. In addition to this, cortisol strengthens the action of glucagon and epinephrine on glucose metabolism to divert energy away from growth and towards body maintenance in stressful times (Dukes, 2015a).

2.3 Ante-mortem stressors

2.3.1 The ante-mortem stress-inducing effects of fasting and hunger

Loss of live mass begins when the allocated group of cattle are fasted prior to transport. A fasting time of 24h is recommended for both sheep and cattle in order to optimise bleeding and carcass cleanliness, to decrease the risk of rupturing the digestive tract during evisceration and to prevent contamination of the carcass with faecal and digestive contents (Agbeniga, 2011). When cattle are deprived of food and water it affects rumen function, tissue homeostasis and the digestive tract, ultimately resulting in a loss of live mass. Some of this live mass loss can be attributed to cessation of feed and water intake, but the major losses arise from catabolic processes which take place at a declining rate (Hogan *et al.,* 2007). When animals are subjected to stress, physical exertion and environmental discomfort, the bodies demand for energy as well as the frequency of urination and defecation increases (González *et al.,* 2015). When fasted, no external food source is available to meet the increased energy requirements, and body reserves start being mobilised (González *et al.,* 2015). This negative energy balance leads to protein catabolism - reflected by elevated levels of plasma lactate and proteolytic enzymes (Schaefer *et al.,* 2001) – as well as mobilisation of fat reserves, evident by the

increase in plasma-free fatty acid concentrations (O'Neill, 2019). Animals experience this negative energy balance as hunger; a basic need that causes further physiological distress as it cannot be resolved during fasting (O'Neill, 2019). Hunger and satiety are controlled by hormones and neurotransmitters produced in the central nervous system (CNS) and periphery, the main hormones being leptin and ghrelin (Hogan *et al.,* 2007). Leptin is secreted to inhibit the action of neuropeptides that stimulate eating and finds its origin in full or filling adipose tissue (Dukes, 2015A). Ghrelin is found in the abomasum of ruminants and acts as an appetite stimulant; therefore, the abomasum can be considered the source of hunger signals in fasted animals (Hogan *et al.,* 2007). These hunger signals coincide with the lower volume and acidity of secreted gastric juices and reflects the decreased flow of volatile fatty acids (VFA) from the rumen through the omasum. In an acylated form, ghrelin functions as an endogenous ligand for the growth hormone secretagogue receptor. Increases of ghrelin therefore result in a dose dependant release of growth hormone. During fasting, plasma concentrations of leptin fall, and an increase in plasma concentrations of ghrelin, as well as non-esterified fatty acids (NEFA) is observed, consequentially growth hormone levels are also heightened. How much the stress induced increase in blood cortisol levels influence ghrelin and leptin is, however, not very well described (Hogan *et al.,* 2007). Furthermore, catecholamine secretion results in significant changes in energy metabolism, including lipolysis, glycogenolysis in muscle and gluconeogenesis (O'Neill, 2019). Epinephrine secreted during antemortem stress increases the rate of lipolysis, causing elevated levels of free fatty acids in the plasma of cattle (Schaefer *et al.,* 2001). Epinephrine also effectively initiates glycolysis through a series of biochemical changes that amplify the cAMP-mediated pathway, allowing for rapid activation of phosphorylase (O'Neill, 2019). As blood glucose is the main energy source for the brain, the glycolysis-activating effect of catecholamines play a crucial role in supplying the brain with sufficient energy (O'Neill, 2019). Withdrawal of food and water in combination with stressors such as transport and miTong Xing during lairage increases the demand for catecholamines, implying that feed restrictions may result in shortages thereof, decreasing an animal's resistance to stress (O'Neill, 2019). Gregory (1998) states that cattle can lose around 7% of live mass during the first 12 hours of fasting, 9% after 24 hours and 11% by 72 hours (Gregory, 1998).

2.3.2 The ante-mortem stress inducing effects of transport and handling

Transportation of cattle is an unavoidable component of the beef production chain. Physiological and physical stressors associated with transport affect both the performance and carcass characteristics of beef cattle, and therefore also influences the economics of the industry (Deters & Hansen, 2019). The coping mechanisms an animal

develops in order to handle the stressful environment leads to alterations in their physiological processes which causes a change in the normal bodily dynamics (Chulayo *et al.,* 2016). During transport animals are deprived of food and water and are often exposed to environmental challenges such as extreme heat and high humidity. These animals also experience physical exertion during transport and loading which contributes to the lower available energy for maintenance and therefore tissue shrink. Likewise, long periods of standing and attempting to maintain balance could result in muscle fatigue and damage (Deters & Hansen, 2019). Muscle fatigue is defined as the decreased ability to generate appropriate amounts of contractile force and can occur either shortly after the onset of exercise or after a prolonged duration of exercise (Deters & Hansen, 2019). Bruising is also a common occurrence during transport, especially when over- or underloading occurs. In addition to this, heat stress, oxidative stress and dehydration are all associated with transportation and will be discussed individually below.

(a) The effects of transport induced heat stress

Hyperthermia, or heat stress (HS), commences when external factors raise the core body temperature of homeothermic animals above that of the resting state (Belhadj Slimen *et al.,* 2015), resulting in an imbalance between heat gained from the environments and that lost to the environment (Brown-Brandl, 2018). Core body temperature is thought to be a very consistent indicator of hyperthermia, however describing it is somewhat problematic due to the inconsistency of an available definition (Lees *et al.,* 2018). Hyperthermia commonly occurs throughout the summer months when heatwaves frequently arise, resulting in great economic losses for the producer as a result of increased mortalities, decreased production performances (Brown-Brandl, 2018) and the effect thereof on live mass losses prior to slaughter. Heat gain and loss from the body is constantly taking place (Lees *et al.,* 2018), and if the heat gained exceeds the species-specific thermoneutral range of livestock, it results in a greater total heat load than the animal has capacity to dissipate (Belhadj Slimen *et al.,* 2015), ultimately rising its core temperature (Lees *et al.,* 2018).

Animals can experience heat stress in a variety of degrees ranging from mild to severe depending on the specific environmental conditions, the individual animal susceptibility, and the management practices for heat alleviation (Brown-Brandl, 2018). Various parameters are used to describe heat stress, including relative humidity, exposure to solar radiation, wind-speed as well as ambient temperature – the primary parameter used to determine the level of HS an animal experience (Brown-Brandl, 2018). The main mechanism by which animals maintain homeothermy during hot environmental

conditions is evaporative cooling (Gaughan *et al.,* 2014). Cattle primarily make use of sweating as an evaporative cooling mechanism, but in extreme conditions it might not be sufficient to dissipate the required body heat and therefore they resort to panting (Gaughan *et al.,* 2014). When high temperatures are combined with high humidity, the collective effect is even greater due to the reduced ability of the animal to dissipate heat by means of evapotranspiration (Belhadj Slimen *et al.,* 2015). To quantify this combined effect, a Temperature Humidity Index (THI) is used. The THI index is used to provide guidelines for livestock management during heat stress conditions, with a THI of <74 being normal, a 74<THI<79 being alert, a 79< THI <84 being dangerous and THI >84 being an emergency (Brown-Brandl, 2018). In a study done on North Korean indigenous cattle, a significant increase in serum cortisol levels and Heat Shock Protein 70 (HSP70) levels was seen at THI of 84.05. Similarly, (GOT) and glutamic pyruvic transaminase (GTP) – markers used to identify liver damage - as well as NEFA – produced due to lipolysis - levels were shown to be significantly higher at THI of 84.05, while serum glucose levels were significantly lower at THI of 82.92 (Won *et al.,* 2018).

Some species and breeds within species have a greater tolerance to heat stress, or a larger thermoneutral zone when compared to others (Belhadj Slimen *et al.,* 2015). They are physiologically better equipped for heat dissipation, with traits including larger skin area to live mass ratio, pigmented skin and eyelids, light coloured coats, and shielded eyes (Belhadj Slimen *et al.,* 2015; Brown-Brandl, 2018). The physiological state of the animal is also important as it dictates the magnitude of the animals response to heightened temperatures. Studies on the effect of HS on the cells date back to 1970 and 1980, where it was found that both fluidity and stability of cellular membranes are affected by HS. In addition to this, HS was shown to inhibit receptors and the function of transmembrane transport proteins. Protein synthesis as well as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerization are compromised under heat stress. In addition to this, HS is also responsible for protein denaturation and aggregation thereof into the nuclear matrix, increasing its concentration and altering molecular functions such as DNA synthesis, replication, repair, cell division and the function of DNA polymerase (Belhadj Slimen *et al.,* 2015). When exposed to elevated temperatures, expression of a small group of polypeptide proteins, namely Heat Shock Proteins (Hsp) take place. They function in binding with denatured proteins to prevent further denaturation and also work together with ATP to renature partly denatured proteins as soon as stress levels return to baseline values (Chulayo *et al.,* 2016).

Heat shock proteins bind to ATP in times of stress, and in this ATP-bound state it shows a low exchange substrate affinity which stimulates the hydrolysis of ATP, leading to a depletion of muscle glycogen and an increase in ultimate pH (pH_u). This negative relationship can be attributed to the hydrolysis of ATP to adenosine diphosphate (ADP), as well as glycolysis that takes place in muscle as a response to stressful circumstances. HSPA1A (HSP family A member A1) is therefore synthesized by cells in an attempt to maintain intracellular protein homeostasis (Chulayo *et al.,* 2016). When environmental temperatures range within the thermoneutral zone, cattle will not experience heat stress and no additional energy above that required for maintenance is needed to cool the body; cattle can then thermoregulate efficiently with low bodily demands (Belhadj Slimen *et al.,* 2015). Therefore, heat stress contributes to live mass loss as a result of greater energy expenditure, but also due to the greater proportion of respiratory loss at the expense of tissue moisture (Coffey *et al.,* 2001). Tissue losses therefore appear to contribute a greater proportion to the total LW loss in conditions of higher ambient temperature, as can been seen in a report by Philips *et al.,* where urine and faecal output during high ambient temperatures (18 ºC to 34ºC) accounted for only 21.5 and 16.4% of the lost live mass, in comparison to the 35.7 and 29.8% lost when temperatures were low (-16 ºC to -6 ºC) (Coffey *et al.,* 2001).

Animals experiencing heat stress also experience alterations in their energy metabolism, hormone parameters and blood metabolites (Won *et al.,* 2018). Catecholamines, for example, are released from the adrenal medulla during heat stress, with greater quantities of epinephrine than norepinephrine being released. In skin blood vessels, epinephrine has a vasodilatory effect whereas norepinephrine has a vasoconstrictor action; this means that heat-stress induced release of catecholamines result in epinephrine-mediated vasodilation as a means of heat dissipation (Gregory, 1998). Heat stress affects feed intake by upregulating the secretion of two adipokines, leptin and adiponectin, as well as the expression of their receptors. Adiponectin regulates feeding behaviour through peripheral and central mechanisms; it stimulates the oxidation of fatty acids in skeletal muscle and inhibits the production of glucose form the liver, improving the whole-body energy homeostasis. Leptin stimulates the HPA axis, resulting in a depression of feed intake. These mechanisms restrict caloric intake as a means of reducing heat generation (Belhadj Slimen *et al.,* 2015).

The duration of heat stress is important to consider, as this influences protein metabolism of hyperthermic livestock. Acute HS increases the rate of protein catabolism – which increases plasma uric acid levels – and reduces synthesis of proteins and

nitrogen (N) retention. Plasma concentrations of various amino acids are also decreased during acute HS (Belhadj Slimen *et al.,* 2015). When chronic HS is experienced, the physiological expression thereof is different than with acute HS. Protein synthesis in various muscles is decreased, but so is protein catabolism. This results in lower plasma levels of amino acids (Belhadj Slimen *et al.,* 2015). The activity of lipolytic enzymes is reduced whilst the activity of lipoprotein lipase of the adipose tissue is increased in conditions of HS. This suggests that hyperthermic animals have a greater storage capacity of intestinal and hepatic triglycerides (Sanders *et al.,* 2009).

Blood glucose levels are also influenced by the duration and intensity of HS. In acute HS conditions, various studies on broilers and rats showed no difference in blood glucose concentrations, however chronic HS conditions resulted in decreased levels of circulating glucose in Holstein bull calves and heifers, as well as broilers, even though intestinal glucose absorptive capacity of these animals were elevated. This suggests a higher rate of glucose leaving the blood pool during HS, implicating glucose as the favoured fuel source of HS animals and therefore they become increasingly dependent on glucose for their energy needs (Belhadj Slimen *et al.,* 2015). It has also been reported that HS can increase insulin receptor abundance, as shown by a study done on Holstein cows. Due to the increased levels of glucose in the blood during HS, the greater number of receptors are required for decreasing the circulating levels of glucose, increasing insulin sensitivity (Belhadj Slimen *et al.,* 2015).

The tricarboxylic acid cycle (TCA) cycle is also influenced differently by chronic and acute HS, where acute HS accelerates the TCA cycle and increases the quantity of skeletal muscle messenger RNA (mRNA) of pyruvate dehydrogenase kinase 4 (PDK4) and lactate dehydrogenase (LDH) whilst simultaneously decreasing urinary excretion levels of succinate, citrate and 2-oxyglutarate. Chronic HS increases skeletal abundance of PDK4 in ruminants and the ratio of $NADH/NAD^+$, as well as the lactate/pyruvate ratio (Belhadj Slimen *et al.,* 2015). Therefore, entry of pyruvate into the TCA cycle through the pyruvate dehydrogenase complex (PDH complex) is altered by HS. The rate of glycogen breakdown has been shown to increase in hot environment, indicating a greater utilization of glycogen during stress. Both acute and chronic HS results in increased glycogenolysis, however during chronic HS, the rate of gluconeogenesis will also increase, as opposed to the decrease in the rate of gluconeogenesis observed during acute HS (Belhadj Slimen *et al.,* 2015).

In addition to this, an important and understudied aspect of the overabundance of catabolic biochemical events during antemortem stress is heat production. The

biochemical events, that occur upon activation of the HPA axis in response to stress, significantly increase heat production and increases energy expenditure. Antemortem stress results in an initial increase of radiated heat, followed by a gradual decrease after a few hours, depending on factors such as how far the animals travelled, their pre-fasting diet and the extent to which they perceived the stress. An infrared thermogram of the same animal before and after a 1.5h transport showed that post transport body temperatures were greater than body temperatures pre-transport, making this animal a greater risk for producing dark, firm and dry (DFD) meat, as animals that are hotter or colder at slaughter have a greater probability for this (Grumpelt *et al.,* 2015).

(b) Oxidative stress resulting from transport and heat stress

The combination of stressors experienced by an animal in the pre-slaughter period – especially heat stress and transportation stress - activate several biological pathways known to induce oxidative stress, with consequences on livestock health, efficiency, and meat quality (Deters & Hansen, 2019). Oxidative stress occurs when the oxidant-antioxidant equilibrium is disturbed in favour of the oxidants, leading to an overproduction of free radicals and reactive oxygen species (ROS) (Belhadj Slimen *et al.,* 2015), ultimately resulting in the disruption of redox signalling, molecular damage, and loss of physiological function (Sies and Jones, 2007; Sordillo and Aitken, 2009). When antioxidants are decreased or oxidants are increased – or a combination of the two - the steady state concentration of oxidants is disrupted and enlarged. This disenables the antioxidant defence system to counterbalance it and subsequently causes different physiological outcomes that those under normal cellular conditions (Deters & Hansen, 2019). The extent to which the physiological outcomes will be different depends on the intensity and duration of the antioxidant-oxidant imbalance (Lushchak, 2014). Low intensity oxidative stress will elicit an adaptative response where upregulation of antioxidant enzymes take place by the redox regulation of transcription factors responsible for the upregulation (Sundaresan *et al.,* 1995; Bae *et al.,* 1997). Intermediate intensity oxidative stress also elicits an adaptative response, however it may involve damage to the protein, lipid, and nucleic acid components of cells via oxidation thereof. The oxidation of these molecules further aggravates oxidative stress and must be either repaired or degrade via ATP-dependant processes (Lehnman, 1974; Grune *et al.,* 1997; Menoni *et al.,* 2007). At high intensity levels of oxidative stress, also referred to as oxidative distress, cell death can occur by either apoptosis, necrosis or both (Saito *et al.,* 2006). The intensity of oxidative stress can be determined and measured by the presence and quantity of ROS, by the oxidative modification to proteins, the presence of lipids and nucleic acids, the activity of antioxidant enzymes

and the concentration of nonenzymatic antioxidants (Deters & Hansen, 2019). These ROS originate either within the cells or come from exogenous sources such as exposure to ultraviolet (UV) light and certain pollutants (Schröder and Krutmann, 2005). Endogenously, ROS come from the mitochondria, the peroxisomes, and other enzymatic systems. Superoxide radicals ($O_2^{\bullet-}$) form when electrons from coenzyme Q leak onto oxygen in the electron transport chain (ETC), rendering the mitochondria the greatest source of ROS (Turrens *et al.,* 1985). Because the mitochondria are the cellular compartments responsible for the highest proportion of ROS production, they are the first compartments to be damaged upon disturbance of the steady state of ROS (England *et al.,* 2004). This reduces electron flow and causes a decline in ATP synthesis, downregulating cellular energy production (Zhao *et al.,* 2006). Even though $O_2^{\bullet-}$ are not permeable to membranes and relatively unreactive, they serve as precursors to most other ROS and generate oxidative chain reactions (Deters & Hansen, 2019). Upon dismutation (simultaneous oxidation and reduction) of $O_2^{\bullet-}$ – which can occur spontaneously or by enzymatic processes – hydrogen peroxide is formed. Hydrogen peroxide (H_2O_2) has a relatively long half-life, and also has the ability to transverse across membranes, making it a suitable signalling molecule (Reth, 2002). The H_2O_2 can undergo Fenton reactions and Haber-Weiss reactions if iron (Fe) and copper (Cu) are present, resulting in the production of highly destructive hydroxyl radicals, a molecule with a very short half-life and the ability to oxidise most classes of biomolecules (Halliwell and Gutteridge, 1992). Transporting cattle can therefore be considered to increase oxidant levels which then activate ROS-producing biological pathways in response (Deters & Hansen, 2019).

