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Farm to abattoir conditions and their subsequent effects on behavioural and physiological changes and the quality of beef from extensively-reared Nguni and non-descript steers

By

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Thesis submitted in fulfilment of the requirements for the degree of

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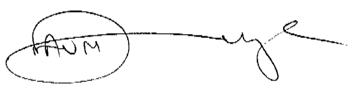
Declaration

I, **Yonela Zifikile Njisane**, vow that this thesis has not been submitted to any other University, it is my original work conducted under the supervision of Prof. V. Muchenje. All assistance towards the production of this work and all the references contained herein have been fully accredited.

09 January 2016

Miss Yonela Zifikile Njisane **Date**

Approved as to style and content by:



Prof V Muchenje

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Abstract

Farm to abattoir conditions and their subsequent effects on behavioural and physiological changes and the quality of beef from extensively-reared Nguni and non-descript steers

The main objective of the study was to determine the effect of farm to abattoir environmental conditions and their subsequent effects on behavioural and physiological responses, as well as the quality of meat from Nguni (NG) and non-descript (ND) beef steers reared extensively on natural pastures. The forty 16 – 19 months old steers (20 ND and 20 NG) used in the current study were grouped together, medically treated, allowed three weeks acclimatizing period and were used in this trial over a four-month period. The weather and periodical variations influence on time budgets and body weights of these steers were determined. Furthermore, the effects of on-farm successive handling on behavioural scores and physiological responses of the same steers were determined. Later in the trial, some pre-slaughter effects on response-behaviour, bleed-out times and selected blood physiological responses were determined. Finally, the effect of genotype, muscle type, lairage duration, slaughter order and stress responsiveness on pH_{24}, temperature, colour (L*, a*, b*, C, HA), thawing (TL) and cooking (CL) losses and Warner Bratzler Shear Force (WBSF) of the meat harvested from the same steers were determined. The daily time budgets of steers in natural pastures changed with temperature, humidity, observation week and time of the day. The grazing behaviour was observed throughout the observation days (> 37%); though it was reduced (26.9±2.64%) on days with higher temperatures and low humidity. Higher proportions of drinking (1.5±1.04%) and standing (20.8±4.63%) behaviours prolonged in such weather conditions, which were mostly during midday. The avoidance-related behaviour of the steers during handling varies, with the steers showing more avoidance and aggression in other weeks than some. These variations could however be traced back to the events of that particular day/time of handling. Only Weighing Box (WBS) and stepping (SS) scores differed (P<0.05) with genotype; with more calm NG steers (> 40%) and not kicking than the ND steers that were more vocal (20–60%) and kicking (> 5%). In addition, the weekly behavioural responses were reflected (P<0.05) in the measured cortisol, glucose and lactate. However, regardless of the prominent negative behaviour seen over time, the levels of the measured blood constituents continued to drop. Furthermore, steers of different genotypes displayed similar (P>0.05) response to the identical pre-slaughter conditions they were exposed to. However, steers that were Transport Group 1 (TG1) showed more avoidance (63.2%) pre-slaughter than those in TG2 (23.9%). Furthermore, all the steers that were in slaughter Group 2 (SG2) showed less avoidance behaviour than those in other groups. Vocalization was observed only for ND steers (5%), in TG1 and SG2. Some connections between the observed pre-slaughter activities and some behavioural and physiological changes of these steers were established; with TG1 and SG1 steers showing higher cortisol (140±14.50 and 175.9±17.24 nmol/L, respectively) and lactate (12.4±0.83 and 13.5±1.12 mmolL) levels than the other groups. Lastly, the muscle type, genotype, lairage duration, slaughter order and stress responsiveness have an effect on some meat quality characteristics of the two genotypes; with the *L. dorsi* muscle having highest WBSF (38.0±1.35N) than the Superficial pectoral muscle (Brisket muscle) (30.7±1.35N). Additionally, steers lairaged for a shorter time produced a *L. dorsi* with higher WBSF (41.6±2.34N) and a Brisket with lower TL (2.7±0.24%). It can therefore be concluded that the conditions and activities at the farm, during transportation, lairaging and slaughter at the abattoir have an influence on some behavioural and physiological changes and the quality of beef harvested from the Nguni and non-descript steers that were extensively-reared in natural pastures. However, the relationship patterns between these different conditions are not clear. In addition, the genotype difference

Key words: Feeding behaviour, Animal-human interaction, Avoidance-related behaviour, Lairage duration, Slaughter order, Beef quality.

Dedication

This Thesis is dedicated to the sovereign God, for without his great plan for my life, none of this would have been possible. All glory belongs to him. To the Njisane clan, this is my gift to you, may this be something you take pride in. Finally to the youngsters out there, aspiring to achieve greatness regardless of their background, may you be inspired and motivated.

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List of symbols

mmol/L – Micromole per litre

N – Newton

n – Sample number

nmol/L – Nanomole per litre

s – Seconds

List of abbreviations

a* – Redness

b* – Yellowness

C – Chroma

CL – Cooking loss

DFD – Dark firm and dry

ES – Entry Score

ESS – Exit Speed Score

HA - Hue angle

L* – Lightness

LG – Lairage Group

M1 – Muscle 1 (Superficial pectoral muscle/Brisket)

M2 – Muscle 2 (*Longissimus dorsi*/Loin)

ND – Non-descript

NG – Nguni

NRF – National Research Foundation

pH_{24} – pH at 24 hours

RMRDT – Red Meat Research and Development Trust

RS – Race Score

SG – Slaughter Group

SS – Stepping Score

TG – Transportation Group

TL – Thawing Loss

WBS – Weighing Box Score

WBSF – Warner Bratzler Shear Force

Chapter 1
General Introduction

1 Background of the study

South African beef-producing farms mostly use the extensive production system. This type of set up is mostly preferred for its allowance towards good animal welfare (Lee *et al*., 2013). However, it is also characterised by minimal interaction between stockman and the animals, except during managerial routines. These routines often involve activities such as weighing, castration, dipping, branding, and vaccination, among others; which are recognized to be unpleasant for the animals (Raussi, 2003; Probst *et al*., 2013). Subsequently, in trying to cope with a situation, animals are subjected to behavioural and physiological changes (Grandin, 1997; Boissy *et al.*, 2001; Terlouw, 2005; Broom and Fraser, 2007).

The beef production chain also exposes slaughter animals to other characteristic conditions like transportation and the abattoir. Transportation often includes exposure to novel and tense conditions such as crowding, noisy vehicles, and elongated periods of food and water or space to rest deprivation, pre-transport management, vibrations, social regrouping, restraint, loading and unloading, transportation duration and climatic factors (Schaefer *et al*., 1997; Mitchell and Kettlewell, 1998; Swanson and Morrow-Tesch, 2001; Kadim *et al*., 2006). It was described to be the link between all meat production activities and was found to significantly contribute to pre-slaughter stress (Tarrant *et al*., 1990; 1992; Mota-Rojas *et al*., 2006; Minka and Ayo, 2013; Miranda-de la Lama *et al*., 2014).

Similarly, the abattoir is characterised by conventional architectural criteria, such as space optimization to facilitate human movement and not much consideration of the behavioural characteristics of the animals has been given in these plans (Miranda-de la Lama *et al*., 2010). In addition, the abattoir also consists of a number of unfamiliar workers and events, as opposed to the mostly ‘normal” farm exposures. While Vimiso *et al*. (2012) reported that animal handling differs between the farm and the abattoir, Grandin (2006) recounted that cattle perceive the abattoir environment in the same way as at the farm during procedures like vaccination and other managerial routines. Exposure to unfamiliar situations such as transport, pre-slaughter treatment and handling at the abattoir, can be extremely stressful to beef cattle (Grandin, 1997; Probst *et al*., 2013).

The previous exposure and experiences of the animals, together with their individual phenotypic and genotypic characteristics are believed to have a great impact on how they respond to pre-slaughter activities at a later stage, resulting to alterations in the quality of meat produced (Grandin, 1980; McGreevy, 2003; Raussi, 2003; Ferguson and Warner, 2008; Muchenje *et al*., 2008; 2009a, 2009b; Hemsworth *et al*., 2011; Cetin *et al*., 2012). The sudden, intense or prolonged elicitation of fear has serious implications on the livestock welfare, farmers' profit and consumers' perception (Hemsworth and Coleman, 1998; Terlouw, 2005; Waiblinger *et al*., 2006; Ferguson and Warner, 2008). Additionally, the animals' characteristics also greatly contributes to its perception and performance (Hansen *et al*., 2001; Dodzi and Muchenje, 2011; Njisane and Muchenje, 2013a, 2013b).

For instance, the Nguni (NG) beef cattle, indigenous to South Africa is known for its continuous excellent performance in any kind of condition or purpose (Muchenje *et al*., 2009; Ndlovu *et al*., 2009); while the non-descript (ND) cattle that are dominating the developing farming sector (Scholtz *et al*., 2008) are basically crossed animals, with unidentified genes and/or characteristics. Therefore, to ensure maximum product quality, this knowledge should be of importance to everyone in the production chain; from the stockman, farmer, transporter, abattoir worker, and the designers of animal facilities (Broom and Fraser, 2007). Hence, the objective was to determine the effect of stress resulting from environmental change from the farm to the abattoir on behavioural and physiological responses and the quality of meat from slaughter cattle.

1.1 Problem Statement

Moving slaughter animals from the farm to the abattoir is a very important and frequently performed operation in the meat industry. This involves forcefully moving animals from the normal/usual environment (farm) to a totally new set up at the abattoir and throughout the process; there is exposure to novel situations. This act has been reported to induce stress to the animals (Ferguson and Warner, 2008). In addition, factors such as exposure to handling (loading, offloading and at the abattoir), loud noises, human-animal contact and other alien activities (to the animals) during transportation and at the abattoir contribute to the induction of stress. Grandin (1999) reported that animal responses differ with diverse handling techniques and systems, as well as the animal's previous farm exposure. Between the farm and the abattoir, these techniques and systems are bound to be different. This comes about by that the two operations have different agendas and objective, time schedules and chasing a different motive in a day. In addition, Grignard *et al*. (2001) reported that genetic characteristics of an animal influences how the animal responds to handling. When animals are stressed, both behaviour and physiological responses of the animals are tempered with (Bourguet *et al.*, 2011). It has been reported by researchers in other countries that good animal-human interactions at an early stage at the farm can improve how they perceive human encounter, improving the handling procedure and reducing handling stress while meat quality is improved (Markowitz *et al*., 1998; Probst *et al*., 2012; 2013). However, this could be almost impossible to achieve in a real life situation and a beef producing system. To meet the high consumer demands for animal protein source, beef producers operate on a large scale, either in feed lots or natural pastures systems. Stress prior to slaughter is an unwanted factor, for it results to reduced welfare of the animal and the quality of the meat produced, thus profit loss.

1.2 Justification

Animals are moved from the farm to the abattoirs for slaughter every day. There is however little scientific material documented on the effects of this on the animals and consequently, meat productivity in South Africa. The meat production chain involves the farm where animals are born, reared, fattened, transported to the slaughter-house, slaughtered and then converted to meat to be distributed to consumers through retailers. All these issues in the meat production system have been separately covered without clearly encompassing the whole production chain. However, it has been reported that the animal perception and response to the adverse conditions at the abattoir depends on its background and previous experience (Grandin, 1993; Terlouw, 2005). Studies that have successfully linked animal exposure and product quality have been done in dairy herds (Dodzi and Muchenje, 2011; Mounier, 2011). Information on both behavioural and physiological responses of slaughter animals and their effects is very crucial nowadays especially with the increasing concerns on animal welfare and production of quality meat from meat consumers. Hence; the current study sought to look at conditions in all areas (Farm, Transport and Abattoir) of production in the meat production chain and relating them to animal responses and the quality of meat produced. The study is one of its kind, especially in the beef industry and in South Africa, hence its' potential to positively contribute to the beef producing sector.

1.3 Objectives

The main objective of the study was to determine the effect of farm to abattoir environmental-conditions and their subsequent effects on some behavioural and physiological responses of Nguni and non-descript beef steers reared extensively on natural pastures.

Specific objectives were:

1. To determine the effects of on-farm successive handling on some behavioural and physiological responses of Nguni and non-descript beef steers reared extensively on natural pastures
2. To determine the effects of some weather and periodical variations on time budgets and body weights of Nguni and non-descript steers reared extensively on natural pastures
3. To determine the effects of pre-slaughter on and off-loading, lairage duration and stunning effects on bleed-out times, response-behaviour and some blood physiological responses of Nguni and non-descript beef steers
4. To determine pre-slaughter activities and their subsequent effects on meat characteristics and beef quality of Nguni and non-descript beef steers reared on natural pastures

1.4 Hypothesis

The null hypothesis states farm to abattoir environmental-conditions have no subsequent effects on some behavioural and physiological responses of Nguni and non-descript beef steers reared extensively on natural pastures.

Specific hypothesis states that:

1. On-farm successive handling has no effect on some behavioural and physiological responses of Nguni and non-descript beef steers reared extensively on natural pastures
2. Some weather and periodical variations have no effects on time budgets and body weights of Nguni and non-descript steers reared extensively on natural pastures

3. Pre-slaughter on and off-loading, lairage duration and stunning have no effects on bleed-out times, response-behaviour and some blood physiological responses of Nguni and non-descript beef steers
4. Pre-slaughter activities have no subsequent effects on meat characteristics and beef quality of Nguni and non-descript beef steers reared on natural pastures

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Chapter 2
Literature review

(*Submitted to Asian Australasian Journal of Animal Science*)

2.1 Introduction

According to Boissy and Erhard (2014), animal welfare is a result of how animals identify with their surroundings and previous experiences. Different environments in the meat production chain, the factors around them and their effects on animal welfare and beef production have been reported in recent research outputs and reviews. Specifically, Waiblinger *et al*. (2006) looked at the interaction between humans and animals at the farm, concluding that it is a significant factor to consider in improving the welfare of farmed animals and the stockpersons. Good stockmanship has been reported to improve productivity (Grandin, 2003), and this kind of treatment should begin at early stages of life (Probst *et al*., 2013; 2014).

However, Ndou *et al*. (2011) highlighted that the welfare of the animal and its further effects on product quality are highly considered in the developed world than the developing ones. They further hinted on the on-farm multipurpose cattle production systems and their consequences on the quality of beef. Work has been done on animal transportation conditions and how they affect the welfare of the animal and the quality of its meat (Ferguson and Warner, 2008; Miranda-de la Lama *et al*., 2014), while Grandin (2014) covered different livestock welfare issues at the farm and abattoirs, further relating them to consumer concerns. Moreover, Vimiso *et al*. (2012) reported that the African perspective on meat production and quality, particularly of the rural consumers have received little attention from the research areas.

All these issues in the meat production system have been separately covered without encompassing the whole production chain. However, the series of processes involved in meat production begins at the farm where animals are born, reared, fattened, transported to the slaughter-house, slaughtered and then converted to meat to be distributed to consumers through retailers. Miranda-de la Lama *et al*. (2014) described this phenomenon as the meat supply chain. Studies that have been independently conducted on each of these events; predominantly the pre-slaughter occasions, which proved to induce stress to slaughter animals (Grandin, 1997; de Passille and Rushen, 2005; Terlouw, 2005; Mota-Rojas *et al*., 2006; Muchenje *et al*., 2009ab; Chulayo *et al.*, 2012).

Some parts of the developed world have done a lot to develop measures to improve the animals' livelihood, together with ensuring good quality animal products for the consumers. However, some parts of the developing world such as Africa have been dragging behind due to numerous factors which include traditional customs and beliefs practised by different ethnic groups (Ndou *et al*., 2011). To ensure maximum product quality, this knowledge should be of importance to everyone in the production chain; from the stockman, farmer, transporter, abattoir worker, and the designers of animal facilities (Broom and Fraser, 2007). Therefore; knowledge of animal stress inducers, animal response-behaviour and its subsequent effects on meat product quality is of importance to ensure an efficient and economic enterprise.

2.2 What happens when an animal is exposed to change?

In trying to cope with a given situation, animals exhibit behavioural changes (Table 2.1) which can be used as apparent animal welfare indicators (Broom, 2000; Broom and Fraser, 2007) and can further reflect on biochemical changes (Figure 2.1). Environmental unsettle activates the hypothalamic-pituitary-adrenal activity due to fear (Ferguson and Warner,

Table 2. 1: Some qualitative and quantitative descriptors to consider in examining animal response behaviour

Qualitative behaviour		Quantitative behaviour
Positive	**Negative**	
Active, Relaxed, Calm, Content, Friendly, Playful, Positively occupied, Lively, Inquisitive, Happy, comfortable, Placid, Settled, Un-phased	Fearful, Agitated, Indifferent, Frustrated, Bored, Irritable, Uneasy, Apathetic, Distress, Nervous, Scared, Avoiding, Panicked, restless	standing immobile, approaching person, in contact with person, sniffing person, vocalising, sniffing the environment and moving away from person; flight zone, exit speed, stepping and/or kicking

Modified from: Wemesfelder *et al*. (2001); Waiblinger *et al*. (2003); Minero *et al*. (2009); Mounier (2011); Bourguet *et al.* (2011); Dodzi and Muchenje (2011); Stockman *et al.* (2012).

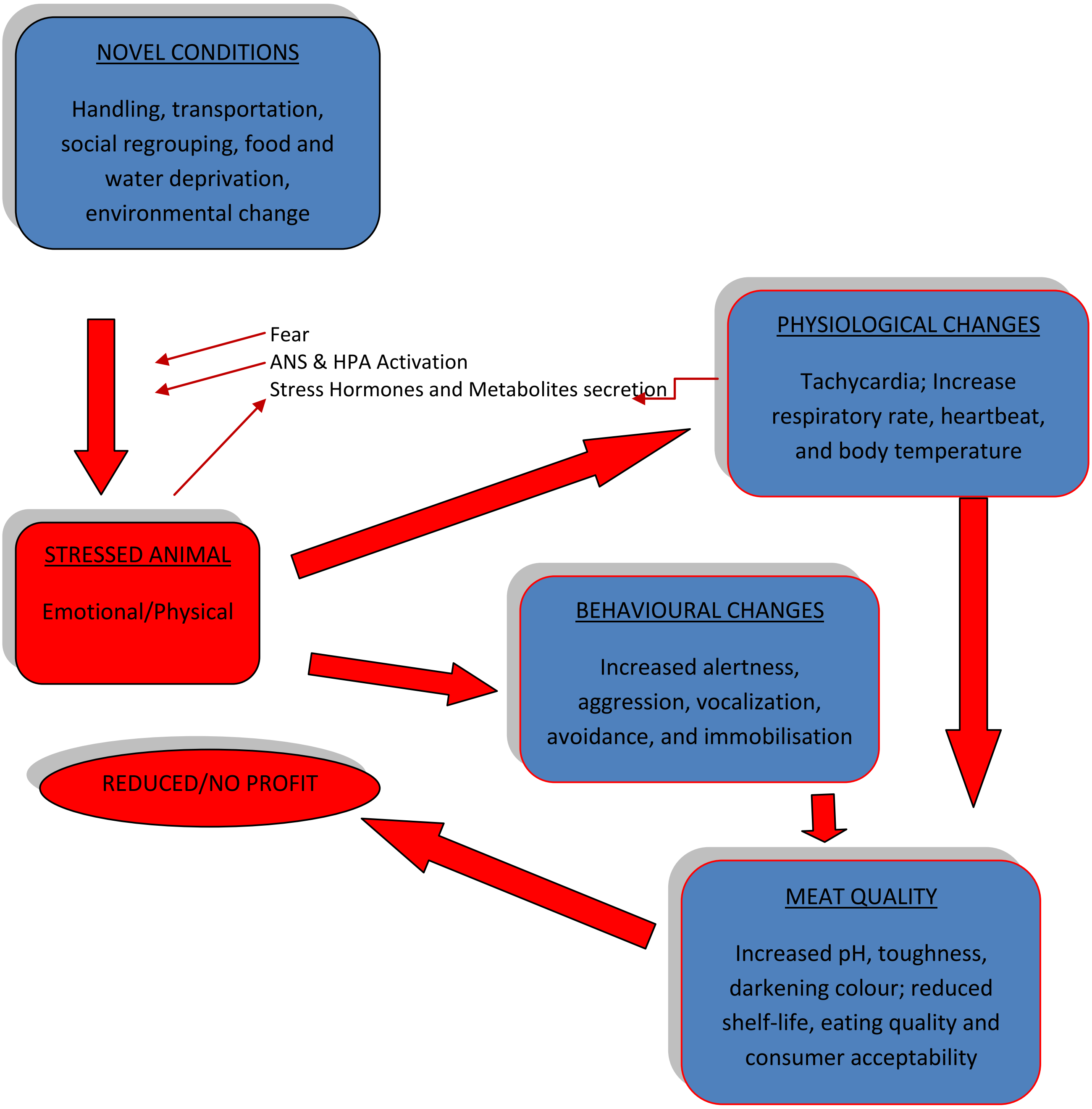

Figure 2. 1: A summary diagram showing the effects of the prior and slaughter process on the animals and the quality of meat, as described in literature. (ANS-Autonomic nervous system; HPA-Hypothalamic pituitary adrenal)

2008), thus leading to some biochemical changes (Hemsworth *et al*., 2011). Furthermore, Bourguet *et al.* (2011) observed that exercise and psychological stress just before slaughter increases muscle metabolic activity, which may continue after death, resulting in faster post-mortem pH decline and thus decreased meat quality (Grandin, 1997).

Moreover, animal-human interaction relationships have been reported to be an influential factor and a determinant of how animals will recognize pre-slaughter stress. Boissy (1998) reported that animals usually perceive human-interaction as a predatory encounter, to such an extent that they would panic and try to avoid human beings out of fear. Fear of humans by cattle can thus compromise animal welfare prior to slaughter, resulting in reduced productivity of quality meat (Raussi, 2003). However, good stockmanship at the farm is said to improve this problem such that animals are familiar with handling even prior to slaughter. In contrast, removal of animals from one environment to another for the purpose of slaughter has a potential to alter this perception.

Miranda-de la Lama *et al*. (2014) singled out transportation to be the link between all the activities that take place before slaughter. It has been found to greatly contribute to pre-slaughter stress (Tarrant, 1990; Tarrant *et al*., 1992; Mota-Rojas *et al*., 2006; Minka and Ayo, 2013; Miranda-de la Lama *et al*., 2014). Additionally, the effects of speed at which the transportation vehicle travels, the design of the vehicle (size, space, surface) and the stocking density during transportation were observed to contribute greatly to pre-slaughter stress (Tarrant *et al*., 1992). Broom and Fraser (2007) reported that lack of control in the animals' environment may result in strange behaviours in trying to adjust to physiological changes.

In addition, the abattoir environment offers the animals a new and different experience to that of the farm, with more confined structures and interactions thus behavioural and physiological adjustments required to get comfortable. Researchers have evaluated lairage conditions, slaughter processes-with or without stunning, bleed-out times and positions, duration between stunning and exsanguination together with their effects on animal welfare and meat quality (Grandin, 1998; 2001; Gregory, 2005; 2008; Gregory *et al*., 2010; 2012; Ndou *et al*., 2011; Agbeniga and Webb, 2012; Njisane and Muchenje, 2013ab; Farouk, 2013; 2014; 2015). Nevertheless, animal age, gender, breed, and species also play a role in the way animals individually respond to stress thus production (Grandin, 1980; McGreevy, 2003; Raussi, 2003; Dodzi and Muchenje, 2011; Muchenje *et al*., 2009).

2.3 The impact of the animals' background and characteristics on welfare thus production

Across species, the main contributing factor in selecting meat producing animals has always been the ability of the animal to produce a profitable quantity of quality meat in a given time. However, through continuous research, there has been a reported significant link between the animal's perception and response (behavioural and physiological) towards challenging situations it is exposed to during the production cycle and the quality of the product it produces. In addition, temperamental cattle have been reported to be difficult to handle and are thus susceptible to handling stress, resulting in poor meat quality (Grandin, 1993; Voisinet *et al.*, 1997). An in-depth understanding reveals that the animals' intrinsic factors such as type of species, breed/genotype, age and gender contribute greatly to this perception and response (Hansen *et al.*, 2001; Dodzi and Muchenje, 2011; Njisane and Muchenje, 2013ab) thus meat quality (Grandin, 1980; McGreevy, 2003; Raussi, 2003; Muchenje *et al*., 2009).

Furthermore, older animals have been reported to respond better (calm) than younger ones (Lambe *et al.*, 2001; Vierin and Bouisson, 2003; Dodd *et al.*, 2012; Njisane and Muchenje, 2013ab). This may be due to their elongated exposure and experience, later developing some sense of tolerance. Studies on sheep reported that castrates were more calm and less fearful compared to ewes (Strappini *et al.*, 2010; Dodd *et al.*, 2012; Njisane and Muchenje, 2013a). However, Dweyer (2009) reported that male animals show more aggression, while the female species only engage in such when competing for limited resources. Grandin (1993) also reported that animals that went through market-handling settle better in stressful environments like the abattoir compared to those sourced directly from the farm.

2.4 On-farm exposure conditions and activities

Cattle farming has greatly contributed towards the success of the meat industry. Beef producing cattle are normally reared extensively during their early stages of life and then sometimes transferred to intensive systems during the fattening and finishing stages (Probst *et al*., 2013). These extensive conditions are characterised by free ranging and uninterrupted time budgeting, which can be classified as “normal behaviour” of cattle. Time-budgeting is an act of performing various activities like satiating hunger or thirst (Breed and Moore, 2016) animal-animal interactions for survival for survival and pleasure purposes. This concept has been greatly established in the dairy production system, relating it to milk production. However, the meat producing sector has given this very little attention even though it has a potential to determine and produce solutions to some of the challenges experienced, like in the dairy.

On-farm human-animal interactions, including time to time managerial activities and/or routines have, however been ventured. Good stockmanship has been reported to improve productivity through reduction of fear and promotion of ease handling (Grandin, 2003; Hemsworth *et al.*, 2011). In addition, it has been recommended that farm animals be subjected to human contact from an early age in order to accustom them to human company for better handling at a later stage (Markowitz *et al.*, 1998; Probst *et al.*, 2012; 2013).

However, this may be a challenge to achieve in some countries, considering the large and extensive herd production of beef cattle in order to maximize production and supply the meat industry with enough quantities to meet consumer demands and generate profit. This concept accommodates mostly dairy farmers whose production is already day to day, the small-scale stall farming systems and small units for research purposes.

Higher cortisol levels during restraint are likely to be found in the extensively raised and less accustomed to handling beef cattle compared to dairy cows (Lay *et al.*, 1992). General human-animal interactions seen in beef farming include occasional managerial routines such as weighing, castration, dipping, branding, and vaccination, among others. Raussi (2003) and Probst *et al.* (2013) reported these events to be unpleasant for animals, such that fear towards humans may be developed. Animals often recognise contact with human beings as destructive (Boissy, 1998). In order to deal with situations, animals may respond and even develop certain behaviours or strategies in trying to cope (Boissy *et al.*, 2001). Furthermore, cattle and sheep can remember an aversive experience for many months after it occurs (Hutson, 1985; Pascoe, 1986). Therefore, the "pre-slaughter" preparation of cattle then remains an un-resolved problem.

2.5 Pre-slaughter events and their impact on animal welfare and productivity

Pre-slaughter events begin the moment animals are led and loaded on a truck/vehicle at the farm to be sent for slaughter. Ferguson and Warner (2008) described pre-slaughter as the conditions and practices during which animals are moved at the farm through to the knocking box at the abattoir. At this stage animals are exposed to extra physical activity, way more than normal animal-human interaction and multiple unfamiliar persons. Grandin (1997) and Probst *et al*. (2013) reported that situations unfamiliar to the animals such as transport, pre-slaughter treatment and handling at the abattoir, can be extremely stressful to beef cattle. In addition, the abrupt change in their social or physical settings exacerbates trauma (Hemsworth and Coleman, 1998; Terlouw, 2005; Ferguson and Warner, 2008).

2.5.1 Transportation

Miranda-de la Lama *et al*. (2014) described transportation as the key component joining the events that take place in the pre-slaughter logistics chain. This process is largely an exceptionally stressful event in the animals' life (Warriss, 2000; Ferguson and Warner, 2008). It often involves novel and tense exposures such as crowding, noisy vehicles with food and water or space (to rest) deprivation, pre-transport management, vibrations, , social regrouping, , restraint, loading and unloading, transportation duration and climatic factors (Schaefer *et al.*, 1997; Mitchell and Kettlewell, 1998; Swanson and Morrow-Tesch, 2001; Kadim *et al.*, 2006). However, the extent of animal welfare alteration and what can be measured as positive or negative behaviour, if there is any, during this stage has not been clearly defined.

Tarrant *et al.* (1992) reported that inability to move and face the preferred direction during transportation caused cattle to lose balance and even fall. However, maintaining balance in a moving vehicle, which is a new experience, while standing and sometimes with little space to move may be hard to achieve. It was also reported that long transportation hours in poor condition transportation vehicles may be unfavourable to animal welfare (Tarrant *et al*., 1992); while it has also been pointed out that prolonged experience of the same stressor results in familiarity (Knowles, 1999; Honkovaara *et al.*, 2003; Knights and Smith, 2007; Leme *et al.*, 2012). However, Mota-Rojas *et al*. (2006) reported that transportation to the abattoir should not take more than 16 hours.

Pre-slaughter stress during transportation has also been reported to influence the immune responses of cattle (Hulbert *et al.*, 2011) through increasing susceptibility to respiratory diseases in cattle (Sporer *et al.*, 2007; Duff and Galyean, 2007), which may result to release of stress hormones (Odore *et al.*, 2004). Moreover, transportation at high stocking density (above 550 kg/m^2) was reported to elevate plasma cortisol (Tarrant *et al.*, 1988; Tarrant *et al.*, 1992; Kadim *et al.*, 2009). However; validation of these points through blood or urine extraction for hormonal analysis is questionable. The stress hormones pick in these samples may be due to handling or the novel environment (vehicle of arrival at abattoir) during the sampling. Nevertheless, ensuring good transportation does not only ensure good animal welfare and meat quality, it is also of economic importance (Whiting, 2000).

2.5.2 Abattoir conditions and impacts

Miranda-de la Lama *et al.* (2010) described the abattoir design as normally based on conventional architectural criteria, such as space optimization or how to facilitate human movement, and not on the behavioural characteristics of the animals. As opposed to the green grass at the farm, abattoirs are mainly concrete and “unnatural”. The animal perception and response to the adverse conditions at the abattoir depends on its background and previous experience (Grandin, 1993; Terlouw, 2005). However, animal response behaviour can also be influenced by unfamiliar environments such as concreted abattoir, many abattoir workers (Terlouw and Porcher, 2005) and other animals from different farms and of other species (Hemsworth and Coleman, 1998; Ferguson and Warner, 2008). For instance, cattle may struggle to adapt with the loud squealing sounds made by pigs during lairaging. Moreover, McGreevy (2003) stated that the loud noise at the abattoir affects the animals’ response, as opposed to the quiet environment at the farm.

Grandin (2006) also reported that cattle perceive the abattoir environment in the same way as at the farm during procedures like vaccination and other managerial processes that involve moving animals through the race. However, there is need to clearly classify to what extant can this be expressed. Vimiso *et al*. (2012) reported that animal handling differs between the farm and the abattoir and Grandin (1999) reported that animal respond differently to varying handling techniques and systems. In addition to that, animals would have been exposed to extensive stressors pre-slaughter compared to just farm managerial procedures. The presence of physical distractions (e.g. shiny objects, dangling chains), humans, and change of either dark or light in the race frightens the animal, resulting in anxiety (Grandin, 2006; Bourguet *et al.*, 2011). However, it was indicated that the animals are not aware that they will die at the abattoir (Grandin, 2006). Grandin (1998; 2001) also reported that use of electric pokes, skidding in the stunning box, and missed stuns, sharp edges on equipment or excessive pressure from a restraint device encourages vocalization in beef cattle at the abattoir.

