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Cumulative incidence and associated risk factors of dark, firm and dry (DFD) meat in South African beef carcasses

by

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BSc Agric (Animal Science) UP

Submitted in partial fulfilment for the degree

Magister Scientiae Agriculturae in Animal Science with specialization in Production Physiology and Product Quality

Department of Animal Science

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University of Pretoria

Pretoria

December 2021

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Declaration

I, Elrie Botha hereby declare that this thesis, submitted in partial fulfilment of the requirement for the degree MSc Agric (Animal Science) Production Physiology and Product Quality, in the Department of Animal Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other university.

E. Botha
December 2021
Pretoria

ACKNOWLEDGEMENTS

I wish to thank the following people and institutions for their contributions to the completion of this research:

Prof E.C Webb, my supervisor and mentor for his expert advice, guidance and enthusiasm. It was a privilege to complete this project with him.

My family for their unconditional love and being my number one supporter in all my dreams I want to achieve.

Andries Masenge of the Department of Statistics, University of Pretoria, for his assistance in the statistical analysis of the data.

Michaela Faulhaber for her supportive assistance with the data sheets and advice in beef processing.

Dr Kabols le Riche for granting me the opportunity to conduct this research project.

Irene Nel from the Agricultural Research Council (ARC), for her assistance with the climatology data.

My fellow students Alexis, Annette, Ashlyn, Danielle, Guillaume, Jaydi, Johannes, Jonathan, Kaylee, Liza, Miche'and Zander for their willingness to help.

Red Meat Research and Development of South Africa (RMRD SA) for their financial support towards the project.

Lastly, my Father in Heaven for providing me with opportunities to learn and grow each day.

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ABBREVIATIONS

ATP	**Adenosine triphosphate**
ß-AA	Beta-adrenergic agonists
DFD	Dark, firm and dry
DCB	Dark-cutting beef
H^+	Hydrogen ions
Mb	Myoglobin
PSE	Pale, Soft and Exudative
THI	Temperature-humidity index
Ultimate pH	pH_u

ABSTRACT

Cumulative incidence and associated risk factors of dark, firm and dry (DFD) meat in South African beef carcasses

The present study investigated the cumulative incidence and associated risk factors of dark, firm and dry (DFD) meat, also described as dark-cutting beef (DCB) or dark-cutters, in South African beef carcasses. The aim was to analyse possible causative risk factors associated with DFD beef in South African conditions, through a comprehensive retrospective review of production data from feeding systems, transportation, lairage, slaughter and cooling to retail.

The studied material consisted of 29,787 cattle from 52 suppliers, distributed over Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West and Western Cape provinces in South Africa and different production systems (Angus = 2,116; Commercial = 10,580; Free-range = 12,976) for the period from January 2020 to December 2020 from a typical high throughput abattoir. Climatological data were obtained from the closest meteorological stations to the loading location of cattle at the farms and the unloading location at the slaughter plant, which were combined with the large data set from the abattoir that included the following information per individual animal: farm production system, estimated standing time, breed type, age class, gender, cattle conformation, live weight, carcass weight, fat content and post-mortem carcass pH-temperature measurements. Because of the large variation in pH values at which DFD beef is identified between geographical locations and from previous studies, the 24-hour pH values post-mortem were divided into risk categories namely: Normal ($pH_u < 5.8$), Intermediate (pH_u $5.8 \leq X < 6.2$) and High ($pH_u \geq 6.2$). The effects of pre-slaughter stress factors on the risk of DFD beef were analysed by means of the General linear mixed model procedure in SPSS IBM Statistics version 27 (2089, 2020).

The overall estimated incidence of DFD beef at a South African abattoir was 0.40% high-risk ($pH_u > 6.2$), 43.2% intermediate risk (pH_u $5.8 \leq X < 6.2$) and 56.4 % ($pH_u < 5.8$) low-risk DFD carcasses. The proportion estimate effect of the extrinsic and intrinsic pre-slaughter stress factors on the pH_u was significant but small. However, combining the estimated effects of all factors on individual cattle significantly increased the risk of DFD beef by c.a 17%. Because the pre-slaughter stress factors are confounded by one another by minimising the effect of one factor, there may be a reduced effect of other related factors.