The cytotoxic reaction of HS results in alteration of biological molecules, the modification of metabolic reactions, disturbance of cellular functions and cell damage that activates apoptotic and necrotic pathways (Du *et al.,* 2008; Pandey *et al.,* 2012). In a study done by Hsu *et al* and Lewandowska *et al* (2006) it was found that the morphology of mitochondria in the skeletal muscle of heat stressed rodents presented many abnormalities (Hsu *et al.,* 1995; Lewandowska *et al.,* 2006). Furthermore, Song et al. (2000) observed alterations in locations and structure, such as broken cristae, low matrix density and swelling. The lipid peroxidation and protein denaturation of the mitochondria as a result of HS could therefore be responsible for the inability of such cells to meet the increased energy demand of HS animals. Heat stress is also responsible for disturbing the synthesis of proteins, with the exception of Hsp's. These have a chaperone function, ensuring that folding, unfolding, and refolding of stress-denatured proteins occur. During HS the synthesis of two specific HSPs - namely HSP70 and HSP90 - are increased in

reaction to the increased damage to proteins as a means of cellular defence and/or repair (Belhadj Slimen *et al.,* 2015).

The cellular antioxidant defence system is comprised of enzymatic and non-enzymatic antioxidant, both endogenously synthesized, as well as antioxidants obtained exogenously (Deters & Hansen, 2019). Glutathione and ascorbic acid (vitamin C) are the two non-enzymatic, endogenously synthesised antioxidants. Glutathione is oxidised upon donation of a hydrogen (H) atom by the thiol group of its cysteine to neutralise ROS (Meister & Anderson, 1983). Glutathione is then regenerated to its reduced form by glutathione reductase using nicotinamide adenine dinucleotide phosphate (NADPH) (Deters & Hansen, 2019). Unlike in humans and pigs, the enzymes responsible for catalysing the final step of ascorbic acid synthesis is functional in mammalian livestock species, allowing for the conversion of glucose to ascorbic acid, the major water-soluble antioxidant in cells (Deters & Hansen, 2019). It reduces ROS by two consecutive electron donations, the first resulting in the formation of ascorbyl free radicals. These free radicals then undergo a second electron donation to produce dehydro-ascorbic acid (Bendich *et al.,* 1986). Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase are the enzymatic, endogenously synthesized antioxidants. Superoxide dismutase catalyses the dismutation of $O_2^{\bullet-}$ to form H_2O_2 and water (H_2O) (Deters & Hansen, 2019). Three isoforms exist for SOD, each requiring a different metal cofactor and cellular localization for activity. Copper and Zink are cofactors required by both SOD1 and SOD3, however they differ in location as SOD1 is found in the cytosol and intermembrane space of the mitochondria (Weisiger & Fridovich, 1973; Okado-Matsumoto & Fridovich, 2001), and SOD3 is located in the extracellular space (Marklund *et al.,* 1982). SOD2 is found in the mitochondrial matrix and is dependent on the cofactor manganese (Mn). Because of its location, Mn-SOD represents a crucial first line of antioxidant defense, as the mitochondria are the primary endogenous source of $O_2^{\bullet-}$ that give rise to ROS (Deters & Hansen, 2019). The H_2O_2 produced form the dismutation of $O_2^{\bullet-}$ by SOD can further be reduced to water by the action of either GPX or catalase. Glutathione peroxidase also has two major isoforms in mammals, both dependant on Se as a cofactor and both using the reducing power of glutathione, however differing in location, as GPX1 is cytosolic and GPX3 is membrane associated (Margis *et al.,* 2008). Catalase, the third endogenously produced enzymatic antioxidant, is dependent on the cofactor Fe and found in greatest capacity in peroxisomes where high concentrations of H_2O_2 is produced, however they are distributed throughout mammalian cells (Deters & Hansen, 2019).

Exogenous antioxidants enter the body either by consumption or by parental delivery. They include tocopherols such as vitamin E, and carotenoids (Deters & Hansen, 2019). The lipid-soluble nature of the major antioxidant vitamin E gives it a crucial role in maintaining the integrity of the cellular membrane (Burton *et al.,* 1982). Vitamin E has the ability to donate a phenolic hydrogen to lipid peroxyl radicals within the membrane and other ROS, and in this lies its antioxidant function. Donating a phenolic hydrogen produces a relatively unreactive tocopheroxyl radical that can be reduced by vitamin C or irreversibly oxidised to tocopherolquinone (Deters & Hansen, 2019). Even though lipid-soluble carotenoids such as B-carotene and lycopene have been shown to have antioxidant activity they are present in tissue at notably lower concentrations than vitamin E and therefore it is assumed that its cellular antioxidant defense activity is not as significant (Burton & Ingold, 1984). Even though the antioxidant defense system is in place to prevent cells from going into oxidative stress, it becomes extremely redundant in its protection against the detrimental effects of oxidative stress when last mentioned is already present (Jacob, 1995). In certain cases, nutritional intervention can be used as a means of improving the antioxidant status of an animal prior to exposure to oxidative stress-inducing activities, such as loading and transport (Deters & Hansen, 2019).

Any deficiencies of the vitamins and trace minerals that support the antioxidant defense will result in a decreased ability to maintain the oxidant-antioxidant balance and thereby decrease immunocompetence, resulting in a variety of oxidative stress-induced pathological conditions in livestock (Fernandes *et al.,* 1979; Bendich *et al.,* 1986; Smith *et al.,* 1987). Transported animals have a lower antioxidant capacity and greater lipid peroxidation than their non-transported counterparts, as suggested by two previous studies that compared transported and non-transported beef calves (Deters & Hansen, 2019). The study done by Urban-Chmiel (2006) showed that lipid peroxidation and antioxidant capacity of leukocytes – immune cells that produce ROS as part of their phagocytic mechanism – isolated from transported calves was increased by 71% and 12% post transit. This implied that transportation resulted in oxidative damage that may have stimulated upregulation of cellular antioxidants. Samples isolated from non-transported calves had an antioxidant capacity of 186% greater and lipid peroxidation of 218% lesser than in leukocytes of transported calves. Similar findings were seen by Chirase et al. (2004), who observed an increase of 177% lipid peroxidation and an 11% decrease in antioxidant capacity in the serum of beef calves post transport compared to pre-transport values (Deters & Hansen, 2019). Additionally, the ROS produced by immune cells as part of their function to eliminate pathogenic bacteria can potentially

damage and kill pulmonary cells and result in respiratory inflammation and dysfunction and subsequently lead to pulmonary adhesions at slaughter (Deters & Hansen, 2019).

In order for oxidatively damaged molecules to be repaired and for production of cellular antioxidants to be increased, ATP is required, making transit-induced oxidative stress energetically expensive (Deters & Hansen, 2019). Not only are the energy requirements of animals experiencing oxidative stress greater, but oxidative stress itself hinders the production of cellular energy by diverting substrates away from glycolysis and towards the pentose phosphate pathway to produce NADPH (and reducing equivalents) which are crucial to maintain the antioxidant defense system (Deters & Hansen, 2019). This could result in a negative overall energy balance and mobilisation of body tissue to compensate for the energy redirected towards management of oxidative stress. A protein carbonyl assessment showed that oxidative damage of proteins was greatest in the breast, leg, heart, liver, gut and lymphocytes of broilers with low feed efficiency when compared to those with high feed efficiency, as well as greater mitochondrial ROS production (Bottje *et al.,* 2006). A greater Mn-SOD and GPX concentration was also observed in steers with low feed efficiency, indicating that such animals use a greater proportion of energy to combat oxidative stress than their counterparts, and therefore less energy can be directed to promote tissue accretion than feed efficient animals (Russell *et al.,* 2016). When the HPA-axis is stimulated to maintain a long-term stress response, a cascade of hormonal event take place that results in directing of released nutrients to the liver for enzyme synthesis and gluconeogenesis (Deters & Hansen, 2019). When these substrates are absorbed by other tissues, mitochondrial derived ROS are increased plainly due to the greater electron flow through the ETC and the leaking of electrons to oxygen impairing physiological function (Deters & Hansen, 2019).

During the pre-slaughter period, animals are completely deprived of food, and partly deprived of water – for at least the duration of travel. This necessitates the use of body reserves to meet any energy demands the animal might have (Deters & Hansen, 2019). With no food to replenish glucose supplies, gastrointestinal availability thereof declines, and liver glycogen stores are depleted. This leads to lower secretions of insulin and subsequently an increase in glucagon secretions from the pancreas. Glucagon stimulates the liver to increase gluconeogenesis in an attempt to maintain blood glucose concentrations, and also stimulates the hydrolysis of triglycerides to glycerol and NEFA for mobilization from adipose tissue to be used as a metabolic fuel source in other tissues such as muscle and the liver. These substances undergo aerobic metabolism and thereby further increase mitochondrial ROS production, thereby fasting contributes to

transport-induced oxidative stress and physiological tissue loss (Deters & Hansen, 2019).

Physical exertion during transport and loading occurs due to the long periods of standing and attempting to maintain balance, resulting in muscle fatigue (Schwartzkopf-Genswein, 2014). Skeletal muscle energy expenditure represents 25-30% of the total body expenditure of cattle, therefore muscle derived ROS possibly greatly contribute to the overall body oxidative status. The general consensus regarding this is that exercise induced ROS production occurs mainly by contraction of the heart and skeletal muscle (Deters & Hansen, 2019).

(c) The stress-inducing effects of dehydration resulting from transport

In addition to the time animals spend deprived of food, they also undergo periods of water deprivation, such as during transport and loading (Warris,1990). Animals that are well hydrated have been found to be more resistant to challenging environments than their dehydrated counterparts (Dukes, 2015b). This is because water is an indispensable component of the body. It accounts for more than half of the body mass, acting as a solvent for the transport of essential chemicals to the required locations by means of diffusion (Dukes, 2015b). Therefore, depriving an animal of food and water challenges its ability to maintain homeostasis. Osmotic pressure, pH and acid base balance are all controlled by the movement of ions - namely potassium (K^+), sodium (Na^+), bicarbonate (HCO_3^-), and chloride (Cl^-) - through the intracellular fluid (ICF) and extracellular fluid (ECF) (Hogan *et al.,* 2007). The levels and metabolism of these ions are regulated by aldosterone, a mineralocorticoid controlled by angiotensin. Aldosterone is secreted upon stimulation of the adrenal glomerulosa by angiotensin II, a converted form of angiotensin I which originates from angiotensinogen. Angiotensinogen is a glomerular blood protein released by the liver and transformed to angiotensin I by an enzyme called renin, which increases whenever a decrease in arterial blood pressure to levels below normal occurs. In addition to stimulating secretion of aldosterone, angiotensin II has an independent effect whereby it causes widespread vasoconstriction, as well as constriction of the efferent arterioles in the kidneys, in order to raise the blood pressure and maintain renal glomerular perfusion (Dukes, 2015b). When aldosterone is secreted, it stimulates the reabsorption of sodium in the ascending loop of Henle, the collection ducts and in the distal renal tubules with chloride passively following the sodium to maintain electro neutrality. Furthermore, renal secretions of K^+ are also increased (Dukes, 2015b). Due to its lipophilic nature, aldosterone easily diffuses into target tissue, where it binds to a nuclear receptor and induces transcription and translation of the Na^+ ion channel proteins

in the apical membrane and the Na^+/ K^+ pump proteins in the basolateral membrane of the tubular epithelium. This allows the active reabsorption of Na^+ from the renal tubular fluid, after which it is pumped into the interstitial fluid. This process stimulates renal conversion of sodium and therefore also the conservation of water, as water follows Na^+ passively. Therefore, it helps to determine the concentrations of Na^+ and water in the body, but it responds to angiotensin II and not the actual sodium levels in the blood. The control of blood- Na^+ concentrations is performed by the osmoreceptors in the hypothalamus, which senses the level of Na^+ in the blood and controls it by secreting antidiuretic hormone (ADH) to either conserve or cause loss of water, in order to prevent fluctuations in the Na^+ levels (Dukes, 2015b).

The hydrophobic nature of cell membranes prevents the effortless diffusion of water into cells. Therefore, water and water-soluble substances can only enter and exit a cell via diffusion through the protein channels found embedded within the phospholipid bilayer. The nature of the water-soluble molecules determines whether diffusion through specific channels will take place or not. Some channels are specific to certain molecules, others to a certain molecule size or a specific molecular charge. The facilitated diffusion of such molecules occurs with the concentration gradient and requires no energy. Some molecules need to be transported actively against their natural diffusion pathway, necessitating additional energy and proteins that act as carriers (Dukes, 2015b). This is crucial to prevent the loss of certain molecules, such as glucose, and to maintain certain balances, such as Na^+ and K^+. Diffusion of the water is called osmosis; this occurs over a semi-permeable membrane from a low effective osmotic pressure (where the non-diffusible particles are least) to a high effective osmotic pressure (where the non-diffusible particles are most), until equilibrium is reached. This tone of solution and the presence or absence of a hydrostatic pressure gradient is what regulates the fluids within the various fluid compartments and results in movement of water between the various compartments (Dukes, 2015b).

The inability of the animal to restore water losses challenges its capacity to regulate homeostasis and results in cellular and extracellular dehydration (Hogan *et al.,* 2007). This induces thirst by stimulating the thirst centre in the hypothalamus and the brain structures mediated by angiotensin II (Hogan *et al.,* 2007). In normal conditions, animals would be prompted to drink water to restore homeostasis, but under the stressful conditions associated with fasting and transport this might very well not be the case (Hogan *et al.,* 2007). Evidence has been found that production of cortisol during such stressful times could inhibit the renin-angiotensin-aldosterone axis. Therefore, the

possibility exists that animals under transport show increased water loss through cortisol-induced diuresis without experiencing the angiotensin induced sensation of thirst (Hogan *et al.,* 2007). Depriving an animal of water could lead to significant loss of live and carcass mass, of which a large contribution could be from the interstitial space. Therefore, provision of water pre- and post-transport would result in smaller deviation from the normal physiology and improved live and carcass mass. The volume and distribution of fluids throughout the compartments within the body are mostly regulated by electrolytes and their influence on osmotic forces. The composition of electrolytes varies between the different body compartments, remaining in osmotic equilibrium and thereby freely allowing water to pass between them. The main determinant of intracellular and extracellular fluid volume, Na^+ and K^+ respectively, are lost during times of antemortem stress. Dehydration results in the loss of sodium via the kidneys as a means of preventing hyperosmolality of plasma. Potassium is rapidly lost when animals are fasted, due to the low efficiency of the kidneys to conserve it and the slow adaptation to body depletion. These physiological mechanisms of maintaining homeostasis of body fluids are therefore impaired in the pre-slaughter period. In normal circumstances, any abnormalities of the water volume are balanced by changes in the sodium appetite and renal sodium excretion, and osmolality of body fluids is regulated by balancing thirst and renal water excretions. However, when either food or water, or both, are not available, regulation is impaired.

Stressed animals have increased pituitary-adrenal secretions causing a natri-orexogenic effect. The secretion of aldosterone in response to the stress-induced secretion of glucocorticoids is responsible for the heightened Na^+ appetite and Na^+ retention. Extracellular fluid Na^+ is considered the main regulator of homeostasis, which is therefore threatened by food and water deprivation (FWD), however the relative significance of Na^+ and K^+ in maintaining homeostasis is still to be determined. Uncertainty with regards to the source of water loss during fasting exists. Daily water gain normally balances daily water loss in order to keep the water content of the body relatively constant on a day-to-day basis. The intake and output of water all depends on how much water is needed at cellular level. Higher producing animals have higher caloric expenditure, a faster metabolism and higher water outputs, meaning they will have much greater need for water gain (Dukes, 2015b). This water can come from either ingestion of water, or from metabolic water, which is produced when cellular metabolism occurs, and hydrogen combines with oxygen at the end of the ETC.