2.6 The impact on the quality of meat

Handling stress prior to slaughter does not only affects the welfare of the animals, but also to a greater extent has an impact on the quality of meat produced from that animal (Ferguson and Warner, 2008; Muchenje *et al.*, 2008; 2009ab; Hemsworth *et al.*, 2011; Cetin *et al.*, 2012). Lawrie (1966) recounted that the quality of meat is highly affected by behavioural and physiological response of the animal before slaughter. Pre-slaughter handling affects meat quality traits, such as colour, pH, as well as texture (Lahucky *et al.*, 1998; Muchenje *et al.*, 2008; 2009ab). Stress-related behavioural and physiological changes have been reported to reduce the quality of meat (Warriss, 1992; Young *et al.*, 2009) through glycogen depletion and elevated ultimate pH (Wood *et al.*, 1998; O'Neill *et al.*, 2006; Muchenje *et al.*, 2009).

Rapid depletion of muscle glycogen during handling, transportation, pre-and post-slaughter results to low lactic acid production, thus DFD meat produced (FAO, 2001). Glucose in the blood and glycogen in the muscle promotes glycolysis, and thus the formation of lactic acid (Choe *et al.*, 2009) thus tougher meat with higher cooking losses (Warner *et al.*, 2007; Gruber *et al.*, 2010). Furthermore, Gajana *et al.* (2013) reported that extended transportation period and higher stocking density affected pH_u and thus reduced meat quality. Warner *et al.* (1998) related dark cutting in beef with the time spent in lairage pre-slaughter. While the animal's genetic background and its exposure prior to slaughter determines its behavioural and physiological responses when encountered with stressful situations, this also has an implication on muscle metabolism (Terlouw, 2005).

2.7 Consumer concerns, laws and regulations governing meat production in the developing world

The developing world such as Africa shows a lot of potential in meat production and export exchange, particularly for beef, due to its ability and resources to accommodate and nurture both indigenous and exotic cattle breeds. Scholtz *et al*. (2011) reported that the climatic and agricultural conditions in this part of the world allow for many areas of compatible interest and opportunities, regarding beef cattle production. Bello *et al*. (2013) reported that there has been an information gap between the developing and the developed world. Even so, it is also important to realise the geographical, climatic and systematic differences of the two worlds. Therefore there is need to intensify research in this regard and come up with findings that are suitable and complement the developing world conditions.

Despite the laws and regulations that govern food animals (Animal Protection Act, 1962 and 1935), meat production (The Meat Act, 2000) and consumption, Ndou *et al*. (2011) described the developing world as giving low priority to the welfare of animals due to factors such as traditional customs, limited knowledge in handling of animal and sub-standard handling facilities. This may then make it hard for these countries to compete with the rest of the world due to high prevalence of food insecurity and poverty (Ndou *et al*., 2011), thus intensifying socio-economic challenges and constraints (Scholtz *et al*., 2011). Furthermore; the elevated concerns from consumers on how the animal was treated before it was slaughtered as well as how it was processed (hygiene) affect the way they perceive meat.

Bello *et al*. (2013) discovered that some abattoirs in Nigeria neglect the practice of regular *ante-mortem* and *post-mortem* inspection of slaughter animals, conventional sanitation practices in operation and post-operation cycle thus putting public health in jeopardy due to unsafe meat production. It was reported that these shortcomings threatened achievement of sustainable food safety. Furthermore, Font-I-Furnols *et al*. (2011) reported that meat consumers were more concerned of the product's place of origin than its price or the feed the animal took; and they were more comfortable with locally produced meat. However a study in South Africa revealed that rural consumers were more concerned about the price of the product than any other factor (Vimiso *et al*., 2012). The current status of the developing world regarding animal welfare awareness and meat quality concerns puts it on the edge relating to import and export participation with the rest of the world through the meat industry in improving the economy. Ferguson *et al*. (2014) concluded in a review that the industry should pay attention and even respond to the consumer and societal demands for more sustainable and ethical animal farming systems and practices.

2.8 Some possible measures to adjust in order to improve animal welfare and meat quality production

Through some trials done to investigate the pre and post slaughter exhibition, conclusions and recommendations have been reported in trying to minimise pre-slaughter stress and thus improve meat quality. Some recommendations that have been drafted are described in Table 2.2.

2.8.1 Knowledge empowerment and enforcement

Through further research on the gaps identified in this paper, more knowledge generated can be of great addition. Furthermore, ease of information transformation from researchers to

Table 2. 2: Some proposed methods to improve the welfare of slaughter animals

Recommendation	Description	References
Supplementation with: **1. Magnesium** **2. Tryptophan** **3. Electrolytes**	1. Stress reduction and improves meat quality 2. Minimize stress 3. Increases carcass yield	Ferguson and Warner (2008)
Nutrition modulation and electrolyte therapy	Reduction of stress during transportation and handling thus improving meat quality	Schaefer *et al*. (1997)
Use of proper facilities and handling techniques	Allows good management and improved welfare and production	Ferguson and Warner (2008); Grandin (2003); Petherick (2005)
Stockmanship improvement	A good relationship between farm animals and humans reduces animals fear and allows ease during handling	Grandin (2003); Waiblinger *et al*. (2003); Ndou *et al*. (2011); Hemsworth *et al*. (2011)
Practise early life animal-human interactions	Encourages good relationship even at the later stage thus good production	Probst *et al*. (2012; 2013)
Selection for temperament	Use of less aggressive breeds thus improved handling and meat quality	Ferguson and Warner (2008)

farmers in an understandable and simple manner still requires establishing. Methods like regular workshops, magazine articles, blogging and social networks may assist. Sequentially, this knowledge may be easily imparted to the stockman and everyone else involved in animal handling in both farms and abattoirs. To ensure maximum product quality and economic returns, everyone in the production chain (the stockman, farmer, transporter, abattoir worker, the designers of animal facilities and consumers) must be well-informed of animal welfare and its subsequent effects on meat product quality (Broom and Fraser, 2007).

2.8.2 Merging farms with abattoirs

In addition, to eliminate the pre-slaughter transportation stress, bringing the fattening/feedlot facilities closer to the slaughter house (Figure 2.2) may improve the situation. Animals can be transported to these facilities at least 2-3 months prior the slaughter date such that herding them by foot to the abattoir would be possible. The abattoirs may adopt this either individually or co-joined with their regular suppliers. Though literature has reported that cattle prefer extensive conditions over confinement (Krohn *et al*., 1992; Legrand *et al*., 2009; Lee *et al*., 2013), this has a potential to improve animal adaptation to the abattoir workers, surrounding conditions, as well as increasing profit. A similar scenario can be observed in pasture-based dairy farms, where animals are hoofed for 1-3 km to the milking parlour once or twice a day for milking. Some abattoirs in South Africa already have farms where they fatten their constant supply of beef cattle. However, just like privately owned farms, they are situated remotely from the abattoir and thus require the use of transportation vehicles to move animals before slaughter. Though feedloting was reported to be unfriendly towards animal welfare due to confinement (Lee *et al*., 2013), Vimiso and Muchenje (2013) reported that animals that were hoofed to the abattoir had lower bruising scores compared to those that were transported either directly from the farm or through auctions.

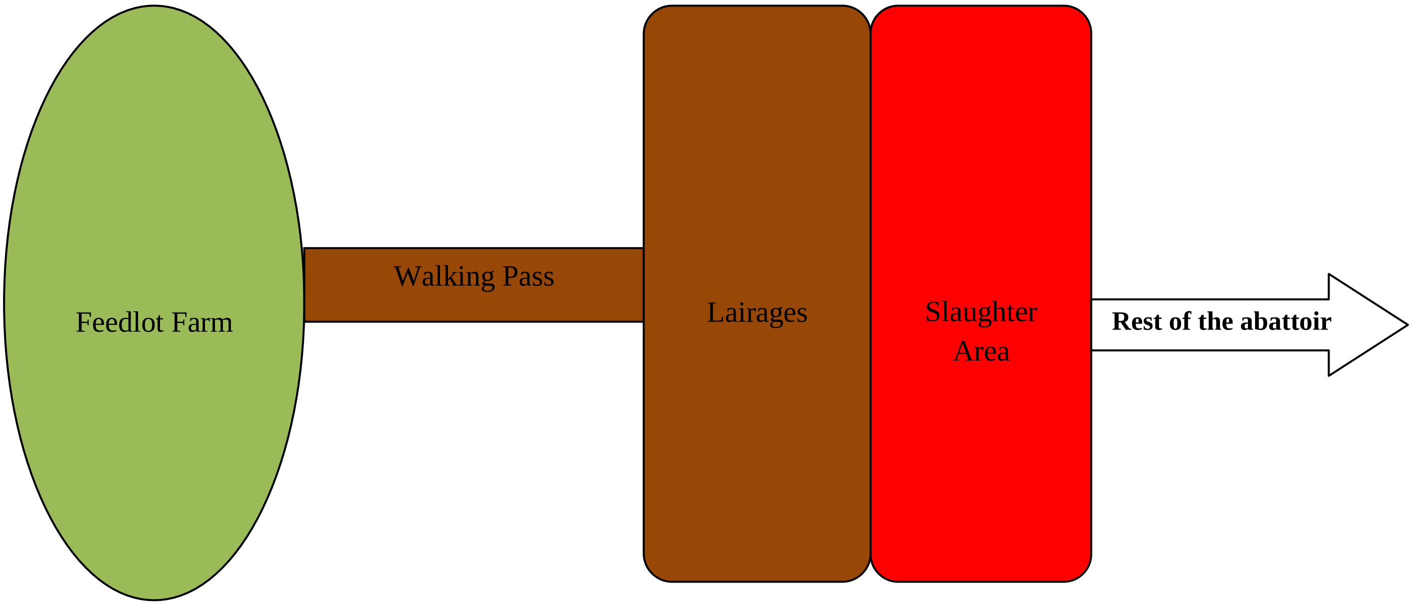

Figure 2. 2: A proposed model for bringing the feedlot farms closer to the abattoirs in trying to minimize transportation stress

2.8.3 Technology advancement in abattoirs

Livestock of all species often react and refuse to move in handling facility due to stress and anxiety if they spot a distraction or separated from their groups (Grandin, 1993; 2006; Bourguet *et al.*, 2011). However as opposed to using electric probes to motivate animal movement, upgrading and use of automated machinery to convey animals might be helpful. Some commercial abattoirs use conveyer restrainer for pigs and sheep (Njisane and Muchenje, 2013b), which reduce strain on both animals and handlers during heading. Furthermore, Gregory (2008) reported that for effective and animal welfare friendly results, it is important to understand the causes of variation in captive bolt gun performance, the efficiency of poll instead vs frontal shooting and the prevalence of false aneurysms in carotid arteries during shechita and halal slaughter.

In addition, post slaughter electrical stimulation is the major method that has been derived to assist the process of converting muscle to meat and trying to improve the major eating qualities, like tenderness. Electrical stimulation has been used post-slaughter to improve meat colour and tenderness (Biswas *et al.*, 2007; Strydom and Frylinck, 2014). Cetin *et al.* (2012) reported that electrical stimulation increases meat quality through pH decrease and tenderness, colour and sensorial improvement. Kadim *et al.* (2009) suggested electrical stimulation to decrease pH and shear force, to produce longer sarcomeres, and to increase juiciness, muscle fiber intensity and lightness. However, minimal electrical stimulation was found to be effective in low stressed and well fed cattle (Strydom and Frylinck, 2014).

2.9 Conclusions

The welfare of the animal and the quality of meat produced by farmed animals are dependent on all the chain activities to which they are subjected to from birth till slaughter. However, there is lack of clear definition in some areas. There is still need to further investigate this area, interlinking the independent discoveries and information that has been found through studies on some of the contributing factors. Furthermore, the developing world needs to fully investigate, adopt and commit to some of the world's standard on animal management and meat production thus improving its food security and economy through the use of its maximum potential. It is also important to note that what works for the developed world might not be working in the developing world, hence the need to intensively investigate the matter.

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Chapter 3
Some weather and periodical variations in time budgets and body weights of Nguni and non-descript steers reared extensively on natural pastures

(*Under review: International Journal of Biometeorology*)

Abstract

Time budgets of Nguni (NG) and Non-descript (ND) steers were determined by analysing the time spent grazing, drinking, browsing, lying down, standing, walking and social-interactions among the steers using the focal-animal and instantaneous sampling methods. Four focal-steers per genotype were observed by four observers throughout the trial. Observations were done during the first 15 minutes/hour from 06:00am to 08:15pm fortnightly over a four months period (February-May). The observations were further split into morning, midday and sunset sessions. Hourly temperature and humidity were recorded using a portable data logger. There were no significant (P>0.05) genotype effects observed on time budgets. Observation weeks had an effect on time spent grazing (P<0.0001), drinking (P<0.0001) and standing (P=0.0218). Week 2 had the highest temperatures (32°C), lowest humidity (34%), thus the least time spent grazing (26.9±2.64%), with the highest proportions of drinking (2.7±0.45%) and standing (24.0±1.28%). Significant differences were observed on time spent browsing (P=0.0164), drinking (P=0.0012), standing (P=0.0262), lying (P=0.0030) and social-interactions (P=0.0418) among the observation times of the day. Browsing (0.7±0.67%) and social-interactions (0.7±0.46%) among the steers were prolonged in the morning, while lying down was prioritised both in the morning (30.0±4.51%) and sunset (34.9±5.37%). Time spent standing was extensive in the morning (19.8±3.96%) and at midday (20.8±4.63%), while drinking time peaked at midday (1.5±1.04%). Body weight correlated with social-interactions (r=-0.75) among the steers, temperature (r=0.65) and humidity (r=-0.67). Regardless of the genotype differences, the observed steers showed similar time budget patterns. In conclusion, the daily time budgets of steers in natural pastures changed with temperature, humidity, observation week and time of the day.

Keywords: Feeding behaviour, Focal-animal, Temperature and Humidity, Correlations, Extensive beef farming, Natural pastures

Introduction

Time budgets of beef cattle have received very little attention over the years compared to dairy cattle (Gomez and Cook, 2010; Dodzi and Muchenje, 2012) and other species such as sheep and goats (Jian-Bin *et al*., 2006; Bakare and Chimonyo, 2011; Pokorna *et al*., 2013). This knowledge limitation is especially affecting cattle reared under extensive production systems compared to intensive systems like housing. The quality and quantity of natural rangelands varies according to seasons. Thus there may be variations in grazing behaviour as well. The amount of time invested in grazing, to an extent, may contribute to the quality and quantity of the muscle produced. Animals undertake various tasks that are essential for survival, such as to satiate hunger or thirst, through time budgeting (Breed and Moore, 2016). Hence time budgets of cattle are important to measure the animals' well-being.

Different animal species in various production systems exhibit unique time budget patterns. However, they do not have control over what takes place in periods of handling and confinement for certain farm management routines such as milking (Gomez and Cook, 2010), health examinations and others. Provenza *et al*. (2003) reported that different farm management systems affect how animals feed. Baumont *et al*. (2000) stated that animals develop their own diverse behavioural patterns in pasture-based systems compared to indoors; they search and harvest their own food. However, Gomez and Cook (2010) described the free-stall system as allowing free movement, adequate rest and readily available water and feed. In addition, housed or confined cows were found to prioritise lying down (over 50%) compared to other activities (Jansen *et al*., 2005; Munksgaard *et al*., 2005; Grant, 2007; Krawczel and Grant, 2009), while cows kept in cultivated pastures generally spent more time grazing across seasons (Dodzi and Muchenje, 2012).

Due to divergent morphological build and physiological function of individual animals, even closely related animals can exhibit different feeding habits (Provenza *et al*., 2003). Arnold (1984) reported that horses grazed more at night and spent little time (1 hour) lying down, while cattle and sheep filled up in the morning and afternoon with more resting hours of 11.6 and 10.5 hours, respectively. In addition, Breed and More (2016) reported that even within a species, animals may vary in their responses to a particular set of circumstances. Dodzi and Muchenje (2012) reported that the Jersey breed were mostly grazing than lying down during the cool–dry season, compared to the Friesland and their crosses. Stressful climatic conditions, either day-to-day or seasonal, may result to altered activities thus failure to balance nutrient and energy requirements (Jian-Bin *et al*., 2006).

Changes in weather patterns experienced in different seasons, days and specific times of each day may have a significant influence on the behavioural habits of an animal. Dodzi and Muchenje (2012) found that cows spent more time in a standing position during the hot-wet season, while they grazed more in other seasons. Also, a study on goats found that 62.4% of the time was spent grazing compared to walking (7.8%), standing (10.6%) and sitting (19.2%) across season (Solanki, 2000). Furthermore, animal daily activities may vary with different times of the day. Bakare and Chimonyo (2011) reported that the three genotypes of goats observed browsed more in the morning and grazed more in the afternoon. Ruminants were reported to prioritise grazing during the day (Decruyenaere *et al*., 2000). In addition, Baumont *et al*. (2000) highlighted that two major grazing periods for pasture-based animals being sunrise and sunset.

Amongst the various cattle breeds and genotypes found in South Africa is the hardy indigenous Nguni breed (NG) which has been reported to perform better than other present breeds and genotypes even in poor quality feed (Ndlovu *et al*., 2009) and during handling at slaughter (Muchenje *et al*., 2009). Another common genotype is the non-descript (ND), containing multiple unidentified genes due to uncontrolled mating of cattle, mostly in communal areas (Bester *et al*., 2001). Scholtz *et al*. (2008) reported that this genotype comprise 35% of bulls found in the developing farming sector. Therefore the current study sought to quantify time investments of NG and ND steers to activities like grazing, drinking, browsing, lying down, standing, walking and any social-interactions amongst the steers while they run free on natural pastures over a period of time.

3.1 Materials and Methods

All procedures conducted for the purpose of this research were done meeting the accepted standards on ethical handling of animals. Consent to carry out the study was approved by the University of Fort Hare Ethical Clearance Committee (Reference Number: MUC03S1NJI01).

3.1.1 Study site and its weather patterns

The study was conducted at the University of Fort Hare's Honeydale Research Farm. It is situated 120 km inland from the coastline, in the False Thornveld of the Eastern Cape of South Africa. It is located at 32.78° S and 26.85° E, at an altitude of 520 m above sea level. The topography of the area is generally flat with few slopes. The mean annual temperature of the farm is 18.7°C. The area receives low annual rainfall of approximately 480 mm per annum both between and within seasons. The vegetation is a mixture of several trees, shrubs and grass species. Plant species, such as *Acacia karroo*, *Themeda triandra*, *Panicum maximum*, *Digitaria eriantha*, *Eragrostis sp.*, *Cynodon dactylon* and *Pennisetum clandestinum* are the predominant species. Temperature and humidity patterns (Figures 3.1

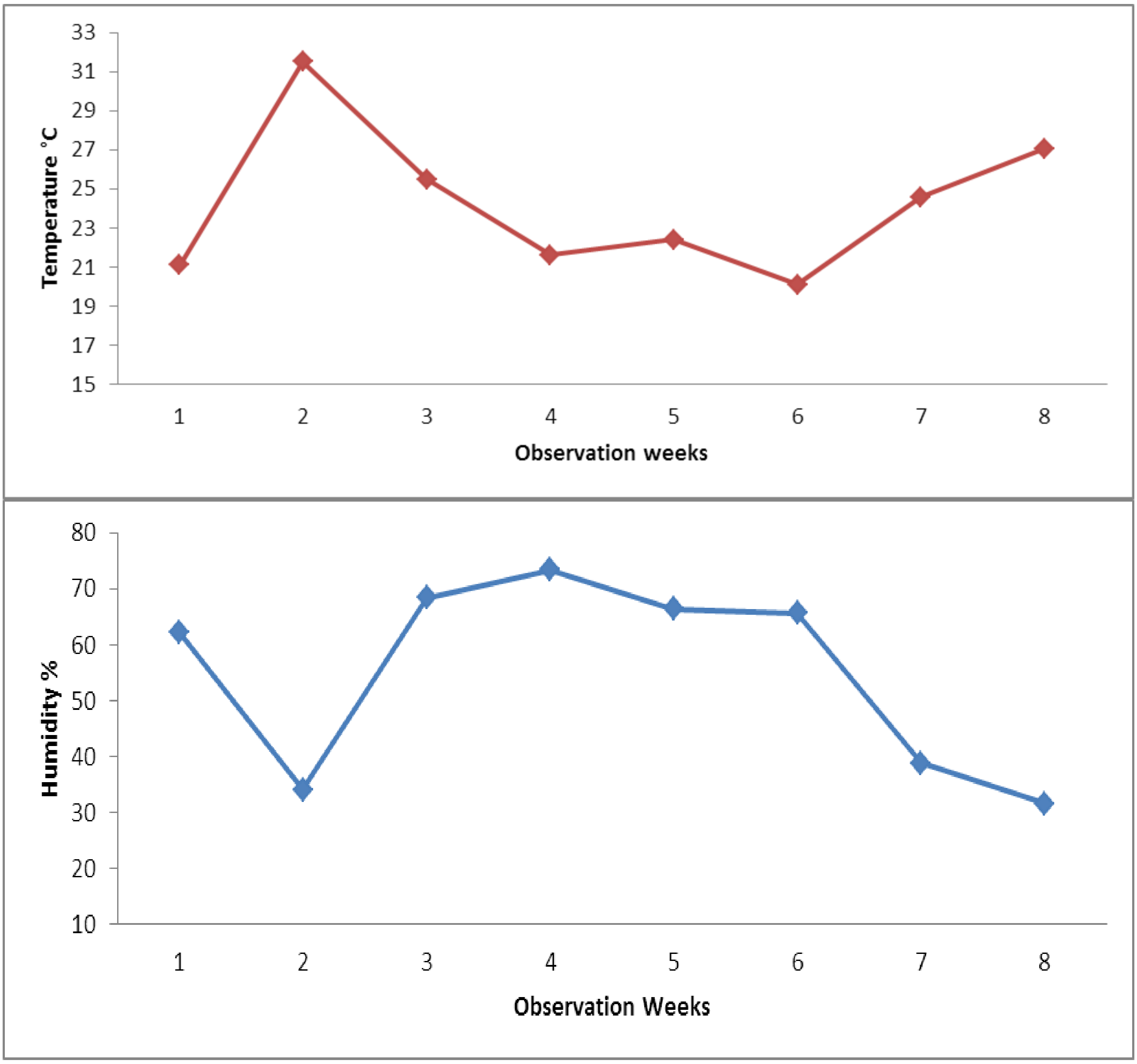

Figure 3. 1: Temperature (°C) and Humidity (%) patterns according to observation weeks

and 3.2) of the area were recorded using a portable data logger (MT669, Major Tech, South Africa) during the sampling and observation period.

3.1.2 Animals and observers descriptions

A group of 40 steers comprised of 20 Nguni (NG) and 20 Non-descript (ND) weaner steers were used in this study. Before the commencement of the trial, the ±12 month's old steers were vaccinated, drenched, dipped, ear-tagged and allowed 3 weeks to acclimatize. The group grazed together on natural pastures with access to water points throughout the trial. The initial weights of these steers ranged between 120-250 kg, with the ND steers generally weighing more than the NG steers. Eight focal-animals were randomly selected from the group, with four steers representing each of the two genotypes. Four observers monitored the same steers over a four month period; with each observer watching one steer of each genotype. All the observers received the same training on all aspects of the trial.

3.1.3 Observations

Direct observations were done to record time budgets of the two groups of steers using the focal-animal and the instantaneous sampling methods as described by Altmann (1974). Generally, the two methods took note of sequential constraints, percent of time, rates, duration, nearest neighbour relations, synchrony and subgroups of the specific steers. This set of response observations was recorded to represent the "free-conditions" that animals are subjected to in the paddocks at the farm. Observations were done fortnightly from February to May (eight observation sets). Data on grazing, drinking water, lying down, standing, walking and any social-interactions (grooming, head butting etc.) among the steers was observed for. The observations were done during the first 15 minutes of every hour, from 6:00 am to 8:15 pm.

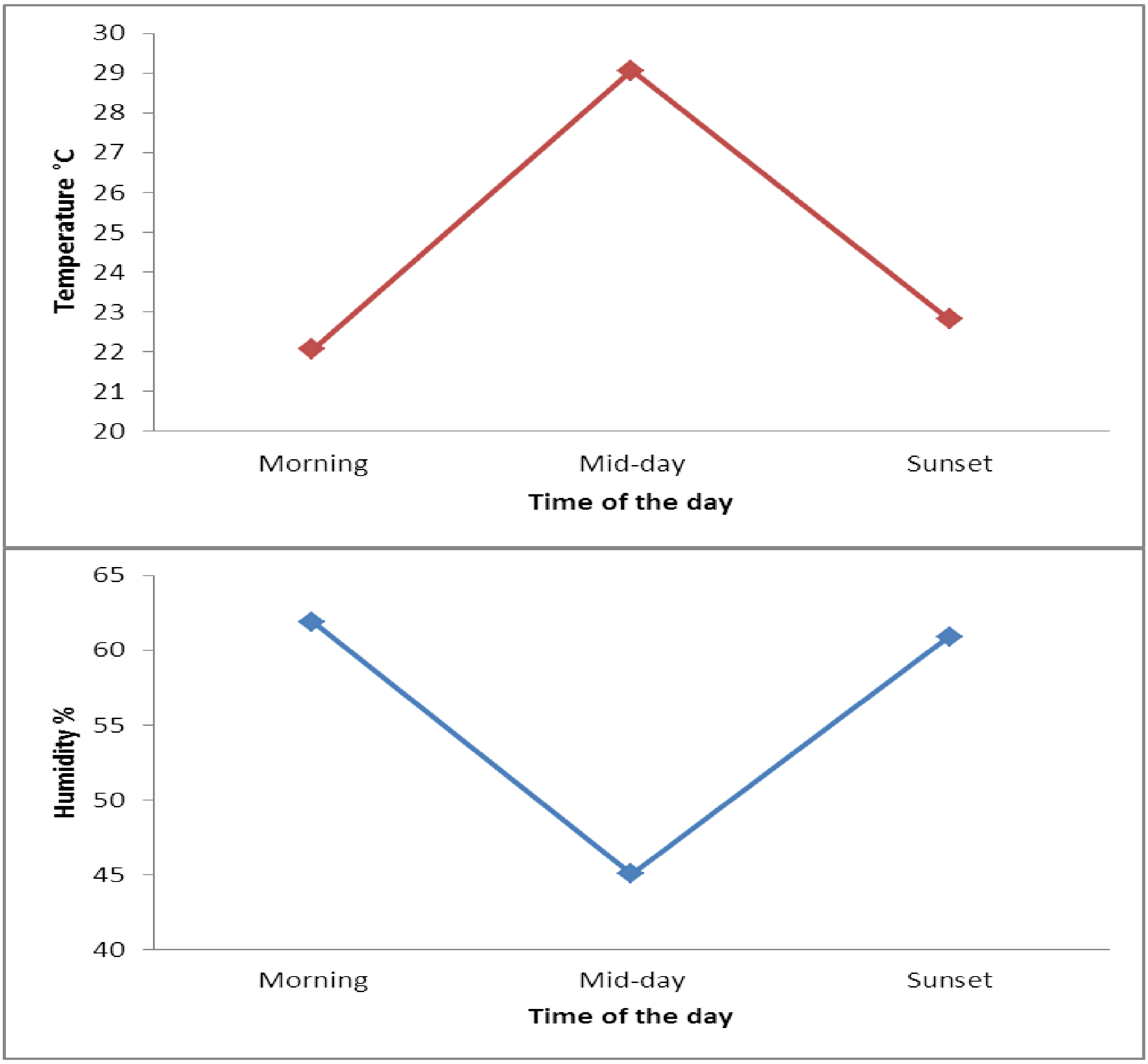

Figure 3. 2: Temperature (°C) and Humidity (%) patterns according to time of the day

The time was limited to these hours because the animals were on natural grazing paddocks, away from any source of light for visual observations during the night. In addition, attempt to use any form of light tampered with the steer's natural behaviour, they became inquisitive of the light. The total observation time for each observation week was 225 minutes (15min/hour × 15 hours/fortnight/observation week). The observation weeks were also split into time/sessions of the day; where morning was from 6:00 am to 10:15 am (total=75 minutes); midday from 11:00 am to 15:15 pm (total=75 minutes) and sunset from 16:00 pm to 20:15 pm (total=75 minutes). The time spent on each activity was expressed as a proportion of the total time spent on various activities observed (Dodzi and Muchenje, 2012).

3.1.4 Statistical analysis

The data was tested for normality using proc univariate. PROC GLM test (SAS, 2003) was used to test the effect of genotype, observation week, time/session of the day and their interactions (genotype and observation week; genotype and time/session of the day) on grazing, drinking water, lying down, standing, walking and social-interactions between the steers. Differences between means were evaluated using Tukey's test. The model used was: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + e_{ijkl}$ where, Y_{ijkl} is the response variable (grazing, drinking water, lying down, standing, walking and social-interactions between the steers); μ is the mean; α_i is the effect of observation week , β_j is the effect of time of the day; γ_k is the effect of genotype; $(\alpha\beta)_{ij}$ is the interaction between genotype and observation weeks; $(\alpha\gamma)_{ik}$ is the interaction between genotype and time of the day and e_{ijkl} is the standard error. Pearson's correlation coefficients between the variables were also determined (SAS, 2003).

3.2 Results

3.2.1 Time budgets according to genotype and observation weeks

The grazing patterns of the two genotypes were similar (P=0.4399) and there were no significant interactions (P=0.9735) between the observation weeks and the two genotypes on this activity. Figure 3.3 shows significant (P<0.0001) proportions of time that the steers spent grazing over the eight weeks of observations. Weeks 1, 4, 6, 7 and 8 showed the same grazing patterns and were also similar to week 5 which had the highest grazing proportion. The steers were on average observed to be grazing for 38% (ND) and 39% (NG) of the observation time. However, the lowest grazing proportion was observed on week 2; while from week 3 to week 6, a gradual and significant increase in grazing proportions was observed. Nevertheless, a drop was observed in week 7 before the rise in week 8.

Furthermore, the two genotypes showed similar patterns (P=0.8426) on time spent drinking and there were no significant interactions (P=0.9145) amongst the observation weeks and the two genotypes regarding this activity. Figure 3.4 shows significant variations (P<0.0001) on time spent drinking over the observation weeks. The highest proportion of time spent drinking was 3% and was recorded in week 2 for both genotypes. In week 3, there was no record of drinking for the ND steers, while this was observed for the NG steers in week 6. The drinking activity increased between weeks 4 and 5 before dropping in week 6. A proportion of 0.3% was recorded for the ND and 1% for the NG steers on week 7, with a drop of proportions observed in week 8.