In summary, suppliers had a significant effect (0.044%) on the incidence of DFD beef along with production systems (Angus = -0.508%; Commercial feedlots = 0.054%; Free-range = 0%) and climatic factors (Tx_FARM - Tx_ABATTOIR = 0.017914; Tn_FARM - Tn_ABATTOIR = 0.016504; RHx_FARM - RHx_ABATTOIR = 0.001758). Although climatic conditions had a small but meaningful effect on increasing the risk of DFD meat, it cannot be compared with the variation in pH_u

caused by the other factors as it cannot be controlled. Transportation distances did not affect the pH_u post-mortem, but loading density (0.002%) significantly increased the risk of DFD beef. Furthermore, the study suggests that cattle should be rested after transportation (1 day = 2.651 %; 2 days = 2.147 %; 3 days = 0 %) but more research is required to quantify a general estimated resting time according to the pre-slaughter stress factors cattle experience before slaughter. Cattle breed did not have a significant effect on DFD beef, however, gender (Cow = -0.027; Heifer = -0.474; Steers = 0 %) and age (A = -0.811 %; AB = -0.794 % ; B = -0.858 % ; C = 0 %) significantly affected the pH_u post-mortem. Furthermore, the outcome of this study indicates that the seasonal variation in pH_u (Autumn = 1.176%; Spring = -0.969%; Summer = -1.528%; Winter = 0%) was mainly determined by the variation in cattle body conformation (live weight, warm carcass mass and fat content) throughout the year. Based on the findings of this study, a DFD beef window was defined under South African conditions, in which cattle have a lower body conformation (live weight (< 458 kg) and low warm carcass mass (< 281 kg)) compared to the rest of the year and therefore responded more severely to the extrinsic and intrinsic pre-slaughter stress factors, increasing the risk of DFD. Thus, body conformation can be used as the main determining factor to identify individual cattle at risk to produce DFD beef prior to slaughter, especially in the DFD window period.

This study indicates that it is possible to decrease the risk of DFD by focussing on the factors that can be controlled, such as nutritional management, low-stress management techniques especially during transportation, avoiding the mixing of unfamiliar cattle, reducing the loading density and standing period and increasing carcass weight. One of the biggest concerns of the effect of pre-slaughter stress factors on an individual animal is the lack of knowledge by suppliers and abattoirs to use basic skilful management techniques to minimise the risk of DFD beef. Therefore, it is important that more awareness campaigns and training of suppliers, advisers and policymakers should occur to explain the key impact each section in the supply chain has on meat quality attributes. Then only can dark-cutting beef be minimised to a manageable extent by skilful manipulation of cattle management techniques in beef production.

CHAPTER 1

INTRODUCTION AND MOTIVATION

Dark, firm and dry (DFD) meat, also described as dark-cutting beef (DCB) or dark-cutters is a persistent quality defect faced by the beef industry worldwide, which has major financial implications (Tarrant, 1989; Viljoen, 2000; Bekhit, 2003; Adzitey & Nurul, 2011; Neethling *et al.*, 2017; Lu *et al*., 2018; Ijaz *et al*., 2020). For instance, abattoirs in South Africa estimated a loss of roughly R1 million/ year due to DFD meat (Botha & Webb, personal communication). As soon as the retailers identify DFD beef in a shipment, the whole batch is often returned to the abattoir. This may consequently increase the financial loss to the abattoirs, by up to 10-fold, and add reputational damage to the suppliers (Viljoen, 2000).