When water loss exceeds water gain, dehydration occurs. Firstly, water from the extracellular compartments is lost. This causes a hydrostatic gradient, resulting in a shift of water out of the cells and into the extracellular compartments. Electrolytes are excreted along with the water to keep the concentrations thereof in the body constant. During dehydration, blood pressure decreases and stimulates the posterior pituitary in the hypothalamus to secrete ADH or vasopressin, increasing the permeability of the collecting ducts and as a result more water is reabsorbed and less expelled. In addition to this, thirst is stimulated to increase the water intake. This stimulation occurs when the osmoconcentration of the thirst cells increase, as well as when angiotensin II is secreted by the kidneys in response to low blood pressure. Angiotensin II also results in salt retention. After drinking water, temporary thirst relief is experienced as a result of the stomach distending and the pharynx and mouth being wetted. The water absorbs after some time, increasing the blood pressure back to normal and lowering the osmoconcentration (Dukes, 2015b).

Deprivation of food and water leads to dehydration of body fluid components from muscle and non-muscle tissue, which enhances the adrenocortical response to stress. Moisture lost from tissue cannot be regained immediately upon free access to water during lairage, as dehydrated animals can take several weeks to properly adjust and regain moisture lost from tissue (Schaefer *et al.,* 2001). In addition to losing moisture, dehydration is accompanied by a shift in the electrolyte balance that result in a change to the anion gap measurements. Changes in such measurements are often expressed as high bicarbonate levels and depleted cation levels, especially potassium (Schaefer *et al.,* 2001). Dehydration can be determined by measuring the concentrations of total protein in the plasma, plasma osmolality and packed cell volume (Gregory, 1998).

2.3.3 The ante-mortem stress inducing effects of lairage

Lairage is defined by the Oxford English dictionary as a place where livestock may be rested on the way to market or slaughter (Oxford Dictionary, 2021). Livestock are held in abattoir lairage yards prior to slaughter in order to:

- Rest the animals post transport
- Allow for greater reduction of the GIT content before slaughter
- Allow resorting of animals into lines consistent with the markets available to the meat processor, and
- Prevent interruptions in the supply of livestock to the slaughter floor (Gregory, 1998).

The current knowledge on the effects of lairage time on animal welfare and meat quality in domestic livestock is limited, with contradicting results appearing in literature. Several authors believe that lairage time post transport potentially allows domestic animal to decrease the concentrations of cortisol, replenish muscle glycogen concentrations and reduce dehydration of body tissues and the loss of carcass mass (Warriss *et al.,* 1984; Mounieret *et al.,* 2006) as they have time to rest and drink water, which could alleviate some of the effects of dehydration (Gregory, 1998). Other authors believe that the lairage environment itself may hinder the animals ability to recover from the effects of feed and water restriction and contributes to the stress the animal is experiencing, as animals are introduced to new and unfamiliar environments and often mixed, meaning that (Jarvis *et al.,* 1996) extended lairage time would be associated with a decrease in carcass and meat quality (Liu *et al.,* 2011).

In a study by Warriss *et al.,* (1995) pigs were mixed upon loading on the farm to determine whether immediate slaughter upon arrival, or slaughter after three hours of lairage would be the better option. The three-hour holding period would provide animals the opportunity to rest and recuperate after the journey, or it might give the mixed animals the chance to fight, adding to the already high levels of stress. Cortisol and lactate concentrations were then tested for as cortisol would indicate generalized stress and lactate would indicate anaerobic muscle exercise. The results from this study showed significantly greater levels of cortisol and lactate in the immediate-slaughter group when compared to their three-hour rested counterparts, leading to the conclusion that a three-hour lairage provided sufficient rest for stress levels to return to those that would be seen in groups that were not mixed (Gregory, 1998).

Mohan *et al.,* (1992) reported a variety of physiological changes that resulted from pre-slaughter miTong Xing in both cattle and pigs (Box 1). MiTong Xing as a pre-slaughter stressor can lead to dominance aggression and physical exertion due to mounting, fighting, and chasing (Mohan Raj *et al.,* 1992; Gregory 1998). This has been found to be detrimental to meat quality as increased muscle glycogen is utilized to meet the increased energy demands, leading to poor acidification of meat and concluding in DFD. DFD can be prevented by managing the muscle glycogen reserves in an animal:

- Minimising preslaughter exercise and stress
- allowing repletion of glycogen
- Supercompensation
  (Gregory, 1998)

Supercompensation is a process human athlete often use to boost their muscle glycogen reserves during training. This comprises a dietary change from low to high carbohydrate intake shortly before competitions, in order to double muscle glycogen content. A version of supercompensation can be used for pigs at lairage, where they are provided with a sugar solution to add to their muscle glycogen reserves. Replenishing muscle glycogen in cattle after exhaustive exercise, however, takes at least 24 hours and up to 48 hours (Gregory, 1998). In one study, the effects of different lairage times after 8 hours of transport on some blood indicators of animal welfare and meat quality in sheep indicated that a 48-hour lairage period resulted in greater loss of live mass, lower carcass mass, and lower dressing percentages. The results indicated that lairage contributed to tissue catabolism, or possibly dehydration, furthering pre-slaughter live mass loss, or shrink (Liu *et al.,* 2012). Similar results were seen by Ferguson and Warner (2008), who concluded that any duration of food and water deprivation beyond 24 hours caused tissue catabolism and dehydration that increased their contribution to live mass loss, however cooking loss was not affected. Cooking loss of beef was also unaffected by lairage times of 3 hours and 18 hours (Ferguson et al. 2007). Consequently, it can be deduced that the 24 to 48 hours required for muscle glycogen repletion in cattle to increase meat quality would possibly decrease meat quantity.

Box 1 Physiological alterations in cattle and pigs resulting from miTong Xing prior to slaughter.

- ↑ enzyme CK
- ↑ FFA
- ↑ BHB
- ↑ LDH
- ↑ GOT
- ↑ Cortisol
- ↓ Plasma lactate

Results obtained for cattle from Warriss *et al.,* (1984) and for pigs from Moss & McMurray, (1979).
CK: creatine kinase; FFA: free fatty acids; BHB: β-hydroxy butyrate; LDH: lactate dehydrogenase; GOT: glutamate-oxaloacetate-transaminase; ↑: increase, ↓: decrease

The question therefore at hand is what the optimum lairage time would be for maximum meat quality, quantity, and animal welfare. Length of transportation seemingly has no

effect on recovery period, as reported by Knowles et.al 1993, who showed that hill lambs transported 9 and 14 hours respectively had similar blood parameters (Gregory, 1998). Management and handling play a larger role in recovery time as they can greatly contribute to stress. Some slaughterhouses are considering reducing lairage time or avoiding it to increase utilization capacity by facilitating greater throughput (Ferguson *et al.,* 2007; Liu *et al.,* 2012). However, this could risk animal welfare and meat quality as an adaption period to recover homeostasis and minimise stress seems to be crucial post transport (Liu *et al.,* 2012).

2.4 Additional factors that could influence physiological tissue losses

2.4.1 Effects of β-adrenergic agonists on tissue loss during transportation

β-adrenergic agonists (βAA, or generally referred to as β-agonists) are naturally occurring catecholamines found in the plasma membranes of most tissue cells that stimulate energy-releasing processes to the essential organ systems involved in responding to stress (Steenkamp, 2014). This energy comes from the catabolism of glycogen and lipids through glycogenolysis and lipolysis. The βAA bind to and activate specific β-adrenergic receptors in fat and muscle tissue, resulting in increased lipolysis and protein accretion, and decreased lipogenesis and protein degradation, whilst reducing blood flow to the GIT and increasing flow to the skeletal muscle. This ensures that the organs involved in the stress response receive optimum levels of oxygen and energy (Hossner, 2005).

Synthetic βAA have been synthesised to mimic this response for the purpose of improving animal production efficiency (Montgomery *et al.,* 2009), by partitioning nutrients towards protein synthesis and muscle growth, rather than fat deposition. The pharmacological and chemical characteristics of these βAA are very similar to that of natural occurring catecholamines (Steenkamp, 2014). Various synthetic βAA exist, including ractopamine hydrochloride, clenbuterol, R-salbutamol and zilpaterol hydrochloride - commercially available in South Africa as Zilmax (NRC, 1994; Montgomery *et al.,* 2009). Due to the extensive research done in South African conditions and the favourable outcomes obtained, only the use of zilpaterol hydrochloride will be discussed further in this section.

Generally, zilpaterol hydrochloride is supplemented to feedlot cattle being finished off for slaughter (Van Bibber & Drouillard, 2012) as a means of improving carcass characteristics, meat quality and overall feedlot performance (Vendaño-Reyes *et al.,* 2016). Upon binding of βAA to B-adrenoreceptors - which are widely distributed within

the cells of the mammalian body – protein kinase A is activated to alter the synthesis and degradation of skeletal muscle (Vendaño-Reyes *et al.,* 2016). Various studies have shown that animals given a zilpaterol hydrochloride supplement yielded heavier carcasses than their counterparts (Vendaño-Reyes *et al.,* 2016; Van Bibber & Drouillard, 2012), demonstrating its potential to increase protein accretion through increased protein synthesis, particularly in fast glycolytic muscle fibres, by changing the transcriptional activity of myosin heavy-chain isoforms in these muscle fibres (Rathmann *et al.,* 2009). The increased protein synthesis occurs at the expense of fat deposition, as energy and nutrients from fatty acid biosynthesis for adipose tissue is redistributed to muscle accretion, resulting in reduced fat deposition in carcasses (Vendaño-Reyes *et al.,* 2016; Van Bibber & Drouillard, 2012).

A study done by the Kansas Agricultural Experiment Station showed how zilpaterol hydrochloride alters the blood constituents of finishing cattle. They found that glucose concentrations – the energy source for maintaining several bodily functions – and plasma urea nitrogen concentrations – an indicator of protein catabolism – were both significantly decreased (P>0.01) in the whole blood. The decreased circulating concentrations of plasma urea nitrogen suggested that protein catabolism could potentially be inhibited in animals receiving zilpaterol hydrochloride (Van Bibber & Drouillard, 2012). Whether this affect plays any role in physiological tissue loss is, however, still unknown.

2.4.2 Effects of body condition score (BCS) on tissue losses during transportation

Body condition scoring has been used effectively for many years as a means of evaluating and describing the energy reserves of cows in the various stages of the production cycle. Conventionally, these scores serve as indicators of the likeliness of successful rebreeding, with conception rates decreasing for overly thin or fat cows (Smith, 2017). In South Africa, body condition scoring is based on a five-point scale. A scoring of “1” represent an extremely thin animal and a scoring of “5” represents a grossly fat animal, with a “3.5” being the ideal score pre-conception to ensure optimal rebreeding rates (ARC, 2013). Despite its regular use for reproduction purposes, very limited information exists regarding the correlation between the body condition and physiological tissue losses of cattle transported for slaughter in South Africa. Most studies regarding transport losses are based on the effect of fasting time on the overall loss of BM, with very few, if any, dedicated towards determining the role of body composition in that loss. This study will aim to evaluate whether body condition, or the

level of fatness, affects the percentage of physiological tissue loss of feedlot cattle transported to the abattoir.

2.4.3 Effects of animal sex on tissue losses during transportation

Hormones, and the rate of release of these hormones, are the primary mediators of the physiological-nutritional interactions that determine the body composition of an animal. The sex of the animal, degree of maturity and external environmental inputs all influence the type of hormones and the rate of release of the composition-determining hormones (Webster, 1986). Intact males generally tend to be leaner than castrated males and females, with both subcutaneous and intramuscular fat levels being lower (Fisher *et al.,* 1985). This difference in body composition can possibly contribute to the variation seen in the percentage of physiological tissue loss of transported cattle. Treatment of castrated animals and females with anabolic steroids can increase their growth rate to levels comparable of that of intact bulls, however this will not necessarily affect body composition (Fisher *et al.,* 1985).

2.4.4 Effects of animal type on tissue losses during transportation

The type of animal being transported for slaughter could potentially influence the amount of physiological tissue loss that occurs during transportation. Many differences in the morphology (Jian *et al.,* 2013), temperament (Voisinet *et al.,* 1997), and endocrine balance (Hammond *et al.,* 1998) exists between B. taurus and B. indicus cattle. These animals behave differently when faced with various stressors, such as heat stress or handling, and are differently adapted to cope with various stress factors due to the above-mentioned differences.

A key example of type differences that could lead to variation in the amount of physiological tissue loss that occurs is the regulation of heat. Cattle maintain their body temperature by controlling the balance of heat gain to heat loss (Hahn 1999). The main means of cooling down is through evapotranspiration mechanisms, such as sweating and panting (Kadzere *et al.,* 2002). Skin morphology is therefore a crucial part in heat dissipation, and therefore management of heat stress. Many studies have confirmed the morphological skin differences of B. indicus and B. taurus animals, concluding that B. indicus animals have both a higher volume and density of sweat glands that are located closer to the skin surface than that of their counterparts. This results in a higher sweating rate, and consequentially more rapid cooling. In addition to this, the density of hair follicles is also higher in B. indicus compared to B. taurus animals, resulting in better

protection against radiant heat (Jian *et al.,* 2013). This makes B. indicus animals much more tolerant to heat than B. indicus animals, which could lead to variation in transport losses.

Temperament and the stress response animals have to human handling also varies between cattle types (Wells, 2020). Studies have revealed that animals with excitable temperaments show reduced performance, decreased initial and final body mass, lower ADG and hot carcass mass as well as less marbling and fat thickness when compared to their calmer counterparts (Reinhardt *et al.,* 2009). The Brahman influence was found to be particularly strong in terms of temperament and excitability, with Brahman crosses showing greater mean temperament ratings than cattle with no Brahman influence (Voisinet *et al.,* 1997). This poor temperament is thought to be as a result of the increased levels of cortisol of Brahman cattle (Hammond *et al.,* 1998). This could result in variation in transport losses.

2.5 Pre- and post-slaughter stress indicators

The stress animals experience during transport and handling results in changes in that animal that can be quantified by certain physiological, behavioural, and pathological indicators (Larios-Cueto *et al.,* 2019). The degree of change can be determined by measuring blood hormone levels, blood metabolites and by observing animal behaviour (Njisane & Muchenje, 2014). The breed and age of an individual animal and especially the type and duration of the stressor can be considered some of the greatest factors determining the degree of physiological alteration an animal would experience (Hernández-Cruz *et al.,* 2016). Another important aspect is the previous experiences of that animal; animals that have had positive experience with humans throughout their lifetime have shown to be less stressed at slaughter than their counterparts with negative experiences (Reichea *et al.,* 2019). A pain stimulus would have nonspecific plasma indicators, however if it results in muscle activity or fear, the plasma indicators for them can be monitored

Some of the main stress indicators in animals are the changes in live mass and changes in the concentrations of cortisol, glucose, and free fatty acids (Larios-Cueto *et al.,* 2019), however lactate and plasma glucose are also often used (Gregory, 1998). A list of some of the important pre-slaughter stressors and their plasma indicators are given in table 2.1. Animals that are stressed also show changes in their behaviour, such as increased vocalisation, heightened aggression, and an attempt to kick (Njisane & Muchenje, 2014). Another common sign of fear in cattle is increased urination and defaecation, which is

due to activation of the vagus nerve in the parasympathetic nervous system (Gregory, 1998). Standing time could be a very important behavioural indicator for heat stress as standing allows for the maximum skin area to be exposed for heat loss while also being the more favourable position for respiration (Won *et al.,* 2018). Animals spending additional time standing as opposed to lying down could therefore be perceived as experiencing heat stress (Won *et al.,* 2018).

Table 2.1 Potentially useful stress indicators in plasma

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

Adapted from (Gregory, 1998; Agbeniga, 2011)

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

The hormones epinephrine, norepinephrine and beta-endorphins are also present in stressed animals and can therefore serve as indicators of stress (Micera *et al.,* 2007), however they are not as suitable to serve as indicators as corticosteroids due to their short half-life in the circulatory system (Agbeniga, 2011; Gregory, 1998). Despite the fact that norepinephrine has a half-life of only about 2 minutes, it can serve as an indicator of stress when measuring its metabolic and physiologic effect, such as the heart rate and packed cell volume response to the hormone (Agbeniga, 2011). The release of corticosteroids from the adrenal cortex has much more of a delayed effect, with its half-life being closer to 20 minutes (Agbeniga, 2011).The presence of blood glucocorticoids such as cortisol at increased would be characteristic of a stress response, with their main function being (Gregory, 1998):

- To stimulate proteolysis
- To stimulate gluconeogenesis
- Anti-inflammatory effect

However, as blood sampling is considered an invasive procedure – shown to have the ability to increase circulating glucocorticoid levels in as little as 3 minutes – an alternative, non-invasive method of testing glucocorticoid levels is measurement of faecal glucocorticoid metabolite levels (Hernández-Cruz *et al.,* 2016). This will reflect how much glucocorticoids were present in the hours and days prior to excretion. This could provide a reflection of the physiological state of a group of animals and their ability to respond to individual, or a combination of stressors. Higher levels of cortisol would be expected when animals are introduced to new and unfamiliar environments, undergo dietary changes, or are subjected to unaccustomed interactions with people (Hernández-Cruz *et al.,* 2016) – typical pre-slaughter events.