Similar patterns (P=0.7973) of time spent standing were observed for the two genotypes. There were no significant interactions (P=0.8336) between the observation weeks and the two genotypes in response to standing behaviour. Figure 3.5 shows significant variations (P=0.0218) on time spent standing over the observation weeks. There was an increase in time

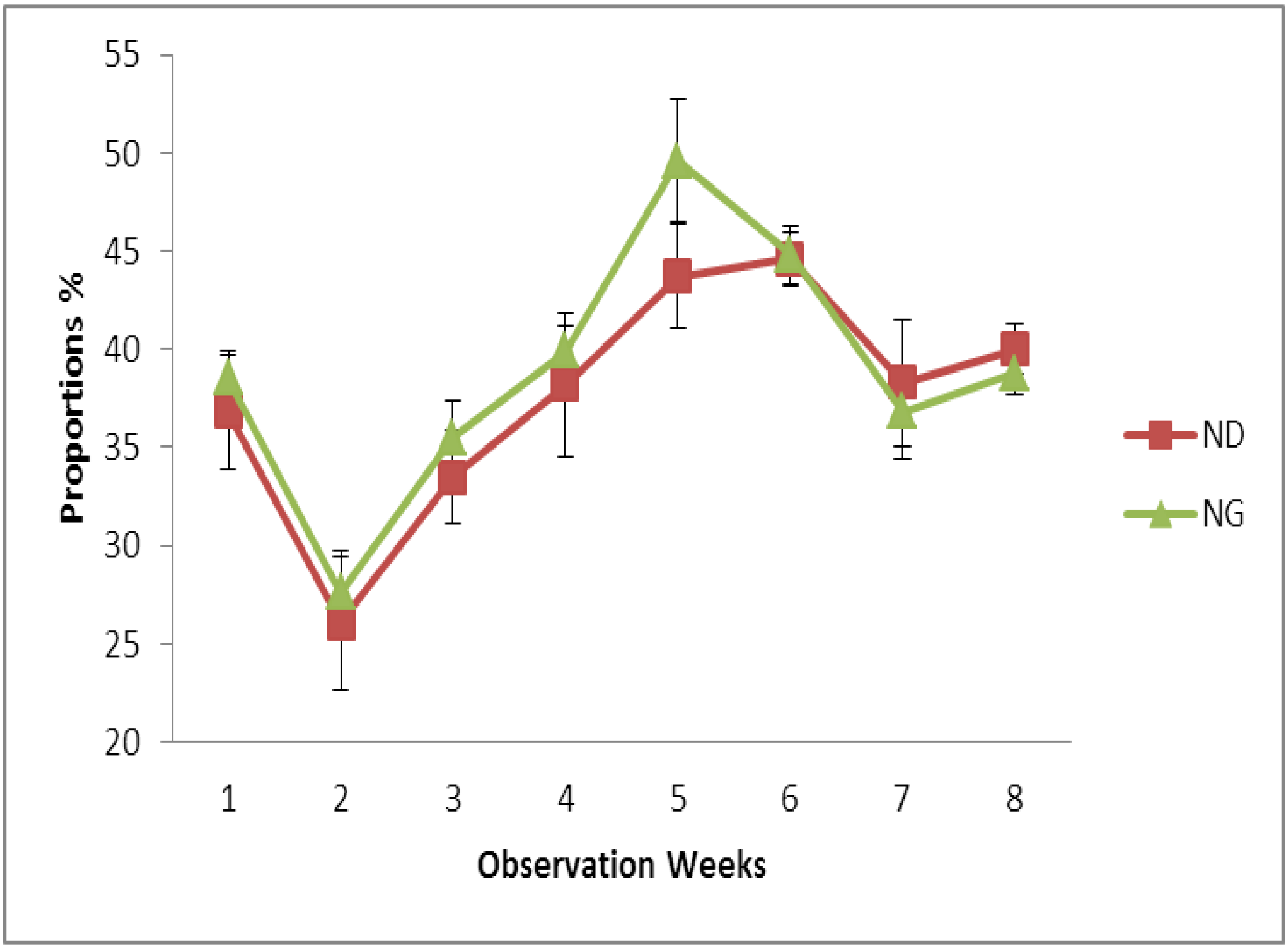

Figure 3. 3: The proportions of time spent grazing during different weeks by the two genotypes

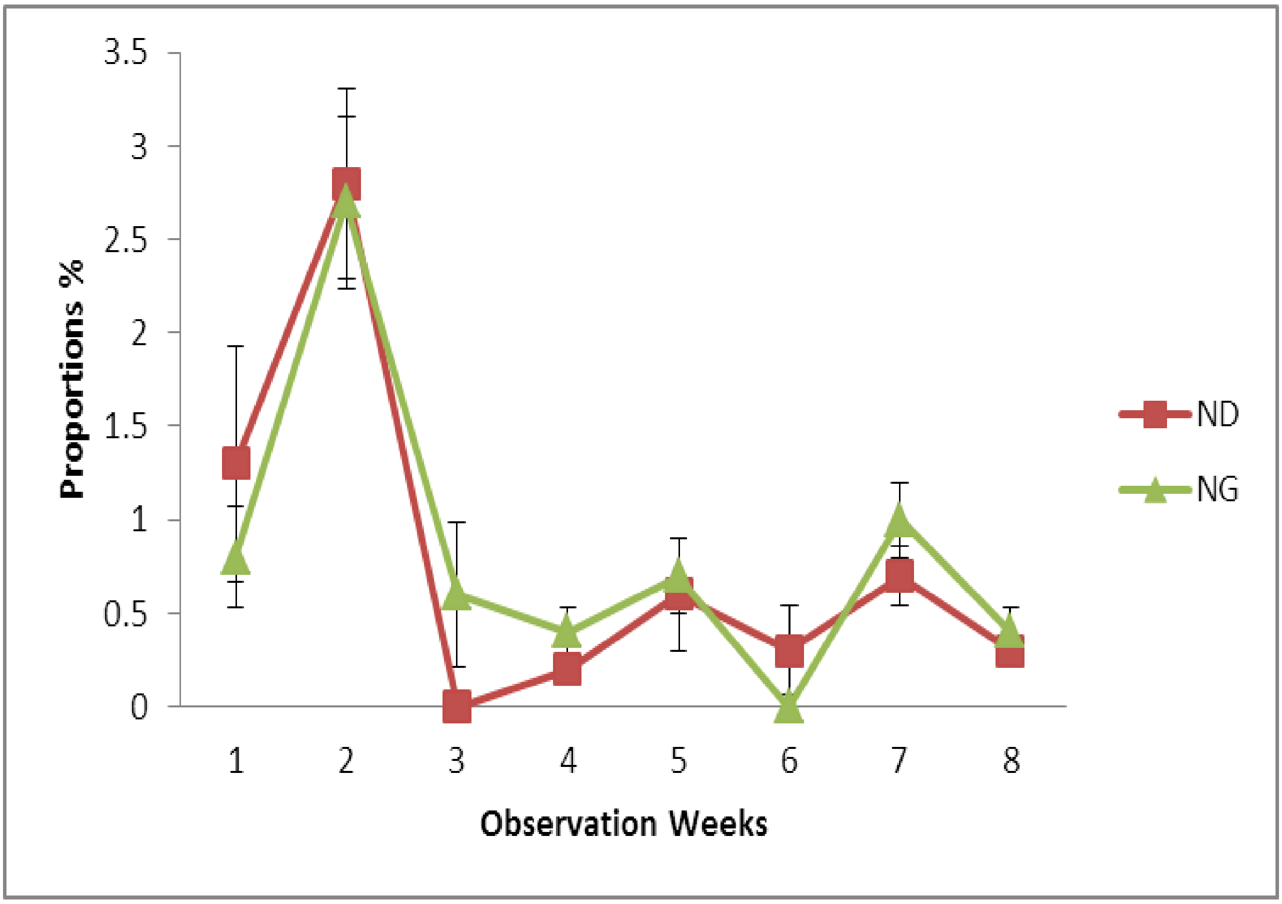

Figure 3. 4: The proportions of time spent drinking during different weeks by the two genotypes

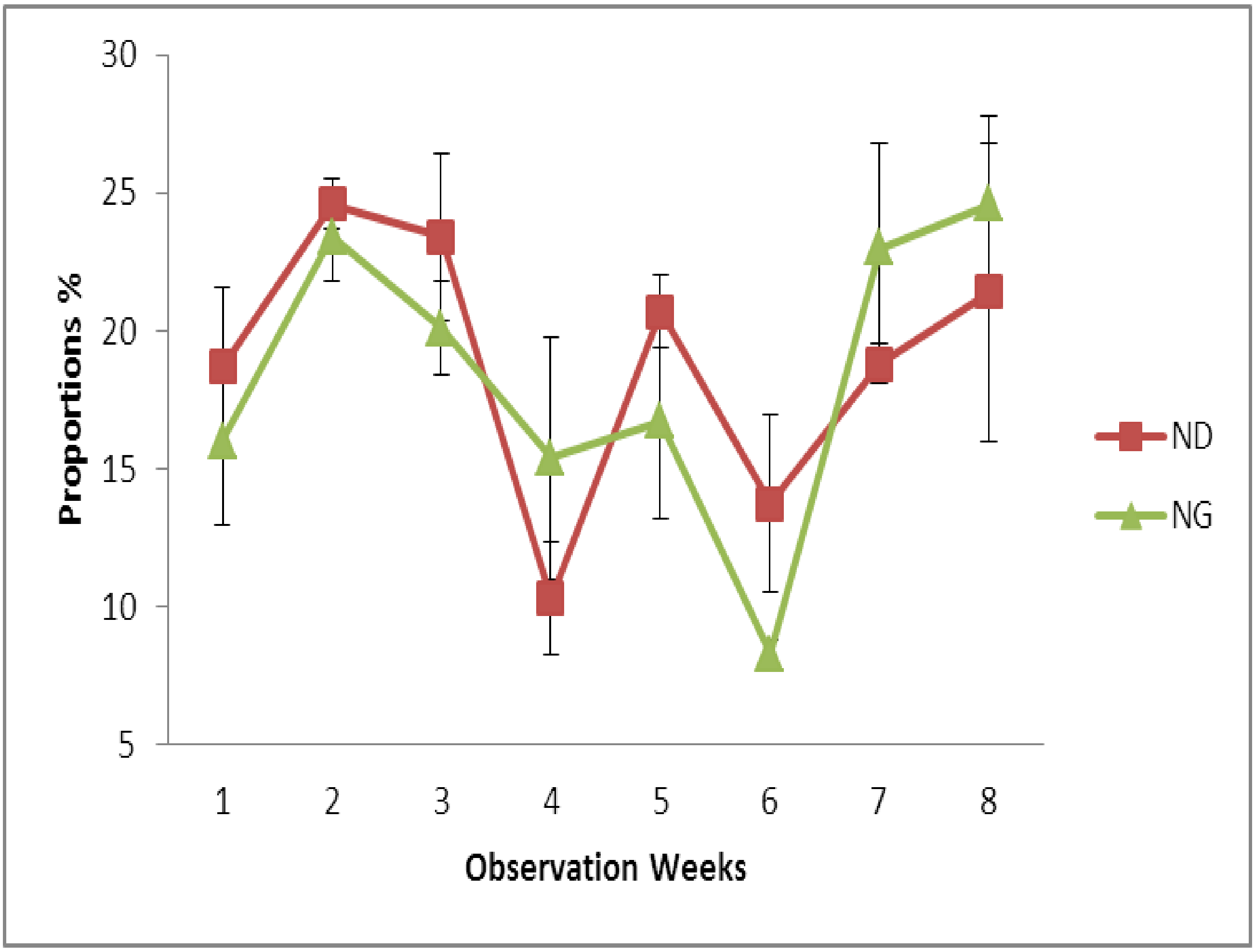

Figure 3. 5: The proportions of time spent standing during different weeks by the two genotypes

spent standing from week 1 to week 2. However; the proportions dropped from week 3 to week 4. The pattern picked up in week 5, before a significant decrease in week 6. Another increase was observed in weeks 7 and 8.

3.2.2 Time budgets of genotype during different times of the day

There were no genotype effects found on time spent grazing (P=0.3412), browsing (P=0.9301) and drinking (P=0.8439) throughout the different times of the day. Furthermore, in Table 3.1, time of the day had no significant effect (P=0.4393) on time spent grazing while it had an effect on time spent browsing (P=0.0164) and drinking (P=0.0012). More browsing was recorded during the morning session compared to other times of the day. A similar trend was observed during midday (ND - 0.1% and NG - 0.2%) and sunset (ND - 0.1% and NG - 0.3%). The steers drank more during midday compared to the morning and sunset sessions, which were significantly the same. There were no interactions between genotype and session on grazing (P=0.6730), browsing (P=0.5571) and drinking (P=0.6544).

There were no genotype effects found on time spent walking (P=0.2699), standing (P=0.7106), lying down (P=0.5076) and interaction with other steers (P=0.7156) throughout the different times of the day. Table 3.2 shows that time spent walking was not affected (P=0.5515) by the sessions of the day. However, standing activity was influenced (P=0.0262) by time of the day with higher proportions observed during midday, followed by the morning, then the sunset sessions. Furthermore, the lying down activity also differed (P=0.0030) with time of the day. The steers were lying down more at sunset, followed by the morning and the midday sessions, respectively. Social-interactions amongst the steers were mostly observed in the morning, during sunset, and then in midday. There were no interactions between genotype and session on walking (P=0.0.8674), standing (P=0.8508), lying down (P=0.9313) and social-interactions (P=0.4889).

Table 3. 1: Effect of time of the day on time (%) spent grazing, browsing and drinking water (Mean±SE)

Parameters	Grazing%		Browsing%		Drinking%	
	ND(n=4)	NG(n=4)	ND(n=4)	NG(n=4)	ND(n=4)	NG(n=4)
Session						
Morning	36.9^{a}±4.51	36.4^{a}±3.89	0.8^{a}±0.78	0.5^{a}±0.55	0.3^{b}±0.27	0.6^{b}±0.51
Midday	38.4^{a}±5.91	42.3^{a}±7.05	0.1^{b}±0.12	0.2^{b}±0.23	1.7^{a}±1.20	1.4^{a}±0.87
Sunset	37.5^{a}±4.16	38.9^{a}±3.81	0.1^{b}±0.17	0.3^{b}±0.28	0.4^{b}±0.42	0.5^{b}±0.37

Means in the same column with different superscripts are significantly different at $p<0.05$; Non-descript (ND); Nguni (NG)

Table 3. 2: Effect of time of the day on time (%) spent walking, standing, lying down and social interacting (Mean±SE)

Parameters	Walking%		Standing%		Lying%		Social Interaction%	
	ND (n=4)	NG (n=4)	ND (n=4)	NG(n=4)	ND(n=4)	NG(n=4)	ND(n=4)	NG(n=4)
Session								
Morning	$10.8^{a}±1.90$	$11.7^{a}±2.28$	$19.7^{ab}±4.27$	$19.9^{ab}±3.71$	$30.5^{ab}±5.22$	$29.6^{ab}±3.75$	$0.5^{a}±0.32$	$0.8^{a}±0.58$
Midday	$11.06^{a}±2.32$	$12.6^{a}±2.22$	$21.8^{a}±4.23$	$19.7^{a}±5.04$	$26.8^{b}±18.7$	$24.1^{b}±5.99$	$0.3^{b}±0.21$	$0.2^{b}±0.15$
Sunset	$10.4^{a}±2.12$	$10.8^{a}±2.34$	$15.0^{b}±5.25$	$15.0^{b}±4.46$	$35.3^{a}±5.96$	$34.5^{a}±4.82$	$0.3^{b}±0.37$	$0.3^{b}±0.38$

Means in the same column with different superscripts are significantly different at $p<0.05$; Non-descript (ND); Nguni (NG)

3.2.3 Correlations amongst the tested variables

Table 3.3 shows significant and non-significant relations between temperature, humidity, weight gains and the time budget activities. The grazing behaviour was observed to be negatively correlated with drinking (P<0.0001), walking (P<0.0001), lying down (P<0.05) and temperature (P<0.01). A positive correlation existed (P<0.01) between grazing and humidity percentage. Browsing positively correlated (P<0.01) with walking, while drinking positively correlated with temperature of the day. The standing behaviour negatively correlated with lying down (P<0.0001) and humidity (P<0.01), while it positively correlated with temperature (P<0.05). The interaction behaviour amongst the steers was positively related to temperature (P<0.0001) and negatively correlated to humidity (P<0.0001). The weight gains of the steers were negatively related to temperatures (P<0.01) of the day and the social-interactions of steers (P<0.0001) during the day.

Table 3. 3: Correlations amongst the time budget activities, temperature, humidity and weight of the animals

Variables	Grazing	Browsing	Drinking	Walking	Standing	Lying	Soc. Interact	Temp °C	Humid%	Weight kg
Grazing	-	-0.33	-0.59***	-0.56***	-0.18	-0.26*	0.15	-0.35**	0.36**	-0.01
Browsing		-	0.34	0.67**	-0.26	-0.17	0.38	-0.002	0.12	-0.35
Drinking			-	0.26	0.03	0.26	-0.43	0.46**	-0.21	-0.07
Walking				-	0.004	-0.22	0.16	0.16	-0.05	-0.19
Standing					-	-0.70***	0.24	0.28*	-0.40**	-0.15
Lying						-	-0.31	-0.04	0.06	0.27
Soc. Interact							-	0.65***	-0.67***	-0.75***
Temp °C								-	-0.71***	-0.45**
Humid%									-	0.23
Weight kg										-

Significance at *(P<0.05), **(P<0.01), ***(P<0.0001), Temp °C (Temperature °C), Humid% (Humidity %), Soc. Interact (Social Interaction)

3.3 Discussion

3.3.1 The genotype effect on cattle time budgeting

There were no genotype differences observed on time budgeting of the tested group of steers because they were introduced to the same group at an early age, subjected to the same treatments and thus were always moving together. This is in contrast with reports that even closely related animals can show different response patterns to specific stimuli (Provenza *et al.*, 2013; Breed and More, 2016). However, Grandin and Deesing (2014) reported that cattle and sheep are herd animals and thus perform as such. Therefore, the time spent by an individual steer on definite activities during their free time was strongly determined by the rest of the group. In a study by Falu *et al*. (2014), cattle and sheep exhibited a corresponding adaptive feeding behaviour at specific sites. In addition, time spent walking did not change with any of the time period variations. In a study on free-ranging goats, Solanki (2000) found that time spent walking was constant throughout the day. In addition, pasture-based systems generally have more space to move around, allowing limitless free-ranging. Walking is an empirical activity for selection and concentrated feeding for free-ranging animals (Solanki, 2000). However; some activities were determined by temperature, humidity, time of the day and observation weeks on specific levels.

3.3.2 Time budgets according to observation weeks

The non-significant difference observed in browsing, walking, lying down and interaction behavioural trends could be further attributed to the similar weather outlines during the observation period. Schlecht *et al*. (2004) reported that seasons did not significantly influence the daily patterns of the combined activities. In addition, though there were weekly differences observed on time spent grazing, drinking and standing behaviours; similar trends of these activities were still picked across the weeks. However, one particular week (week 2) exhibited very unique weather patterns of about 32 °C and 34% humidity on average, and thus less grazing, more drinking and standing behaviour compared to the other weeks. In this dry and hot week, the steers spent more time standing in one place near the water source and drinking. Solanki (2000) reported that animals change their activity patterns as an adaptation strategy, in response to the environment and to avoid climatic stress. Furthermore, these results concur with the current correlations results that temperature increase resulted in reduced time spent grazing and increased time spent drinking and standing. Breed and Moore (2016) described feeding and drinking as essential tasks for survival, therefore required in any kind of situation.

3.3.3 Time budgets according to time of the day

The steers were equally grazing and walking regardless of time/session of the day (Morning, midday or sunset). Dodzi and Muchenje (2012) reported that cows kept in cultivated pastures prioritised grazing throughout the seasons. In contrast, Arnold (1984) and Solanki (2000) reported that cattle and goats were grazing more in the morning and afternoon sessions. Additionally, it was reported in a study that goats grazed more in the afternoon, while they browsed more in the morning (Bakare and Chimonyo, 2011). These results are also in agreement with the current results that the steers spent more time browsing in the morning. More plant categories were consumed in the morning compared to other times of the day (Solanki, 2000). This could also be because the steers spent the night in more bushy areas for warmth and canopy over-night, thus more exposed to shrubs in the morning. They were also observed to spend more time in one place, which mostly they would have spent the night, either lying down or standing during this time of the day before they start moving around feeding.

Similarly, the steers spent more time lying down at sunset and this could be marking the end of an eventful day of feeding and moving up and down thus full and tired, ready to rest.

Decruyenaere *et al*. (2000) reported that ruminants principally invest time in grazing behaviour during day light. In addition, Solanki (2000) reported that goats sitting time progressively increased as the day advanced to noon. Furthermore, the morning session was seen to result in social-interactions (grooming and head butting) amongst the steers as they gathered in one place for the night till the next morning. Further, these social-interactions positively correlated with temperature, thus more grooming and head butting in high temperatures while the steers stood together, mostly ruminating. There is no apparent explanation for the negative correlation between social-interactions and animal weight gain. However, an assumption can be made that the more time the steers spent interacting reduced the time they allocated towards feeding thus low nutritional fill for growth and development.

3.3.4 Further correlations

Lower weight gains were associated with higher temperatures, which also negatively correlated with grazing. In hot weather, the steers spent more time standing in one place during higher temperatures, thus reduced time allocated for other activities like feeding. It was reported that cattle priorities shade during high temperatures and show reduced productivity (Blackshaw and Blackshaw, 1994). Gibb *et al*. (1998) also reported that cows were reluctant to graze before 16:00 on an extremely hot day (28°C) thus spent more time ruminating. In addition, more time spent drinking was also observed during the midday session, during which temperatures were high, with low humidity. Similar results were observed that drinking was higher in the hot-wet season compared to other (Dodzi and Muchenje, 2011). There is need to re-hydrate during hot weather.

Current results also confirmed that the standing behaviour was prolonged during high temperature periods and reduced moisture; which was the case at midday and on week 2. Pasture-based dairy cows (Dodzi and Muchenje, 2012) and free-ranging goats (Bakare and

Chimonyo, 2011) spent more time standing during the hot-wet season. Additionally, elongated time spent standing was equivalent to reduced time spent lying down, allowing airing and freshening to all measures of the body. These results agree with those reported by Dodzi and Muchenje (2012) in the hot-wet season, which they related to thermoregulation through the four routes of heat exchange, transmission, convection radiation and evaporation. Time spent walking also triggered time spent browsing and this could be that the steers were merely exploring as they walked past the bushes that occupied their habitat. However, Solanki (2000) found insignificant relations between the tested activities.

3.4 Conclusion

The time budgets of steers in the same group are the same, regardless of the genotype differences. Some of the time-budget activities of beef steers raised in natural pastures change with temperature, humidity, observation week and time of the day. Correlations exist between some time budget activities, weather readings and body weights. Body weight gains were not influenced by some time budget activities but social-interactions between the steers and temperature. The daily time budgets of steers in natural pastures changes with temperature, humidity, observation week and time of the day, regardless of the genotype differences.

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Chapter 4:
On-farm successive handling effects on some behavioural and physiological responses of Nguni and non-descript beef steers reared extensively on natural pastures

(*Under review in Applied Animal Behaviour Science*)

Abstract

The behavioural responses of Nguni (NG) and Non-descript (ND) steers during eight weeks of continuous handling were determined by recording avoidance-related scores; Race Score (RS), Entry Score (ES), Weighing Box Score (WBS), Stepping Score (SS), and Exit Speed Score (ESS). Furthermore, blood samples were collected from the jugular vein using two specialised vacutainer tubes and a needle mounted on a holder for glucose, cortisol and lactate analysis. Both WBS (P=0.0001) and SS (P=0.0011) were associated with genotypes. Most NG steers were more calm than the ND steers, with the latter also vocalizing and aggressively kicking. However, genotype did not (P>0.05) influence RS, ES, ESS, cortisol, glucose and lactate levels. Observation/sampling week had an effect on RS (P<0.0001), ES (P<0.0024), WBS (P<0.0001), glucose (P<0.0001), cortisol (P=0.0005) and lactate (P<0.0001) levels. The avoidance-related behaviour of the steers during handling varied, with the steers showing more avoidance and aggression in other weeks than some. Similarly, the levels of cortisol, glucose and lactate varied in a decreasing order over time, with strongly defined variations for cortisol and lactate levels. The weekly behavioural responses were clearly reflected in the measured cortisol, glucose and lactate. However, regardless of the prominent negative behaviour seen over time, the levels of the measured blood constituents continued to drop.

Keywords: Avoidance-related behaviour, behaviour scores, Animal-human interaction, Cortisol, Beef cattle, Genotype

4 Introduction

Beef producing cattle are normally reared extensively during their early stages of life and are sometimes transferred to intensive systems during the fattening and finishing stages (Probst *et al*., 2013). Most South African beef farmers use the extensive production system which allows them to supply the meat industry with enough quantities to meet the consumer demands. This production system makes it impossible for some of the suggested (Markowitz *et al.*, 1998; Probst *et al*., 2012; 2013) day to day and close interactions between animals and the stockman to improve their relation and promote ease of handling in the long run. Beef cattle reared extensively normally interact with humans during management routines. Unlike dairy cows, beef cattle are less accustomed to being handled and are thus a little less experienced with human interactions. General farm procedures that require close human-animal interactions include time to time managerial activities and/or routines such as castration, dipping, branding, vaccination and sampling for research purposes.

Raussi (2003) and Probst *et al*. (2013) reported that animals perceive these events as unpleasant, to an extent that fear towards humans (who carry out these routines) may be developed. In addition, cattle and sheep can remember an aversive experience for many months after it occurs (Hutson, 1985; Pascoe, 1986). Therefore, in order to deal with certain situations, animals may respond and even develop certain behaviours or strategies (Boissy *et al*., 2001) (i) towards each other during resting and feeding in the kraals/feedlots and, (ii) during management routines, which involves human interaction such as feeding or any other activity. Broom (2000) described animal behaviour as the utmost evident indicator that can be used to assess if an animal is struggling to cope with a given situation. In addition, Waiblinger *et al*. (2003) reported that farm animal behavioural tests may be more informative and reliable than stockman tests when assessing animal welfare. For instance, results in

Chapter 3 showed that the steers spent drank more and spent more time either standing in one place or lying down during midday when the sun was hot the most.

In the presence of potential stressors, animals do not only change in behaviour, but also some physiological changes occur. Animal handling, transport and slaughter were observed to be stressors that produced significant changes in blood-hormonal levels in animals (Grandin, 1997; de Passille and Rushen, 2005; Terlouw, 2005; Mota-Rojas *et al*., 2006; Muchenje *et al*., 2009; Chulayo *et al*., 2012; Seshoka *et al*., 2013; Jama, 2014). Disturbance in the environment activates the hypothalamic-pituitary-adrenal activity due to fear, thus leading to catecholamines and cortisol discharge (Ferguson and Warner, 2008; Muchenje *et al*., 2009; Hemsworth *et al*., 2011) and further elevates blood lactate and glucose. Blood, urine and saliva have been used to extract hormones and metabolites such as catecholamines, cortisol, blood lactate and glucose and others for determination of stress levels. According to Canario *et al*. (2013), highly adaptable animals may perform better with varying conditions than animals of low adaptability.

Grignard *et al*. (2001) reported that genetic characteristics of an animal influences how the animal responds to handling. The variety of beef breeds reared in South Africa may as well react differently from one another. Amongst other breeds used in South Africa, there is the hardy indigenous Nguni breed (NG) and the non-descript (ND) genotype. The NG breed has functional characteristics that allow it to perform better than other breeds even during the dry season with poor quality feed (Ndlovu *et al*., 2009) or during handling at slaughter (Muchenje *et al*., 2009). The ND genotype on the other hand may contain multiple unidentified genes and it comprises 35% of bulls found in the emerging farming sector (Scholtz *et al*., 2008). Knowledge and understanding of different genotype responses to stress allows farmers to select for animals with better temperament in order to maximise product quality and good economic refunds (Broom and Fraser, 2007; Ndou *et al*., 2010).

On-farm behaviour and animal-human interaction assessments, regarding welfare and product quality, have mostly been conducted on dairy cows (Waiblinger *et* al., 2003; Dodzi and Muchenje, 2011; Mounier, 2011) compared to beef cattle; also in other production systems and countries. Therefore, the current study sought to assess some on-farm behavioural and physiological responses of Nguni (NG) and non-descript (ND) beef steers reared on natural pastures in relation to successive handling.

4.1 Materials and Methods

Ethical clearance and consent to carry out this study was as described in section 3.1.

4.1.1 Study site and its weather patterns

The study description is as described in section 3.1.1. The temperature and humidity patterns recorded during the sampling and observation period are shown in Figure 4.1.

4.1.2 Animal description and procedure

The animal description is the same as described in section 3.1.2. Animals were brought from the grazing pastures to the holding pens for treating, examination, behaviour scoring and blood sampling around 7am fortnightly over a four-month period.

4.1.3 Avoidance Behaviour Scoring

Observations began when each animal was manually separated (either out of free will or encouraged) from the rest of the group in the race, individually moved along towards the weighing box and the holding crush. The weights and other examinations were done and recorded. Then blood samples were taken before the animal was freed from the holding crush. The avoidance-related scores were adapted and modified from various similar studies (Grandin, 1993; Breuer *et a*l., 2000; Gibbons *et al*., 2009; Petherick *et al*., 2009ab; Ndou *et al*., 2010; Dodzi and Muchenje, 2011).

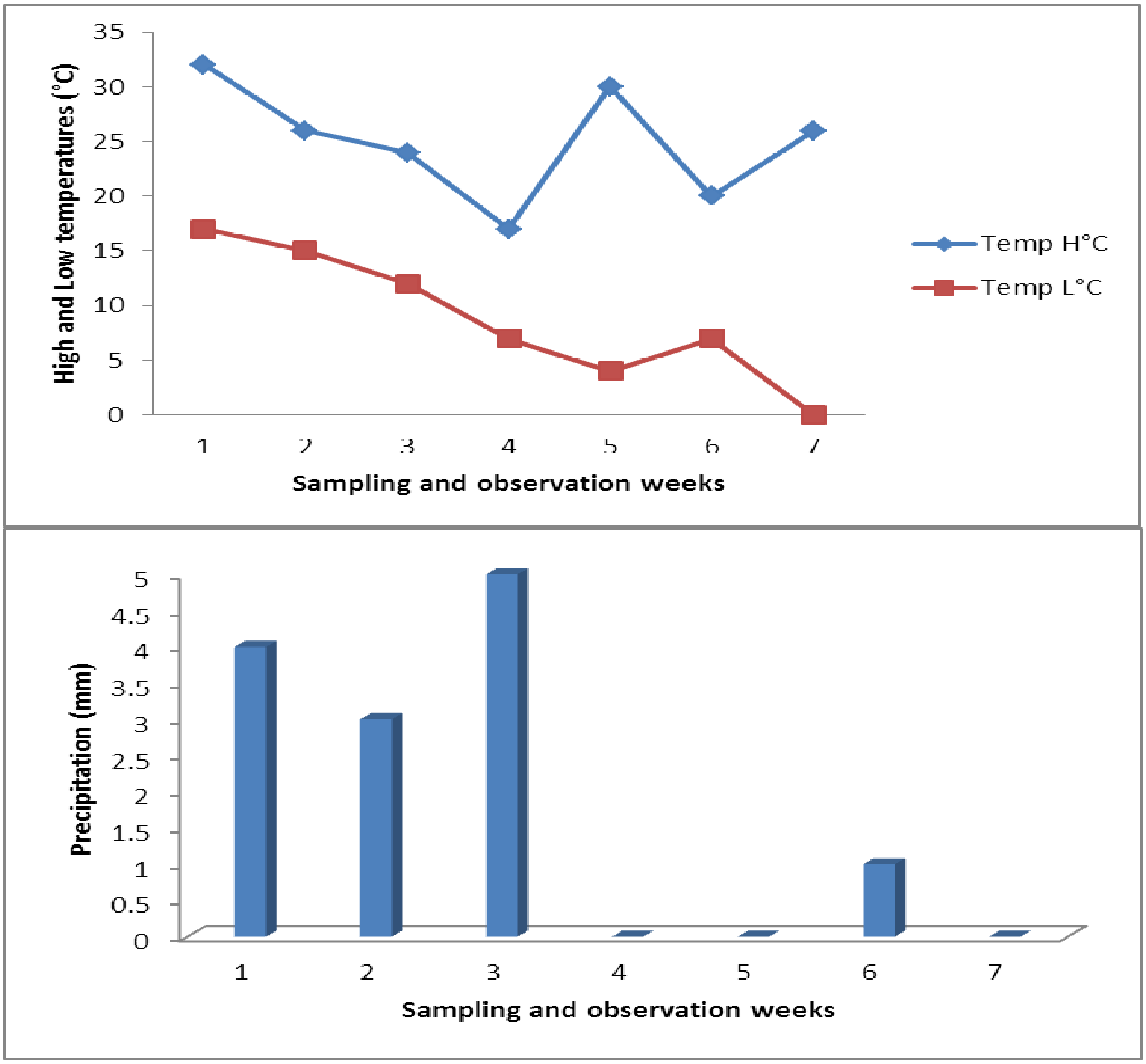

Figure 4. 1: Temperature and precipitation patterns during the data collection periods (Source: http://www.accuweather.com/en/za/fort-hare)

The Race Scores (RS) were recorded as 1- Not-stopping (walks through the race towards the weighing box without stopping) or 2- Stopping (reluctant to move, sometimes even turning to face the opposite direction) while in the race; Entry Score (ES) was measured by 1- No encouragement (required to move the animal), 2- Slight encouragement (vocal instruction), 3- Some encouragement (vocal and prodding), 4- Force (pushing the animal to move); Weighing Box Score (WBS) was whether the animal was 1- Calm (just standing), 2- Agitated (in panic, shaking the box-trying to escape) or 3- Vocalizing (making sounds); Stepping Score (SS) was determined by 1- Not kicking, 2- Light kick (minor and gently) and 3- Aggressive kick (violent); and Exit Speed Score (ESS) was scored as 1- Walked (normal walk), 2- Trotted (rushing/jumping out) and 3- ran (running out). Higher scores determined high avoidance behaviour.