It is well known that DFD meat is the consequence of pre-slaughter stress, associated with certain genotype-environmental interactions in both cattle raised on pastures as well as intensively fed feedlot cattle, that results in the depletion of muscle glycogen storage prior to slaughter (Viljoen *et al*., 2002; Adzitey & Nurul, 2011; Frylinck *et al.*, 2015; Neethling *et al*., 2017; Pannampalam *et al*., 2017; Loudon *et al.*, 2018; Lu *et al*., 2018; Loredo-Osti *et al*., 2019; Ijaz *et al*., 2020). The depletion of muscle glycogen storage leads to lower production of hydrogen ions (H^+) and Pi during post-mortem muscle metabolism, so the carcass does not acidify correctly and produce high pH meat (Viljoen *et al*., 2002; Neethling *et al*., 2017; Pannampalam *et al*., 2017; Loredo-Osti *et al*., 2019). High pH meat can be identified as a pH > 5.7 (McGilchrist *et al.*, 2012; Loudon e*t al.*, 2018), pH ≥ 5.71 (Warner & Tarr, 2018), pH > 5.8 (Viljoen, 2000; Mahmood *et al.*, 2016; Loredo-Osti *et al.*, 2019), pH ≥ 5.87 (Page *et al.*, 2001), pH > 5.9 (Augustini & Fischer, 1979; Ferguson *et al.*, 2001), pH > 6.0 (Fischer & Hamm 1980; Tarrant & Sherington, 1980; Kadim *et al.*, 2004; Apple *et al.*, 2006; Apaoblaza *et al.*, 2020) or a pH > 6.2 (Taylor & Shaw, 1977; Fjelkner-Modig & Ruderus, 1983a; Fjelkner-Modig & Ruderus, 1983b; Muchenje *et al.*, 2008). The pH value at which DFD beef is identified differs in previous studies, therefore more research is required to identify the pH at which DFD beef becomes a serious meat quality risk. Furthermore, DFD meat is characterized by a dark red colour, which consumers find unsatisfactory and discriminate against (Viljoen *et al*., 2002; Ponnampalam *et al*., 2017). Dark-cutting beef has a reduced shelf-life and the quality aspects of DFD beef are unacceptable to consumers, which leads to major financial losses in the meat industry (Adzitey & Nurul, 2011; Loredo-Osti *et al*., 2019).

The DFD condition has been previously researched in South Africa (Viljoen, 2000; Viljoen *et al.*, 2002; Neethling *et al*, 2014; Neethling *et al* 2017) and across the world (Mach *et al.*, 2008; Warriss & Brown, 2008; McGilchrist *et al.*, 2012; Moore *et al.*, 2012; Warner *et al.*, 2014). Although the physiology of the condition is well understood, the causative risk factors of DFD meat and their efficient management remains problematic.

In all stages of the beef production cycle, cattle may experience physiological stress, which is caused by several factors associated with the occurrence of DFD (Loredo-Osti *et al*., 2019). These causative risk factors can be categorized into extrinsic (seasonal effects, feeding systems, administration of hormone implants and growth promoters in cattle, pre-slaughter animal handling, transportation, lairage, electrical stimulation and storage temperature) and intrinsic (genetic diversity between and within breeds, gender differences, animal age, muscle-specific variation and muscle fibre types) factors (Neethling *et al*., 2017; Xing *et al*., 2019). The incidence of dark cutting meat can be prevented if producers comprehend, specify and manage the factors optimally to minimize the physiological stress cattle endure (Pannampalam *et al*., 2017). However, because of genetic variation between animals, there will always be a proportion of cattle that are more susceptible to physiological stress than others. In addition, managing the environmental effect on the occurrence of DFD may be problematic, particularly the fluctuations in weather conditions between seasons (Ponnampalam *et al*., 2017; Neethling *et al*., 2014; Neethling *et al*., 2017), along with increased worldwide temperatures and more recurrent heat waves due to global warming (Gonzalez-Rivas *et al*., 2019). Furthermore, it is evident that DFD meat results from a combination of factors contributing to pre-slaughter stress (Pannampalam *et al*., 2017), however, more research is required to understand the causative risk factors since the causation is not scientifically well quantified.