Free fatty acids, amino acids and low blood glucose concentrations are all typical indicators of fasting. This is due to the lower availability of glucose as a source of energy that then results in breakdown of lipid and protein tissue, causing FFA and amino acids (AA) to enter circulation. Amino acids are deaminated to form ammonia and then enzymatically converted to its safer form, glutamate dehydrogenase (GLDH) (Gregory, 1998).

2.6 Effects of stress on meat quality

During farm to abattoirs transport, animals are exposed to various stressors (Box 2) that stimulate the neuroendocrine system and affect physiological and metabolic functions (Agbeniga, 2011; Tong Xing *et al.,* 2019). The inevitability of antemortem stress not only

affects meat yield by increasing drip-loss, thawing loss and evaporative loss (Agbeniga, 2011), but it also affects meat quality by altering the biochemical and metabolic processes that take place in the early post-mortem period, seriously affecting the important meat quality attributes (Tong Xing *et al.,* 2019). These factors also contribute to potential alterations of the aspects affecting the conversion of muscle to meat, including energy homeostasis, intracellular ion dynamics, protease systems and proteins in the skeletal muscle (Ouali *et al.,* 2006; Scheffler & Gerrard, 2007). Ante-mortem stress results in the rapid release of enzymes that deplete the glycogen stores in the body, namely dehydrogenase and creatine lactate. This results in the reduced synthesis of post-mortem lactic acid, a greater ultimate pH, higher water-holding capacity, and an unideal meat colour (Birhanu *et al.,* 2019).

Box 2 Pre-slaughter stressors affecting meat quality attributes.

- Uncommon noises
- Vibration,
- Confinement,
- Fasting
- Changes in social grouping,
- MiTong Xing with unfamiliar animals,
- overloading,
- additional human contact
- stunning
- dehydration
- motion sickness
- physical exertion
- temperatures above the thermo-neutral zone

(Adzitey, 2011; Birhanu *et al.,* 2019)

These stress-induced physiological changes in combination with inappropriate handling, incorrect loading densities and harsh road conditions during transportation results in the DFD meat and causes carcass damage through bruising, ultimately downgrading the value of the carcass and causing huge economic losses (Tong Xing *et al.,* 2019; Birhanu *et al.,* 2019). Dark firm and dry meat are one of the main meat quality concerns worldwide and can be defined as any muscle tissue with an ultimate pH of higher that 5.7 (Coombes *et al.,* 2014). Meat that falls into this category has a reduced shelf life, is often bland and

varies in its tenderness, making it unacceptable for consumers and retailers alike. This ultimate pH is determined by the concentration of glycogen within muscle at the time of slaughter. Following exsanguination of an animal, the environment for glycolysis shifts from aerobic to anaerobic as oxygen and nutrient supplies are depleted. In the new physiological environment glycogen is metabolised to form lactate and hydrogen ions, ultimately decreasing the intracellular pH of the muscle from around 7 to between 5.4 – 5.7 within the first 24 to 48 hours post-mortem (Tong Xing *et al.,* 2019; Coombes *et al.,* 2014). This process only occurs when sufficient glycogen concentrations are present in the muscle at slaughter in order for adequate quantities of lactate to form to drop the pH_u to the required levels post slaughter (Coombes *et al.,* 2014). Muscle glycogen concentration at slaughter can be determined as follows:

Glycogen at slaughter = glycogen synthesized – glycogen utilized

The quantity of glycogen synthesized will be dependent upon the nutrition an individual animal received on the farm, while that utilized would be dependent upon the energy requirements for muscle contractions and responding to stress in the pre-slaughter period (Coombes *et al.,* 2014). However, even in animals of the same breed, managed under similar conditions, receiving the same diet and transported to the same abattoir, great variability among the incidence of DFD meat has been reported for both sheep and cattle (Tong Xing *et al.,* 2019). This has been attributed to individual animal susceptibility to stress and different handling practices (Ponnampalam *et al.,* 2017). Numerous studies have shown that the individual or combined effect of stressors result in ante-mortem behavioural and physiological changes that affect the metabolites of muscles and therefore the meat properties (Tong Xing *et al.,* 2019). Pre-slaughter stress in cattle also results in greater clearing of the caecum and large intestine, thereby increasing the concentration of salmonella and risk of carcass contamination and infection of other animals (Agbeniga, 2011).

In addition to this, perimortem oxidative stress possibly affects post-mortem oxidative stress and meat quality. This is due to the accumulation of ROS in muscle due to its inability to maintain the cellular antioxidant defense system after slaughter, resulting in many quality defects due to lipid and protein oxidation. Lipid and protein oxidation have been indicated as one of the major nonmicrobial causes of meat quality deterioration (Tong Xing *et al.,* 2019). Oxidised AA residues and protein backbone, formation of protein-carbonyls and protein-protein crosslinks are the result of oxidative modifications of the structure and function of proteins, which negatively affects the water-holding capacity of meat. Meat tenderness is also at risk as calpains – the major enzyme

responsible for post-mortem proteolysis and therefore meat tenderness – are very susceptible to oxidation and subsequent inactivation (Deters & Hansen, 2019).

Calpains are crucial for the proteolysis of the myofibrillar structures – which partly contributes to the changes of muscle structures – and thereby influences meat tenderness and water holding capacity (Tong Xing *et al.,* 2019). Water holding capacity is also impaired by lipid peroxidation, which also causes meat discolouration, loss of nutritive value and the potential production of highly toxic compounds in the meat that could adversely affect human health (Tong Xing *et al.,* 2019). Meat is very susceptible to oxidative processes due its high levels of unsaturated fatty acids, therefore oxidation in seen as a major risk factor for protein functionality, nutrition quality and shelf-life of meat products (Tong Xing *et al.,* 2019). Muscle oxidation can be determined by measuring the amounts of malondialdehyde (MDA), protein sulfhydryl, and carbonyl groups. These reflect the degree of protein and lipid oxidation. (Deters & Hansen, 2019). At present the exact mechanisms linking stress with meat quality is still largely unknown, however knowledge of such pathways can promote an understanding of the variation that exists in meat quality attributes, allowing for a theoretical basis that could lead to improving meat quality and reducing the incidence of undesirable meat (Tong Xing *et al.,* 2019).

CHAPTER III: MATERIALS AND METHODS

3.1 Introduction

This study was conducted to determine if the percentage (%) of physiological tissue loss occurring in beef cattle being transported for slaughter will significantly change as a result of variation of certain fixed factors. These factors are listed below:

- Animal breed type
- Pre-slaughter mass
- Carcass classification
- Fat %
- Treatment with a β-agonist
- ADG
- Dressing %
- CCM
- FCR

This was done by analysis of two datasets. Data previously collected from a feedlot and its corresponding abattoir was used to compile the first dataset, which will be referred to throughout this dissertation as ‘‘dataset 1’’. This is in interest of the contributing parties (feedlot and abattoir), who agreed to the use of the data on the condition that they remain anonymous. Data obtained from the Hatfield Feedlot Challenge of 2020 and the corresponding abattoir was also used in this study to compile the second dataset. This dataset will be referred to throughout this dissertation as “dataset 2”, as the abattoir also required anonymity. All the animals used for data collection was subject to typical feedlot conditions and are representative of animals used in South African feedlots. More research would need to be done to determine if these results are applicable to other forms of cattle production, such as extensive systems, or other species, like sheep, pigs, and broilers. Since only secondary data previously collected is used and no live-animal trial took place, this was not considered an experimental study.

3.2 Ethics approval

An application for ethical clearance was submitted to the Animal Ethics Committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria to justify and approve the use and analysis of slaughter data collected over a period of ten years

from various different feedlots. The application, with reference number NAS348/2020, was approved and conformational letter sent on 09 April 2021.

3.3 Analysis of dataset 1

3.3.1 Site of collection

The data used for this study was obtained from a Gauteng based feedlot and a high throughput (A-grade) commercial abattoir with a slaughter capacity of over a thousand heads of cattle per day, both of whom wishes to remain anonymous. The climate in this province is classified as mild, with low average humidity. Temperatures vary a lot between the summer and winter months, with sub-zero (ºC) temperatures possible mid-winter, and high 30's (ºC) often seen in summer times. The average rainfall is around between 600mm and 700mm per annum, depending on the location within the province. Most of the rain falls during the summer months.

3.3.2 Animal management

A total of 77 male cattle typical of those used in South African feedlots were randomly allocated to one of five treatment groups after a 30-day adaptation period in the feedlot. The treatments included a negative control group and four groups treated with various inclusion levels of a β-agonists, given for various durations. Data regarding the final mass and pre-slaughter mass, ADG's, FCR's, fat percentages and subcutaneous fat thickness was collected from all the cattle. For statistical analysis, only the data from 73 out of the 77 available cattle was used, after exclusion of four outliers. Upon arrival at the abattoir, all the bulls were tagged and assigned a treatment. The tag number and corresponding treatment was recorded in Microsoft Excel.

The base diet for all the cattle was identical and provided by the same supplier, with the β-agonist treatment being mixed into the feed as part of the finisher ration. A good quality drinking water was freely available and a three-day β-agonist withdrawal period applied to all animals prior to slaughter. Feed and water were available ad libitum for the full duration in the feedlot, excluding the fasting period prior to transportation. A fasting time of 24h is normally recommended in order to optimise bleeding and carcass cleanliness, to decrease the risk of rupturing the digestive tract during evisceration and to prevent contamination of the carcass with faecal and digestive contents (Agbeniga, 2011). Only animals that fall in the "A" age group, roughly 12 months of age, was obtained for use in this study, as classified in accordance with the South African Beef Classification System (Webb, 2015) (Table 3.1).

Their fatness was mainly a two or a three (lean to medium), as described by the current South African Beef Classification System (Table 3.2), however a few animals had a fatness of 4 (fat).

Table 3.1: Classification by Age

A	0 teeth
AB	1 to 2 teeth
B	3 to 6 teeth
C	more than 6 teeth

Table 3.2: Carcass Fat Codes

Fat Class	Description	Subcutaneous fat %	Fat thickness (mm)
0	No fat		0
1	Very lean	3,3	<1
2	Lean	4,1	1 to 3
3	Medium	5,2	>3 and ≤5
4	Fat	6,3	>5 and ≤7
5	Overfat	7,3	>7 and ≤10
6	Excessively fat	7,8	>10

(Webb, 2015)

All the animals were individually weighed on a livestock scale (max 5000 kg) prior to departure and after arrival at the abattoir, after which the live mass of each bull was recorded in kilograms. Using Google maps, the distance travelled between the feedlot and abattoir was obtained. Animals travelled by truck and transportation occurred without rest stops or other delays, and transport conditions were as close as possible to those normally used in industry. All the bulls were familiar with humans and handled by experienced personnel in a humane way.

3.3.3 Data collection

Capturing, sorting, and processing of the data from dataset 1 was done in Microsoft Excel (Microsoft Office 365), after which all outliers, incorrect and incomplete data was removed.

Individual mass was recorded on several occasions, by methods mentioned above. This was used to calculate %TL. Feed intake for the duration of the experimental period was measured, recorded, and used to calculate the ADG and FCR for each bull.

All the bulls were slaughtered according to acceptable slaughtering procedures, the carcass marked according to its corresponding tag number and chilled below 5ºC. Carcass mass (WCM and CCM), dressing % and the carcass classification of each carcass was obtained from the abattoir. Prime rib-cut samples of *m. longissimus dorsi* were taken 24 hours post-mortem and analysed for SCF thickness and carcass composition, including carcass fat %. Measurement of the SCF thickness was done using a vernier caliper at the 13th rib, 5cm from the medial plane (Steenkamp, 2014). After slaughter, each carcass classified based on the age and SCF % as an A2, A3 or A4 carcass. Throughout this dissertation, the carcass classification might sometimes be referred to as “grade”, as this was how the carcass fatness data was captured during the animal trials.

3.3.4 Calculations

Physiological tissue losses have been extensively discussed in this dissertation, and as mentioned before a degree of loss is expected when animals are transported for slaughter. The mass lost during transport was calculated for each bull by subtracting the post-transport mass from the pre-transport mass. This value was then divided by the pre-transport mass and multiplied by 100 to give the %TL, or physiological tissue loss. An average and standard deviation was calculated in SPSS.

Dressing percentage is defined as the percentage of live animal that ends up as carcass (Whiteheart, 2012). The dressing percentage is normally taken directly after skinning and evisceration and is normally known as the hot hanging mass (Whiteheart, 2012). In this study, the dressing percentage was calculated as the hot carcass mass divided by final body mass, multiplied by 100 to give a percentage. This was, however, calculated and forwarded by the abattoir.

Average daily gains and FCR’s are measures of feedlot performance. The ADG for each bull was calculated by subtracting its initial mass from its final mass and dividing that value by the number of days in feed (Wells, 2019). This will give a value in kg/day. Feed conversion ratio is a measure of the efficiency of converting feed into meat (Kahl, 2018), and was expressed as the amount of feed (in kg) needed for 1kg of gain.

3.4 Analysis of dataset 2

3.4.1 Site of collection

Individual data from a total of 120 animals off different genotypes and different sexes was obtained from the Hatfield Feedlot Challenge of 2020, which was held on the University of Pretoria's Experimental farm. The farm is situated in Pretoria, Gauteng, where the climate is classified as warm and temperate. Average summer temperatures range between 17.5ºC and 28.5ºC, however temperatures upwards of 36ºC have been measured mid-summer. Average winter temperatures range between 6.9ºC and 19.2ºC, with July being the coldest month of the year. The average rainfall is around 661mm per annum, most of which falls during the summer months.

3.4.2 Animal management

Of the 120 animals used for data collection, only he data from 113 was used for analyses. Two mortalities occurred, the first due to a urinary tract infection and the second as a result of bovine malignant catarrhal fever, more commonly known as "snotsiekte". Four additional animals were removed due to error in the recording of their pre-slaughter mass, leading to incorrect calculations which were therefore not useable. The final animal removed from the dataset fell in the "AB" age group and was removed to prevent skewing of the results, as "AB" animals are atypical to most feedlots in South Africa. The animals removed came from pens 1, 2, 5, 7 and 19. The remaining cattle were all typical of feedlot cattle slaughtered in South Africa, and all of then fell in the "A" age group, as classified in accordance with the Beef Carcass Classification System of South Africa (Table 3.1).

During their time in the feedlot, all cattle were individually weighed on a livestock scale (max 5000kg) for a total of 9 times. Departure time from the farm and arrival time at the abattoir were recorded, after which the duration of transportation was calculated as the difference between arrival and departure times. Distance travelled was obtained from the transport vehicles using the odometer reading. The animals were transported by truck for 40km to the Onderstepoort Campus, where they stood for about 12 hours awaiting evaluation. Afterwards they were transported again for another 40 km to the abattoir for slaughter. Animals travelled by truck and transportation occurred during the cooler times of the day, with the distance and average travel time recorded. No rest stops or other delays occurred on the journey.

The animals were treated humanely, by experienced personnel, and were all accustomed to being handled. Travelling conditions were as close as possible to those normally used in industry. All the animals used were subjected to very similar diets - all typical feedlot diets - with comparable protein, fat and fibre contents, for the duration of their time in the feedlot. Prior to transport, all the animals were subjected to a fasting period. A fasting time of 24h is recommended for reasons mentioned above, and was adhered to. Water was available ad lib for the full duration of pre-transport fasting and for the time spent at Onderstepoort. Immediate slaughter occurred upon arrival at the abattoir, no resting time was spent in lairage.

3.4.3 Data collection

The effect of breed on carcass losses was assessed by collecting data from the 113 animals of different breeds and crossbreeds from the Hatfield Feedlot trial on the University of Pretoria's Experimental farm in June 2020. Each animal was individually tagged and its breed recorded upon arrival at the feedlot. Fifteen different breeds and crossbreeds were observed and accordingly recorded, however for simplification of the statistical analyses, these breeds were categorized according to their Type as either Bos taurus, Bos indicus, hybrid or composite animals. In this study, composite animals refer synthetic lines obtained by crossbreeding of purebred animals to the point of creating a new breed and achieving hybrid vigour without further need for crossbreeding. Hybrid animals refer to any crossbred animals not formally recognized as a composite breed. Statistical analyses were performed to determine whether different types of cattle vary in the amount of physiological tissue loss that occurs when transported for slaughter to the abattoir.