4.1.4 Blood Sampling and analysis

The jugular vein was used to source the central circulatory blood from the steers through a puncture using a needle connected to the specialised (for specific analysis) vaccutainer tube and its holder. This was done while the animal was restrained in a holding crush, also using a nose grip to hold the steer in position while availing the vein for sampling. The blood samples were stored in a cooler box with ice until separation of serum through centrifuging using Model 5403 Centrifuge (Gatenbay Eppendorf GmbH, Engelsdorp, Germany) for 15 min at 10°C and 3000 rpm before analysis for cortisol, glucose and lactate was carried out. The procedure used to analyse the samples were as described by Chulayo et al. (2015) and the analysis were done in University of Pretoria, Pathology lab (Cortisol and Lactate) and National Health Laboratories (glucose).

4.1.5 Statistical analysis

A Chi-square test (SAS, 2003) was used to assess for existing associations between observation week, genotype and behaviour scores. Frequencies were calculated using Proc freq procedure of SAS (2003). A repeated measure model, with cortisol, glucose and lactate as repeated measures and animal as a random variable was fitted in SAS (2003). Comparison of means was performed using Tukey's test. Differences were considered significant at $P<0.05$. The model used was: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$ where, Y_{ijk} is the response variable (cortisol, glucose and lactate); μ is the mean; α_i is the effect of sampling week; β_j is the effect of genotype; $(\alpha\beta)_{ij}$ is the interaction between variables and e_{ijk} is the standard error.

4.2 Results

4.2.1 Behavioural Changes according to observation week and genotype

Observation week was found to have an effect on RS ($P<0.0001$). However, there were no genotype effects ($P>0.3108$) found on RS. In addition, an association ($P<0.02$) between the observation week and genotype was found. Figure 4.2 show that the curves for the two genotypes resemble a similar trend over time. In week 1, the RS frequencies of steers that were confident to move in the race were 53% (ND) and 65% (NG); while the rest were more reluctant (ND-47% and NG-35%). However, these scores changed in week 2, with reluctance proportions rising. The ND reluctance curve continued to rise in week 3 before significantly dropping to in week 4; while that of NG dropped in week 3 and then rose to in week 4. In weeks 5 and 6, all the steers of both genotypes were not willing to move in the race. The same trend was seen in NG steers in week 7 while the proportion dropped for ND steers.

The ES was not influenced ($P=0.3108$) by genotype differences, both genotype curves resembled the same pattern. However, an observation week effect ($P<0.0024$) was found. There were no associations ($P=0.2877$) between the observation week and genotype. Figure 4.3 shows a 0-5% frequency of NG steers that required no encouragement while entering the weighing box across the observation weeks. While some of the ND steers required some form of encouragement, about 5% (week 2), 30% (week 4) and 10% (week 7) of ND steers moved

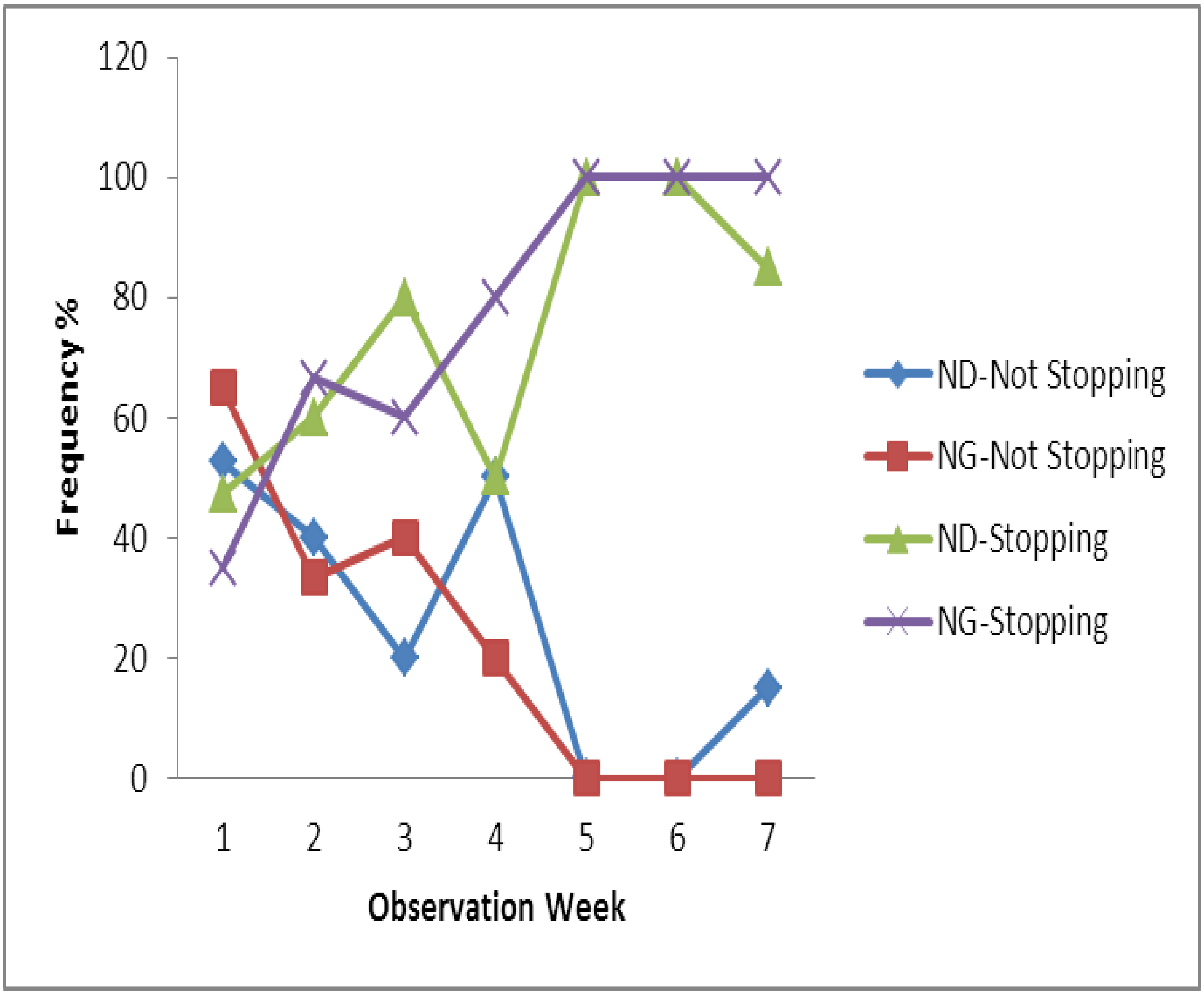

Figure 4. 2: Race score (RS) response-behaviour frequencies according to observation week (P=0.001) and genotype (ND-Non-descript and NG-Nguni)

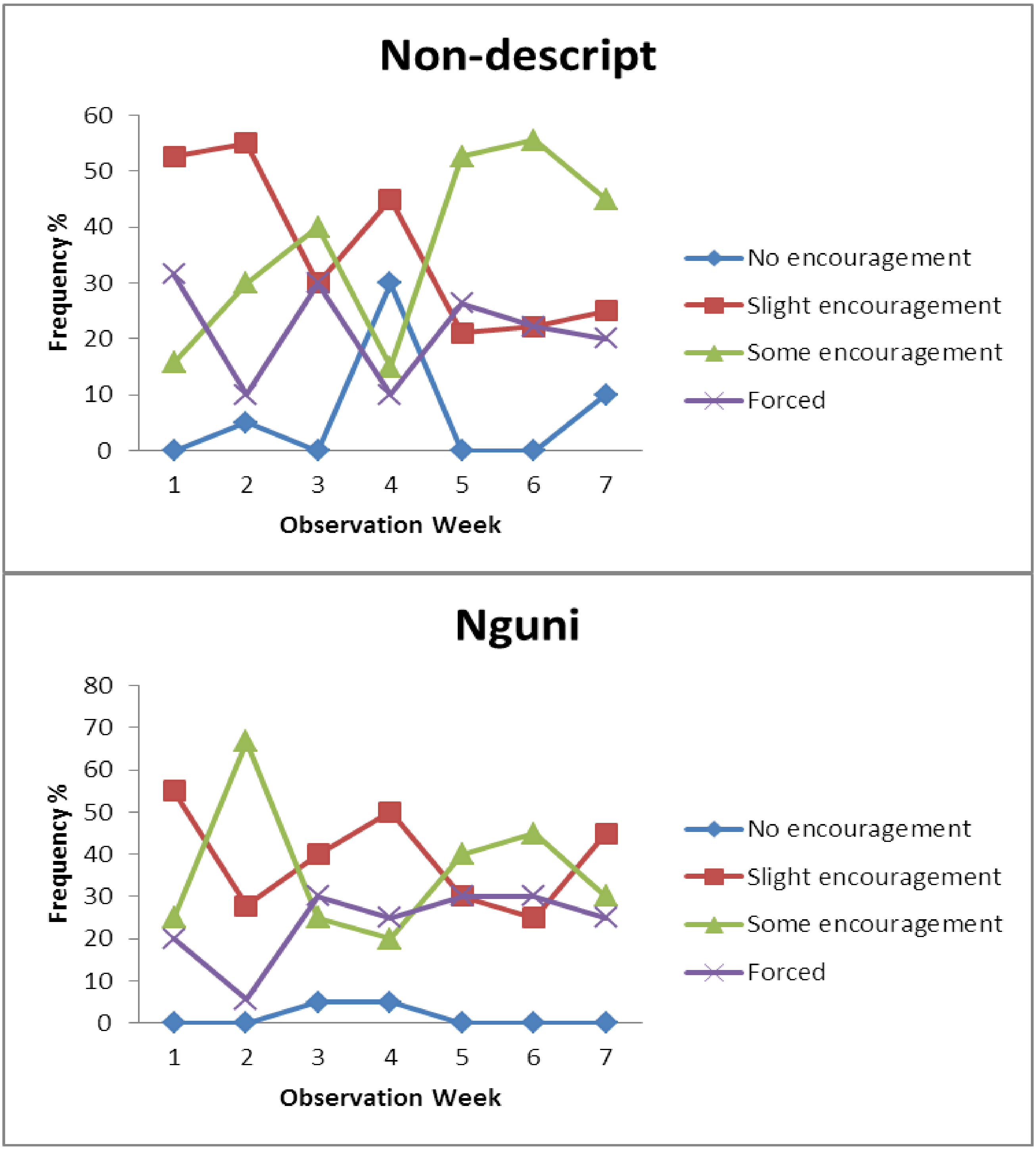

Figure 4. 3: Entry score (ES) response-behaviour frequencies according to observation week (P=0.0024) and genotype (P>0.05)

into the weighing box voluntarily. In week 1, most steers of both genotypes required only vocal (slight) encouragement and 31% ND had to be physically forced to move. In week 2, more ND steers required slight encouragement, while some (vocal and prodding) encouragement was necessary to move most of NG steers.

Week 3 was characterised by both genotypes being forced to enter the weighing box with only 5% of NG needing no encouragement. Most of the steers of both genotypes required only vocal reinforcement to move, with 25% of NG physically forced in week 4. In week 5, vocal plus prodding was used on 53% of ND and 40% of NG steers. In the same week, 30% of NG steers were physically forced as opposed to 26% ND, and 30% NG were vocally cheered compared to 21% ND. More NG steers still required physical forcing in weeks 6 and 7 compared to the ND steers. In the same weeks, vocal encouragement plus prodding was used to move most ND steers, while 10% moved on their own. Furthermore, 45% NG steers were vocally assisted, while vocal plus prodding and/or physical force was used on some.

The WBS was found to be affected by both genotype ($P=0.0019$) and observation week ($P<0.0001$). Furthermore, there were no associations ($P=0.2795$) found between genotype and observation week. Figure 4.4 shows that NG steers were generally more calm compared to ND steers, with the latter having high vocalizing frequencies. In Week 1, most animals across the genotypes were calm. However in the same week, 45% of NG were agitated; while 32% and 21% of ND steers were agitated and vocalizing, respectively. Week 2 was characterised by most steers of both genotypes being calm; though some were also agitated, with more ND steers vocalizing compared to the NG steers. Similarly, in Week 3, majority of the NG and ND steers were calm, with some agitated and vocalizing. Week 4 frequencies were the similar between the two genotypes. In both weeks 5 and 6, the ND steers showed constant results. More NG steers were calm in week 5 compared to week 6.

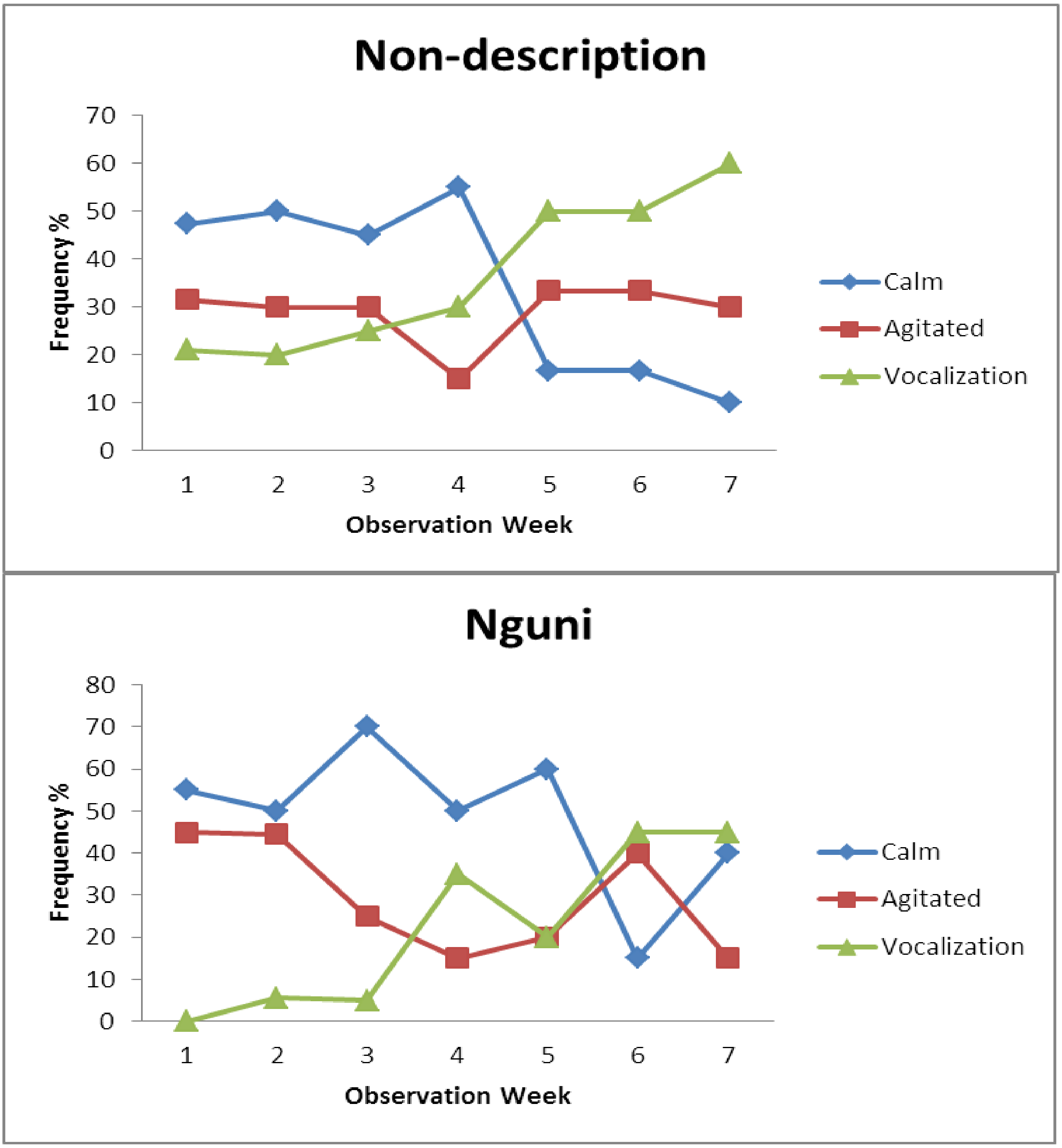

Figure 4. 4: Weighing box score (WBS) response-behaviour frequencies according to observation week (P=0.0001) and genotype (P=0.0019)

Calm ND steers continued to drop in week 7, while the proportion rose for the NG steers.

The SS frequencies were found to be influenced (P=0.0011) by genotype differences. Figure 4.5 shows that there were no observation week effects (P=0.5382) were found nor associations with genotype (P=0.0955). Generally, both genotypes were highly represented by calm steers across the observation weeks. However, more ND steers were seen kicking compared to NG steers. Across all observation weeks, over 85% of NG steers were found to not kick at all and none was found aggressively kicking during handling. However, between 5-6 percent of ND steers were found to kick aggressively across observation weeks. In addition, only between 0-15 percent of NG steers were found to slightly kick, while a variety of ND steers were found in this category, with a maximum of 40% in the last week of observations.

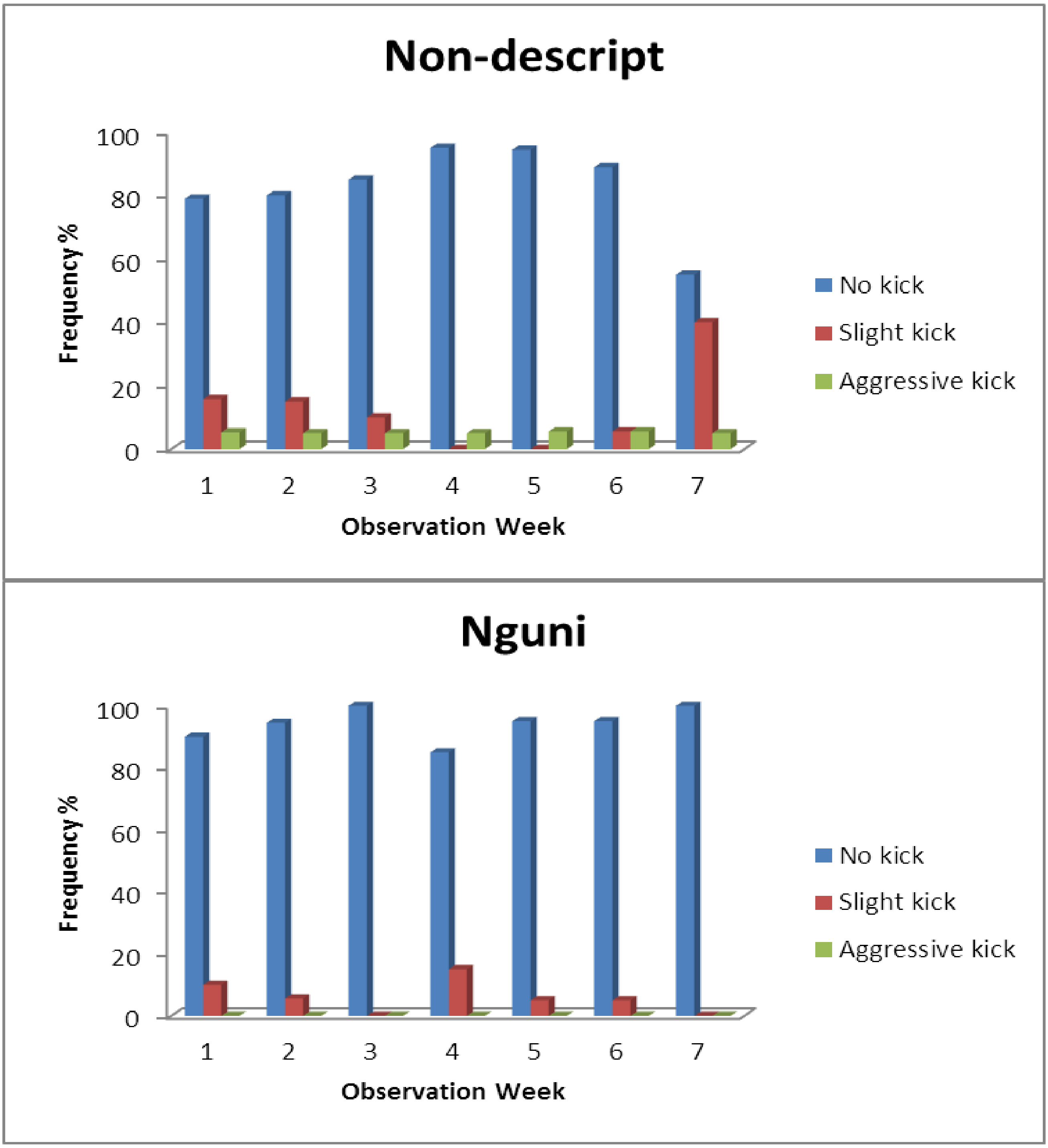

Figure 4. 5: Stepping score (SS) response-behaviour frequencies according to observation week ($P>0.05$) and genotype ($P<0.0011$)

4.2.2 Some physiological changes according to sampling week and steer genotype

Genotype was found not to affect either cortisol (P=0.9125), glucose (P=0.0773) nor lactate (P=0.7215) level. In addition, there were no interactions observed between the genotype and the sampling week. However, there were changes in these levels over time. Figure 4.6 shows a gradual drop in average cortisol levels of both ND and NG steers from week 1 to week 2. However, there was a peak on week 3 before they started dropping again through to week 5. Another rise was observed in week 6 and then dropped again in week 7.

Figure 4.7 shows a significant drop in glucose levels of the two genotypes, with the NG group having slightly higher levels than the ND group in weeks 1 (4.72 and 4.41mmol/L, respectively). However, just as with cortisol levels (Figure 4.6), there was a slight increase in week 3 before the levels similarly dropped from week 4 to week 7.

In Figure 4.8, the Week 1 lactate levels for both the ND and NG steers dropped in week 2. However, an insignificant rise for ND steers was observed in week 3, while it was a drop for the NG steers. For the NG steers, the lactate levels continued to significantly drop through to Week 6, before rising in Week 7. The ND group also recorded decreasing levels from week 4 to week 5. However, there was a significant increase in weeks 6 and 7.

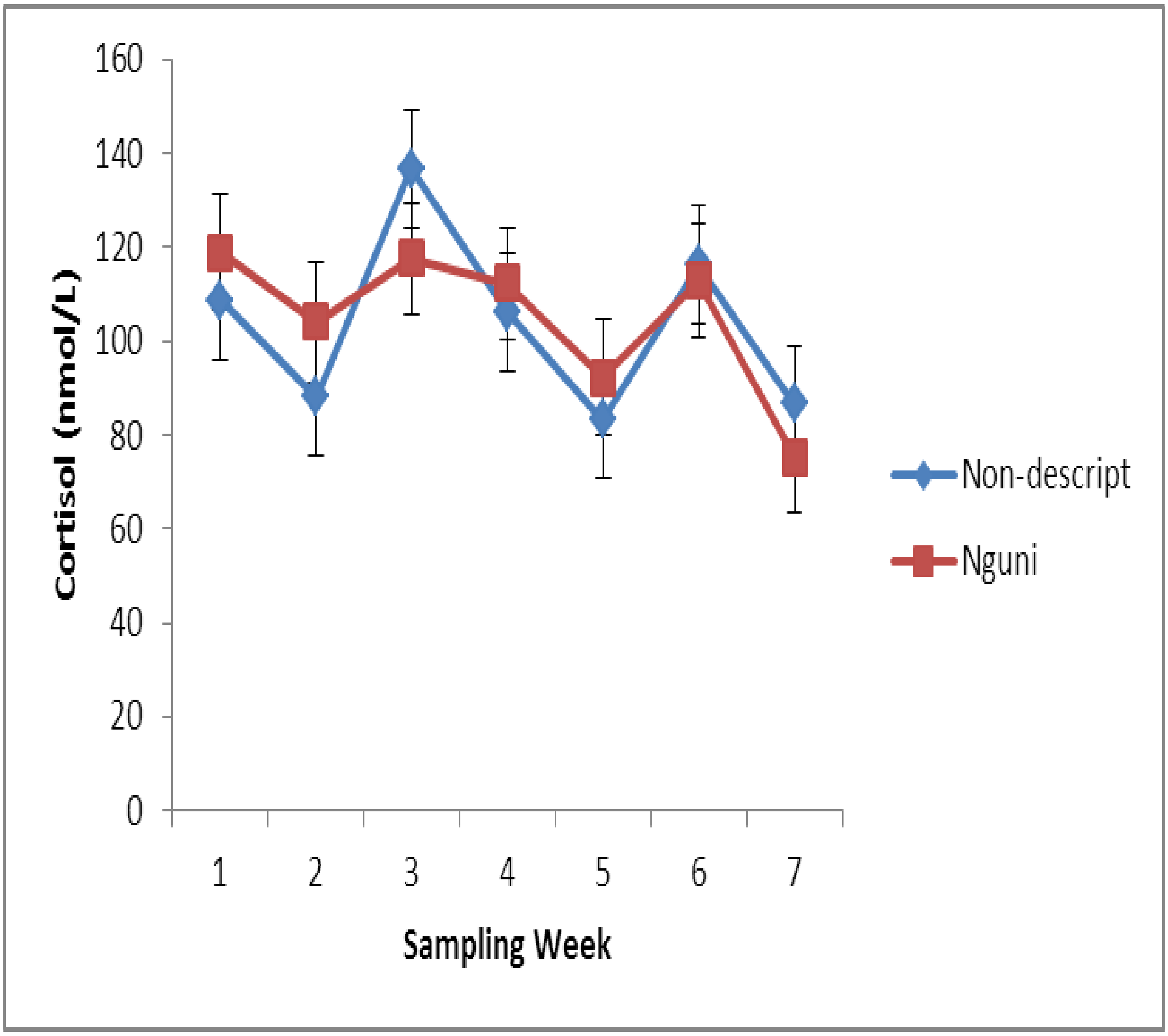

Figure 4. 6: Weekly changes in serum cortisol levels according to genotype

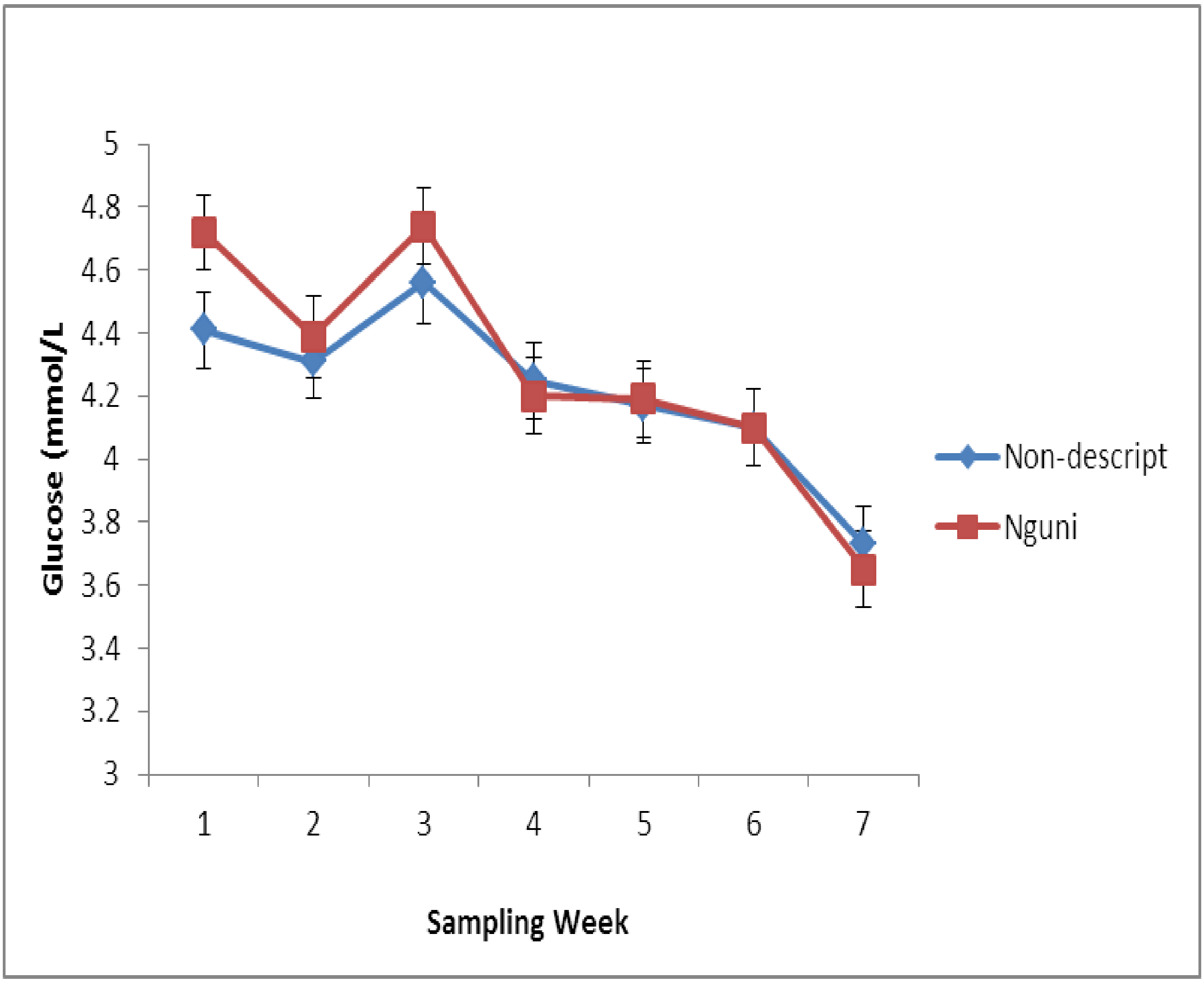

Figure 4. 7: Weekly changes in blood glucose levels according to genotype

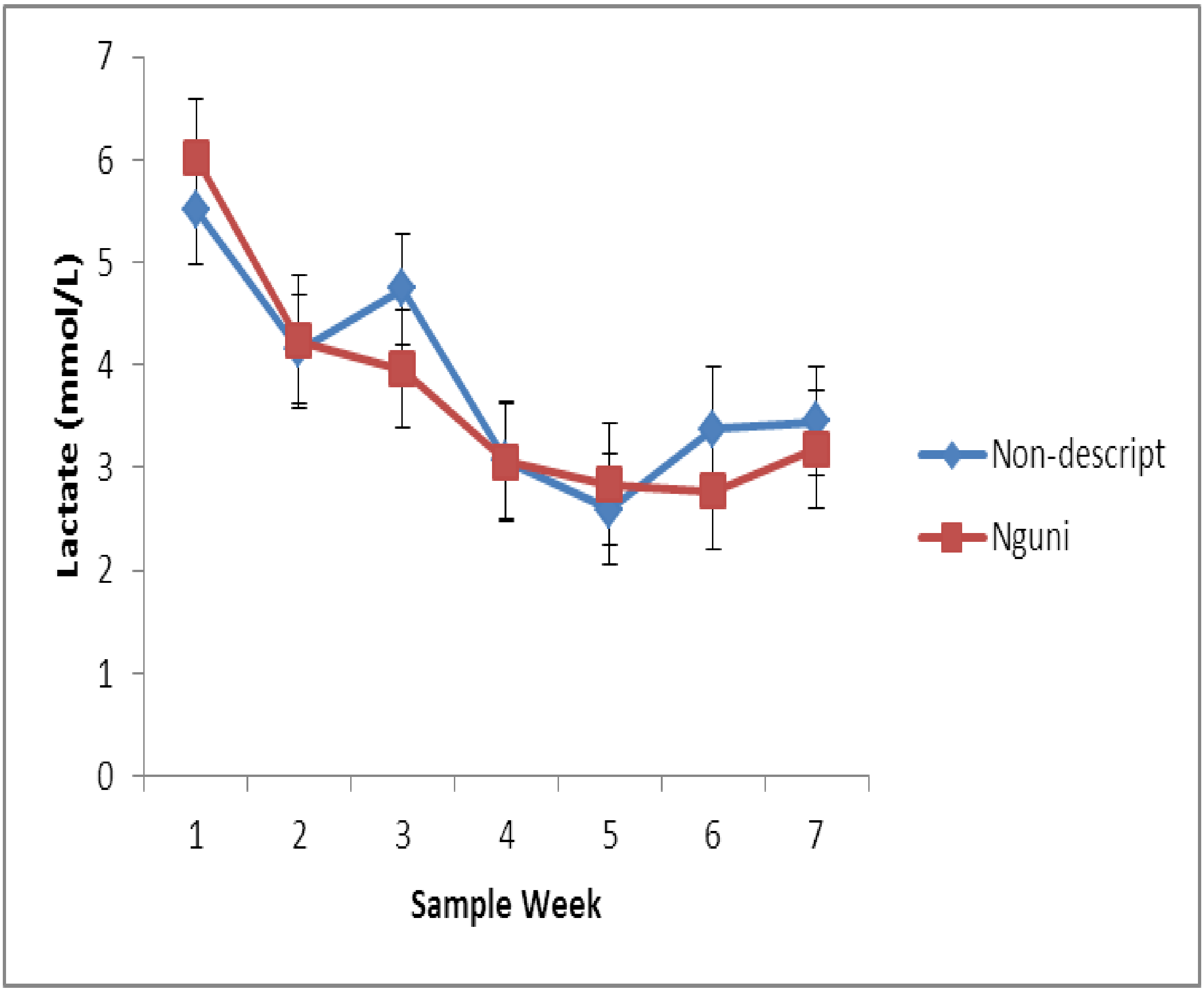

Figure 4. 8: Weekly changes in serum Lactate levels according to genotype

4.3 Discussion

The avoidance related behaviour of the steers observed in the current study varied with observation weeks. However, the behavioural response of animals to handling and to a certain environment is expected to improve with time of exposure to that condition, as it is believed that they get used to the effects. In addition, Grandin (1993) reported that the tendency to become behaviourally agitated stabilizes over time in certain individual animals. The response behaviour seen in this study can be strongly related to specific conditions and events observed on the sampling day/week. Particularly, the increased reluctance for both genotypes (RS and ES) observed in weeks 3 and 6 may be attributed to the unforeseen activities that took place on the sampling days.