The current study analysed large data sets obtained from large commercial abattoirs receiving cattle from feedlots and pasture rearing systems for a period between January 2020 to December 2020 in South Africa. Data from conception to dispatching to retail outlets were included to predict the possible causative risk factors associated with DFD beef in South Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 DARK, FIRM AND DRY MEAT

2.1.1 A brief description of Dark, Firm and Dry (DFD) meat

Dark, Firm and Dry (DFD) meat, also described as dark-cutting beef (DCB) or dark-cutters is a persistent quality defect that the beef industry is facing worldwide (Table 2.1) (Tarrant, 1989; Viljoen, 2000; Adzitey & Nurul, 2011; Lu *et al*., 2018; Ijaz *et al*., 2020).

Table 2.1: The occurrence of DFD meat in beef carcasses across several countries.

Country	**% Dark-cutting**	**Reference**
South Africa	11.8% (n= 22178)	Viljoen (2000)
USA	3.2% (n= 9802)	Moore *et al.* (2012)
Australia	5.45% (n= 1157781); 2.6-24.6% (n= 1512)	McGilchrist *et al.*, 2012; Warner *et al.* (2014)
Spain	13.9% (n= 5494)	Mach *et al.* (2008)
United Kingdom	8.8% (n= 717)	Warriss & Brown (2008)

It is well known that DFD meat is the consequence of chronic pre-slaughter stress, associated with certain genotype-environmental interactions in both cattle raised on pastures as well as intensively fed feedlot cattle, that result in the depletion of muscle glycogen storage prior to slaughter and lead to high pH meat (Viljoen *et al*., 2002; Adzitey & Nurul, 2011; Frylinck *et al*., 2015; Neethling *et al*., 2017; Ponnampalam *et al*., 2017; Loudon *et al*., 2018; Lu *et al*., 2018; Gonzalez-Rivas *et al*., 2019; Loredo-Osti *et al*., 2019; Ijaz *et al*., 2020). The pH value at which DFD beef is identified differs between geographical locations and from previous studies (Table 2.2). According to Tarrant (1981), the precise ultimate pH (pH_u) value at which a carcass is considered dark-cutting ranges between 5.8 to 6.3 and mainly depends on the processing and marketing factors, along with the type of beef. Moreover, Ponnampalam *et al*. (2017) reviewed that the actual pH at which meat is defined as dark-cutting is caused by a combination of intrinsic and extrinsic factors contributing to pre-slaughter stress. However, it is known that as the pH_u value increases, the DFD condition progressively worsens (Tarrant, 2012). Moreover, Tarrant (2012) mentioned that giving a precise definition of DFD beef in terms of pH_u is problematic thus, more research is required to specify the definite pH_u at which DFD meat can be determined in specific locations with different genotype-environmental interactions.

Table 2.2: The ultimate pH (pH_u) of DFD beef.

High pHu meat	Location	Reference
pH > 5.7	Australia	McGilchrist *et al.*, 2012; Loudon e*t al.*, 2018
pH ≥ 5.71	Australia	Warner & Tarr, 2018
pH > 5.8	South Africa, Canada, Mexico	Viljoen, 2000; Mahmood *et al.*, 2016; Loredo-Osti *et al.*, 2019
pH ≥ 5.87	United States	Page *et al.*, 2001
pH > 5.9	Germany, Australia	Augustini & Fischer, 1979; Ferguson *et al.*, 2001
pH > 6.0	Germany, Ireland, Saudi Arabia, United States	Fischer & Hamm 1980; Tarrant & Sherington, 1980; Kadim *et al.*, 2004; Apple *et al.*, 2006; Apaoblaza *et al.*, 2020
pH >6.2	United Kingdom, Sweden, South Africa	Taylor & Shaw, 1977; Fjelkner-Modig & Ruderus, 1983a; Fjelkner-Modig & Ruderus, 1983b; Muchenje *et al.*, 2008