Individual mass data was collected from the 113 feedlot trial cattle. All the animals were individually weighed multiple times on a livestock scale (max 5000 kg), after which the live mass of the animals was recorded in kilograms. Upon arrival at the abattoir, the animals were weighed a final time and the masses recorded as the pre-slaughter mass. In order to determine if live mass prior to slaughter affects the amount of physiological tissue loss, the 113 animals used were categorised into 1 of 5 groups, based on their pre-transport mass, from the lightest to the heaviest animal (mass category 1 through to 5). The mass categories, and not individual mass, was then compared and analysed to determine if differences in mass lead to significant differences in physiological tissue loss.

After slaughter, the carcasses were classified based on the age and SCF% (Table 3.2). The animals from this dataset were classified as either an A1, A2, A3 or A4 animal,

based on their level of fatness. The dressing % was calculated as mentioned above and sent forth from the abattoir along with the WCC and CCM of each carcass.

3.4.4 Calculations

Percentage physiological tissue loss and % were calculated by the same methods mentioned above. The average %TL and a standard deviation was calculated in SPSS.

3.5 Statistical analysis

This study is a retrospective review of data collected over a period of ten years from three different feedlots. Two large data sets were used to calculate the live mass losses and tissue losses of transported cattle in Microsoft Excel. The data was first checked for outliers and normal distribution. Variables that are not normally distributed was analysed by non-parametric methods.

After checking for errors, the datasets were individually analysed in SPSS (version 27) using a Generalised Linear Mixed Model (GLMM) procedure. This was done to determine whether the fixed factors from dataset 1 (Box 3) and the fixed factors from dataset 2 (Box 4) had a statistically significant effect on the percentage of physiological tissue and moisture loss of transported cattle. The effects of these factors were assessed at a significance level of $p<0.05$.

The analysis of both datasets was done via GLMM procedure due to the presence of random factors. The mixed model allows for determination of the significance of the random factors on the target variables. Various models were initially compared using both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), which implicated the GLMM as the best model.

Box3 Fixed factors: Dataset 1

- β-agonist treatment
- Fat %
- SCF – thickness (mm)
- Carcass classification
- Dressing %
- CCM

Some of the datasets were altered to facilitate easy analysis, including the removal of the breed effect from dataset 2 in favour of the Type effect, as well as grouping of BW into categories instead of using individual body mass. For dataset 2, treatments were numbered 1 – 5, however treatment names cannot be disclosed.

Box 4 Fixed factors: Dataset 2

- Type
- Carcass classification
- Mass Category
- ADG
- FCR
- CCM
- Dressing %

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Introduction

Two datasets from typical beef feedlot systems were analysed to evaluate body mass and tissue losses. In the first dataset (Dataset 1), the effects of treatment of cattle with a beta-agonist, fat percentage, SCF thickness, carcass conformation, dressing % and CCM on mass losses during transportation were assessed. In the second dataset (Dataset 2) the effects of breed type, carcass classification, pre-transport mass, feed conversion ratio, average daily gains, and CCM on mass losses during transportation were assessed. Dataset 2 was altered to facilitate easy analysis of the data, including the classification of breeds into various “types” and categorization of individual mass into “mass categories”, thereby replacing individual breed and body mass variables.

After conducting a Mixed model analysis, the model summary indicated a smaller information criterion value for a General Linear Model (GLM), which verifies it as the best model for accurate analysis of both datasets.

Table 4.1: Model Summaries

Model Summary Dataset 1		
Target	%Transport kg loss	
Probability Distribution	Normal	
Link Function	Identity	
Information Criterion	Akaike Corrected	20,54
	Bayesian	24,59
Information criteria are based on the -2 log likelihood (16,34) and are used to compare models. Models with smaller information criterion values fit better.		
Model Summary Dataset 2		
Target	%Transport kg loss	
Probability Distribution	Normal	
Link Function	Identity	
Information Criterion	Akaike Corrected	92,06
	Bayesian	97,13
Information criteria are based on the -2 log likelihood (87,94) and are used to compare models. Models with smaller information criterion values fit better.		

4.2 Descriptive statistics: Dataset 1

The minimum and maximum values, means and standard deviations of carcass parameters and transport losses for cattle as affected by the different factors are summarised in Table 4.1, excluding the string variables (β-adrenergic agonist treatment, carcass classification).

Figure 4.1 shows all the fixed effects analysed for their effects on %TL. This figure illustrates the relationship between each factor and the target variable (% transport loss). Thicker lines were indicative of the greater effects of the factor on %TL, whilst thinner lines indicated smaller effects of the specific factor on %TL. The mixed model analysis for the effects of the fixed coefficients from dataset 1 on % transport losses is summarised in Table 4.2.

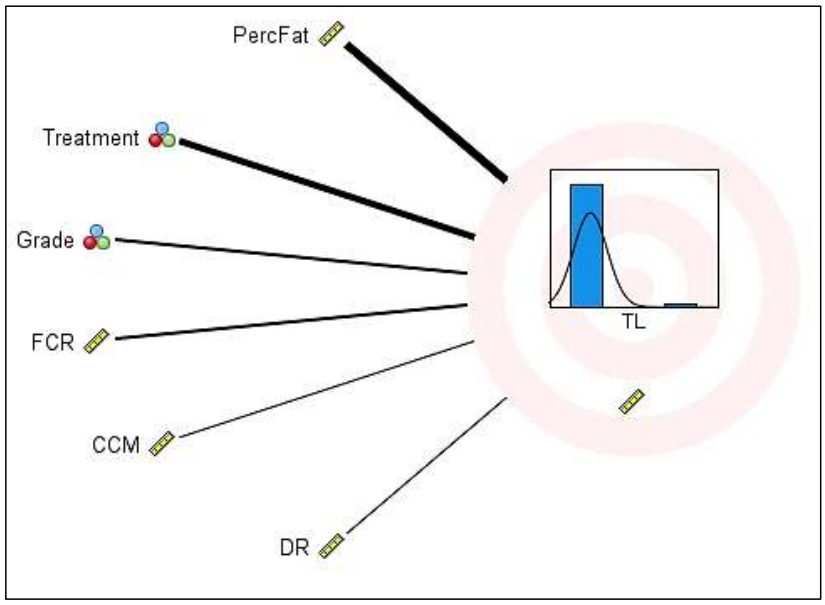

Figure 4.1: Fixed factors Dataset 1

PercFat ~ Subcutaneous fat percentage; Treatment ~ β-Agonist treatment; Grade ~ Carcass fat classification; CCM ~ Carcass mass after cooling, DR ~ Cold carcass dressing %

Table 4.2: Descriptive statistics for Dataset 1

Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
%TL	73	-2,06	-0,75	-2,01	0,20
Dressing %	73	49,00	64,25	57,20	2,88
SCF 13 (mm)	73	2,00	15,00	7,66	3,08
% Fat	73	11,75	33,74	24,76	4,21
CCM	73	232,50	294,60	261,24	15,35
FCR	73	3,26	12,99	7,22	1,71

4.2.1 Descriptive statistics for transportation loss

The target variable for dataset 1 was % transport loss. Cattle from this dataset had a minimum transport loss of 0.75% and a maximum transport loss of 2.06%. The mean %TL was 2.01% ± 0.20% over a distance of less than 100km, with no delays occurring throughout the journey. The results showed that these animals, that were typical South African feedlot cattle under conditions identical to those normally occurring in the industry, did not lose more than 2.06 % of their body mass during transportation.

The effect of the random factor (breed) on the target variable (%TL) was not found to be statistically significant (P = 0.94), however it might have been the cause of some numerical variation in the final mass of animals due to genetic breed differences. The analysis of the effect of breed on %TL is presented in Table 4.3.

Table 4.3: The effect of the random effect (breed) on the % of body mass lost during transportation

Random Effect						
Random Effect Covariance	**Estimate**	**Std. Error**	**Z**	**Sig.**	**95% Confidence Interval**	
					Lower	**Upper**
Var (Breed type)	0,00	0,00	0,08	0.94	$1,5 \times 10^{-15}$	12777952,19

4.2.2 Descriptive statistics for dressing percentage

The carcasses of cattle from dataset 1 had a minimum dressing % of 49.0% and a maximum dressing % of 64.25% (Table 4.2). The mean dressing % for this group of cattle was 57.20%, with a standard deviation was 2.88%. As mentioned previously, a

mean dressing % of 58.5% is normally achieved in a feedlot (Ford, 2017), which is just slightly above the mean calculated for the carcasses used in this dataset. Measurements of the dressing % was obtained from the carcasses of 77 feedlot cattle, however some error in the recording of the final mass of four of these animals resulted in incorrect calculations of their dressing percentages, leading to highly unlikely values that exceeded 65%. All data from these animals were excluded from the analyses of dataset 1, and only 73 animals were used to calculate the mean dressing % for this dataset.

4.2.3 Descriptive statistics for subcutaneous fat thickness

Sub cutaneous fat thickness of the 73 feedlot cattle ranged between 2mm and 15 mm, with a mean and standard deviation of 7.66 ± 3.08mm.

4.2.4 Descriptive statistics for fat percentage

Fat related carcass traits are of importance to the beef industry because of its influence on the value of the meat. Both fat quantity and distribution are key factors affecting carcass and meat quality in beef cattle (Taniguchi *et al*., 2009). The minimum value obtained for the fat % of the 73 cattle used in this study was 11.75% and the maximum value was 33.74%. The mean fat % for these animals was 24.76 %, with a standard deviation of 4.21%

4.2.5 Descriptive statistics for cold carcass mass

The CCM is the mass of the carcass after a period of refrigeration (Department of Agriculture & Rural Development, 2005b) and is also the mass that the carcass is sold at. The CCM of the carcasses from the 73 feedlot cattle ranged between 232.50 kg and 294.60 kg, with a mean CCM of 261.24 kg and a standard deviation of 15.35 kg.

4.2.6 Descriptive statistics for feed conversion ratio

Feed conversion ratio, in terms of beef, refers to the amount of feed consumed (in kg) in order to achieve 1 kg of gain (Coleman, 2015). Animal feed constitutes about 70% of feedlot input costs, therefore FCRs are closely related to the profitability of the feedlot industry (Coleman, 2015). Cattle in feedlots can achieve FCR's of 5.5:1 (Ford, 2017), with even lower values being reached on stud farms. The minimum FCR value for the cattle used to obtain data for dataset 1 was 3.62, while the maximum FCR value was 12.99. The average FCR value for these animals was 7.22, with a standard deviation of 1.71. The mean value of 7.22, or 7.22kg of feed required to obtain 1kg of gain, was moderately higher than the 5.5:1 typically seen in feedlots.

Testing done on a Drakensberg stud in 2015 showed an average FCR of 5.0:1, with individual scores as low as 3.2:1. According to Brahman SA, this breed has an average FCR of 6.70:1 or higher (Brahman, 2019). The majority of animals used in this trial was either Brahman, Drakensberg or had an influence of one of these two breeds. This explains the wide range between the minimum and maximum FCR values.

4.2.7 Descriptive statistics for β-Agonist treatment

One of five treatments was randomly assigned to the 73 cattle used in this trial. Treatment 1 was the control group receiving no β-agonist, whereas treatments 2-5 received β-agonists at various inclusion levels and for different durations. The frequency of assigned treatments is summarised in Table 4.4.

Table 4.4: Treatment frequencies

B-Agonist Treatment		
Treatment Number	**Frequency**	**%**
1	15,00	20,55
2	15,00	20,55
3	16,00	21,92
4	14,00	19,18
5	13,00	17,81
Total	73,00	100,00

A very common effect of supplementation with a β-agonist is the reduction in carcass fat due to decreased lipogenesis and increased lipolysis, and an increase in muscle mass as a result of hypertrophy and decreased muscle degradation. This leads to animals with a greater lean: fat ratio. Overall, feedlot performance and carcass characteristics, such as ADG, FCR and dressing % are all desirably affected by β-agonist supplements (Steenkamp, 2014).

4.2.8 Descriptive statistics for carcass classification

Results for the effect of carcass classification on %TL was obtained by analysis of data from the 73 feedlot cattle and the corresponding abattoir used for slaughter, who, as mentioned previously, wished to remain anonymous. Seventy-three carcasses were individually classified after slaughter based on age and level of fatness as either an A2, A3 or A4 carcass (Table 4.5). This data was then used to determine the effect of carcass classification, or animal fatness, on the percentage of physiological tissue loss.

Table 4.5: Carcass classification frequencies

Carcass Classification	Frequency	%
A2	47	64,38
A3	23	31,51
A4	3	4,12
Total	73	100,00

4.3 Descriptive statistics: Dataset 2

The minimum and maximum values, means and standard deviations of carcass parameters and transport losses for cattle as affected by the different factors are summarised in Table 4.6, excluding the string variables (cattle breed type, carcass classification and mass category).

Figure 4.2 gives a visual representation of the effects of the different factors on transportation losses. It illustrates the relationship between each factor and the target variable (% transport loss). Thicker lines mean that the factor had a larger effect on the target variable than the factors represented by thinner lines

Table 4.6: Descriptive Statistics Dataset 2

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
%TL	113	-2,32	-0,42	-1,51	0,31
ADG Final	113	1,01	2,35	1,64	0,24
CCM	113	207,80	353,20	269,75	30,41
CDR%	113	52,46	64,27	60,71	2,25

4.3.1 Descriptive statistics for percentage transportation loss

The target variable was %TL, which had a minimum value of -2.32%, a maximum value of -0.42% and a mean and standard deviation of -1.51 ± 0.31%. This indicates that a typical feedlot animal transported for less than 100km to the abattoir without delays is likely to lose between 1.51 and 2.32 % of their body mass.

The effect of the random factor (pen number) on the target variable (%TL) was not found to be statistically significant (P = 0.86), however it might have been the cause of some numerical variation in the final mass of animals from pen 1 and pen 3. All animals were male, except for pen 2, which contained female animals that came onto heat and

resulted in a decreased appetite for the male animals in pens 1 and pen 3. This was evident by the decreased feed intake (FI) recorded for these pens after the females came into heat, when being compared to the FI before the females came into heat. The results from the analysis of the effect of pen number can be found in Table 4.7.

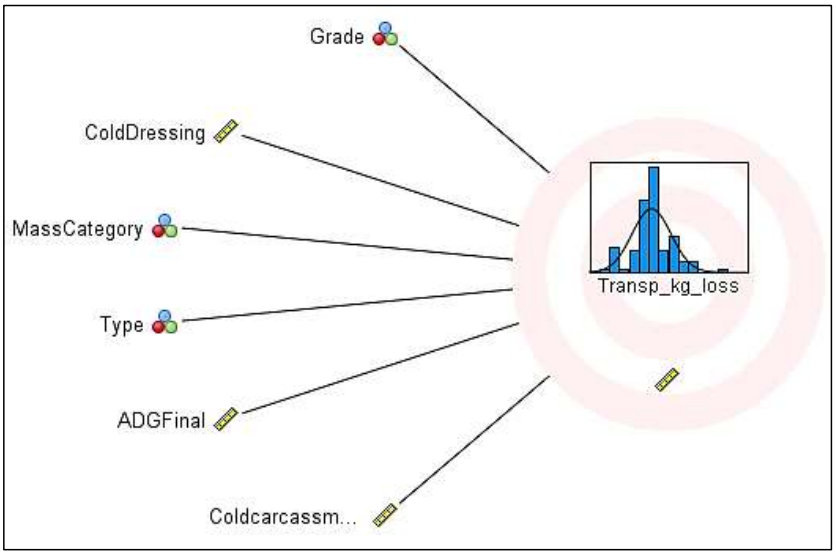

Figure 4.2: Fixed factors for Dataset 2

Grade ~ Carcass fat classification; ColdDressing ~ Cold carcass dressing %; Type ~ Breed type; ADGFinal ~ Final average daily gain; Coldcarcassmass ~ Carcass mass after cooling; MassCategory ~ Categories as described below:

Category 1: < 391kg; Category 2: 391kg – 440kg; Category 3: 441kg – 490kg; Category 4: 491kg – 540kg; Category 5: > 540kg

Table 4.7: The effect of the random factor (pen number) on the % of body mass lost during transportation

Random Effect					
Random Effect Covariance	**Estimate**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
				Lower	**Upper**
Var (Pen#)	0,00	0,01	0,86	0,00	73,80

4.3.2 Descriptive statistics for average daily gain

ADG can be defined as the final mass of the animal - initial mass of the animal divided by the number of days on feed. Average daily gains for the cattle used to compile this dataset ranged between 1.01kg and 2.35kg per day, with a mean and standard deviation of 1.64 ± 0.24kg/day. According to recent feedlot data, the ADG in South African feedlots is 1.7kg/day (Ford, 2017), indicating that the ADG for this dataset is only slightly below that of typical South African feedlot cattle.

4.3.3 Descriptive statistics for cold carcass mass

Cold carcass mass for the cattle used to compile this dataset ranged between 207.80 kg and 353.20 kg, with a mean CCM of 269.75 kg and a standard deviation of 30.41 kg.