In week 3, there was a drastic change of weather during the sampling period, accompanied by rain and very low temperatures which then resulted to an unfavourable working environment for both handlers and animals. It was reported that farm animals develop certain behavioural strategies in order to deal with certain situations at the farm (Boissy *et al*. 2001). In addition, more ND steers were negatively affected by these conditions, particularly in the weighing box during restraint, with higher frequencies of agitation and vocalizing while 70% of NG steers calmed down. There is no apparent explanation for this trend; however Breed and More (2016) reported that within a species, animals may vary in their responses to a particular set of circumstances through personality expression, with some animals in a population behaving more boldly than others. Additionally, different types of animals may react differently to the same the system (Lanier *et al*, 1995).

The similar trend was also seen in hot and dry week 5 where all the steers required vocal, prodding or physical force to encourage them to move into the weighing box. However, the majority of NG steers became calm in the weighing box during restraint, compared to the ND steers. However, the recurrent fear to move forward in the race (RS and ES) observed across the observation weeks in both genotypes may have resulted from separation distress from the rest of the group during individual assessment. It was reported that farm animals are accustomed with herds and separation from their own may result to stress (Grandin and Deesing; 2014). Cattle in this study seemed to do better while they were in their familiar group than when they were alone, especially during interactions with humans.

Furthermore; during week 6 there were multiple events going on around the holding pens; including the presence of unfamiliar animals, humans (regular farm herd and staff) and their noises while other farm routines were conducted. Both the ND and NG steers were similarly affected by these conditions, showing parallel trends in response-behaviour and this was no surprise as the study steers were only familiar and acquainted with their small group at the farm. Grandin (1989) reported that cattle and sheep are highly affected by high frequency noise compared to humans. In addition, as a survival strategy (Siegel and Gross, 2000), livestock species often react and refuse to move in a handling facility due to stress and anxiety if they spot a distraction (Grandin, 2006; Bourguet *et al*., 2011).

In addition, there drastic increase in vocalization observed from week 4 through to week 7 is not fully understood. However, it could be attributed to the previous stressful week 3 experiences reported earlier during which handling the steers was challenging. The steers must have suffered effective stress and pain from the external measures that had to be used to encourage motion and keep the steers in position. Hutson (1985) and Pascoe (1986) reported that for cattle and sheep, an aversive experience can take months to forget. In addition, the steers showed to be more sensitive; more especially the ND steers which recorded higher frequencies of vocalizing compared to NG steers. Grandin (1989) reported that cross-bred cattle are highly excitable and harder to handle compared to English breed.

In addition, cross-bred animals can be more temperamental compared to pure-bred animals due to a mixture of different gene sequences, mainly aimed to producing quality meat product while the behavioural impacts may not be known. Grandin (1993) and Voisinet *et al*. (1997) reported that temperamental cattle are not easy to handle and are thus susceptible to handling stress. The NG breed on the other hand is known for its enduring nature (Muchenje *et al*., 2009; Ndlovu *et al*., 2009), while the ND genotypes' background may be not known. The ND steers discomfort and lack of ability to cope during the process was further shown through frequent aggressive attempts to kick (SS) the handlers compared to NG steers. In contrast to these results, Grandin and Deesing (2014) also reported that small bodied animals with smaller-diameter leg bones may be more fearful.

However with all this, the ESS of the two genotypes was significantly the same and it could be that the steers generally were not comfortable with confinement, regardless of the level of discomfort they were experiencing. Furthermore, the nonexistence of genotype effects on cortisol, glucose, lactate and some response-behaviour scores may be because the steers were still young and introduced to the same group and treatment since day one of the trial. The measured glucose, cortisol and lactate generally reflected the behavioural changes that were observed during handling and sampling each week. Higher levels of these metabolites were detected in the same weeks when the steers displayed higher levels of avoidance behaviour. This is in agreement with reports that increased glucose, cortisol and lactate levels are indicators of stress-related behaviour and excessive muscle activity (Shaw and Tume, 1992; Gruber *et al*., 2010; Leroy *et al*., 2011). These hormones and metabolites are triggered by fear through the activation of the hypothalamic-pituitary-adrenal activity (Ferguson and Warner, 2008) during handling (Tarrant *et al*., 1992; Hulbert *et al*., 2011).

4.4 Conclusion

The avoidance-related behaviour of the steers during handling varied, with the steers showing more avoidance and aggression in other weeks than some. Similarly, the levels of cortisol, glucose and lactate varied in a decreasing order over time, with strongly defined variations for cortisol and lactate levels. The weekly behavioural responses were clearly reflected in the measured cortisol, glucose and lactate. However, regardless of the prominent negative behaviour seen over time, the levels of the measured blood constituents continued to drop. However, more NG steers were calm during handling with light kicking, while more ND steers were vocalizing and kicking aggressively.

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Chapter 5:
Pre-slaughter effects on response-behaviour, bleed-out times and some blood physiological responses of Nguni and non-descript beef steers

(*Submitted to South African Journal of Animal Science*)

Abstract

The objective of this study was to investigate the effect of on- and off-loading, transportation, lairaging and slaughter on response behaviour, bleed-out times and some blood physiological responses of Nguni and non-descript beef steers. Twenty Nguni (NG) and 20 Non-descript (ND) beef steers were loaded at the farm, transported in two groups (morning and afternoon trips) over a 120 km distance to the abattoir, with steers of both genotypes represented in each group. Trip duration, vehicle speed and weather conditions were recorded. Behavioural response and their duration (s) were recorded during on- and off-loading, transportation, lairaging, stunning and exsanguination. The posture of the steers during transportation; time-budgets of the steers during lairaging; avoidance-related behaviour and vocalization scores during stunning were recorded. The steers were slaughtered in four groups (SG1, SG2, SG3 and SG4) and the number of attempts to stun each steer and the slaughter order/group were logged. Blood samples were collected from each steer during the process of exsanguination for cortisol, glucose and lactate analysis. It took less time to load and off-load group 1 (370s and 602s, respectively) than group 2 (420 s and 782 s, respectively). All 40 steers were standing throughout transportation and during the lairage observation period. Avoidance-related behaviour and vocalization in the stunning box were not influenced by genotype. The steers that were in transportation group 1 (TG1) and rested longer showed more avoidance behaviour (63.2%) than those in the second group (TG2 - 23.9%). Steers that were in slaughter group 2 (SG2 - 100%) showed minimal avoidance behaviour than those that were in the other groups. The only steers that vocalized were 5% of the ND group, which were all in

TG1 and SG2. There were no (P > 0.05) genotype and number of attempts to stun effects on bleed-out times, cortisol, glucose and lactate levels. The TG1 steers, with longer lairaging periods had higher cortisol (140.6±14.50 nmol/L) and lactate (12.4±0.83 nmol/L) levels than those in TG2. Similarly, SG1 steers had higher cortisol (175.9±17.24 nmol/L) and lactate (13.5±1.12 mmol/L) levels than in the other 3 SGs'. Cortisol and lactate positively (P<0.001) correlated (r=0.70). In conclusion, steers of different genotypes displayed similar responses to identical pre-slaughter conditions that they were exposed to.

Keywords: on- and off-loading, Transportation, Lairage duration, Stun-attempts, Cortisol, Lactate

5 Introduction

According to chapter 3 findings, beef cattle reared extensively in natural pastures spend most of their "free" time between 6am and 20:15pm feeding (grazing) throughout the different times of day, with some lying down, standing, walking, drinking water and interacting with one another in between. This is expression of their normal behaviour (Lee *et al*., 2013). However, they spend more time showing avoidance-related behaviour leading to biochemical and/or physiological changes in the animal during management procedures (Chapter 4) that involve moving these animals from their habitat to the handling facilities, involving human contact. Towards the end of their production cycle, the pre-slaughter events commence with the removal of slaughter animals from the farm habitat, exposing them to various conditions and handling methods, through to the knocking box at the abattoir (Ferguson and Warner, 2008).

Steers transportation has been described as the key component joining the events involved in the pre-slaughter logistics chain (Miranda-de la Lama *et al*., 2014). Amongst other various modes of transportation used for slaughter cattle, road transport is the most significant and economic type which also allows multiple loads of animals in a single trip (Tarrant, 1990). While the processes of loading and off-loading animals were reported to be more stressful than the journey itself (Agnes *et al*., 1990; Trunkfield and Broom, 1990); transportation has been recounted to largely induce significant stress in animals being transported (Grandin, 1997; Warriss, 2000; Maria *et al.*, 2004; Minka and Ayo, 2007; Ferguson and Warner, 2008). Furthermore, transportation stress was related to health defects such as respiratory diseases (Sporer *et al*., 2007; Duff and Galyean, 2007) and weight losses (Schwartzkopf-Genswein *et al*., 2012).

Confinement of cattle in a moving vehicle was described as being more stressful compared to confinement in a stationery vehicle, during on and off-loading and re-penning (Tarrant, 1990). However, there is limited information quantifying the effects of loading and off-loading processes on animal welfare (Maria *et al.*, 2004). Jacobson and Cook (1998) reported that loading is more stressful than off-loading, while Maria *et al*. (2004) recounted that hurried loading reduced stress metabolites such as cortisol, lactate and creatine phosphokinase. More than anything, the novel conditions that slaughter animals get exposed to prior to slaughter have been generally reported to be stressful thus poor welfare (Grandin, 2001; Ekiz *et al*., 2012; Schwartzkopf-Genswein *et al*., 2012).

While Grandin (2006) reported that slaughter cattle perceive the abattoir environment in the same way as the farm during managerial procedures, moving animals through the race, the abattoir also exposes the animals to a different structure designed more of conventional architectural criteria and less for behavioural characteristics of the animals (Miranda-de la Lama *et al*., 2010). Further, just like transportation, lairaging may be characterised by unfamiliar conditions such as environment, concrete floors as opposed to the natural grassy farm, confinement in lairages, multiple abattoir workers, handling procedures, feed restriction and presence of animals from different farms and species (Hemsworth and Coleman, 1998; Ferguson and Warner, 2008; Terlouw and Porcher, 2005).

Further, lairage duration of over 3 hours was reported to be ideal for slaughter animals to rest and recover from previous stress caused during transportation (del Campo *et al*., 2010). However, it was also reported that feed deprivation during this stage may result to alterations in blood stress indicators such as creatine kinase, lactate dehydrogenase, glucose, cortisol and packed cell volume (Ekiz *et al*., 2012), while longer durations may result to live weight losses through excreting and urinating (Ferguson and Warner, 2008). Multiple indicators such as response behaviour towards certain stimuli and the biochemical changes (in the blood, urine, excreta) have been used to quantify animal welfare in different conditions (Muchenje *et al*., 2009; Gruber *et al*., 2010; Njisane and Muchenje, 2013ab). In addition, avoidance-related behaviour and vocalization have been used to determine animal welfare in abattoirs (Grandin 1998; Manteuffel *et al*., 2004; Hemsworth *et al*., 2011; Njisane *et al*., 2013ab). The objective of this study was therefore to investigate the behavioural, bleed-out times and biochemical responses of Nguni and non-descript beef steers as affected by on- and off-loading, transportation, lairaging and slaughter activities.

5.1 Materials and Methods

Ethical clearance and consent to carry out this study was as described in section 3.1.

5.1.1 Animal and study site description

The animal description is the same as described in section 3.1.2 and part of the study site description is as described in section 3.1.1. In addition, the steers were transported from the farm (section 3.1.1) to the East London Abattoir (High throughput), which is situated in Cambridge, East London, South Africa. Its geographical coordinated are 32.58° S and 27.53° E. Average midday temperatures in East London range between 20˚C (July) to 26˚C

(February) with an annual rainfall of about 593 mm mostly occurring during the summer months. The abattoir operates under typical commercial conditions and is equipped with modern technology to enhance production. It operates according to standard laws and regulations governing abattoirs such as "The Meat Act, 2000, the Animal Protection Act, 1962 and 1935 for animal welfare maintenance" to ensure public health safety.

5.1.2 On- and off-loading and transportation

The steers were scheduled for transportation a day prior to slaughter, allowing them time to rest overnight at the abattoir lairages. They were brought from the farm grazing pastures into the holding pens at least 90 minutes before loading. Prior to loading, the group was randomly divided into two groups of 19 steers for the morning trip (Transport Group 1 - TG1) and another 21 steers for an afternoon trip (Transport Group 2 - TG2). However, the genotype distribution ratio between the two trips was considered and catered for to make sure that both genotypes were represented in the two trips. Verbal instructions were used to encourage the steers to go into the loading area, then into the truck. Due to practical implications, the structure of the facility and to avoid separation stress, group observations were done as opposed to assessing the animals individually. The variables of interest included were: the time it took to load/off-load each group; whether the steers willingly moved or required encouragement to enter/ exit the vehicle; changing direction (opposite) to escape; loss of balance, slipping or falling; and any aggressive behaviour (Mariah *et al*., 2004; Bourguet *et al*., 2011; Stockman *et al*., 2012). The transportation distance was 120 km and it was covered within 1h30min – 2h in a 2010 Mitsubishi (Model: Fuso FM 16-253), with an average speed of 80 km/h. During transportation, the position and posture (e.g. standing or lying down and direction faced) of animals was observed before leaving the farm and on arrival at the abattoir. All steers in both trips were up on their feet throughout transportation period, facing various directions as allowed by the conditions. The weather on the day of transportation was cloudy and cool in the morning with rain in the afternoon.

5.1.3 Lairaging: Duration and time-budgeting

Upon arrival (both trips) at the abattoir, the steers were separated into groups of ten and confined into four roofed lairage pens (5.3 m × 5.3 m). TG1 was lairaged for 20 hours while TG2 for 15 hours. The steers were fasted during lairaging and had *ad lib* access to water. In the same surrounding were other cattle from other farms, sheep and squealing pigs. Hourly response behaviour (to the new conditions) observations were monitored by four observers allocated to each group. The records were made during the first 15 minutes of every hour, from the first hour after arrival to 12:15 midnight. TG1 was observed for 12 hours, while TG2 was observed for 7 hours. The behaviours of interest included: standing, lying down, drinking and interactions amongst the steers (e.g. grooming/head butting). Further observations were done between 6:00am and 7:15am of the following day until the process of regrouping/re-penning began at 8:00am in preparation for slaughter.

5.1.4 Slaughter: Stunning and Exsanguination

The steers were individually weighed (170-310 kg range) using a digital alleyway scale before they were manually moved to the holding pen, then into the stunning box. A captive bolt pistol was used onto the cattle's forehead to render it unconscious before initializing the exsanguination process. Records on the number of stuns used per animal and the response behaviour of each steer inside the stunning box were noted. The behaviour scores recorded in the box were 0-Calm, 1-Head movement up and down avoiding the stunner, 2-Turning to face the opposite direction and 3- Attempts to jump out of the stunning box (Bourguet *et al*., 2011; Stockman *et al*., 2012). Further, Vocalization was recorded as Yes (score 2) or No (score 1) as adapted from Grandin (1998; 2001). The order of slaughter was also taken note of, with the steers grouped into four Slaughter Groups (i.e. SG1, SG2, SG3, and SG4) of 10 animals as they randomly enter the raceway to the stunning box. Immediately after initializing the bleeding process, two exsanguination blood samples (4 ml each) were collected from each animal for glucose, cortisol and lactate analysis. The blood sample storage and processing is described in section 4.1.4. Further, the time it took for each steer to bleed-out was recorded according to Kirton and Woods (1977).

5.1.5 Statistical analysis

A Chi-square test (SAS, 2003) was used to assess for existing associations between genotype, transport/lairage group and slaughter group with the response behaviour and vocalization scores. Frequencies were calculated using Proc freq procedure of SAS (2003). A PROC GLM test (SAS, 2003) was used to test the effect of genotype, transport/lairage group, slaughter group and the number of stun shots on bleed-out times; and exsanguination blood cortisol, glucose and lactate levels. Differences between means were evaluated using Tukey's test. The model used was: $Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + e_{ijklm}$ where, Y_{ijk} is the response variable (bleed-out times; exsanguination blood cortisol, glucose and lactate levels); μ is the mean; α_i is the genotype effect, β_j is the transport/lairage group effect; γ_k is the slaughter group effect; δ_l is the effect of the number of stun shots; and e_{ijklm} is the standard error. Pearson's correlation coefficients for the relationship between bleed-out times, levels of cortisol, glucose and lactate were also determined (SAS, 2003).

5.2 Results

5.2.1 Loading to lairage general observations

Records in Table 5.1 show that the morning group was loaded over a 370 seconds period, while the afternoon group took 410 seconds to get into the truck. The off-loading times after the journey were longer than the loading times. Transportation Group 1 (TG1) collectively took lesser time to get off than Group 2 (TG2). TG1 spent longer time in the abattoir lairages before slaughter, while Group 2 rested for a shorter period compared to the former group.

Table 5. 1: A summary of general observations recorded during loading and off-loading, transportation and lairaging

Transport Group	Transport session	Loading duration (s)	Weather Conditions	Transport Behaviour	Off-loading duration (s)	Lairage Duration (h)	Lairage Behaviour
1 of 1	Morning	205	Cloudy	Standing	362	20	Standing
2 of 1	Morning	165	Cloudy	Standing	240	20	Standing
1 of 2	Afternoon	180	Rainy	Standing	362	15	Standing
2 of 2	Afternoon	240	Rainy	Standing	420	15	Standing

The steers were standing and vigilant during the observations in the truck and lairage pens, with minimal movements around the pen.

5.2.2 Avoidance-related behaviour and vocalization in the stunning box

All the steers of both genotypes showed some kind of avoidance behaviour inside the stunning box, none of them was calm. However, both avoidance-related behaviour (χ^2= 0.0715) and vocalization (χ^2= 0.3112) were not significantly influenced by genotype (Figure 5.1). Over 50% of the steers of both genotypes were moving their head either up and/or down in trying to avoid the gun stunner pointed to their heads. Some of the steers even turned to face the opposite direction they came from; while some attempted to jump out of the stunning box. Furthermore, 5% of ND steers vocalized while the rest, including all the NG steers did not vocalize.

The avoidance related behaviour was significantly influenced by transportation group (χ^2= 0.0246) while vocalization was not (χ^2=0.2870). Figure 5.2 shows that 36.8% of steers that were in the first trip and lairaged longer were moving their head up and/or down, while about 76.2 % that showed this behaviour were in the second trip and lairaged for a shorter period. In addition, 31.6% of the Group 1 steers turned to face the opposite direction with another 31.6% even attempting to jump out of the stunning box; while only a few of the Group 2 steers behaved as such, respectively. Furthermore, the only steers that were vocal belonged to Group 1.

Figure 5.3 show that avoidance related behaviour was significantly influenced by slaughter group (χ^2= 0.0445) while vocalization was not (χ^2=0.3799). Half of the steers slaughtered in the first group (SG 1) were moving their heads up and/or down avoiding the stunner; while some of the total SG1 steers turned to face the opposite direction and others even attempted to jump out of the stunning box. From Group 2 (SG2), 30% head movements, 30% turned to

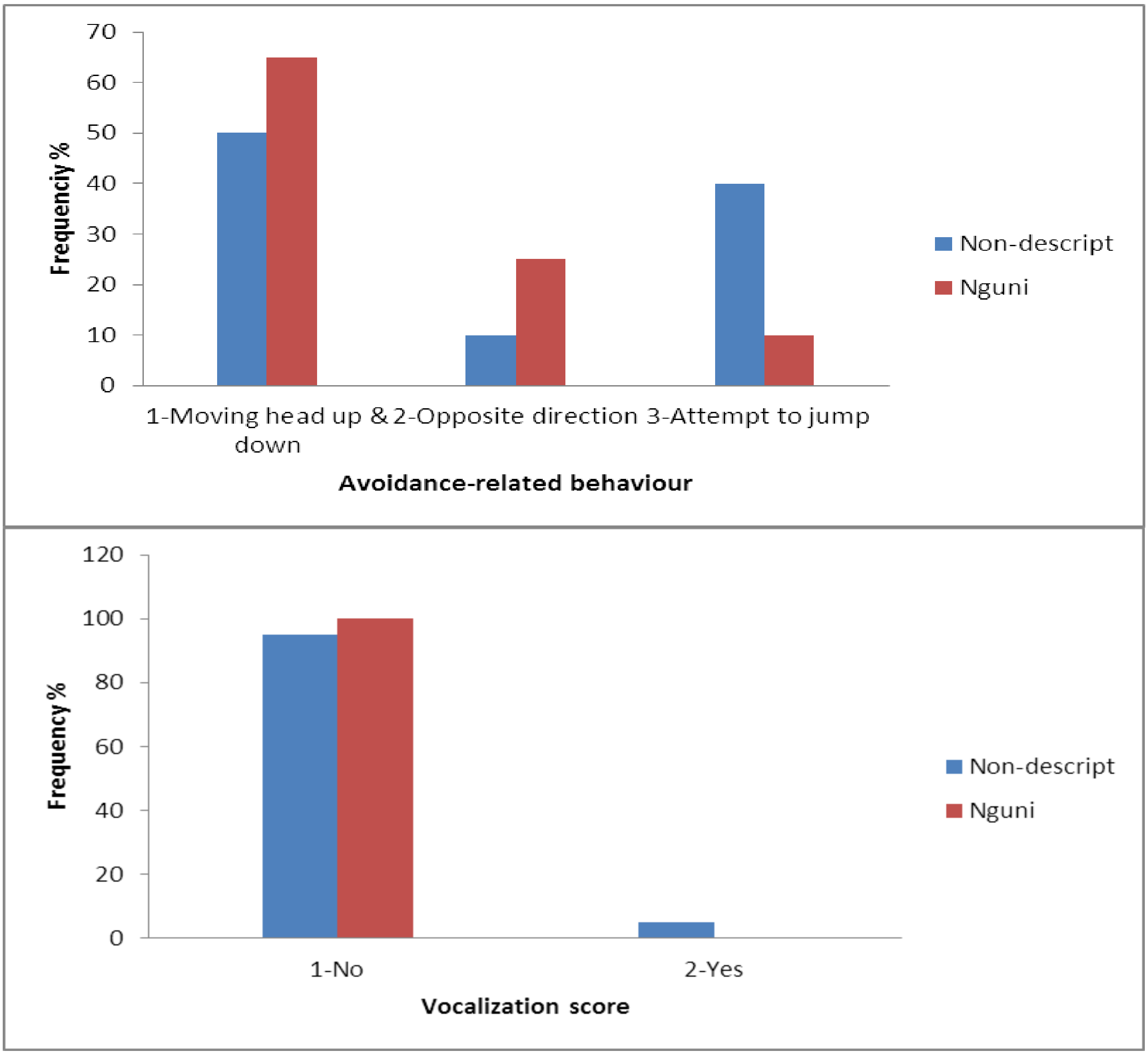

Figure 5. 1: Frequencies of avoidance-related behaviour and vocalization by the two genotypes in the stunning box

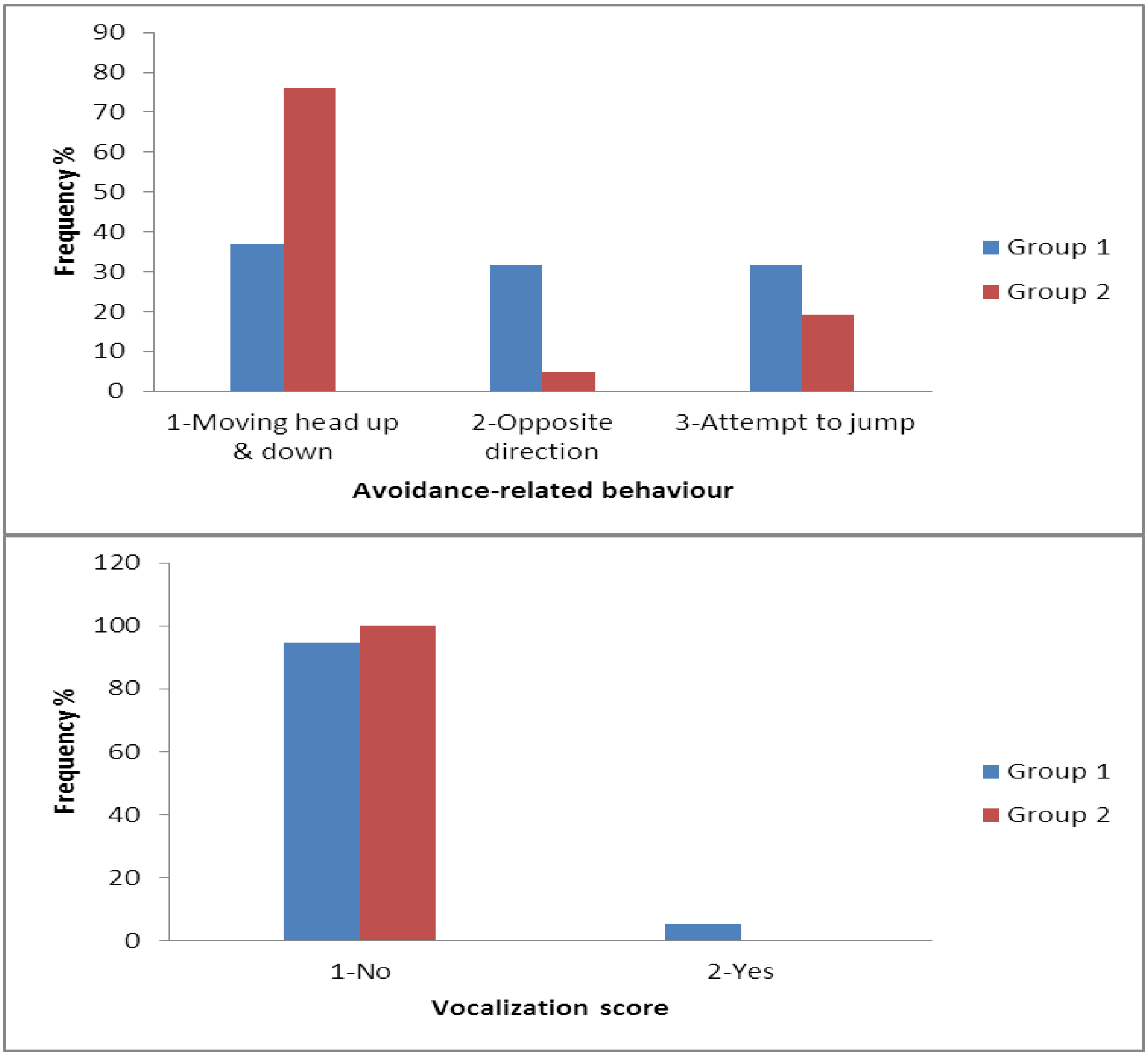

Figure 5. 2: Frequencies of avoidance-related behaviour and vocalization in the stunning box by the two successive groups transported and lairaged

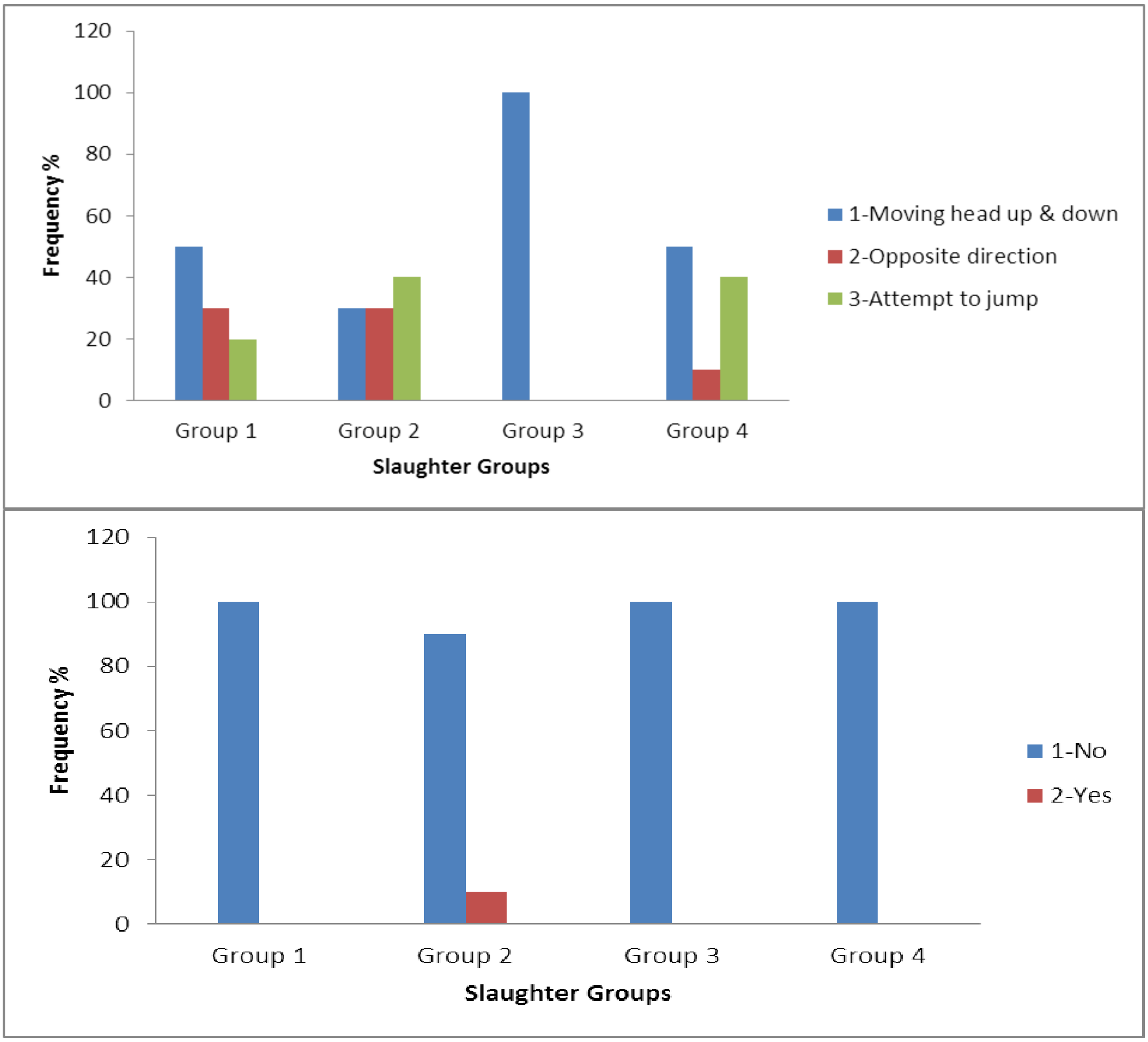

Figure 5. 3: Frequencies of avoidance-related behaviour and vocalization in the stunning box by the four successive groups of slaughter

the opposite direction and 40% tried to jump out were recorded. All the steers that were in Group 3 only moved their heads, avoiding the stunner. The half of the last group (Group 4) showed head avoidance, with 10 % facing the opposite direction and 40% trying to jump out of the stunning box. Vocalization was only recorded in Group 2, while the rest in other groups did not vocalize at all.