DFD meat has major meat quality defects that consumers, retailers and food services find unsatisfactory and discriminate against, therefore leading to financial implications (Ferguson *et al*., 2001; Viljoen *et al*., 2002; Bekhit, 2003; Neethling *et al*., 2017; Gonzalez-Rivas *et al*., 2019; Ijaz *et al*., 2020). Over several years abattoirs noticed that a dark-cutting syndrome exists between cattle. However, this problem was never quantified, nor qualified, and the causative risk factors and their efficient management affecting the occurrence of DFD were not known, but only left to speculation (Viljoen, 2000). Ponnampalam *et al*. (2017) reviewed that there is no single production factor that results in dark-cutting, therefore it is a complex condition to be resolved.

2.1.2 The biochemical causes of dark-cutting beef

Cattle are exposed to various intrinsic and extrinsic stressors from the farm to the abattoir (Ferguson & Warner, 2008; Xing *et al*., 2019; Birhanu, 2020). A combination of factors and interactions can disrupt the animals' homeostatic balance and to restore the physiological equilibrium, the stress response is initiated (Ferguson & Warner, 2008; Xing *et al*., 2019). The stress response has two mechanisms, namely the defence- (short term stress) and adaptive (long term stress) responses, that lead to physiological and behavioural changes (Birhanu, 2020). Glycogen is the main storage carbohydrate in animal cells (Pethick *et al.*, 1995; Pearson, 2014), which is utilized as an energy source for muscle relaxation and contraction (Pethick *et al.*, 1995; Miller, 2007). Ultimately this stimulation results in the depletion of muscle- and liver glycogen (Ponnampalam *et al*., 2017). The intensity, type and duration of pre-slaughter stressors determine the animals' response (Ferguson *et al.*, 2001; Ferguson & Warner, 2008; Xing *et al.*, 2019).

In summary, the stress response affects physiological and metabolic functions that control the post-mortem biochemical reactions and has adverse effects on meat quality (Ferguson & Warner, 2008; O'Neill & Webb, 2012; Xing *et al.*, 2019; Birhanu, 2020), such as pale, soft and exudative (PSE) meat and DFD meat (Figure 2.3) (Neethling *et al*., 2017). Acute or short term stress can cause PSE meat, whereas chronic or long term pre-slaughter stress may cause DFD meat (Adzitey, 2011). Figure 2.1 summarizes the effect of pre-slaughter stress on cattle and subsequently the negative effect on meat quality, which has serious financial implications for the meat industry.