4.3.4 Descriptive statistics for cold dressing percentage

As mentioned above, a mean dressing percentage of 58.5% is normally achieved in a feedlot (Ford, 2017). However, the dressing percentage does vary depending on the conditioning of the animal. For lean animals, it can be as low as 49% and it can increase to more than 60% at a high level of finish (Department of Agriculture & Rural Development, 2005a). Cold DR% for the animals used to compile this dataset ranged between 52.45% and 64.27%, with a mean of 60.71 ± 2.25%. This was just slightly higher than the 58.5% normally achieved in South African feedlots. These results were, however, expected due to the higher level of finish of these animals.

4.3.5 Descriptive statistics for breed type

Results for the effects of breed and type on %TL was obtained by analysis of Dataset 2. This data was collected from the Hatfield Feedlot trial on the University of Pretoria's Experimental farm in 2020. All animals were of similar age and subjected to similar diets during their time in the feedlot. The animals were transported by truck for 40km to the Onderstepoort Campus, where they stood for about 12 hours awaiting evaluation.

Afterwards they were transported again for another 40 km to the abattoir for slaughter. A total of 113 animals were used to determine the effect of fifteen different breeds and crossbreeds (Table 4.8) of cattle on the percentage of body mass lost during transportation (%TL). For simplification of the analyses, these breeds were categorized according to their type as either Bos taurus, Bos indicus, Hybrid or Composite animals (Table 4.9)

The mixed model analysis for the effects of the fixed coefficients from dataset 2 on % transport losses is summarised in Table 4.13.

Table 4.8: Breed frequencies

Breed	Frequency	%
Afrikaner	6	5,30
Bonsmara	6	5,30
Bonsmara cross	5	4,40
Bonsmara x Afrikaner	12	10,60
Bonsmara x Afrikaner x Brahman	6	5,30
Boran	10	8,80
Boran cross	3	2,70
Brahman	8	7,10
Brahman (red)	5	4,40
Drankensberger	5	4,40
Santa Gertrudis	12	10,60
Simbra	6	5,30
Simbra cross	11	9,70
Simmental	7	6,20
Tuli	11	9,70
Total	114	100,00

Table 4.9: Type frequencies

TYPE	FREQUENCY	%
B. indicus	13	11,50
B. taurus	47	41,60
Composite	16	14,20
Hybrid	36	33,70
Total	113	100,00

4.3.6 Descriptive statistics for mass category

Results for the mass category effect on transportation losses was obtained by analysis of Dataset 2. The individual mass of 113 cattle was measured before transportation to the abattoir. Those measurements were then used to determine the effect of animal mass on %TL.

For simplification of the analyses, all individual masses were categorized as either 1, 2, 3, 4 or 5 (Table 4.10) to determine if differences in pre-slaughter mass affected the percentage of physiological tissue loss that took place.

Table 4.10: Mass category frequencies

Mass Category	Frequency	%	
1	7	5,90	Category 1: < 391kg
2	49	41,50	Category 2: 391kg – 440kg
3	35	29,70	Category 3: 441kg – 490kg
4	24	20,30	Category 4: 491kg – 540kg
5	3	2,50	Category 5: > 540kg

4.3.7 Descriptive statistics for carcass classification

Results for the effect of carcass classification on %TL was obtained by analysis of Datasets 1 and 2. Dataset 1 consisted of data from 73 bulls from an anonymous feedlot. The bulls were classified based on their level of fatness as either A2, A3 or A4 (Table 4.11A) Information for dataset 2 was obtained from both the Hatfield Feedlot trial on the University of Pretoria's Experimental farm in 2020 and the corresponding abattoir used for slaughter. The abattoir wishes to remain anonymous. One hundred and fourteen animals were individually classified after slaughter based on the level of fatness as either A1, A2, A3 or A4 carcass (Table 4.11B). One AB animal was present; however, AB animals are atypical of feedlots and it was therefore removed to prevent skewed results. Only data from 113 animals was therefore used to determine the effect of carcass classification, or animal fatness, on the percentage of physiological tissue loss.

Table 4.11A Carcass classification frequencies – Dataset 1

Carcass Classification	Frequency	%
A2	47	64,40
A3	23	31,51
A4	3	4,12
Total	73	100,00

Table 4.11B Carcass classification frequencies – Dataset 2

Carcass Classification	Frequency	%
A1	1	0,90
A2	102	80,30
A3	9	8,00
A4	1	0,90
Total	113	100,00

Table 4.12 Summary of the effects of the fixed coefficients of dataset 1 on the percentage of transport losses of feedlot cattle transported for slaughter

Model Term	Coefficient	Std. Error	t	Sig.
Intercept	-1.29	0.55	-2.35	0.02
Treatment 1	0.10	0.08	1.25	0.22
Treatment 2	-0.07	0.08	-0.92	0.36
Treatment 3	-0.13	0.09	-1.46	0.15
Treatment 4	-0.04	0.08	-0.54	0.59
Treatment 5	0.00	.	.	.
Fat %	-0.02	0.01	-2.83	0.01
SCF	0.00	0.01	-0.07	0.95
A2 Carcass	-0.22	0.14	-1.55	0.13
A3 Carcass	-0.12	0.13	-0.88	0.38
A4 Carcass	0.00	.	.	.
Dressing %	-0.01	0.01	-0.75	0.45
CCM	0.00	0.00	1.04	0.30

Table 4.13 Summary of the effects of the fixed coefficients of dataset 2 on the percentage of transport losses of feedlot cattle transported for slaughter

Model Term	Coefficient	Std. Error	t	Sig.
Intercept	-2.98	0.99	-3.00	0.00
A1 Classification	-0.55	0.46	-1.19	0.24
A2 Classification	-0.27	0.33	-0.82	0.41
A3 Classification	-0.28	0.34	-0.82	0.41
A4 Classification	0.00	.	.	.
Dressing %	0.02	0.02	1.20	0.23
Mass Category 1	-0.01	0.48	-0.01	0.99
Mass Category 2	-0.11	0.39	-0.29	0.77
Mass Category 3	0.02	0.31	0.05	0.96
Mass Category 4	-0.07	0.24	-0.28	0.78
Mass Category 5	0.00	.	.	.
Type: Bos indicus	-0.05	0.11	-0.46	0.65
Type: Bos taurus	0.01	0.07	0.15	0.88
Type: composite	-0.02	0.11	-0.19	0.85
Type: hybrid	0.00	.	.	.
ADG	0.04	0.16	0.25	0.80
CCM	0.00	0.00	0.23	0.82

4.4 The effect of fat percentage on percentage transportation loss

The body composition of an animal, and therefore its fat %, is largely dependent on the interactions between genetic, environmental and nutrition factors (Webster *et al.,* 1986). The effect of fat % on the % of physiological tissue loss of the 73 bulls from dataset 1 was analysed using a mixed model approach in SPSS, with breed type as a random factor. The results show that fat % had a statistically significant (P = 0.007) effect on %TL of these animals. The relationship between fat % and %TL was found to be negative, with a coefficient of -0.022. A regression plot was generated to illustrate the relationship between fat % and %TL (Figure 4.3).

For the 73 bulls used in this trial, no dietary difference besides the inclusion levels of a β-agonist existed, which is the main reason for variation in fat %. Genetic differences can also explain some of the variation in fat %, as animals of different breeds were used. Environmental variation was limited and therefore an unlikely cause of variation in fat %. All 73 bulls were kept at the same location for the same duration and handled, transported, and slaughtered in the same manner.

The finding of statistical significance indicates that the mean % transport losses of these bulls were influenced by the level of fatness of each individual animal. This suggests that, for well-conditioned cattle in typical feedlot conditions, the amount of physiological tissue loss that occurs upon transportation to the abattoir will be greatly influenced by each animal's level of fatness.

The negative relationship that was seen between fat % and %TL signifies that an increase in fat % will result in a decrease in physiological tissue loss. More specifically, for every 1% increase in fat, a decrease of 0.022% in tissue losses occurred. Fatter animals therefore showed a lower loss of moisture and tissue during transportation than leaner animals.

As mentioned before, % transport loss essentially refers to the amount of moisture and tissue an animal loses upon transportation. Fat tissue is known to have much lower water content than muscle tissue, explaining why the total body water (TBW) of very fat animals is up to 30% less than that of very lean animals. Therefore, the volume of moisture in the body is largely dependent on the amount of fat in the body (Dukes, 2015a). Consequently, the greater the lean: fat ratio is, the greater potential for moisture loss there is.

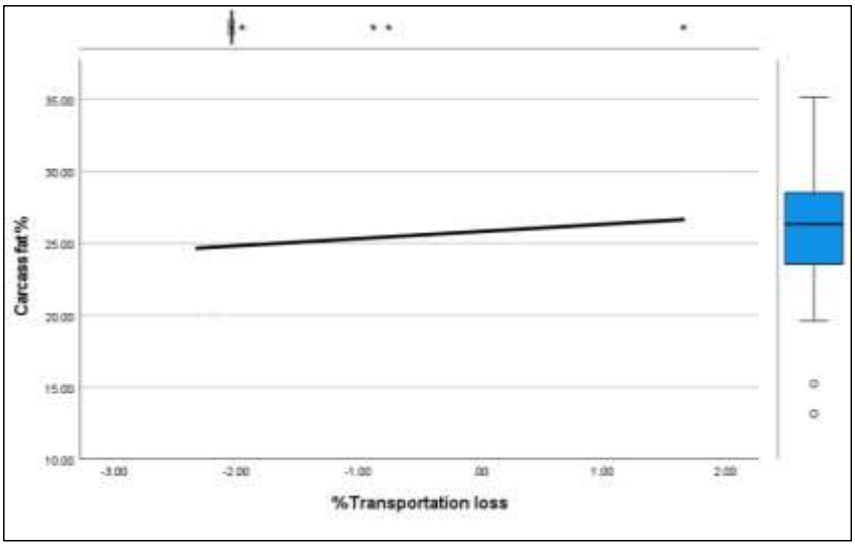

Figure 4.3: A graphic representation showing the effects of carcass fat % (Y-axis) on the % of transportation loss (X-axis)

4.5 The effects of carcass classification on percentage transportation loss

The effect of carcass classification on the percentage of physiological tissue loss that occurs when feedlot cattle are transported to the abattoir was determined by analysis of two datasets in SPSS 27. In both cases, a generalised linear mixed model approach was used, with “breed” as a random factor in dataset 1 and “pen number” as a random factor in dataset 2.

The results from dataset 1 indicate that carcass classification did not significantly (P = 0.160) affect the percentage of physiological tissue loss of the 73 bulls used for this trial. This indicated that the variation seen in % transport losses was statistically no different among the different carcass classifications of animals being transported. Table 4.14 contains a summary of the mean %TL and standard error for each carcass classification from dataset 1.

A pairwise comparison of the %TL between the carcass classifications revealed that no significant differences in percentage of physiological tissue loss between any of the carcass classifications existed. For all comparisons, (P > 0.05). This further supports the above results that carcass classification had no statistically significant effect on the %TL that occurred during transportation to the abattoir. A summary of all the pairwise carcass

classification comparisons from dataset 1 and their corresponding P values can be found in Table 4.15 and a graphical representation thereof in Figure 4.4.

Table 4.14 Mean %TL for each carcass classification - Dataset 1

Mean %TL: Carcass classification - Dataset 1		
Carcass classification	**Mean**	**Std Error**
A2	-2,04	0,032
A3	-1,94	0,048
A4	-1,82	0,132

Table 4.15 Carcass classification pairwise contrast – Dataset 1

Pairwise Contrast: Carcass classification – Dataset 1			
Carcass classification Pairwise Contrasts	**Contrast Estimate**	**Std. Error**	**Adj. Sig.**
A2 - A3	-0,10	0,06	0,11
A2 - A4	-0,22	0,14	0,13
A3 - A2	0,10	0,06	0,11
A3 - A4	-0,12	0,13	0,38
A4 - A2	0,22	0,14	0,13
A4 - A3	0,12	0,13	0,38

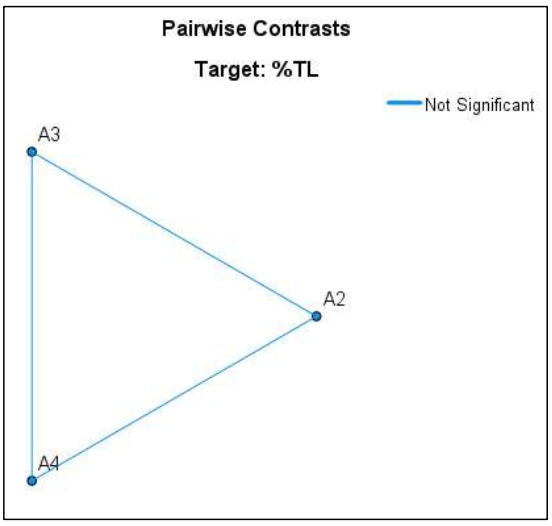

Figure 4.4: Carcass classification pairwise contrast: Dataset 1

Carcass classification (A2, A3, A4 as described previously); %TL ~ % physiological tissue loss

The results from dataset 2 also indicated that carcass classification had no statistically significant (*P* = 0.698) effect on the percentage of physiological tissue loss that occurred upon transportation to the abattoir. These findings suggested that the mean % transport loss for both datasets was not meaningfully affected by the variation in carcass classification. Table 4.16 contains a summary of the mean %TL and standard error for each carcass classification from dataset 2.

Table 4.16: Mean %TL for each carcass classification – Dataset 2

Mean %TL: Carcass classification – dataset 2				
Carcass classification	**Mean**	**Std. Error**	**95% Confidence Interval**	
			Lower	**Upper**
A1	-1,78	0,33	-2,42	-1,13
A2	-1,50	0,07	-1,63	-1,36
A3	-1,50	0,13	-1,76	-1,25
A4	-1,23	0,33	-1,87	-0,58

A pairwise comparison of the difference in % transport losses for cattle of different carcass classifications revealed that no significant differences in these losses occurred between the animals of different classifications. For all comparisons, (*P* > 0.05). This further supports the above results that carcass classification had no statistically significant effect on the %TL that occurred during transportation to the abattoir. A summary of all the pairwise carcass classification comparisons from the cattle used to compile dataset 2 and their corresponding P values can be found in Table 4.17, with a graphical representation thereof in Figure 4.5.

In both datasets, it was found that % transport loss was not significantly affected by the variation in carcass classification. However, despite the statistical non-significance, a distinct downward trend in % transport loss was seen with an increase in carcass fatness (Figures 4.6 and 4.7). From both datasets, higher numbered carcass classifications showed a lower mean %TL. For dataset 1, bulls that had A2 carcasses showed the greatest mean loss of physiological tissue (2.04%), followed by bulls with A3 carcasses, with a mean loss of (1.94%), and finally bulls with A4 carcasses, who had a mean loss of (1.82%).

For dataset 2, cattle with A1 carcasses showed the greatest mean %TL (1.78%) followed by animals with A2 and A3 carcasses (1.50%), and finally cattle with A4 carcasses, who had the lowest mean %TL (1.23%).

Table 4.17: Carcass classification pairwise comparison – Dataset 2

Pairwise Contrast: Carcass classification – Dataset 2			
Carcass classification Pairwise Contrasts	**Contrast Estimate**	**Std. Error**	**Adj. Sig.**
A1 - A2	-0,28	0,32	0,39
A1 - A3	-0,27	0,34	0,43
A1 - A4	-0,55	0,46	0,24
A2 - A1	0,28	0,32	0,39
A2 - A3	0,01	0,12	0,97
A2 - A4	-0,27	0,33	0,41
A3 - A1	0,27	0,34	0,43
A3 - A2	-0,01	0,12	0,97
A3 - A4	-0,28	0,34	0,41
A4 - A1	0,55	0,46	0,24
A4 - A2	0,27	0,33	0,41
A4 - A3	0,28	0,34	0,41

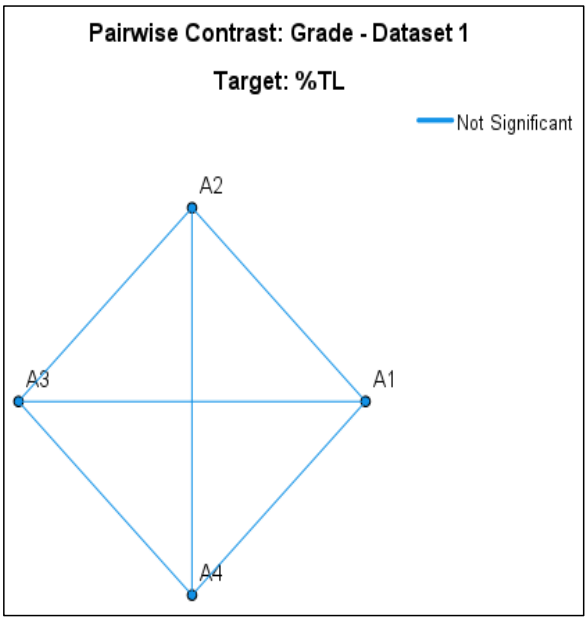

Figure 4.5: Carcass classification pairwise contrast – Dataset 2

Carcass classification (A1, A2, A3 and A4, as defined previously); %TL ~ % Physiological tissue loss

Figures 4.6 and 4.7 show that despite its non-significance, carcass classification possibly contributed to the 2.01% and 1.51% variation seen in the %TL of datasets 1 and 2 respectively. Cattle whose carcasses were classified as A3 and A4 therefore did numerically lose more mass during transport than those classified as A1 and A2, however the differences were not enough to be statistically significant.