5.2.3 Effect of pre-slaughter exposure on the bleed-out times and the blood metabolites

Genotype had no effect on bleed-out times (P=0.9787), cortisol (P=0.7940), glucose (P=0.1556) and lactate (P=0.7659). Both ND and NG steers on average took 76±7.90 seconds to bleed-out. The average serum cortisol levels were 114.5±16.55 nmol/L (ND) and 120.9±15.60 nmol/L (NG). Similarly, the ND steers had 5.3±0.40 mmol/L glucose and 10.6±0.98 mmol/L lactate, while the NG steers had 6.2±0.38 mmol/L glucose and 11.0±0.93 mmlo/L lactate levels.

Furthermore, the bleed-out times (P=0.7191) and glucose (P=0.1523) levels did not differ with transportation group and lairage time. However, Table 5.2 shows that cortisol (P=0.0307) and lactate (P=0.0069) were affected by transportation group and lairage time. Cortisol levels were higher for steers in Group 1 than those in Group 2. Similarly, the lactate levels were higher for steers in Group 1 than those in Group 2.

Similarly, the bleed-out times (P=0.7755) and glucose (P=0.0827) levels did not differ with slaughter group. However, Table 5.3 shows that cortisol (P=0.0031) and lactate (P=0.0408) were affected by slaughter group. Cortisol levels were higher for steers in Group 1, followed by Groups 3 and 4, and then Group 2 had the lowest levels. Additionally, the lactate levels were higher for steers in Groups 1 and 3, then Groups 2 and 4 (8.7 mmol/L).

The number of attempts to stun each steer had no significant effect on bleed-out times (P=0.1185) and the levels of serum cortisol (P=0.2627), glucose (P=0.5743) and lactate

Table 5. 2: Effect of transportation and lairage grouping on bleed-out times (s) and the levels of cortisol (nmol/L), glucose (mmol/L) and lactate (mmol/L) from exsanguination blood (Mean±SE)

Variables	Group 1	Group 2	P-value
Bleed-out times (s)	73.5±8.09	77.6±7.69	0.7191
Cortisol (nmol/L)	140.6^{a}±14.50	92.8^{b}±15.38	0.0307*
Glucose (mmol/L)	5.4±0.38	6.2±0.40	0.1523
Lactate (mmol/L)	12.4^{a}±0.83	9.0^{b}±0.88	0.0069*

Means in the same row with different superscripts are significantly different at P<0.05

Table 5. 3: Effect of slaughter grouping on bleed-out times (s) and the levels of cortisol (nmol/L), glucose (mmol/L) and lactate (mmol/L) from exsanguination blood (Mean±SE)

Variables	Group 1	Group 2	Group 3	Group 4	P-value
Bleed-out times (s)	71.7±11.30	85.5±11.30	70.3±11.30	75.1±11.30	0.7755
Cortisol (nmol/L)	175.9^{a}±17.24	83.3^{c}±17.24	91.2^{b}±19.28	115.8^{b}±22.26	0.0031*
Glucose (mmol/L)	5.1±0.47	5.3±0.50	6.8±0.56	6.5±0.61	0.0827
Lactate (mmol/L)	13.5^{a}±1.12	9.6^{b}±1.12	10.5^{ab}±1.26	8.7^{b}±1.45	0.0408*

Means in the same row with different superscripts and P-values are significantly different at P<0.05

(P=0.2243). However, though it was not statistically significant, the steers that were successfully stunned on first attempt bled longer (79.9±5.71 s) compared to the 2nd (45.0±19.51 s) and 3rd (46.5±23.89 s) attempts, and had the lowest cortisol levels (112.1±11.77 nmol/L) compared to the other two groups (2 attempts=149.6±37.23 nmol/L and 3 attempts=205.0±64.48 nmol/L). Similarly, the former group had the lowest serum lactate levels (10.5±0.70 mmol/L) compared to the 2nd (11.8±2.20 mmol/L) and 3rd (17.1±3.81 mmol/L) attempts. Lastly, the blood glucose levels were more similar with 1st attempt value being 5.8±0.30 mmol/L, 2nd attempt at 6.4±0.93 mmol/L and 3rd attempt at 4.5±1.61 mmol/L.

5.2.4 Correlations amongst the tested variables

There were no significant relationships between the bleed-out times, cortisol, glucose and lactate levels. However, a positive correlation ($r = 0.70$ at $P < 0.0001$) was found between cortisol and lactate levels at the abattoir. This relationship was also observed from the results obtained at the farm throughout the trial; week 1 ($r = 0.49$ at $P = 0.0038$), week 2 ($r = 0.32$ at $P = 0.0507$), week 3 ($r = 0.41$ at $P = 0.0122$), week 4 ($r = 0.62$ at $P < 0.0001$), week 5 ($r = 0.49$ at $P = 0.0021$), week 6 ($r = 0.34$ at $P = 0.0424$), week 7 ($r = 0.39$ at $P = 0.0157$), week 8 ($r = 0.47$ at $P = 0.0024$). In addition, cortisol levels obtained at the abattoir (week 9) positively correlated to week 3 ($r = 0.36$ at $P = 0.0444$) at the farm; while lactate level obtained in week 9 positively correlated with week 3 ($r = 0.41$ at $P = 0.0240$) and week 5 ($r = 0.35$ at $P = 0.0515$) at the farm. Glucose levels from the abattoir did not relate with any levels measured at the farm.

5.3 Discussion

5.3.1 Genotype effect on behaviour, vocalization, Bleed-out times, Cortisol, Glucose, Lactate

The similar response behaviour and physiological changes between the two genotypes may be attributed to the fact that the steers used were introduced to the same treatment and conditions at an early age. They had been running together as one group at the farm since the beginning of the trial until slaughter. These results concur with those reported by Probst *et al*. (2014) that there were no breed effects on any of the stress-indicating traits they measured. In addition, this might also be related to the report that cattle show more of group behaviour than individual, they are herd animals (Grandin and Deesing, 2014). A similar pattern was also seen in two other preceding experiments on the same animals during handling behaviour and blood analysis at the farm (Chapter 4), as well as on time-budgets of these steers (Chapter 3), there were no breed effects picked up. In addition, the steers standing position throughout transportation may be related to the short distance travelled to the abattoir (120 km); while their standing in lairages may have been due to discomfort in a new exposure with concrete and wet floors (raining). Seshoka *et al*. (2013) reported an increase in salivary cortisol during shorter distances travelled than longer.

Though not statistically significant in the current study, ND steers exhibited more avoidance behaviour compared to the NG steers. Some similar results were also obtained in Chapter 4. In addition, the recorded vocalization was from the ND steers. Cross-bred cattle have been reported to have high excitability and hard to handle, compared to pure bred (Grandin, 1989) thus susceptible to stress. In addition, Grandin (2001) reported that vocalization is associated with aversive and physiological measures of stress. Furthermore, the non-significant effect of genotype on the bleed-out times and the exsanguination serum levels of cortisol, glucose and lactate confirm the results obtained on the behavioural observations. Increased levels of cortisol, glucose and lactate are expected to reflect stress-related behaviour and excessive muscle activity (Shaw and Tume, 1992; Gruber *et al*., 2010; Leroy *et al*., 2011). In addition, the relationship observed between abattoir cortisol and lactate levels together with weeks 3 and 5 at the farm agrees with the fact that (Chapter 4) metablolites reflect stress-related behaviour.

5.3.2 Transportation/Lairage group effect on behaviour, vocalization, Bleed-out times, Cortisol, Glucose, Lactate

Though it took shorter time to on- and off-load, TG1 showed more signs of stress compared to TG2 (Figure 5.2 and Table 5.2). This is in contrast with reports that quick loading minimise stress (Maria *et al*., 2004). In addition, it was reported that it takes longer to load and is more stressful than off-loading (Maria *et al*., 2004). The opposite was recorded for the current study, where longer periods were required for off-loading than on-loading. Furthermore, the increased avoidance-related behaviour, as well as the levels of serum cortisol and lactate exhibited by the steers that were transported in the morning (Group 1) and thus lairaged and rested longer at the abattoir was not expected. However, Gruber *et al*. (2010) reported that elevated levels of lactate indicate stress-related behaviour at the abattoir.

The current findings are in contrast with reports that longer resting hours before slaughter improve animal welfare thus better response during slaughter. del Campo *et al*. (2010) reported that resting duration of over 3 hours promotes recovery from the transportation stress. In addition, animals are expected to acclimatize to a specific condition after some time of exposure. However, these reports may be in contrast due to the fact that the abattoir still provides a novel environment to the animals, may even be varying procedures (i.e. frequent handling, concrete floors, food deprivation and noise) from point of arrival through to slaughter, regardless of the resting allowance. Therefore, elongating the time may even exert more stress to the animals. In addition, Probst *et al*. (2014) reported that cattle that spent longer times in the stunning box showed higher cortisol levels.

5.3.3 Effect of slaughter group and Stun attempts on behaviour, vocalization, Bleed-out times, Cortisol, Glucose, Lactate

The steers that were in the front and the end of the slaughter line showed varying response behaviour, with more avoidance, and this phenomenon is not clearly understood. However, it could be attributed to being the first ones in their group to enter the dark alley and the other group being left behind with their herd-mates disappearing. Cattle being herd animals (Grandin and Deesing, 2014) and seeing more going in, group 3 showed less avoidance though an increase was seen again in the last group. Similarly, SG1 had the highest cortisol and lactate levels compared to the rest of the groups. However, Stewart *et al*. (2015) reported that "kill-order" may better reflect acute stress, with successive order showing higher pre-slaughter stress indicators.

In addition, Grandin (2006) reported that the animals are not aware that they will die at the abattoir. Furthermore, the number of attempts to stun each animal may have been found to have no influence on bleed-out times and the blood metabolites. However, reports have been made that the stunning process influence bleeding (Lawrie and Ledward, 2006; Agbeniga and Webb, 2012). Furthermore, sheep and cattle that were stunned using captive-bolt method had more blood yield compared to those that were not stunned (Anil *et al*., 2004; 2006). Therefore, minimal bleeding may be associated with stress. However, the number of stun attempts did not relate to any of the measured stress indicators. In addition, samples that measured higher cortisol correspondingly showed elevated lactate levels as well (Table 4). The current results are in agreement with the reports on the subsequent relationship of the two metabolites (Shaw and Tume, 1992; Gruber *et al*., 2010; Leroy *et al*., 2011). This relationship was observed throughout the trial.

5.4 Conclusion

Steers of different genotypes that are reared together under the same conditions responded the same to identical pre-slaughter conditions exposed to. Avoidance-related behaviour, serum cortisol and lactate levels only changed according to transportation/lairage groups and slaughter groups. The steers that were transported in the morning, thus rested longer in the lairages, were similarly distributed across the avoidance behaviour scores at slaughter with higher cortisol and lactate levels, while the latter group was less avoiding. Those that were in the first slaughter group also had higher cortisol and lactate levels compared to other groups; while Slaughter Groups 1, 2 and 4 steers were distributed amongst the behaviour scores. A positive correlation exists between cortisol and lactate levels. There is a connection between pre-slaughter activities and the behavioural and physiological changes of slaughter steers.

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Chapter 6:
Pre-slaughter activities and their subsequent effect on meat characteristics and beef quality of Nguni and non-descript beef steers reared on natural pastures

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Abstract

The objective of this study was to determine the effect of genotype, muscle type, lairage duration, slaughter group/order and stress responsiveness on pH 24 hours post-mortem (pH_{24}), temperature (T°C), colour (L*, a*, b*, C, HA), thawing (TL) and cooking (CL) losses and Warner Bratzler Shear Force (WBSF) of two muscles from non-descript (ND) and Nguni (NG) steers. Two lairage duration times (20 and 15 hours) were used, Group 1 and 2 (LG1 and LG2); and the steers were slaughtered in four slaughter groups (SG1, SG2, SG3 and SG4). The measurements were done on the Superficial pectoral muscle (Brisket muscles) (M1) and the *longissimus dorsi* (Loin) (M2) of 20 non-descript and 20 Nguni steers following the standard procedures. Some of the selected meat quality characteristics of the two muscles did not differ (P>0.05). However, M1 had the highest CL (29.6±0.51%) and WBSF (30.7±1.35 N), while M2 had the highest HA (39.9±0.57). There were no genotype effects (P>0.05) on the quality of M1; while genotype influenced L* (ND = 33.3±0.74 and NG = 36.8±0.72) and HA (ND = 38.1±0.68 and NG = 41.6±0.66) colour coordinates of M2. Lairage duration influenced the WBSF of M1 (P=0.0335), with steers that were lairaged shorter showing higher force (41.6±2.34 N) than the longer time (34.2±2.40 N). Also, the M2 of the steers that were lairaged longer had a lower TL (2.7±0.24%) than those of shorter time (3.6±0.24%). Slaughter order only affected the TL and WBSF of M1. The last group slaughtered had the lowest TL (2.7±0.39%) compared to the other 3 groups. Slaughter group 3 produced the toughest meat (48.5±2.96 N), with the other groups requiring similar force ranging from 33-36 N. Significant correlations were not obtained between the stress response variables and meat quality characteristics of the ND steers. However, NG cortisol and glucose from exsanguination blood negatively correlated to pH_{24} (r=-0.49) and a*(r=-0.48), respectively. Further correlations were found between the stress response metabolites obtained fortnightly at the farm and some of the selected meat quality characteristics, particularly of the non-descript steers. It was concluded that, muscle type, genotype, lairage duration, slaughter order and stress responsiveness had effects on some of the meat quality characteristics of non-descript and Nguni steers.

Keywords: Lairage duration, slaughter order, stress responsiveness, farm-exposure, beef quality

6 Introduction

The developing world, particularly South Africa, retains climatic and agricultural conditions that support many areas of compatible interest and opportunities such as beef farming (Scholtz *et al*., 2011). In addition, multiple beef cattle genotypes are successfully reared in these conditions; with the Nguni breed slowly gaining recognition for its ability to perform better than other breeds during poor quality feed seasons, at slaughter and for its lean meat (Muchenje *et al*., 2009; Ndlovu *et al*., 2009). Another common genotype, predominantly amongst communal farmers is the non-descript line, which is basically a cross-bred containing unidentified genes due to extensive and uncontrolled mating (Bester *et al*., 2001).

The extensive type of farming, common in South Africa, allows production of multiple animals at a given time, with the aim to meet the increasing demand for protein supply. However, the resulting minimal human-animal interaction at the farm produces animals that are less adapted to handling. Beef production is no exception to this system, either in commercial or communal setups. In a previous experiment (Chapter 3), steers that were subjected to the extensive setup showed normal time budgeting patterns that they developed themselves (Baumont *et al*., 2000), based on environmental conditions, during their roaming time in the paddocks. However in Chapters 4 and 5, these steers had no control over what took place during handling (Gomez and Cook, 2010) and there responded as such towards the stimuli to find balance.

Regardless of the animal's experience at the farm, slaughter cattle get exposed to different conditions from the farm to slaughter. Pre-slaughter events, inclusive of handling and transportation, have been reported to give rise to animal welfare issues and thus poor carcass and meat quality, further causing economic losses (Grandin, 2006). Maria *et al*. (2004) reported that poor meat quality attributes may be a consequence of unacceptable pre-slaughter handling. In addition, the major pre-slaughter stressors may be both psychological through restraint and novel conditions, as well as physical such as hunger or thirst thus; often leading to meat of poor quality (Gregory *et al*., 1998; Muchenje *et al*., 2009). Ultimately, the quality of the meat is dependent on how the slaughter animals respond to the pre- and slaughter process (Lawrie, 1966). Subsequently, Terlouw (2005) reported that these reactions have influence on the ante- and post-mortem muscle metabolism and cause glycogen breakdown and pH variations.

According to Muchenje *et al*. (2009), the acceptable meat pH range is 5.4 – 5.8, with higher values resulting in dark, firm and dry (DFD) meat. Furthermore, the quality attributes of meat like pH, colour, tenderness and water holding capacity are related to some biochemical and physiological changes in the blood and muscle (Muchenje *et al*., 2008a; 2009; Warner *et al*., 2007; Gruber *et al*., 2010). However, most of the results and conclusions were made concentrating only on the loin muscle, even though all the muscles are generally prepared and sold for human consumption. In addition, for quality, quantity and profit protection, the abattoirs sometimes allow sampling of other muscles instead of the loin muscle for research purposes, like the brisket muscle. It is a “minor” cut that is supports movement and exercise and may thus be susceptible to and relevant for pre-slaughter stress studies. Klont *et al*. (1998) reported on varying muscle quality characteristics within an animal; hence it is of importance to study the different muscles and their relationships. Furthermore, some studies have also revealed that pre-slaughter stress may impact the welfare of the animals without necessarily affecting the quality of meat.

In this regard, Maria *et al*. (2004) reported that transportation stress affected the animals' well-being but not the quality of meat harvested from those animals. In addition, though lairaging is beneficial for fatigued animals (Diaz *et al*., 2014), longer lairage time is associated with weight loss (Ferguson and Warner, 2008), low carcass and meat quality (Gallo *et al*., 2003). However, the resulting weight losses that were observed in pigs were reported to have no effect on pork quality (Panella-riera *et al*., 2012). On the other hand, lairaged lambs and rabbits produced low quality meat compared to the ones slaughtered on arrival (Liste *et al*., 2011; Diaz *et al*., 2014). However, these authors further recounted that the meat of lower quality also improved after several days of refrigerating. Therefore the current study sought to determine the effect of genotype, lairage duration, slaughter group/order and stress responsiveness on pH_{24}, temperature, colour (L*, a*, b*, C, HA), thawing (TL) and cooking (CL) losses and Warner Bratzler Shear Force (WBSF) of two muscles from non-descript (ND) and Nguni (NG) steers.

6.1 Materials and Methods

Ethical clearance and consent to carry out this study was as described in section 3.1.

6.1.1 Animal handling and slaughter procedure

The animal description is the same as described in section 3.1.2 and parts of the study site description is as described in section 3.1.1 and 5.1.1. In addition, the steers were kept at the abattoir overnight in groups of 10 (5.3 m × 5.3 m) per pen. The morning group (LG1) was lairaged for 20 h, while the afternoon group (LG2) had a 15 h lairage period. The slaughter procedure began with weighing each steer on the morning of slaughter using a digital alleyway scale and the average slaughter weights ranged between 170-310 kg. The selection of slaughter groups for determination of slaughter order effect is described in section 5.1.4.

Stunning was done using a captive bolt pistol on the cattle's forehead. Exsanguination blood samples (3×4 ml) were collected from each steer for cortisol, glucose and lactate analysis. The blood sample storage and processing is described in section 4.1.4. In addition, eight sets of results, obtained fortnightly from live animals (procedures described in section 4.1) at the farm for cortisol, glucose and lactate results prior slaughter were also captured for used in the current study (Table 6.1). The results showed no genotype effects observed on cortisol (P=0.9125), glucose (P=0.0773) and lactate (P=0.7215); and there were no interactions between genotype and the weekly levels of cortisol (P=0.8605), glucose (P=0.4331) and lactate (P=0.9375).

6.1.2 Meat Sampling and measurements

After dressing, the carcasses were chilled at 4°C for 24 hours before harvesting the meat samples. The 40 carcasses were classified as AB class, with a body conformation score of 3 and a 0 - 1 fat content range. The cold carcass mass ranged between 78.4 – 163.9 kg. Two samples (i.e. *longissimus dorsi* and the Brisket – superficial pectoral muscles) of 200 g were obtained from the left side of each carcass for meat quality analysis. The following were measured from each sample:

Table 6. 1: Average fortnight cortisol, glucose and lactate levels (Mean±SE) obtained at the farm prior slaughter

Week		Cortisol		Glucose		Lactate	
	Genotype	ND	NG	ND	NG	ND	NG
1		127.6^{a}±12.56	125.8^{a}±14.27	4.7^{a}±0.13	5.1^{a}±0.13	5.7^{a}±0.54	5.5^{a}±0.68
2		108.6^{a}±12.56	118.8^{a}±12.25	4.4^{ab}±0.12	4.7^{b}±0.12	5.5^{a}±0.54	6.0^{a}±0.58
3		87.9^{b}±12.22	103.9^{ab}±12.95	4.3^{b}±0.12	4.4^{bc}±0.13	4.2^{b}±0.53	4.2^{ab}±0.64
4		136.8^{a}±12.56	117.6^{a}±11.94	4.6^{a}±0.13	4.7^{b}±0.12	4.7^{a}±0.54	4.0^{bc}±0.57
5		106.2^{a}±12.56	112.3^{a}±11.94	4.3^{b}±0.12	4.2^{c}±0.12	3.1^{bc}±0.57	3.1^{bc}±0.57
6		83.2^{b}±12.56	92.2^{ab}±12.25	4.2^{b}±0.12	4.2^{c}±0.12	2.6^{c}±0.54	2.8^{c}±0.6
7		116.2^{a}±12.59	112.8^{a}±11.94	4.1^{b}±0.12	4.1^{c}±0.12	3.4^{c}±0.6	2.8^{c}±0.57
8		86.6^{b}±12.22	75.2^{b}±11.94	3.7^{c}±0.12	3.7^{d}±0.12	3.5^{c}±0.53	3.2^{b}±0.57

Table adapted from Chapter 4 results. ND (non-descript), NG (Nguni); means in the same column with different superscripts are significantly different at $P<0.05$.

6.1.2.1 Ultimate pH and Temperature

Meat pH_{24} and temperature (°C) were measured using a portable pH meter (Crison pH 25, Crison instruments, S.A., Alella, Spain). The pH meter was calibrated using pH 4 and 7 standard solutions before taking the actual readings.

6.1.2.2 Colour

Meat colour coordinates L^* (lightness), a^* (redness) and b^* (yellowness) were measured using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine (illuminant: D65; Visual angle: 10º; diameter measurement: 20 mm). Calibrations were done using the white standards prior to measurements. Three different locations of the sample were measured by rotating the machine and the averages were recorded as the final values for statistical analysis. Further, Chroma (C) and hue angle (HA) values were calculated as C = $(a^{*2}+b^{*2})1/2$ and HA = tan−1 (b* / a*), respectively. Before further measurements, the samples were frozen at -4°C for a total of 26 days.

6.1.2.3 Thawing and cooking losses

Frozen weights were measured before thawing the samples at room temperature (±25°C). Weighing was done using a PGW 753i Adam weighing scale. The samples were weighed again after complete defrosting. Percentage thawing loss (TL %) was calculated as:

$$Thawing\ Loss\ (TL)\% = \frac{Weight\ before\ thawing - Weight\ after\ thawing}{Weight\ before\ thawing} \times 100$$

Then the samples were cooked in a water bath at 85 °C for 45 minutes, cooled at room temperature and then weighed again. Percentage cooking loss (CL %) was calculated as:

$$Cooking\ Loss\ (CL)\% = \frac{Weight\ before\ cooking - Weight\ after\ cooking}{Weight\ before\ cooking} \times 100$$

6.1.2.4 Warner Bratzler Shear Force

The cooked and cooled samples were further analysed for tenderness using Warner Bratzler Shear Force (WBSF), measured in Newton (N). Three sub-samples of each sample harvested from each animal were cored parallel to the grain of the meat thus making a total of 60 (3 subsamples × 20 steers/genotype) subsamples per genotype. These samples were sheared perpendicular to the fibre direction were subjected to a Warner Bratzler (WB) shear device, mounted on a Universal Instron apparatus (Model 3344; cross head speed: 400mm/min; one shear in the centre of each core). 3 subsamples were cored from each of the animals/genotype,. The maximum mean load recorded for the three cores represented the WBSF value of each sample.

6.1.3 Statistical analysis

PROC GLM test (SAS, 2003) was used to test the effect of genotype, lairage duration, slaughter group/order and pre-slaughter stress physiological response on pH24, temperature, colour (L*, a*, b*, C, HA), thawing (TL) and cooking (CL) losses and Warner Bratzler Shear Force (WBSF). Comparison of means was performed using Tukey's test. Differences were considered significant at P<0.05. The model used was: $Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + e_{ijklm}$ where, Y_{ijk} was the response variable (L*, a*, b*, C, HA, TL, CL and WBSF); μ was the mean; α_i was the genotype effect, β_j was the lairage duration effect; γ_k was the slaughter group/order effect; δ_l was the muscle effect; $(\alpha\beta)_{ij}$ was the interaction between genotype and lairage duration; $(\alpha\gamma)_{ik}$ was the interaction between genotype and slaughter order; $(\alpha\delta)_{il}$ was the interaction between genotype and muscle type; and e_{ijklm} is the standard error. Pearson's correlation coefficients for the relationship between levels of cortisol, glucose and lactate from different stages of pre-slaughter handling, and the meat quality variables were also determined (SAS, 2003).

6.2 Results

The selected meat quality characteristics, namely pH, T°C, L*, a*, b*, C and TL of the two muscles used in the current study were the same (P>0.05). There were no significant interactions (P>0.05) between the measured variables. However, Table 6.2 shows that the *L. dorsi* muscle had a higher HA colour variable and WBSF than those of the Brisket muscle. Additionally, the Brisket muscle had the highest CL than the *L. dorsi* muscle. Genotype did not influence (P>0.05) the pH_{24}, Temperature (°C), Colour variables (L*, a*, b*, C and HA), thawing loss (TL %), cooking loss (CL %) and tenderness (WBSF) of the Brisket muscles.

Though most of the selected meat characteristics of the *L. dorsi* muscle were also not affected (P>0.05) by genotype, Table 6.3 shows that the Nguni (NG) steers had lighter meat than that of non-descript (ND) steers. Similarly, the NG steers produced meat with a higher Hue angle than that of the ND steers.

Table 6.4 shows that lairage duration had no effect on meat pH_{24} (P = 0.6888), T°C (P = 0.8396), L* (P = 0.5732), a* (P = 0.8264), b* (P = 0.7162), C (P = 0.8507), HA (P = 0.7791), TL (P = 0.6076) and CL (P = 0.1961) of the Brisket muscle. However, lairage duration influenced the WBSF, with steers that were lairaged for a shorter period (LG2) requiring more force to shear than the meat from those lairaged for a longer time.

Table 6.5 shows that lairage duration had no effect on meat pH24 (P = 0.1637), T°C (P = 0.5591), L* (P = 0.9760), a* (P = 0.4559), b* (P = 0.3897), C (P = 0.3953), HA (P = 0.8865), CL (P = 0.7528) and WBSF (P = 0.6475) of the *L. dorsi* muscle. However, the TL was influenced by lairage duration, with steers that were lairaged for a longer period (LG1) having meat with a higher TL than the meat from those lairaged for a shorter time (LG2).

Table 6. 2: Least square means and standard errors of means of the selected meat quality characteristics of the Superficial pectoral muscle (Brisket) and the *L. dorsi* muscles from non-descript and Nguni steers

Variables	Brisket (n=40)	*L. dorsi* (n=40)	P-value
pH_{24}	5.8±0.02	5.8±0.02	0.3233
Temperature (°C)	11.3±0.36	10.7±0.36	0.2408
L* (Lightness)	34.3±0.56	35.1±0.56	0.3185
a* (Redness)	14.4±0.35	14.0±0.35	0.4103
b* (Yellowness)	11.2±0.32	11.7±0.32	0.3442
C (Chroma)	18.4±0.44	18.3±0.44	0.9131
HA (Hue angle)	37.8^{b}±0.57	39.9^{a}±0.57	0.0106*
Thawing loss (TL) %	3.6±0.19	3.1±0.19	0.1122
Cooking loss (CL) %	29.6^{a}±0.51	22.7^{b}±0.51	<.0001*
WBSF (N)	30.7^{b}±1.35	38.0^{a}±1.35	0.0003*

Means in the same row with different superscripts are significantly different at *P<0.05; WBSF- Warner Bratzler Shear Force

Table 6. 3: Least square means and standard errors of means of the selected meat quality characteristics from the *L. Dorsi* muscle of the non-descript and Nguni steers

Variables	Non-descript (n=20)	Nguni (n=20)	P-Value
pH_{24}	5.8±0.02	5.8±0.02	0.4680
Temperature (°C)	10.7±0.55	10.7±0.55	0.9500
L* (Lightness)	33.3^{b}±0.74	36.8^{a}±0.72	0.0017*
a* (Redness)	14.9±0.56	13.2±0.55	0.442
b* (Yellowness)	11.7±0.47	11.7±0.46	0.9360
C (Chroma)	18.9±0.70	17.7±0.68	0.2243
HA (Hue angle)	38.1^{b}±0.68	41.6^{a}±0.66	0.0008*
Thawing loss (TL) %	3.1±0.26	3.2±0.26	0.9514
Cooking loss (CL) %	21.8±0.77	23.7±0.75	0.0831
WBSF (N)	31.0±1.06	30.4±1.03	0.6655

Means in the same row with different superscripts are significantly different at $P<0.05$; WBSF- Warner Bratzler Shear Force

Table 6. 4: Least square means and standard errors of means of the selected meat quality characteristics of the Superficial pectoral muscle (Brisket) muscle by lairage duration

Variables	LG1 (n=19)	LG2 (n=21)	P-value
pH_{24}	5.8±0.02	5.8±0.02	0.6888
Temperature (°C)	11.2±0.46	11.4±0.46	0.8396
L* (Lightness)	34.6±0.76	34.0±0.74	0.5732
a* (Redness)	15.1±0.19	15.0±0.19	0.8264
b* (Yellowness)	11.1±0.46	11.4±0.45	0.7162
C (Chroma)	18.3±0.55	18.4±0.54	0.8507
HA (Hue angle)	37.6±0.86	37.9±0.84	0.7791
Thawing loss (TL) %	3.7±0.29	3.5±0.28	0.6076
Cooking loss (CL) %	30.2±0.65	29.0±0.63	0.1961
WBSF (N)	34.2^{b}±2.40	41.6^{a}±2.34	0.0335*

Means in the same row with different superscripts are significantly different at *P<0.05; LG1- Lairage group one (duration-20 h); LG2- Lairage group 2 (duration-15 h); WBSF- Warner Bratzler Shear Force

Table 6. 5: Least square means and standard errors of means of the selected meat quality characteristics of the *L. Dorsi* muscle by lairage duration

Variables	LG1 (n=19)	LG2(n=21)	P-value
pH_{24}	5.8±0.02	5.8±0.02	0.1637
Temperature (°C)	10.5±0.55	10.9±0.55	0.5591
L* (Lightness)	35.0±0.85	35.1±0.82	0.9760
a* (Redness)	14.3±0.59	13.7±0.57	0.4559
b* (Yellowness)	12.0±0.46	11.4±0.45	0.3897
C (Chroma)	18.7±0.71	17.9±0.69	0.3953
HA (Hue angle)	39.9±0.79	39.8±0.77	0.8865
Thawing loss (TL) %	3.6^{a}±0.24	2.7^{b}±0.24	0.0209*
Cooking loss (CL) %	22.9±0.80	22.6±0.78	0.7528
WBSF (N)	31.0±1.06	30.3±1.03	0.6475

Means in the same row with different superscripts are significantly different at *P<0.05; LG1- Lairage group one (duration-20 h); LG2- Lairage group 2 (duration-15 h); WBSF- Warner Bratzler Shear Force

Slaughter order did not influence (P>0.05) the pH24, Temperature (°C), colour variables (L*, a*, b*, C and HA), thawing loss (TL %), cooking loss (CL %) and tenderness (WBSF) of the *L. dorsi* muscle. Though most of the selected meat characteristics of the Brisket muscle were also not affected (P>0.05) by genotype, Table 6.6 shows that TL and WBSF were both influenced (P = 0.0492 and P = 0.0025, respectively) by this order. Slaughter groups (SG) 1, 2 and 3 showed similar TL, while the last group (SG4) had the lowest percentage. Furthermore, SG1, SG2 and SG4 had similar WBSF, while SG3 had the highest force. With the exception of pH_{24} and the cortisol levels, as well as the a* colour variable and the glucose levels of Nguni steers (NG), there were no further significant (P>0.05) correlations obtained between the stress response variable (cortisol, glucose and lactate) and the selected meat quality characteristics (pH_{24}, temperature, colour variables, thawing loss, cooking loss and tenderness) of the NG and the non-descript (ND) steers. NG cortisol negatively correlated (r = -0.49) to pH_{24} (P = 0.0409), as well glucose negatively correlating (r = -0.48) to the meat redness (a*) of the loin muscle (P = 0.0501).

Table 6.7 shows significant negative correlations between ND cortisol levels at the farm during handling and meat pH_{24} in weeks 5 (P = 0.0330), 7 (P = 0.0077) and 8 (P = 0.0078); L* (P = 0.0083) in week 7; and HA in weeks 2 (P = 0.0020), 7 (P = 0.0046) and 8 (P =0.0217). From the NG steers, negative correlations were obtained between cortisol and the meat T°C (P =0.0351) in week 1; L* (P = 0.0494) in week 3; and a positive correlation with T°C (P = 0.0270) in week 8. The rest of the tested variables had no significant (P > 0.05) correlation to one another.

Table 6. 6: Least square means and standard errors of means of the selected meat quality characteristics of the *L. Dorsi* muscle by slaughter order

Variables	SG1 (n=10)	SG2 (n=10)	SG3 (n=10)	SG4 (n=10)	P-value
pH_{24}	5.8±0.03	5.8±0.03	5.9±0.03	5.8±0.04	0.7115
Temperature (°C)	10.7±0.61	11.0±0.61	12.5±0.61	11.0±0.68	0.1871
L* (Lightness)	35.8±1.03	33.4±1.03	34.1±1.03	33.8±1.09	0.3886
a* (Redness)	15.1±0.27	15.0±0.27	15.2±0.27	15.0±0.28	0.9159
b* (Yellowness)	11.7±0.64	10.9±0.64	11.3±0.64	11.0±0.68	0.8172
C (Chroma)	18.8±0.77	18.2±0.77	18.3±0.77	18.0±0.82	0.9189
HA (Hue angle)	38.6±1.20	36.7±1.20	38.1±1.20	37.6±1.26	0.6930
Thawing loss (TL) %	3.4^{ab}±0.37	4.1^{a}±0.37	4.0^{a}±0.37	2.7^{b}±0.39	0.0492*
Cooking loss (CL) %	30.7±0.91	28.9±0.91	29.8±0.91	29.0±0.96	0.4897
WBSF (N)	33.0^{b}±2.96	34.3^{b}±2.96	48.5^{a}±2.96	36.0^{b}±3.12	0.0025*

Means in the same row with different superscripts are significantly different at *P<0.05; SG-Slaughter groups; WBSF- Warner Bratzler Shear Force

Table 6. 7: Correlations between fortnight cortisol levels during handling at the farm and the selected meat quality characteristics of the non-descript and Nguni steers

Variables	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Genotype				**Non-descript**				
pH_{24}	0.39	0.24	-0.01	0.45	0.07	-0.11	0.35	0.20
Temperature (°C)	-0.32	-0.11	-0.14	-0.39	-0.50*	0.71	-0.62**	-0.59**
Lightness (L*)	-0.45	-0.25	-0.09	-0.10	-0.23	0.01	-0.62**	0.31
Redness (a*)	0.38	0.21	0.27	0.24	0.29	-0.14	0.40	0.33
Yellowness (b*)	0.16	-0.15	-0.07	-0.01	0.07	-.033	0.02	0.04
Chroma (C)	0.31	0.08	0.20	0.15	-0.22	-0.22	0.26	0.23
Hue angle (HA)	-0.39	-0.68**	-0.34	-0.46	-0.38	-0.36	-0.65**	-0.52*
Thawing Loss (%)	0.09	-0.04	-0.08	-0.31	-0.13	0.25	-0.02	0.04
Cooking Loss (%)	-0.03	0.02	0.10	0.20	0.12	-0.12	-0.06	-0.03
WBSF (N)	-0.16	0.04	0.27	-0.13	0.25	0.37	0.01	0.09
Genotype					**Nguni**			
pH_{24}	-0.57*	0.05	-0.24	-0.02	-0.10	-0.04	-0.30	-0.04
Temperature (°C)	0.23	0.21	0.24	0.22	0.31	0.04	-0.05	0.51*
Lightness (L*)	0.29	-0.08	-0.48*	-0.22	-0.24	-0.11	-0.06	-0.40
Redness (a*)	-0.15	-0.11	0.09	-0.34	-0.28	0.08	0.02	-0.01
Yellowness (b*)	0.22	-0.11	-0.17	-0.40	-0.35	0.08	0.05	-0.28
Chroma (C)	0.02	-0.11	-0.02	-0.39	-0.33	0.08	0.03	-0.13
Hue angle (HA)	0.45	-0.02	-0.31	-0.03	-0.06	-0.04	0.02	-0.41
Thawing Loss (%)	0.21	-0.04	-0.07	-0.32	-0.25	-0.05	0.06	-0.12
Cooking Loss (%)	-0.26	-0.31	-0.31	0.02	0.18	0.39	0.03	-0.21
WBSF (N)	-0.15	-0.13	-0.04	0.14	0.17	0.30	0.31	0.08

*P<0.05; **P<0.01; WBSF- Warner Bratzler Shear Force

Table 6.8 shows a significant positive correlation between ND glucose levels at the farm during handling and meat pH_{24} in weeks 5 (P = 0.0162), 7 (P = 0.0229) and 8 (P = 0.0043). In addition, there were negative correlations obtained between glucose and T°C (P = 0.0030) in week 6; and HA in weeks 2 (P =0.0083), 4 (P = 0.0132), 5 (P = 0.0119), 6 (P = 0.0276) and 7 (P = 0.0121). From the meat harvested from NG steers, positive correlations were obtained between glucose and the b* value (P =0.0104), C (P = 0.0274) and CL% (P = 0.0383) in week 4. The rest of the tested variables had no significant (P > 0.05) correlation to one another.

Table 6.9 shows a significant positive correlation between ND lactate levels at the farm during handling and meat pH_{24} in weeks 1 (P = 0.0037), 2 (P = 0.0257), 3 (P = 0.0172) and 4 (P = 0.0187). In addition, there were negative correlations obtained between the same lactate and b* (P = 0.0451) in week 1; and HA in weeks 1 (P =0.0053), 2 (P = 0.0026), 4 (P = 0.0083), 5 (P = 0.0022), 6 (P = 0.0005) and 7 (P = 0.0105). From the NG steers, only C correlated (P = 0.0458) to lactate levels obtained in week 4. The rest of the tested variables had no significant (P > 0.05) correlation to one another.

Table 6. 8: Correlations between fortnight glucose levels during handling at the farm and the selected meat quality characteristics of the non-descript and Nguni steers

Variables	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Genotype				**Non-descript**				
pH_{24}	0.26	0.55	0.20	0.31	0.56*	0.30	0.34*	0.64**
Temperature (°C)	-0.31	-0.32	-0.49	-0.26	-0.10	-0.64**	-0.24	-0.36
Lightness (L*)	-0.09	-0.13	-0.43	0.002	0.01	-0.31	-0.19	-0.38
Redness (a*)	-0.05	-0.10	0.36	0.07	-0.14	0.07	-0.01	0.02
Yellowness (b*)	-0.14	-.041	0.16	-0.24	-0.43	-0.19	-0.32	-0.19
Chroma (C)	-0.08	-0.23	0.30	-0.05	-0.25	-0.03	-0.14	-0.07
Hue angle (HA)	-0.16	-0.60**	-0.35	-0.51**	-0.58**	-0.50*	-0.59*	-0.41
Thawing Loss (%)	0.17	-0.33	-0.05	-0.13	-0.16	-0.29	-0.40	-0.30
Cooking Loss (%)	-0.07	0.06	-0.09	0.03	-0.10	-0.16	-0.23	0.01
WBSF (N)	0.39	-0.11	-0.01	0.24	0.05	-0.24	0.03	-0.34
Genotype				**Nguni**				
pH_{24}	-0.15	-0.20	0.12	-0.31	0.03	0.26	-0.07	-0.22
Temperature (°C)	-0.44	-0.02	-0.31	-0.16	0.09	-0.02	-0.22	0.05
Lightness (L*)	0.08	0.19	-0.13	0.05	0.08	0.15	-0.16	0.09
Redness (a*)	0.01	0.001	-0.12	0.42	0.01	0.05	-0.12	-0.04
Yellowness (b*)	0.15	0.19	-0.17	0.59*	0.20	0.19	-0.11	0.15
Chroma (C)	0.09	0.09	-0.15	0.52*	0.10	-0.12	-0.11	0.04
Hue angle (HA)	0.31	0.36	-0.08	0.17	0.27	0.16	0.10	0.27
Thawing Loss (%)	0.12	0.28	-0.18	0.22	0.17	-0.05	0.01	-0.12
Cooking Loss (%)	0.26	0.08	-0.19	0.49*	0.36	039	-0.04	0.22
WBSF (N)	-0.05	-0.20	-0.14	0.08	-0.18	-0.39	-0.22	0.07

*P<0.05; **P<0.01; WBSF- Warner Bratzler Shear Force

Table 6. 9: Correlations between fortnight lactate levels during handling at the farm and the selected meat quality characteristics of the non-descript and Nguni steers

Variables	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Genotype				Non-descript				
pH_{24}	0.65**	0.52*	0.54*	0.55*	0.30	0.27	0.44	0.02
Temperature (°C)	-0.33	-0.32	-0.32	-0.14	-0.28	-0.35	-0.32	-0.29
Lightness (L*)	-0.18	-0.19	-0.10	-0.19	-0.25	-0.33	-0.40	-0.31
Redness (a*)	-0.18	0.13	0.09	0.26	0.33	0.23	0.28	0.40
Yellowness (b*)	-0.48*	-0.22	-0.11	-0.07	-0.03	-0.17	-0.07	0.15
Chroma (C)	-0.30	0.001	0.02	0.14	0.21	0.09	0.16	0.32
Hue angle (HA)	-0.63**	-0.66**	-0.31	-0.60**	-0.71**	-0.72**	-0.60**	-0.40
Thawing Loss (%)	-0.37	0.23	-0.04	-0.09	0.07	-0.01	-0.02	0.19
Cooking Loss (%)	-0.06	-0.07	0.10	0.11	-0.02	-0.16	0.10	0.10
WBSF (N)	-0.16	-0.05	-0.10	-0.01	0.40	0.14	-0.30	0.29
Genotype					Nguni			
pH_{24}	0.07	-0.003	-0.12	-0.13	-0.14	0.05	0.13	0.03
Temperature (°C)	-0.13	0.13	0.05	-0.03	0.01	-0.20	-0.27	0.02
Lightness (L*)	0.24	0.03	0.11	0.37	0.04	-0.003	-0.21	0.11
Redness (a*)	-0.26	-0.36	-0.24	-0.52	-0.21	-0.24	-0.11	-0.25
Yellowness (b*)	-0.04	-0.27	-0.07	-0.32	-0.12	-0.35	-0.28	-0.21
Chroma (C)	-0.17	-0.34	-0.18	-0.45*	-0.17	-0.30	-0.19	-0.24
Hue angle (HA)	0.24	0.24	0.22	0.42	0.21	-0.12	-0.24	0.11
Thawing Loss (%)	0.39	-0.09	0.23	-0.07	0.02	0.01	0.28	-0.05
Cooking Loss (%)	0.05	0.30	0.25	0.43	0.24	0.18	0.12	0.32
WBSF (N)	-0.26	0.22	-0.38	-0.33	-0.13	-0.07	0.13	-0.26

*P<0.05; **P<0.01; WBSF- Warner Bratzler Shear Force

6.3 Discussion

6.3.1 Quality of the two selected muscles

The current study showed that the two tested muscles (Brisket and *L. dorsi*) had some similar meat quality characteristics. However, even though it was still within an acceptable range, the *L. dorsi* (Loin) muscle was tougher with lower cooking losses (CL) and a more stable colour than the Brisket muscle. It is not clearly understood as to how the Loin muscle would be tougher than the Brisket, given that the former muscle is less exposed to movement/exercise compared to the latter, which according to literature may later result to toughening of meat (Muchenje *et al.*, 2008a; 2009; Young *et al.*, 2009). However, there is a fat cap that is often attached to the brisket muscle, especially the superficial pectoral muscle, thus keeping the meat from drying out during cooking (Prime, 2016). In addition, tougher meat has been associated with higher CL (Warner *et al.*, 2007; Gruber *et al.*, 2010), and yet the opposite was reported in the current findings. There is no apparent explanation for this phenomenon. However, Lawrie (1985) and Klont *et al*. (1998) recounted that due to metabolic and contractile properties of the specific muscle fibres, a large variation exists in the quality of the meat produced within an animal, a breed and across species.

6.3.2 Genotype effect on the quality of the two selected muscles

Though a significant difference in the lightness (L*) of the meat from the two genotypes was obtained, both values were still within the acceptable levels. Muchenje *et al*. (2008a) reported that L* values less than 33 indicate darkness. The *L. dorsi* muscle from the Nguni (NG) steers was lighter, with a more stable (HA) colour compared to the non-descript (ND) steers. In contrast, Muchenje *et al*. (2009) found the meat harvested from the Nguni breed to be less light (L* value) than the other tested beef breeds (Bonsmara and Angus). They further reported these colour variations to be possibly due to other biochemical and physiological factors such as muscle fibre type, sarcomere length and glycolytic potential. These parameters were unfortunately not measured in the current study. Specifically, the current results showed that the pre-slaughter stress responsiveness mostly affected the Brisket muscle to a greater extent than the Loin muscle.

6.3.3 Lairage duration effect on the quality of the two selected muscles

The steers that were lairaged for a shorter period produced a tougher (higher WBSF) Brisket muscle than those of longer lairage duration. These results concur with multiple findings that animals that have had minimal or no resting allowance before slaughter produce poor quality meat (Warner *et al.*, 1998; Gallo *et al*., 2003; del Campo *et al*., 2010; Ekiz *et al*., 2012). However, these results are in contrast with those that lairaged lambs produced poor quality meat than those slaughtered on arrival (Diaz *et al*., 2014). Elongated lairage time may result to prolonged periods of discomfort and anxiety, thus dehydration. In addition, the thawing loss (TL) of the former group was lower than the latter. This could mean that the tougher meat with low TL had limited excess water to eliminate compared to tender and high TL muscle. However, it may also be noted that the difference was 0.9 %. According to Muchenje *et al*. (2008a), a difference of less than 3 units is essentially too small. The rest of the selected meat quality characteristics of both tested muscles were not affected by differences in lairage duration. These results are in agreement with findings that lairage duration may influence weight loss but has no effect on meat quality (Panella-riera *et al*., 2012).

6.3.4 Slaughter order effect on the quality of the two selected muscles

The slaughter order only affected two of the Brisket muscle quality characteristics and not the loin muscle. Different muscles of the same animal are likely to differ in quality parameters (Lawrie, 1985; Klont *et al*., 1998). The highest WBSF was recorded for the third slaughtered group; which from a recent trial (Chapter 5) was amongst those with the least cortisol and lactate levels during slaughter. These results are the opposite of the reports that stress responsiveness towards pre-slaughter stimuli later reflects in the quality of meat (Grandin, 1980; Muchenje *et al*., 2008a; 2009). However they concur with findings which imply that pre-slaughter activities and stress responsiveness may have an effect on the welfare of the animal and not necessarily affect the quality of the meat the animal produces (Maria *et al*., 2004; Liste *et al*., 2011; Panella-riera *et al*., 2012). Furthermore, these results are in contrast to those of TL of steers slaughtered in Groups 1, 2 and 3 were similar

6.3.5 Correlations between the selected meat quality characteristics of the loin muscle and stress responsiveness at the farm and at the abattoir by genotype

Stress responsiveness during slaughter affected the NG steers' meat pH_{24} and C, but not ND meat quality characteristics. These results concur with a report by Muchenje *et al*. (2009) that the relationships between stress responsiveness and certain beef quality characteristics may differ with breed type. The NG meat pH_{24} decreased with increasing cortisol and these results correspond to results reported by Okeudo and Moss (2005) on initial pH of mutton. The redness of the NG loin muscle decreased with increasing glucose and this could be attributed to the fact that colour variations are a result of biochemical and physiological changes (Muchenje *et al*., 2009). These results concur with those reported by Muchenje *et al*. (2008b) on the same breed. They further recounted that inadequate energy intake may affect amount of the glycogen reserves in the muscle due reduced circulatory glucose and cholesterol levels.

In addition, the ND steers muscle was mostly affected during handling at the farm than the NG steers muscle. This could mean that NG steers cope better in a familiar environment (farm) than an unfamiliar one (abattoir), compared to the ND steers. However, previous trials (Chapters 3, 4 and 5) on the same animals, based on response behaviour and blood metabolite analysis showed that NG steers performed better than ND steers both at the farm and at the abattoir. This could then mean that there are variations in terms of relating pre-slaughter stress and meat quality produced; there is no clear pattern. Higher plasma cortisol levels during handling at the farm in weeks 3 and 4 were related to lower L* values on NG and ND loin muscle, respectively and this approve findings that associates increased pH with dark cuttings (Muchenje *et al.*, 2009; Gruber *et al.*, 2010). In addition, the ND meat was also losing colour stability with increasing pH_{24}.

Furthermore, the ND meat temperature drop was related to high cortisol levels obtained in weeks 5, 7 and 8; while that of NG meat rose with higher cortisol in week 8. It could be that some muscles retain temperature better than others due to some inter-animal and across-animals biochemical and physiological factors. Finally, ND meat pH_{24} dropped with increasing cortisol levels obtained in week 1 of handling. The higher ND plasma glucose levels obtained in weeks 5, 7 and 8 at the farm during handling were associated with increased meat pH_{24} levels; reduced colour stability in weeks 2, 4, 5, 6 and 7; and reduced muscle temperature. Choe *et al.* (2009) reported that presence of glucose in the blood and glycogen in the muscle promotes lactic acid formation thus a higher meat pH. It was also reported that DFD meat results from rapid muscle glycogen depletion and low lactic acid production (FAO, 2001).

Furthermore, the NG meat had a more red (b*) and saturated (C) colour with reported elevated glucose levels. This could be a result of other factors such as genetic make-up and not necessarily related to glucose or lactic acid. In addition, the same meat had a higher CL, which is in agreement with results that glucose in the blood results to tougher meat with higher cooking losses (Warner *et al.*, 2007; Gruber *et al.*, 2010). Though it has been reported that lactate levels in the blood results in tougher meat with higher cooking losses (Gruber *et al*., 2010); there were no such relationships obtained in the current study. However, increased lactate in the first four weeks of the farm handling trial and stress responsiveness related to elevated ND meat pH_{24}. However, according to Edwards *et al*. (2010), elevated lactate levels (> 10 mM) in the blood relate to low pH values in the meat. In addition, like glucose and cortisol, increased lactate levels encouraged a decrease in ND meat redness and colour intensity of NG meat. This concurs with reports that meat colour variations result from biochemical and physiological changes (Muchenje *et al*., 2009).

6.4 Conclusion

In conclusion, muscle type, genotype, lairage duration, slaughter order and stress responsiveness have an effect on some of the meat quality characteristics of non-descript and Nguni steers. The relationships between stress responsiveness and the quality of meat produced may vary across genotypes and stage of production, i.e. non-descript meat was not influenced by exsanguination cortisol, glucose and lactate levels; while Nguni meat was not affected by these levels during handling at the farm. In addition, pre-slaughter events may affect the welfare of the animal but still have minimal or no effect on the quality of some meat/muscles harvested from that animal.

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Chapter 7
General Discussion, Conclusions, and Recommendations

7 General Discussion

The main objective of the study was to determine the effect of farm to abattoir environmental conditions on some behavioural and physiological responses, as well as the quality of meat from Nguni (NG) and non-descript (ND) beef steers reared extensively on natural pastures; and their subsequent effect on selected meat quality characteristics. The effects of some weather and periodical variations on time budgets and body weights of Nguni and non-descript steers reared extensively on natural pastures were determined in Chapter 3. Chapter 4 focused on determining the effects of on-farm successive handling on some behavioural and physiological responses of Nguni and non-descript beef steers. The pre-slaughter effects on response-behaviour, bleed-out times and some blood physiological responses of Nguni and non-descript beef steers were determined in Chapter 5. Finally, the meat quality characteristics of Nguni and non-descript beef steers were measured in Chapter 6.

The results obtained in all the experiments show that genotype had no effect on time-budgets; had minimal influence on some response behaviour during handling at the farm and no effect during the pre- and slaughter activities at the abattoir. Furthermore, genotype had no effect on the serum physiological changes at any of the production stages. Grandin and Deesing (2014) reported that cattle and sheep are herd animals and therefore respond as such. Steers in the same herd show more of a group reaction rather than individual. In Chapter 3, it was obtained that some time-budgets of beef steers reared in natural pastures were influenced by temperature, humidity, observation week and time of the day. Literature has also reported that cattle, sheep and goats time-budgets change with seasons (Dodzi and Muchenje, 2012), time of the day (Decruyenaere *et al*., 2000; Baumont *et al*., 2000; Bakare and Chimonyo, 2011), production system (Jansen *et al*., 2005; Munksgaard *et al*., 2005; Grant, 2007; Krawczel and Grant, 2009; Gomez and Cook, 2010; Dodzi and Muchenje, 2012) and type of animal (Provenza *et al*., 2003; Bakare and Chimonyo, 2011; Dodzi and Muchenje, 2012).

In Chapter 4, steers of both genotypes reacted similarly to handling during managerial procedures at the farm. These reactions were clearly reflected in the serum levels of cortisol, glucose and lactate. Increased glucose, cortisol and lactate levels are indicators of stress-related behaviour and excessive muscle activity (Shaw and Tume, 1992; Gruber *et al*., 2010; Leroy *et al*., 2011); mostly triggered by fear through the activation of the hypothalamic-pituitary-adrenal activity (Ferguson and Warner, 2008) during handling (Tarrant *et al*., 1992; Hulbert *et al*., 2011). Similar results were also obtained in Chapter 5, triggered by pre-slaughter handling, transportation and the slaughter process itself. There were no genotype effects on time-budgets in the lairages; avoidance-related behaviour and vocalization in the stunning box; and bleed-out times, cortisol, glucose and lactate levels. However, Steers that spent lesser time in the lairages responded, behaviourally and physiologically, similarly and in some cases even better than those that had longer resting duration. This is in contrast with literature that longer lairage durations improve animal welfare (del Campo *et al*., 2010).

Furthermore, the steers that were at the beginning and the end of the slaughter line showed more avoidance-related behaviour, with higher cortisol and lactate levels compare to those that were in the middle. The reason for this may be that they were not bothered much as they were surrounded by their herd-mates before and after them; while the first steers where only faced with a new environment and the last ones were completely "separated" from the others. However; the quality of meat (Chapter 6) did not reflect these findings as is expected to (Ferguson and Warner, 2008; Muchenje *et al*., 2009; Hemsworth *et al.*, 2011). The middle groups produced tougher meat with higher thawing loss than the other groups. In addition, the only genotype effect was observed on the some colour variables of the loin muscle; with the NG meat being lighter and more colour-stable.

The reason for these variations may have be due to other biochemical and physiological factors (Muchenje *et al*., 2009) in both the blood and the muscle, hence the unclear pattern between the two stages. Furthermore, lairage duration affected the different meat quality variables of the two tested muscles. Though the effect was practically small, the thawing loss of the loin muscle was influenced with shorter lairage periods showing lower loss than the longer lairaged steers. However, the Brisket muscle of the shorter lairaged meat was tougher than that of steers lairage for longer time. These do not reflect the stress responsiveness results obtained in Chapter 5, where shorter lairage periods produced lower cortisol and lactate levels. This implies that stress responsiveness prior slaughter may not necessarily influence the quality of meat produced. However, the current results showed that to an extent, some behavioural and biochemical stress responsiveness at the farm may be used to estimate the quality of meat to be harvested from that animal.

7.1 Conclusion

It can be concluded that the conditions and activities at the farm, during transportation, lairaging and slaughter at the abattoir have an influence on some behavioural and physiological changes and the quality of beef harvested from the Nguni and non-descript steers that were extensively-reared in natural pastures. However, the relationship patterns between these different conditions are not clear. In addition, the two genotypes used in the current study showed similar response patterns to common stimuli, as far as behaviour and blood metabolites. However, a genotype differences were seen on some meat colour variables, with the Nguni meat being lighter and stable that the non-descript. Pre-slaughter effects also show some level of influence towards different muscle types. Lastly, some pre-slaughter effects on the quality of meat from specific breeds may be traced back to stress responsiveness at the farm and not necessarily at the abattoir.

7.2 Recommendations

- From a general South African perspective, there is still need to train farmers on the importance of good stockmanship on all levels of the meat production chain. The stakeholders, especially the employees need to understand the importance of animal welfare with regards to both the animal and the consumer safety.

- There is need to conduct this complete farm to abattoir trial using different time frames, longer and/or seasonally; also considering animal background such as other breeds, gender and age; as well as the production systems so as to attain larger pool of findings and conclusions.

- There is need to do a comprehensive study on meat quality characteristics in this regard, considering other biochemical and physiological impacts such as glycolytic potential, quality of muscle fibres, sarcomere length, water holding capacity, and others.

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Appendices

Appendix 1: Record sheet used to capture time budget activities

Animal Number:				Evaluator Name:				
	Cattle behaviour in a natural grazing paddock							
Time	**Grazing**	**Browsing**	**Drinking**	**Standing**	**Sitting**	**Walking**	**Mounting**	**Other Interactions**
6:00-6:15								
7:00-7:15								
8:00-8:15								
9:00-9:15								
10:00-10:15								
11:00-11:15								
12:00-12:15								
13:00-13:15								
14:00-14:15								
15:00-15:15								
16:00-16:15								
17:00-17:15								
18:00-18:15								
19:00-19:15								
20:00-20:15								

Other interactions: Head butting, grooming, etc.

Appendix 2: Record sheet used to capture avoidance related behaviour at the farm during handling

Tag number	RS	ES	WBS	SS	ESS	Weight

Entry Score (ES): 1- No encouragement (required to move the animal); 2- Slight encouragement (vocal instruction); 3- Some encouragement (vocal and prodding); 4- Force (pushing the animal to move)

Weighing Box Score (WBS): 1- Calm (just standing), 2- Agitated (in panic, shaking the box-trying to escape) or 3- Vocalizing (making sounds)

Stepping Score (SS): 1- Not kicking; 2- Light kick (minor and gently); 3- Aggressive kick (violent)

Exit Speed Score (ESS): 1- Walked (normal walk), 2- Trotted (rushing/jumping out); 3- ran (running out)

Appendix 3: Data sheet used to capture details during loading at the farm and off-loading at the abattoir

Group:	Loading time:	Compartment:
	Number of animals/activity	Other Comments
Stop		
Reversals/change of direction		
Motivation (1/2)		
Slip/loss of balance		
Vocalization (yes/no)		
Elimination (urine/dung)		
Mounting		
Aggression/fights		
Group:	Offloading time:	Compartment:
	Number of animals/activity	Other Comments
Stop		
Reversals/change of direction		
Motivation (1/2)		
Slip/loss of balance		
Vocalization (yes/no)		
Elimination (urine/dung)		
Mounting		
Aggression/fights		

Motivation scoring: 1-Slight (vocal only); 2-Medium (Vocal and physical)

Appendix 4: Data sheet used to record avoidance-related behaviour, vocalization and number of stun attempts inside the stunning box during slaughter

Tag Number	Avoidance Behaviour	Vocalization	Stunning comment

Avoidance Behaviour: 0-Calm; 1-up/down heard movement; 2-turn to opposite direction; 3-attempts to jump.
Vocalization Scores: **1-**No vocal; **2-**Yes vocals.
Stunning Comments: number of stuns

Appendix 5: Data sheet used to record the bleed-out times during exsanguination

Tag number	Initial time (s)	Final time (s)	Actual time

Initial bleed-out time: Start of blood flow, gushing out
Final bleed-out time: Blood flow changed from a constant stream into drips
Actual bleed-out time: Time interval between the initial and final times

Appendix 6: Preliminary paper presented 60th International Congress of Meat Science and Technology, 17-22rd August 2014, Punta del Este, Uruguay

THE EFFECT OF THE FARM AND THE ABATTOIR ENVIRONMENTS ON CATTLE BEHAVIOUR, BLOOD HORMONES AND METABOLITES AS STRESS INDICATORS

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Abstract-The objective of the study was to assess cattle behaviour, blood hormones and metabolites as stress indicators, influenced by the farm and the abattoir activities. Thirty, 12 months old castrates were observed for behaviour response at the farm feedlot and holding pens/crush; and at the abattoir lairage, race. Two sets of blood samples were obtained from each animal, both at the farm (live animals-from the tail) and the abattoir (exsanguination). Glucose, cortisol and lactate levels were analysed from the blood samples. Results showed that the cattle spent most of their time in the feedlot eating and resting. Removal from the feedlot to the holding pens resulted in agitation and trying to escape, frequent urination and loose excreta; which were associated with stress. At the abattoir, the cattle spent all the time standing until the slaughter time; looking around curiously and sniffing on animals in other pens (from different farms). Blood hormones and metabolites concentration registered at the farm did not show differences from that determined at the abattoir. It can be concluded that the abattoir environment and breed type have a different effect on cattle response behaviour compared to the farm. However these two environments had no effect on blood hormones and metabolites.

I. INTRODUCTION

Slaughter animals are subjected to at least three different environments in their lifetime before they are slaughtered and converted to meat. These include the farm where they are reared, which is characterised by minimal activities; the unstable and moving transportation vehicle; and the eventful, loud abattoir/slaughter house. It has been reported that these conditions could induce pre-slaughter stress on animals (1; 2; 3). This may be a great challenge as pre-slaughter stress is associated with reduced meat quality.

Bourguet *et al.* (4) reported that exercise and psychological stress just before slaughter increases muscle metabolic activity, which may continue after death, resulting in faster post-mortem pH decline. This can furthermore be linked to decreased meat quality (1). Knowledge of the response behaviour and physiological response of the animal can be used to minimise any losses or reduction in productivity that may be posed by stress. Moreover; this would result to known animal health status (5); ensure good animal social status and an effective and economical production enterprise (6).