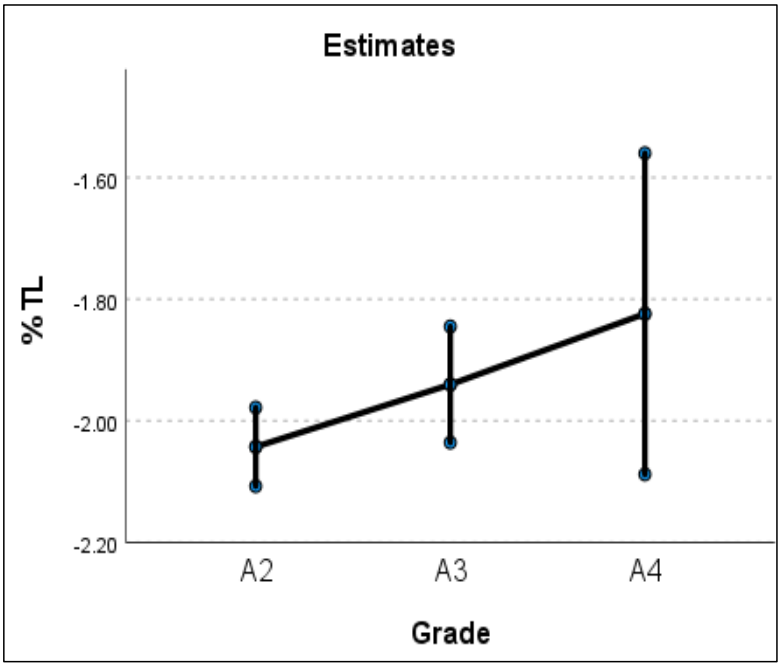

Figure 4.6: Mean %TL for each carcass classification – Dataset 1

Grade ~ Carcass classification (A1, A2, A3 and A4, as defined previously); %TL ~ % Physiological tissue loss

4.6 The effect of subcutaneous fat thickness on percentage transportation loss

The effect of SCF-thickness (measured in mm) on the % of moisture and tissue lost during transport was determined by analysing data from dataset 1. A mixed model approach in SPSS 27 was used, with breed type as a random factor. The results showed that SCF-thickness did not significantly ($P = 0.946$) affect the percentage of physiological tissue loss of the 73 bulls upon transportation to the abattoir.

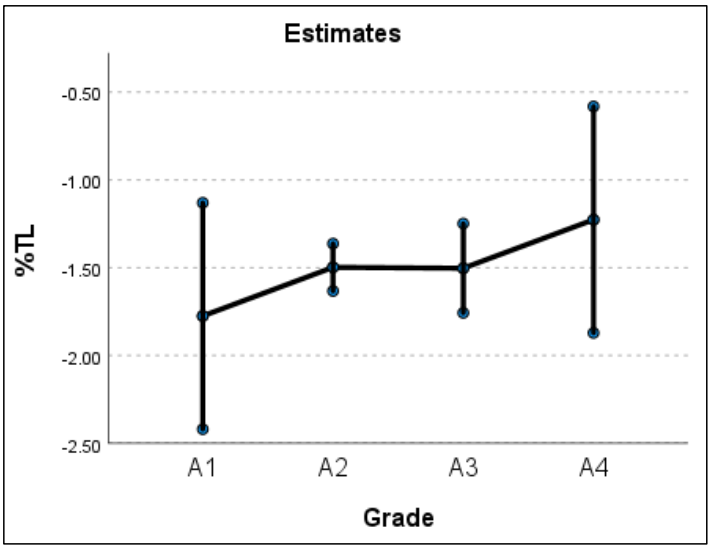

Figure 4.7: Mean %TL for each carcass classification – Dataset 2

Grade ~ Carcass classification (A2, A3, A4 as described previously); %TL ~ % physiological tissue loss

These findings were unexpected due to the very significant effect of fat %, as discussed above. A significant, or at least close to significant, relationship was predicted because of the connection between fat % and SCF-thickness. Despite the insignificance, the relationship between SCF-thickness and %TL was still found to be negative, which implies that an increase in SCF-thickness is associated with a decrease in %TL. However, the variation in % transport loss that can be explained due to the differences in SCF-thickness would be very small.

4.7 The effect of using a β-agonist on % transportation loss

Data from seventy-three bulls of various breeds was used to compile dataset 1. Dataset 1 was then used to determine if treatment with a β-agonist significantly affected the % of transport losses that took place when these animals were transported for slaughter. The results from the GLMM indicated that treatment had a significant effect (P = 0.042) on the percentage of physiological tissue that was lost during transport. This suggests that inclusion level and duration of treatment had an effect on how much tissue loss occurred. The means and standard errors for each treatment are summarized in Table 4.18, and a summary of the treatments with their durations and inclusion levels are given in Table 4.19.

Table 4.18 Mean %TL - Treatment

Mean %TL: Treatment				
Treatment number	**Mean**	**Std. Error**	**95% Confidence Interval**	
			Lower	**Upper**
1	-1,81	0,07	-1,95	-1,67
2	-1,98	0,06	-2,11	-1,85
3	-2,03	0,06	-2,15	-1,91
4	-1,95	0,07	-2,09	-1,81
5	-1,91	0,08	-2,06	-1,76

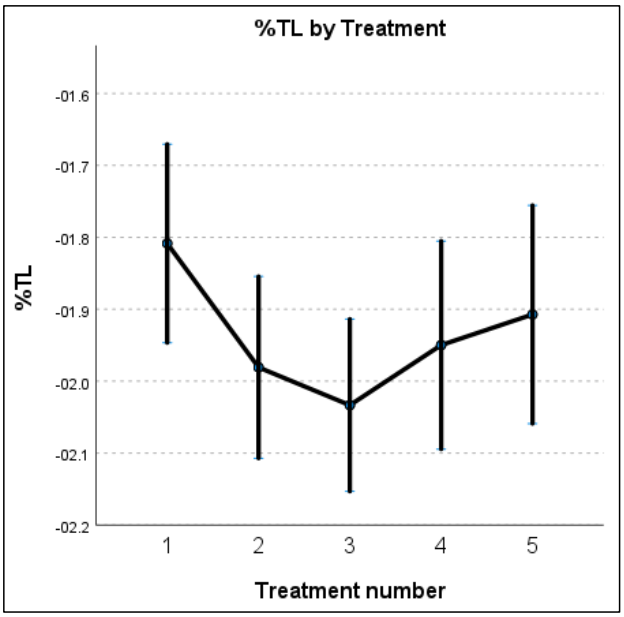

Figure 4.8: Mean %TL for each treatment

%TL ~ % physiological tissue loss

Table 4.19: β-agonist treatment duration and treatment inclusion levels

Treatment number	**Inclusion level (mg/day)**	**Treatment duration (days)**
1 (Control)	-	-
2	120	30
3	120	40
4	90	40
5	60	30

Treatment 1 (the control) had the lowest mean %TL (1.81%) of all five treatments, followed by treatment 5 (1.91%), treatment 4 (1.95%), treatment 2 (1.98%) and finally treatment 3, with the highest mean loss (2.03%). A pairwise comparison of the %TL of each treatment (Table 4.20) showed statistically significant differences in the %TL of treatments 1 and 2, and treatments 1 and 3. A graphical representation of this is given in Figure 4.8

The results can be explained by the variation in inclusion level and duration of the various treatments, as well as the effects of β-agonists on body composition. The control group (treatment 1) that received no β-agonists and showed the least losses would also have been the group with the lowest lean: fat ratio, as previously mentioned. In that way they had a greater proportion of fat compared to bulls from the other treatments. A pairwise comparison (Table 4.20) of each treatment showed significant differences in the %TL of animals in treatments 1 and 2 (P = 0.020), and treatments 1 and 3 (P = 0.003). The differences between animals in treatments 1 and 4 were close to but not statistically significant (P = 0.053). These pairwise comparisons and their significance can also be seen in Figure 4.9.

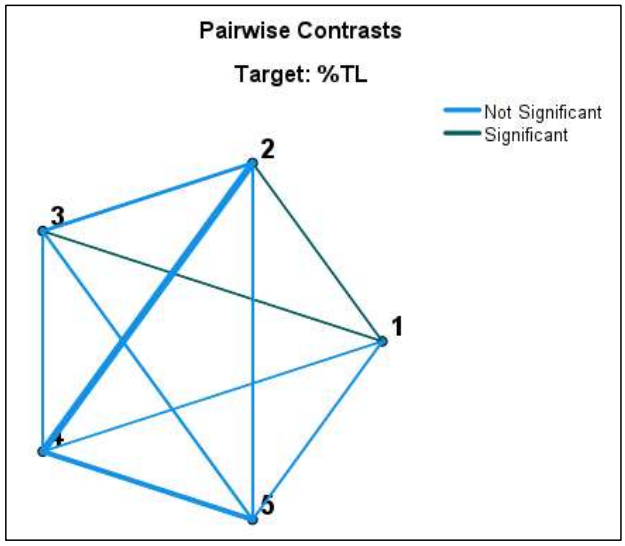

Figure 4.9: Pairwise Contrast – Treatment

%TL ~ % physiological tissue loss; 1,2,3,4,5 ~ Treatment numbers

Results thus far have indicated a tendency for fatter animals to lose a smaller percentage of physiological tissue due to transport, which explains why bulls from treatment 1 had

the lowest reported %TL. The tissue losses from bulls in treatment 1 was also significantly higher than those of treatment 2 and treatment 3, the two treatment groups with the highest inclusion (120mg) of the β-agonists. No significant differences in the losses between the control and treatments 4 and 5 were observed, which were the groups with the lower (90mg and 60mg respectively) inclusion levels of a β-agonists.

The β-agonist given to bulls in treatment 3 was given for 40 days as opposed to treatment 2, who only received it for 30 days. This could explain why treatment 3 showed greater losses than treatment 2 despite receiving the same dose of the β-agonists. Treatment 3 would have had an additional 10 days where protein deposition was favoured above fat deposition, resulting in a greater lean: fat ratio.

Table 4.20: Treatment pairwise comparison

Pairwise Contrasts: Treatment			
Treatment number Pairwise Contrasts	**Contrast Estimate**	**Std. Error**	**Adj. Sig.**
1 - 2	0,17	0,07	0,02
1 - 3	0,22	0,07	0,00
1 - 4	0,14	0,07	0,05
1 - 5	0,10	0,08	0,22
2 - 1	-0,17	0,07	0,02
2 - 3	0,05	0,07	0,46
2 - 4	-0,03	0,07	0,68
2 - 5	-0,07	0,08	0,36
3 - 1	-0,22	0,07	0,00
3 - 2	-0,05	0,07	0,46
3 - 4	-0,08	0,08	0,28
3 - 5	-0,13	0,09	0,15
4 - 1	-0,14	0,07	0,05
4 - 2	0,03	0,07	0,68
4 - 3	0,08	0,08	0,28
4 - 5	-0,04	0,08	0,59
5 - 1	-0,10	0,08	0,22
5 - 2	0,07	0,08	0,36
5 - 3	0,13	0,09	0,15
5 - 4	0,04	0,08	0,59

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4.8 The effect of mass category on percentage transportation loss

The effects of mass category on the % of transport loss that occur when feedlot cattle are transported for less than 100km to the abattoir was determined by analysis of dataset 2. A mixed model approach in SPSS 27 was used, with pen number as a random factor. The results reveal that mass category did not significantly (P = 0.519) affect the percentage of physiological tissue loss that occurred during transportation. This suggests that the mean transport loss (-1.51%) of these cattle was not affected by the variation in animal mass. Table 4.21 contains a summary of the mean %TL and standard error for each mass category.

Table 4.21: Mean %TL – Mass category

Mean %TL: Mass Category				
Mass Category	**Mean**	**Std. Error**	**95% Confidence Interval**	
			Lower	**Upper**
1	-1,47	0,24	-1,95	-0,99
2	-1,58	0,15	-1,88	-1,28
3	-1,45	0,12	-1,70	-1,20
4	-1,53	0,17	-1,88	-1,19
5	-1,47	0,34	-2,14	-0,79

A pairwise comparison of each mass category showed no significant differences in % transport loss of animals in the various categories. For all comparisons, (P > 0.05). This further supports the impression that tissue and moisture losses during transport to the abattoir was not significantly affected by the mass of the animal, because variations in mass could not be linked to variation in %TL.

A summary of all the pairwise comparisons of tissue losses of various mass categories and their corresponding P values can be found in Table 4.22, and a graphical representation thereof in Figure 4.10.

In addition to the non-significance, no trend was observed in the % transport losses within the different mass categories (Figure 4.11). Mass category 2 (391kg – 440kg) showed the greatest mean %TL (1.58%), followed by mass category 4 (491kg – 540kg) with a mean %TL of (1.53%). Mass categories 1 (< 391kg) and 5 (> 540kg) followed with a mean %TL of (1.47%) and finally, mass category 3 (441kg – 490kg) which had the lowest mean %TL of (1.45%). The % of physiological tissue loss did not increase or decrease as the mass of the cattle increased but showed a random dispersal.

Table 4.22 Mass Category pairwise comparison

Pairwise Contrast: Mass Category			
Mass Category Pairwise Contrasts	**Contrast Estimate**	**Std. Error**	**Adj. Sig.**
1 - 2	0,11	0,16	0,49
1 - 3	-0,02	0,22	0,92
1 - 4	0,06	0,32	0,85
1 - 5	-0,01	0,48	0,99
2 - 1	-0,11	0,16	0,49
2 - 3	-0,13	0,12	0,27
2 - 4	-0,05	0,22	0,83
2 - 5	-0,11	0,39	0,77
3 - 1	0,02	0,22	0,92
3 - 2	0,13	0,12	0,27
3 - 4	0,08	0,14	0,56
3 - 5	0,02	0,31	0,96
4 - 1	-0,06	0,32	0,85
4 - 2	0,05	0,22	0,83
4 - 3	-0,08	0,14	0,56
4 - 5	-0,07	0,24	0,78
5 - 1	0,01	0,48	0,99
5 - 2	0,11	0,39	0,77
5 - 3	-0,02	0,31	0,96
5 - 4	0,07	0,24	0,78

The results obtained from the GLMM analysis suggested that mass category had little to no effect on the 1.51% variation seen in the % tissue and moisture losses of the 113 animals from which the data was obtained.

In addition to the non-significance, no numerical pattern was observed between the mean %TL of the various mass categories, leading to the assumption that pre-slaughter mass was not a determinant in the amount of physiological tissue that was lost due to transport.

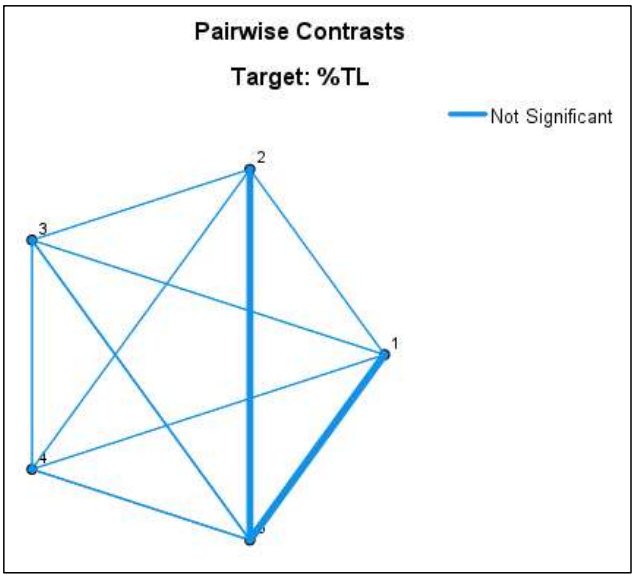

Figure 4.10: Pairwise contrast – Mass Category

Category 1: < 391kg; Category 2: 391kg – 440kg; Category 3: 441kg – 490kg; Category 4: 491kg – 540kg; Category 5: > 540kg

Body composition, instead of mass itself, is the more likely contributor to the variation in %TL seen in this group of cattle. As mentioned previously, the results obtained showed an inclination towards fatter animals losing a smaller percentage of mass during transport, most likely due to the lower water holding capacity of fat in comparison to muscle, or possibly due to the isolating function of fat.

Cattle within the same mass category will have different body compositions (i.e., fat and muscle percentages), and therefore possibly lose different amounts of physiological tissue, despite being of similar mass. This would explain the randomness of the losses seen across the mass categories in Figure 4.11.