Slaughter animals were reported to be unaware of what would happen to them at slaughter; they perceive the pre-slaughter distractions and changes as those of the farm during managerial procedures like vaccinations and dipping (7). This then results to similar behaviours in both environments. However; in addition to the behaviour response, blood hormones and metabolites have been used further as indicators of stress determinants. Ferguson and Warner (8) reported that disturbance in the environment activates the hypothalamic-pituitary-adrenal activity due to fear; resulting in the release of catecholamines and cortisol (9) which furthermore elevates blood lactate and glucose. Therefore the objective of this study was to assess the relationship between cattle response-behaviour, blood hormones and metabolites as stress indicators influenced by the farm and the abattoir activities.

II. MATERIALS AND METHODS

Ethical clearance

All procedures conducted on animals for the purpose of this research were done meeting the worldwide ethical principles considerations. Consent to carry out the study was approved and issued by the University of Fort Hare Ethical

Clearance committee (Reference Number: MUC03S1NJI01).

Study site

The data was collected from two study sites, the farm and the abattoir situated at around 120 km east of Alice Town. These two sites are about 30 km away from each other and are privately owned by the same group of people for business purposes.

Site one (farm): is situated in Berlin, King William's Town and its geographical coordinates are 32˚53'0" South and 27˚35'0" East. The average midday temperatures ranges between 20˚C (July) to 27˚C (February) with an annual rainfall of about 502 mm occurring mostly in summer. The vegetation around this area is comprised of Acacia Karroo bushes and the grasses involve species like *Themeda triandra*, *Eragrostis capensis*, *Heteropogon contortus* and others in a dense and sour-like form.

Site two (East London abattoir): is situated in Cambridge, East London. Its geographical coordinated are 32˚58'0" South and 27˚53'0" East. Average midday temperatures in East London range between 20˚C (July) to 26˚C (February) with an annual rainfall of about 593 mm mostly occurring during the summer months.

The abattoir operates under typical commercial conditions and is equipped with modern technology to enhance production. It operates according to standard laws and regulations governing abattoirs such as "The Meat Act, 2000, the Animal Protection Act, 1962 and 1935 for animal welfare maintenance" to ensure public health safety. According to the description report on different abattoirs by the RMAA (10), the current one is a high throughput abattoir.

Animal Transportation and resting periods

Animals were transported by truck for an hour from the farm to the abattoir and they were slaughtered on the same morning they arrived. They were allowed to rest at the abattoir holding pens (5.3 m × 5.3 m) with 7-9 cattle per pen, and had access to water for about 3 hours before slaughter. There was minimal animal-human interaction as the animals directions were led by the narrow crush connecting the holding pens and restraining or stunning areas.

Animal Description and Data Collection

A group of 30 twelve months old castrates were randomly picked from the farm herd and were used for the current study. These were 7 Angus, 14 Brahman and 9 Brangus breeds. Ear tagging was used to mark and identify.

The cattle were observed for response behaviour as a group at the farm feedlot and holding pens/crush; and at the abattoir lairage and race. The technique used was the direct observation of spontaneous behaviour with 3 assessors taking part. Behaviour assessment looked at the comfortability and the emotional state of the animals by looking at the animal's body language (11). This determined whether the animals were free and happy around each other and in the environment as a whole. Factors described in Table 1 below were noted; where group 1 represented positive behaviour and group 2 being the negative behaviour.

Table 1 Animals emotional state observation at farm

Group 1 (positive behaviour)	Group 2 (negative behaviour)
Active, Relaxed, Calm Content, Friendly, Playful Positively occupied, Lively Inquisitive, Happy.	Fearful, Agitated, Indifferent Frustrated, Bored, Irritable Uneasy, Apathetic, Distress.

Modified from Mounier (11)

Two sets of blood samples were obtained from each animal both at the farm and the abattoir for glucose, cortisol and lactate analysis (blood stress hormones and metabolites). This was done to measure the effect of both on cattle's stress levels.

Central circulatory blood was collected from the tail/caudal vein once at the farm through the palpation of animal's tail, vein identification, then a puncture using a needle connected to the vaccutainer tube and its holder. Blood was drawn and sucked into the vaccutainer tube. The second set of blood samples were collected during the process of exsanguination after the stunning processes. The samples were kept in ice until separation of serum through centrifuging at 21^{o}C for 5 min at 3000 rpm. Analysis of the samples was done in two laboratories; University of Pretoria Pathology lab (cortisol and lactate) and Victoria hospital laboratory (glucose).

Statistical analysis

The effect of environment and breed on behaviour was analysed using PROC FREQ of

SAS (12) statistical package. The effect of the environment and animal characteristics on blood hormones and metabolite levels was analysed by means of Proc GLM for ANOVA.

III. RESULTS AND DISCUSSION

Overall observations on behaviour responses

The cattle in the current study showed positive behaviour at the feedlots (Table 1; Figure 1). This is a normal scenario as reported by literature that cattle spend most of their time feeding and resting at the feedlots (13; 14; 15). Moreover; the animals were calm and content around one another, showing no signs of aggression or fighting. However when they were driven out of the feedlot to the holding pens/race and into the crush, a negative set of response behaviour was observed in some (Figure 1). This movement can be related with the invasion that normally happens when the management activities like vaccination are conducted at the farm. During blood sampling at the holding pens/crush, most of the animals (56.7%) were observed to show aggression (negative behaviour, Figure 1) involving attempt to kick, escape and vocalization. This set of behaviour was also observed when the castrates were moved from the lairage through the race to the stunning box. This could be attributed with the human interference with the animals. Broom and Fraser (6) reported that lack of control or destructions in the animal's environment may result to strange behaviours in response to trying to adjust. In addition, frequent urination and release of loose faeces were observed. This was thought to be related to the adrenalin hormone release due to the aggressive behaviour.

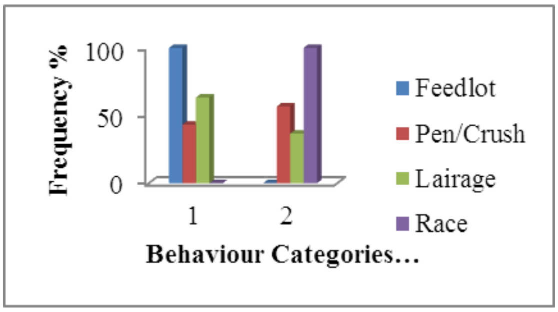

Figure 1. General Frequencies in behaviour scores at the feedlot, holding pen/crush, abattoir lairage and race

However this was not the case at the abattoir lairage. Animals were observed to possess more of the positive (63.3%) behaviour than the negative one (Table 1); they were calm and curious. This was contrary to the expected results that animals would respond better at the farm than the new environment which is the abattoir. Moreover; Grandin (5) reported that from her on-farm (feedlot) and abattoir observations on animal behaviour, she discovered that cattle behaved the same but went on to say that dangling chains and disturbances on their paths around abattoir races resulted to fear thus refusal to move. This could explain the other 36.67% that showed negative behaviour at the lairage. In addition, Bourguet *et al.* (4) also reported that presence of physical distractions like shiny objects, humans, and changes on light intensity from light to dark induce a sense of anxiety.

All breeds were calm at the feedlots and agitated at the abattoir race(Figure 1). Figure 2 shows that all Angus castrates (53.8%) possessed positive behaviour (calm-) at the farm holding pens and in the crush, and at the abattoir lairage; whereas the Brahman (82.4%) showed negative behaviour (aggression, kicking). This was expected because of the Brahman's notorious nature of being disobedient. On the other hand, the crosses between the two (Brangus) were mostly positive in both areas (Pen/Crush-46.2% and Lairage-31.6%). This could be a result of hybrid vigor, which generally allows offspring from two different breeds to perform better than its parents. Moreover; Lanier *et al.* (16) reported that different types of animals may have different physiological and behavioural responses to the same procedure.

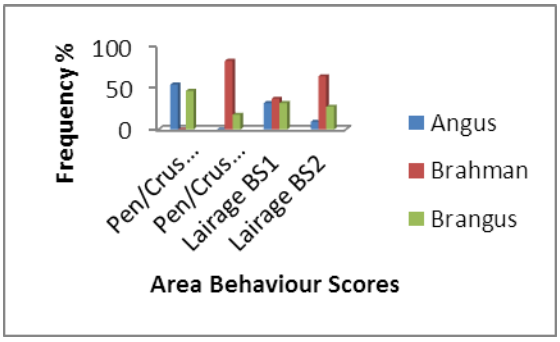

Figure 2. Frequencies across breeds in behaviour scores at the holding pen/crush and abattoir lairage

Blood analysis results

The animals' stress hormones and metabolites did not differ with either environment or animal characteristics. This may have been due to a smaller sample of 40 cattle used so far. Moreover; it could be related to that cattle perceive the transportation and slaughter process the same way they see the management activities (dipping, vaccination) that normally take place at the farm. The slaughter animals do not really know that they will die (5). Hence there is no elevated reaction than normal. It could also be linked to the fact that in both situations at the farm and abattoir, humans were present to influence the animal's response behaviour by moving the animals from one point to another before the sampling took place.

IV. CONCLUSION

It can be concluded that environment and breed differences have an effect on cattle response behaviour. However; the blood glucose, cortisol and lactate do not differ with environmental changes. Moreover; more data samples need to be obtained around the same matter to give room to variations.

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Appendix 7: Preliminary paper presented 61st International Congress of Meat Science and Technology, 23-28th August 2015, Clermont-Ferrand, France (1)

ON-FARM HANDLING BEHAVIOUR, BLOOD GLUCOSE, LACTATE AND CORTISOL LEVELS OF NGUNI AND NON-DESCRIPT BEEF STEERS REARED ON NATURAL PASTURES

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Abstract – The study was conducted to assess the on-farm handling behaviour in relation to the blood glucose, lactate and cortisol levels of Nguni (NG) and non-descript (ND) beef steers reared on natural pastures. Forty castrates of 20 NG and 20 ND were observed monthly for behaviour, recorded as Race Score (RC), Entry Score (ES), Weighing Box Score (WBS), Stepping Score (SS), and Exit Speed Score (ESS). Blood samples were obtained from each animal's caudal vein for glucose, lactate and cortisol analysis. Monthly weights of the animals were recorded. Cortisol did not differ (P>0.05) between genotypes, month and body weight. The NG steers had the highest glucose levels (4.7±0.7) compared to the ND (4.4±0.5). Higher lactate values were recorded in February (5.5±2.5) compared to March (4.2±2.3). There were no associations (P>0.05) between month and all the behaviour observation scores. Genotype was only associated (P<0.05) with the race score behaviour. The NG (21%) showed more confidence to walk through the race without stopping compared to ND steers (14%). Most ND's were reluctant to move forward (stopping) in the race compared to NG's (19%). NG responded better to handling and thus used up less glucose compared to ND. Lactate levels dropped with time of sampling.

Key Words – Genotype, Response behaviour, Stress hormones & metabolites.

V. INTRODUCTION

Cattle farming has greatly contributed towards the success of the red meat industry. There is a vast number of beef breeds reared in South Africa. Amongst them is the hardy indigenous Nguni breed which has functional characteristics which allows it to survive and reproduce in any given environment (1). The ND, also known as cross bred is also contributing greatly to the South African beef industry. Scholtz *et al*. (2) reported a 35% record of ND bulls found in the emerging sector.

Beef producing cattle are normally reared extensively during their early stages of life and are sometimes transferred to intensive systems during the fattening and finishing stages (3). Normal farm operations that require close human-animal interactions include time to time managerial activities and/or routines such as castration, dipping, branding, and vaccination, among others. Cattle destined for beef production are normally weaned and castrated at 7-10 months of age. Raussi (4) and Probst *et al*. (3) reported these events to be unpleasant for animals, to the extent that fear towards humans (who carry out these routines) might develop. In order to deal with certain situations at the farm, animals may respond and even develop certain behaviours or strategies (5) (i) towards each other during resting and feeding in the kraals/feedlots and, (ii) during management routines, when humans are involved through feeding or any other activity that seems to be interfering with their space.

On-farm behaviour and animal-human interaction assessments, regarding welfare and productivity, have mostly been conducted on dairy cows (6; 7) compared to beef cattle. A report by "A Farm Sanctuary Report" from a farmer's point of view stated that, unlike dairy cows, beef cattle are less accustomed to being handled and are thus a little less experienced with human interactions. Ultimately, the underlying factor that has much influence to ensure good quality product from farm animals is to maximize good management in favour of animal welfare at the farm.

In the presence of potential stressors, animals do not only change in behaviour, but also some physiological changes occur. Animal handling, transport and slaughter were noted to be stressors that produced significant changes in blood-hormonal levels in cattle. Disturbance in the environment activates the hypothalamic-pituitary-adrenal activity due to fear (8), thus leading to the release of catecholamines and cortisol (9) and further elevate blood lactate and glucose. Blood, urine and saliva have been used to extract hormones and metabolites such as catecholamines, cortisol, blood lactate and glucose and others for this determination. The current study then seeks to assess the on-farm handling behaviour in relation to the blood glucose, lactate and cortisol of Nguni (NG) and non-descript (ND) beef steers reared on natural pastures.

VI. MATERIALS AND METHODS

Ethical clearance

All procedures conducted on animals for the purpose of this research were done meeting the worldwide ethical principles considerations. Consent to carry out the study was approved and issued by the University of Fort Hare Ethical Clearance committee (Reference Number: MUC03S1NJI01).

Study site

The study was conducted at the University of Fort Hare's Honeydale Research Farm. It is situated 120 km inland from the coastline, in the False Thornveld of the Eastern Cape of South Africa. It is located at 32.78° S and 26.85° E, at an altitude of 520 m above sea level. The topography of the area is generally flat with few slopes. The mean annual temperature of the farm is 18.7°C. The hot-wet season is characterized by hot sunny weather and thunderstorms with average temperature range of 17 °C to 28 °C. The area receives low annual rainfall of approximately 480 mm per annum both between and within seasons. The vegetation is a mixture of several trees, shrubs and grass species. Plant species, such as *Acacia karroo*, *Themeda triandra, Panicum maximum, Digitaria eriantha, Eragrostis sp.*, *Cynodon dactylon and Pennisetum clandestinum* are the predominant species.

Animal Description

From an ongoing study, forty 12-18 month-old Nguni and Non-descript cattle breeds sourced from 3 farms and kept and at the Fort Hare Honeydale farm were used for this study. The bulls were castrated, vaccinated, drenched, dipped, tagged and allowed 3 weeks of acclimatizing before the trial began. The initial weights of these animals ranged between 120-250 kg and they were grazing on natural pastures with access to water points.

Blood Sampling

Central circulatory blood was collected from the caudal vein through a puncture using a needle connected to the vaccutainer tube and its holder. The samples were kept in ice until separation of serum through centrifuging at 21^{o}C for 5 min at 3000 rpm before analysis.

Behaviour Scoring

The Race Scores (RC) were recorded as not-stopping (1) or stopping (2) while in the race; Entry Score (ES) was measured by no encouragement required to move the animal (1), slight encouragement (2), some encouragement (3), Force the animal to move (4); Weighing Box Score (WBS) was whether the animal was calm (1) or agitated (2) in the box; Stepping Score (SS) was determined by no kicking (1), light kick (2) and aggressive kick (3); and Exit Speed Score (ESS) was scored as walked (1), trotted (2) and ran (3).

Statistical analysis

The association between month, breed and the behaviour observation scores (RS, ES, WBS, SS, ESS) was analysed using Chi-square of SAS (10) statistical package. The effect of breed and month and their interaction (month and breed) on cortisol, glucose and lactate was analysed by ANOVA using PROC GLM with weight considered as a covariate. Differences between means were evaluated using tukey's test. The model used was: $Y_{ijk} = \mu + \alpha_i + \lambda_j + (\alpha\lambda)_{ij} + \beta_1 X_1 + e_{ij}$

VII. RESULTS AND DISCUSSION

Table 1 shows that there were no associations (P > 0.05) between month and all the behaviour observation scores. Breed was only associated (P < 0.05) with the race behaviour scores. It was reported that cattle often perceive an encounter with humans as predatory (11) such that they develop certain behaviours or strategies, trying to cope (5). However, the NG steers (21%) showed more confidence (Figure 1) in walking through the race without stopping compared to the ND steers (14%). Most ND steers were reluctant to move forward (stopping) through the race compared to Nguni steers (19%), their avoidance instinct kicked in and hence encouragement was required to move them further.

Table 1: The association between month, breed and handling behaviour observation scores

Variables	*RS*	*ES*	*WBS*	*SS*	*ESS*
Breed	P <0.05	NS	NS	NS	NS
Month	NS	NS	NS	NS	NS

NS-Not Significant, significant difference at P<0.05, Race Scores (RC), Entry Score (ES), Weighing Box Score (WBS), Stepping Score (SS), and Exit Speed Score (ESS)

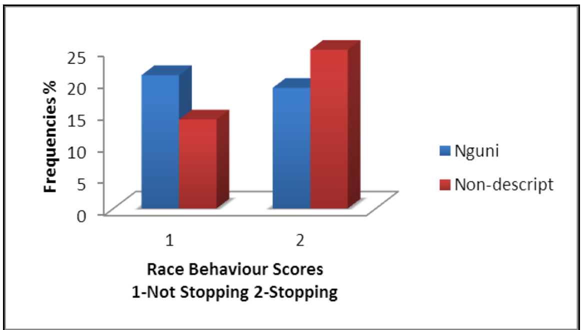

Figure 1: The frequencies of Nguni and Non-descript behaviour scores in the race during handling

Table 2 shows that glucose differed (P < 0.05) with breed. The NG steers had the highest glucose levels (4.7 ± 0.7) compared to the ND (4.4 ± 0.5). Lactate also differed (P < 0.05) with month. The steers recorded higher lactate levels (5.5±2.5) in the beginning of the trial in the month of February compared to March (4.2±2.3). Gruber *et al*. (12) associated raised lactate concentrations with more stress-indicating behaviour. Therefore, the current results show an improvement trend with progressing months. It could be attributed to the steers getting used to handling. Cortisol did not differ (P > 0.05) with breed, month and weight. The weight of the animals had no effect (P > 0.05) on glucose, lactate and cortisol levels.

Table 2: The effect of breed, month and weight on cortisol, lactate and glucose levels

Variables	Breed		Month	
	NG	**ND**	**Feb**	**Mar**
Glucose	4.7^{a} ±0.7	4.4^{b}±0.5	NS	NS
Lactate	NS	NS	5.5^{a}±2.5	4.2^{b}±2.3

Means in the same row with different superscripts are significantly different at P<0.05, NS-Not Significant, NG-Nguni, ND-Non-descript.

Generally, the NG breed is known for being hardy and able to cope in harsh conditions. Muchenje *et al*. (13) reported in a study on three beef breeds that the NG steers had the lowest (P < 0.05) catecholamine concentrations in response to pre-slaughter encounters compared to the Bonsmara and Angus breeds; making them less susceptible to pre-slaughter stress. Moreover, the low glucose levels recorded for ND steers can be related to their reluctance to move through the race and thus more energy utilized, through high muscle activity, while trying to avoid handling and moving forward. Increased physical stress can result to glucose levels decreasing. In contrast, glucose was reported to increase more with handling and transportation on temperamental cattle than on calm ones (14; 15).

VIII. CONCLUSION

The obtained results conclude that the NG steers were less susceptible to stress and thus had higher glucose levels in the blood, compared to the ND steers. The lactate levels for the group dropped with time of sampling.

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Appendix 8: Preliminary paper presented 61st International Congress of Meat Science and Technology, 23-28th August 2015, Clermont-Ferrand, France (2)

BLOOD GLUCOSE, CORTISOL AND SOME MEAT QUALITY CHARACTERISTICS OF COWS FROM BEEF AND DAIRY PRODUCTION SYSTEMS

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Abstract – The objective of the study was to assess blood glucose and cortisol, pH_{24}, colour (L*, a*, b*), thawing-drip-cooking losses and Warner Bratzler Shear Force (WBSF) of slaughter cows from a dairy and a beef production system. Forty four cows from two different breeds (19 Friesland and 25 Bonsmara) were used for the study. Animal behavior was observed during lairaging. Two exsanguination blood samples were obtained from each cow for glucose and cortisol analysis. Furthermore, 44 meat samples were harvested from the Brisket muscle for measurements of pH_{24}, colour (L*, a*, b*), thawing loss (TL), drip loss (DL), cooking loss (CL) and WBSF. Glucose levels did not differ (P>0.05) between breeds, while Cortisol was higher in Bonsmara (273.7±18.0) than Friesland (104.9±20.7). Colour variable b* and TL did not differ (P>0.05) between breeds. The Friesland had higher pH24 (5.9±0.6), CL (37.8±1.1) and WBSF (54.1±2.2) than the Bonsmara (5.7±0.5, 33.6±0.9 and 45.8±1.9, respectively). Variables L*, a* and DL were higher in Bonsmara samples than in Friesland samples. Even though the Friesland breed seemed generally more social towards humans in the lairages and had lower cortisol levels compared to the agitated Bonsmara, it produced meat of lower quality with regard to tenderness, colour and cooking properties than the latter.

Key Words – Production system, Lairage behaviour, Meat Classification.

IX. INTRODUCTION

Different cattle breeds are kept in a wide range of production systems according to their specific traits. Nevertheless, at the end of their production cycle, they are culled, sometimes fattened and slaughtered for meat production regardless of their initial function. Due to different management systems, the meat quality from these cattle is expected to differ. However, the red meat classification system post dressing does not cater for the differences caused by selection and production system, it only addresses what is observed on the carcass. The South African red meat classification criterion includes classes like sex, age, conformation, fat and bruising. The purpose served by this is to allow the consumers to select meat according to their partialities (1). Nonetheless, not all details that could influence the choices are made available.

This practice also occurs for dairy producing cows. Bazzoli *et al.* (2) listed the major reasons for culling dairy cows as reproductive failure, mastitis and udder problems, low milk production, and old age. Rogers *et al.* (3) reported that one of the options dairy farmers have when culling non-productive or diseased cows is to sell them at a salvage value to slaughter plants. Generally, in South Africa, beef producing cattle breeds are kept extensively in either natural or cultivated pastures with the aim of increasing muscle growth as well as meat quality and quantity; while dairy producing cattle breeds are mostly fed supplemental diets to primarily enhance milk production, quality and quantity. Soji *et al.* (4) reported that this practice can influence the carcass (lean-to-fat ratio, dressing percentage and conformation) and meat (taste, colour and texture) quality traits.

Apart from the nutrition differences of the two systems, dairy cattle are more exposed to human interactions on a daily basis during milking and feeding and this may be favourable prior to slaughter by allowing ease during handling. Unlike dairy cows, beef cattle only have minimal contact with humans, mostly during management procedures like weighing, vaccination, regrouping and dipping. Cattle mostly perceive these rare interactions as being hostile (5) thus sometimes resulting to fear of humans (6; 7). In addition, exposure to unfamiliar human beings either at the farm or abattoir is likely to frighten slaughter animals (8; 5). Grandin (9) reported that cattle perceive the abattoir environment in the same way as at the farm during procedures like vaccination and other managerial processes that involve moving animals through the race.

Blood cortisol and glucose levels have been used to determine short-term stress due to handling and pre-slaughter activities in general. Both cortisol and glucose were reported to increase with handling and transportation, and was also observed more on temperamental cattle than on calm ones (10; 11). These biochemical changes in the animal's system, mainly due to stress, then affect the quality of meat produced (12; 13). An increase in blood glucose and cortisol in the muscle results in glycolysis, and thus in the formation of lactic acid and also in decreased meat pH, increased WBSF, cooking loss, drip loss and lightness of the meat (14; 15). The aim of this study was then to assess blood glucose and cortisol, pH_{24}, colour (L*, a*, b* values), thawing loss, drip loss, cooking loss and WBSF of slaughter cows from dairy and beef production systems.

X. MATERIALS AND METHODS

Ethical clearance and study site

Consent to carry out the study was approved and issued by the University of Fort Hare Ethical Clearance committee (Reference Number: MUC03S1NJI01). The data was collected from the East London abattoir. The abattoir is a typical high throughput commercial operation, equipped with modern technology to enhance production efficiency. It operates according to the standard laws and regulations governing abattoirs, under "The Meat Act, 2000, the Animal Protection Act, 1962 and 1935 for animal welfare maintenance" to ensure public health safety.

Animal Description, Lairaging and Slaughter

Two groups of cows of the same age group (class C) from a dairy and beef production systems were selected at the abattoir lairages. The dairy system was represented by 19 Friesland culls and the beef production by 25 Bonsmara cows. Information on the animals' background was accessed through the abattoir manager. These cows were kept in groups of 7-9 animals in a 5.3 m × 5.3 m area space per lairage pen overnight with ad lib access to water.

On the day of slaughter, they were partially observed for their general responses to the presence of humans around the lairage aisles. The Friesland group seemed to be more social towards humans, curious and not bothered by the presence of humans around the lairages. This was not the case for the Bonsmara group: they kept their distance from humans at least 2-3 m away, limited by the enclosure of the lairage.

When moved towards the slaughter floor, there was minimal animal-human interaction as the animals directions were led by the narrow crush connecting the holding pens and restraining or stunning areas. A gun stunner was used to terminate the cattle's consciousness before exsanguination was initiated using a sharp knife to cut the jugular vein across the animals neck. A Horizontal bleeding method was used for all cows.

Blood & Meat Sampling & analysis measurements

Two sets of blood samples were obtained from each animal into two vacutainer tubes during the process of exsanguination after the stunning processes for glucose and cortisol analysis. This was done to determine the stress levels of the two breeds. The samples were kept in ice until separation of serum through centrifuging at 21°C for 10 min at 3000 rpm.

After carcass dressing, forty four meat samples of 100-200 g were collected from the Brisket muscle of each cow. This muscle was made available for sampling by the abattoir, but not the usual *longissimus dorsi* muscle to protect their production quality, quantity and profit. The samples were analysed for pH_{24} and colour (L*, a*, b* values). Further measurements for thawing loss (TL), drip loss (DL), cooking loss (CL) and Warner Bratzler Shear Force (WBSF) were done and analysed.

Statistical analysis

The data obtained was analysed using SAS (16) statistical package. The effect of the breed/background on Glucose and Cortisol, Meat pH, Colour, Thawing, Drip and Cooking Losses and WBSF was analysed by ANOVA using Proc GLM for. Pearson's correlation coefficients between the variables were also determined (16). The model used was: $Y_i = \mu + \alpha_i + e_i$

Table 2: Correlations between blood parameters and some meat quality variables

Variables	*Cortisol*	*pH_{24}*	*L**	*a**	*b**	*TL*	*DL*	*CL*	*WBSF*
Glucose	0.34*	-0.19	-0.15	-0.74	-0.03	0.15	0.34	-0.16	-0.03
Cortisol		-0.29	0.07	0.08	-0.01	0.07	0.28	-0.37**	-0.21
pH_{24}			-0.49***	-0.26	-0.34*	-0.13	-0.48***	0.11	-0.001
Lightness (L*)				0.23	0.45**	0.04	0.24	-0.01	0.02
Redness (a*)					0.81***	-0.16	0.20	-0.32*	-0.09
Yellowness (b*)						-0.03	0.24	-0.14	-0.01
TL (in %)							-0.12	0.29	-0.04
DL (in %)								-0.30*	-0.18
CL (in %)									0.24

Significantly correlated at *P<0.05, **P < 0.01, ***P <0.001, thawing loss (TL), drip loss (DL), cooking loss (CL) and Warner Bratzler Shear Force (WBSF)

XI. RESULTS AND DISCUSSION

Blood Cortisol and Glucose results

Table 1 shows results that glucose levels did not differ (P>0.05) between breeds. However cortisol was higher in Bonsmara (273.7±18.0) than in Friesland (104.9±20.7). Cortisol was reported to increase with handling and transportation more on temperamental cattle than on calm ones (10; 11). This may have been the case with the Bonsmara breed which was observed to keep their distance from human beings at the lairages compared to the more social Friesland breed. Additionally, these observations could be linked to the previous exposures of these animals to handling at the farm, allowing the group from a dairy farm to exhibit better results than the other group.

Table 3: The effect of breed on glucose, cortisol, Meat pH_{24}, colour (L*, a*, b*), TL, DL, CL and WBSF

Parameter	*Bonsmara*	*Friesland*	*P.Value*
Glucose	5.6±0.1	5.4±0.2	NS
Cortisol	273.7±18.0^{a}	104.9±20.7^{b}	P <0.001
pH_{24}	5.7±0.5^{b}	5.9±0.6^{a}	P <0.05
L*	28.9±0.5^{a}	26.8±0.6^{b}	P <0.05
a*	18.8±0.4^{a}	17.4±0.5^{b}	P <0.05
b*	13.9±0.4	13.1±0.5	NS
TL (in %)	5.4±0.4	5.3±0.5	NS
DL (in %)	5.8±0.2^{a}	4.9±0.2^{b}	P <0.05
CL (in %)	33.6±0.9^{b}	37.8±1.1^{a}	P <0.05
WBSF (in N)	45.8±1.9^{b}	54.1±2.2^{a}	P <0.05

Means in the same row with different superscripts are significantly different at P<0.05, P<0.001, NS-Not significant, TL-thawing loss, DL-drip loss, CL-cooking loss and WBSF-Warner Bratzler Shear Force.

Meat pH, Colour, TL, DL, CL and WBSF

Table 1 also reveals that the b* value and TL did not differ (P>0.05) between breeds. However, even though behavior in the lairages and exsanguination blood parameters looked better for the Friesland breed, these animals produced meat with higher pH_{24} (5.9±0.6), CL (37.8±1.1) and WBSF (54.1±2.2) compared to the Bonsmara which had lower values (5.7±0.5, 33.6±0.9 and 45.8±1.9, respectively). The higher WBSF and cooking loss values for the Friesland could be attributed to the fact that for a greater part of the cows' developmental cycle, feeding was focused on enhancing milk production rather than muscle quality. Additionally, dairy animals are normally selected for strong and well attached suspensory ligament in favor of udders. Therefore, the tough muscle is within their genetic makeup. In contrast, Jurie *et al*. (17) found no breed differences on meat tenderness of Holtein (dairy) and Salers (beef) cull cows slaughtered at the same age and fattening level.

The Bonsmara cows produced meat of significantly lower pH_{24} despite their high cortisol levels. This is in contrast with results reported that high cortisol levels in the blood result to glycogen depletion and increase meat pH_{24} thus inducing DFD meat (18). Okeudo and Moss (19) associated cortisol increase with decreased initial mutton pH. In addition, the Bonsmara cows produced meat that is lighter (28.9±0.5), redder (18.8±0.4) and tender (45.8±1.9) than the Friesland cows. These results confirm those that were observed by Wulf *et al*. (20) and Hayes *et al*. (21) that lighter, more red and yellow meat is more tender.

Correlations between the studied variables

Table 2 shows a positive correlation (P<0.05) between blood glucose and cortisol levels.

Both these blood parameters are determinants of short term stress such as handling and transportation. Tarrant *et al*. (10) and Hulbert *et al*. (11) reported that cortisol and glucose increase more with handling and transportation. Cortisol is also correlated negatively (P<0.01) with CL. These results confirm those reported by Okeudo and Moss (19) on a study on sheep and mutton quality. Increased meat pH resulted in elevated glycogen depletion thus Dark-Firm-Dry meat (18).

This is in agreement with the current results that showed a negative correlation between pH_{24} and L* (P<0.001); a* (P<0.05); and DL (P<0.001). Furthermore, DL negatively correlated (P<0.05) with CL. The more water and nutrients lost during thawing, the less there will be available to lose at cooking.

XII. CONCLUSION

The Friesland dairy breed seemed generally more calm and social towards humans in the lairages and had lower blood cortisol levels after slaughter. However, compared to the more agitated Bonsmara beef breed, the meat harvested from Friesland carcasses was of lower quality with regard to tenderness, colour and cooking properties. Additionally, these two groups may have been classified under the same C age class; however they produced meat of different quality.

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