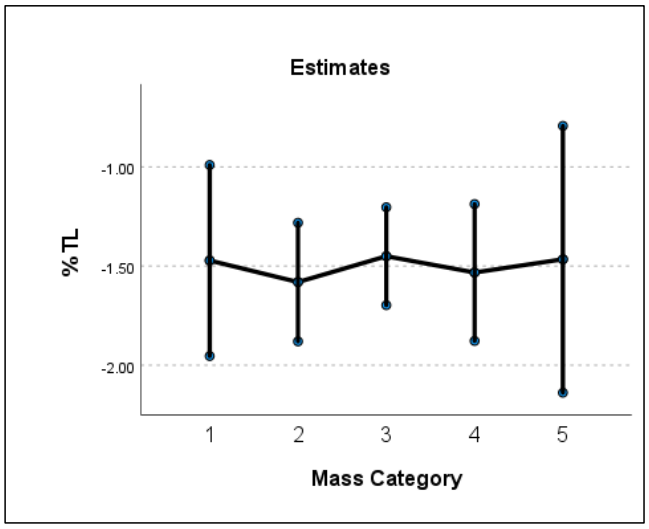

Figure 4.11: Mean %TL for each Mass Category

Category 1: < 391kg; Category 2: 391kg – 440kg; Category 3: 441kg – 490kg; Category 4: 491kg – 540kg; Category 5: > 540kg

The variation in body composition among cattle can be attributed to nutrition and the effect of nutrition on the genetic and physiological factors over a period of time. Given adequate nutrition and no major environmental constraints, animals proceed towards maturity along a sigmoid curve for cumulative growth (Webster, 1986). Stage of maturity - best defined as a proportion of mature lean mass - is the main non-nutritive determining factor and predictor of body composition. Genotypes or breed maturing at a later age will, by definition, be less mature at a given mass or age, given other factors are equal, and therefore tend to be leaner than individuals maturing at a younger age (Webster, 1986). It is necessary to mention that the body composition of any animal at any age, mass or stage of maturity can be influenced to a very great degree by both the quantity and quality of food that it has received. Restricting feed intake during growth will result in reduced gains and a lower fat to protein deposition rate (Webster, 1986), however restricted feed intake is not a problem for animals in feedlots, they receive a scientifically formulated feedlot ration in order to ensure optimal growth.

4.9 The effect of breed type on percentage transportation loss

The effect of breed type on the % of transportation losses that occurs when feedlot cattle are transported to the abattoir was assessed by analysis of dataset 2. This was done

using a mixed model approach in SPSS 27, with pen number as a random factor. The results indicate that animal type did not significantly (P = 0.957) affect the percentage of physiological tissue loss of the transported group of animals. This suggests that cattle type, or the breed of the animal, did not make a large contribution to the variation seen in % transport loss. Table 4.23 contains a summary of the mean %TL and standard errors for each type.

Table 4.23: Mean %TL – Mass category

Mean %TL: Type				
			95% Confidence Interval	
Type	**Mean**	**Std. Error**	**Lower**	**Upper**
Bos indicus	-1,53	0,16	-1,83	-1,22
Bos taurus	-1,47	0,13	-1,72	-1,22
Composite	-1,50	0,14	-1,77	-1,23
Hybrid	-1,48	0,15	-1,75	-1,21

Comparison of the losses among the different cattle breed types revealed no statistically significant differences, for all comparisons (P > 0.05). This further supports the findings that cattle type had little effect on the transport losses of this group of cattle. A summary of all the type comparisons and their corresponding P values can be found in Table 4.24.

Table 4.24: Type pairwise comparison

Pairwise Contrast: Type			
Type Pairwise Comparison	**Contrast Estimate**	**Std. Error**	**Adj. Sig**
Bos indicus - Bos taurus	-0,06	0,11	0,59
Bos indicus - Composite	-0,03	0,13	0,83
Bos indicus - Hybrid	-0,05	0,10	0,64
Bos taurus - Bos indicus	0,06	0,11	0,59
Bos taurus - Composite	0,03	0,11	0,79
Bos taurus – Hybrid	0,01	0,07	0,91
Composite - Bos indicus	0,03	0,13	0,83
Composite – Bos taurus	-0,03	0,11	0,79
Composite - Hybrid	-0,022	0,11	0,83
Hybrid - Bos indicus	0,05	0,10	0,64
Hybrid – Bos taurus	-0,01	0,07	0,91
Hybrid - Composite	0,00	0,11	0,84

Despite the statistical non-significance, numerical differences in %TL among cattle types were observed for this dataset, with a definite trend for physiological tissue loss observed. The mean %TL was greatest for B. indicus animals (1.53%), followed by Composite (1.50%) and Hybrid animals (1.48%), with B. taurus animals having the lowest (1.47%) mean %TL. This is demonstrated in Figure 4.12.

This implies that animal type did contribute to the variation seen in % transport loss, even though the differences were not large enough to be statistically significant.

The variation in the mean %TL resulting from animal type can be attributed to a variety of factors. Firstly, the difference in temperament and excitability among the various breed types. Temperament can be defined as the fear/reactive behavioural response to human handling (Burrow, 1997). B. indicus animals tend to be more excitable with a higher mean temperament rating than their B. taurus counterparts (Voisinet *et al.,* 1997).

This means they have greater cortisol levels and a greater tendency to experience stress. B. indicus animals therefore tend to be more stressed during the pre-slaughter journey than the other types and can therefore experience greater stress-induced losses. More excitable animals have also been shown to have decreased fat thickness compared to their calmer counterparts (Reinhardt *et al.,* 2009), which could also possibly contribute to the greater losses of the Indicus type animal.

Secondly, morphological differences can possibly explain the contribution of breed type to the variation seen in %TL. Cutaneous evaporation is the primary means by which cattle dissipate heat through the involvement of sweat glands and other components of the skin. The difference in the ability of B. indicus and B. taurus animals to tolerate heat can be attributed to the differences in their skin morphology (Finch, 1986; Kadzere *et al.,* 2002; Yousef, 1985). A study done by Jian *et al* in 2014 found that both the density and volume of sweat glands in B. indicus cattle was greater than that found in B. taurus and crossbreeds, leading to a higher sweating rate.

The distinctive humped back and large ears of B. indicus cattle also increase the surface area for sweat glands, allowing for even more evaporative losses as a means of cooling down. This could mean that B. indicus animals suffer from more moisture loss than B. taurus animals, leading to a greater %TL. The study also revealed that crossbred animals will differ in skin morphology, and therefore moisture loss, based on the genetic fraction of B. taurus and B. indicus (Jian *et al.,* 2014).

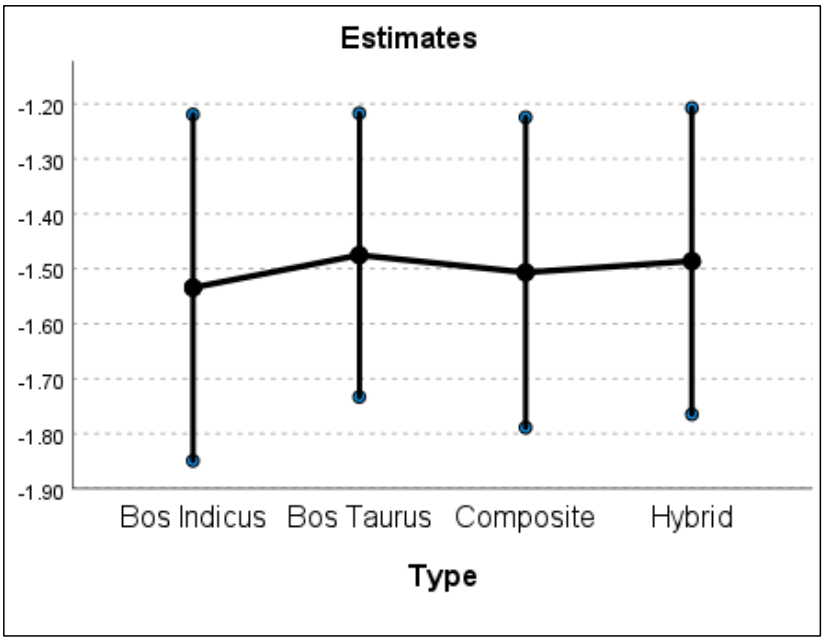

Figure 4.12: Mean %TL for each Type

Type ~ Breed types including:

- B. indicus
- B. taurus
- Composite ~ recognised synthetic breeds obtained from multiple generations of crossbreeding
- Hybrid ~ crossbred animal not recognized as a new breed

Thirdly, the variation in %TL due to type can be explained by the difference in the fat versus lean mass, or carcass composition, of the different types. Beef breeds have a predisposition to lay down fat subcutaneously as a consequence of traditional selection (Webster, 1986), however differences in the mature size among genotypes still results in variation in the fat: lean ratio. B. indicus cattle are slower maturing than B. taurus cattle, meaning that fat deposition occurs at a slower rate and for a longer duration. B. indicus animals will therefore have less subcutaneous fat deposits at slaughter age than their B. taurus counterparts. Studies have also shown that B. indicus animals have less subcutaneous fat at maturity than B. taurus animals (Webster & Wilson, 1980). Analysis of datasets 1 and 2 have shown a trend suggesting fatter animals, not heavier animals,

have less tissue and moisture losses compared to their leaner counterparts, possibly due to the lower water holding capacity of fat compared to muscle, as well as the greater isolating function of fat. A lot of the variation seen in %TL due to differences in the breed of the cattle can therefore be explained by the variation in the subcutaneous fat thickness of the different types.

4.10 The effect of average daily gain on percentage transportation loss

The effect of ADG on the % of physiological tissue loss of transported cattle was determined by analysis of dataset 2, with pen number as a random factor. The results from the GLMM indicate that ADG did not significantly (P =0.802) affect the %TL of the 113 cattle used for this trial. This indicates that the ADG of individual animals is not expected to affect the mean % tissue and moisture loss when being transported to the abattoir. It is therefore unlikely that ADG contributed to the variation seen in %TL of the 113 cattle.

Despite the statistical insignificance, ADG and %TL showed a positive relationship, suggesting that animals with greater ADG's could have had higher transport losses. Based on the results so far, it is evident that fatness, rather than total body mass, is the major factor influencing the percentage of tissue loss that occurs. This is due to the higher potential for moisture loss of lean compared to fatter animals of similar mass. The positive relationship between %TL and ADG thus suggests that muscle gain was somewhat greater than fat gain for the feeding period. The animals with greater ADG's possibly had a greater amount of muscle than those with lower ADG's, thereby having a greater potential for moisture loss.

The results, however, show that the impact of this positive relationship is very insignificant. It appears to have had no practical or statistical effect on the mean %TL. This is possibly due to a variety of other factors, namely the difference in breed (and therefore maturity type), sex (which causes variation in fat deposition) (Webster, 1986), herd dominance, which influences feed intake and in turn ADG, and minor differences in age.

4.11 The effect of feed conversion ratio on % transportation loss

One of the main objectives of a feedlot is to increase the FCR - the efficiency with which feed is converted into meat. Feed conversion ratio is a function of animal genetics, age, condition and the quality and ingredients of the feed given (Kahl, 2018). It represents the efficiency of animal growth with regards to input costs – of which feed makes up the

largest part – and is therefore an important parameter used in determining the profitability of the feedlot (De Lange *et al.,* 2014).

The effect of FCR on %TL was determined by analysis of dataset 1. The results indicate that the FCR of the 73 bulls used in this trial had no significant effect (*P* =0.122) on the percentage of moisture loss during transport to the abattoir. The efficiency with which feed was converted into muscle therefore did not influence the amount of mass lost during transport.

4.12 The effect of cold dressing percentage and cold carcass mass on % transportation loss

The effect of DR% and CCM on the percentage of physiological tissue loss that occurs upon transportation to the abattoir was determined by analysis of datasets 1 and 2. For both fixed factors in both datasets, the results indicated non-significance (*P* >0.05) on %TL. These results are shown in Table 4.25.

Table 4.25: The effects of dressing % and CCM on %

DATASET 1				
Source	**F**	**df1**	**df2**	**Sig,**
DR%	0,57	1	62	0,45
CCM	1,08	1	62	0,30
DATASET 2				
Source	**F**	**df1**	**df2**	**Sig,**
DR%	1,44	1	99	0,23
CCM	0,05	1	99	0,82

The results obtained on the effect of dressing % on %TL were very unexpected; a significant relationship was anticipated due to the highly significant effect of fat % on %TL and the positive relationship between dressing % and fat % (Webb & Erasmus, 2013). Fatter carcasses are generally associated with a higher dressing % (Webb & Erasmus, 2013), which is why the results were especially surprising.

Dressing % is an important concept in the beef industry, as it establishes the mass upon which payment is calculated for animals sold on a live mass basis (The Beef Site, 2006). Greater dressing percentages are therefore associated with higher profit margins and is an important and sought-after trait in the beef production industry, consequently

considering the influence of dressing % on %TL in this study was thought to be a relevant point.

The insignificance of the effect of dressing % on %TL is best explained by the small proportion of the total growth curve under investigation during the feedlotting period in this study, and the minimal variation in the point on the growth curve of all the animals used. As mentioned before, dressing % increases with an increase in live mass, or with an increase in fatness (The Beef Site, 2006). As feedlot cattle move towards finishing mass, the amount of fat increases at a faster rate than any of the other body components, such as muscle and bone. This fat is deposited either subcutaneously, intramuscular, or as visceral fat. Besides the visceral fat, much of the rest remains in the carcass at slaughter, thereby increasing the dressing percentage (The Beef Site, 2006). However, it appears as though the effect of this is not sufficient to cause significant variation in transport losses.

The non-significant effect of CCM on %TL was anticipated, due to the insignificance of the effect of mass category on tissue losses. The live mass of an animal is positively related to CCM, therefore heavier live animals will yield heavier carcasses. However, as established previously, the composition of the carcass and not the mass itself is a greater indicator of how much tissue loss to expect. Therefore, carcass mass is not likely to have had any impact on the percentage of physiological tissue loss that occurs upon transportation to the abattoir.

CHAPTER V: CONCLUSION

The results from this study indicate that typical feedlot cattle in South Africa transported for less than 100km to the abattoir for slaughter will lose an average of 1.51% to 2.01% of their body mass as physiological tissue losses. Despite the clear trends observed for “breed type” and “carcass classification”, the only fixed factors with a statistically significant effect on % physiological tissue loss were fat % and β-agonist treatment. Animal mass, ADG, FCR, CCM and dressing % were shown to be insignificant with no observable trends, and it can therefore be assumed that these factors made no contribution towards the variation seen in tissue losses.

Reflecting on the overall results, it is apparent that animal fatness (fat %) was the main driver behind the variation observed in physiological tissue loss. In itself, fat % was found to be a significant (P = 0.007) contributor to the variation in the % of physiological tissue loss, with an increase in fatness relating to a decrease in tissue losses. Fatter animals can therefore be expected to lose less tissue, or moisture, than leaner animals when being transported for short, uninterrupted distances. This is possibly due to the greater moisture holding capacity of protein as opposed to adipose tissue, resulting in a greater potential for moisture loss in animals with a greater lean: fat ratio.

It also explains the trends observed in the “breed type” and “carcass classification” effects, and why β-agonist treatment was significant (P = 0.042). Subcutaneous fat levels vary due to the genetic differences in breed, meaning that breeds with greater fat deposition would tend to lose less moisture than genetically leaner breeds. This was observed in the results, with the fatter B. taurus types trending towards less moisture loss and the leaner B. indicus types trending towards greater moisture loss despite the insignificance (P = 0.957) of the “breed type” effect. Similar reasoning can be extrapolated to the trend seen in “carcass classification”, where fatter (A3 and A4) carcasses showed lower losses than leaner carcasses (A1 and A2).

The nutrient repartitioning effect of β-agonists resulted in leaner carcasses of equivalent mass when compared to non-treated carcasses, therefore animals in the control group was fatter, though not lighter, than animals received the β-agonists. The tissue losses for the “treatment” effect showed significant increases with increase in treatment duration and inclusion level. The control group, and therefore the fattest group, showed the lowest tissue losses of all the groups. β-agonists treatment can therefore be seen as having indirectly contributed to the variation seen in the % of physiological tissue loss due to its effect on carcass fatness, the direct contributor of the variation.

Tissue losses are of economic importance as it directly affects the dressing percentage. Therefore, the empty body mass was used to calculate physiological tissue losses with complete exclusion of gut losses from all calculations, as is done in normal conditions.

Despite the significance of some of the tested factors, losses still did not exceed 2.32% for either of the datasets, regardless of the low fat % of some of the animals and the high inclusion levels of the β-agonist, or even the great variation in breed types. Feedlot cattle transported for less than 100km to the abattoir for slaughter without delay can therefore be expected to lose on average 1.51% to 2.01% of their body mass, given that their body condition is similar to those normally seen in feedlots.

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