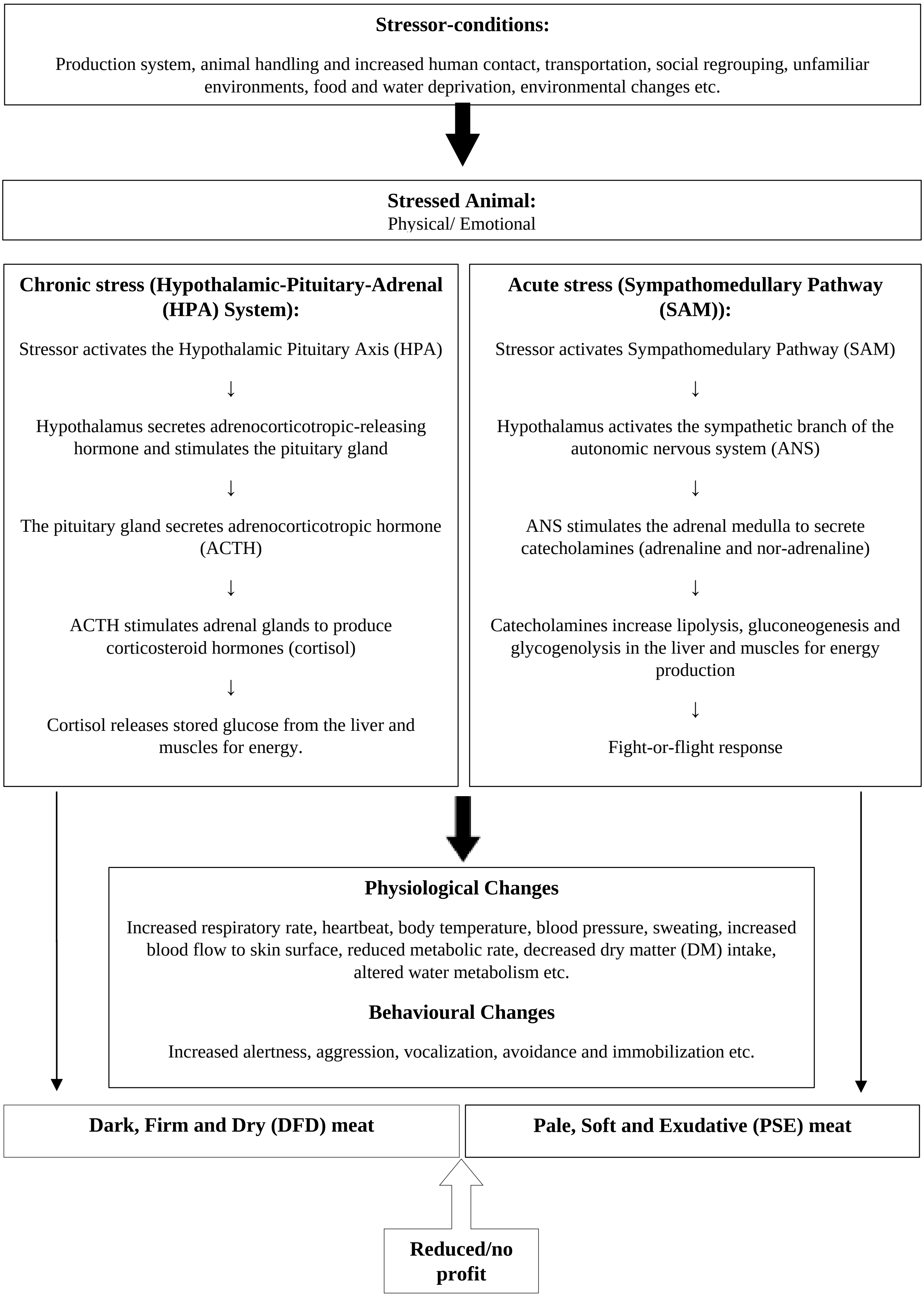


Figure 2.1: The correlation between pre-slaughter stress and meat quality defects (Tarrant, 1989; Pethick *et al.,* 1995; Ferguson & Warner, 2008; Gaughan *et al.,* 2009; Farooq *et al.,* 2010; O'Neill & Webb, 2012; O'Neill *et al.*, 2012; Njisane & Muchenje, 2017; Bozzo *et al.*, 2018; Lu *et al.*, 2018; O'Neill *et al.*, 2018; Biraima *et al.*, 2019; Gonzalez-Rivas *et al.*, 2019; Xing *et al.*, 2019; Birhanu, 2020; Samuelsson, 2020**).**

Post-mortem glycolysis and pH decline are enzymatically regulated, thus it is critical to understand the biochemical regulation of glycolysis, as the quantity of muscle glycogen stored at the time of slaughter is correlated with the pHu, which will have an immediate effect on the meat quality (Figure 2.2) (Pethick *et al.*, 1995; Kadim *et al.*, 2004; Ponnampalam *et al.*, 2017; Chuhan & England, 2018). After exsanguination, blood flow and oxygen supply end, which leads to physical and biochemical transformation in the skeletal muscle and consequently the conversion of muscle to meat. Regardless of these transformations, the skeletal muscle will continue to aim to reach the ante-mortem homeostatic balance. Thus, energy will be metabolized by synthesizing adenosine triphosphate (ATP) through catabolism of preserved glycogen to generate lactate and H^+, which will result in a drop in pH (Figure 2.2) (Chuhan & England, 2018).

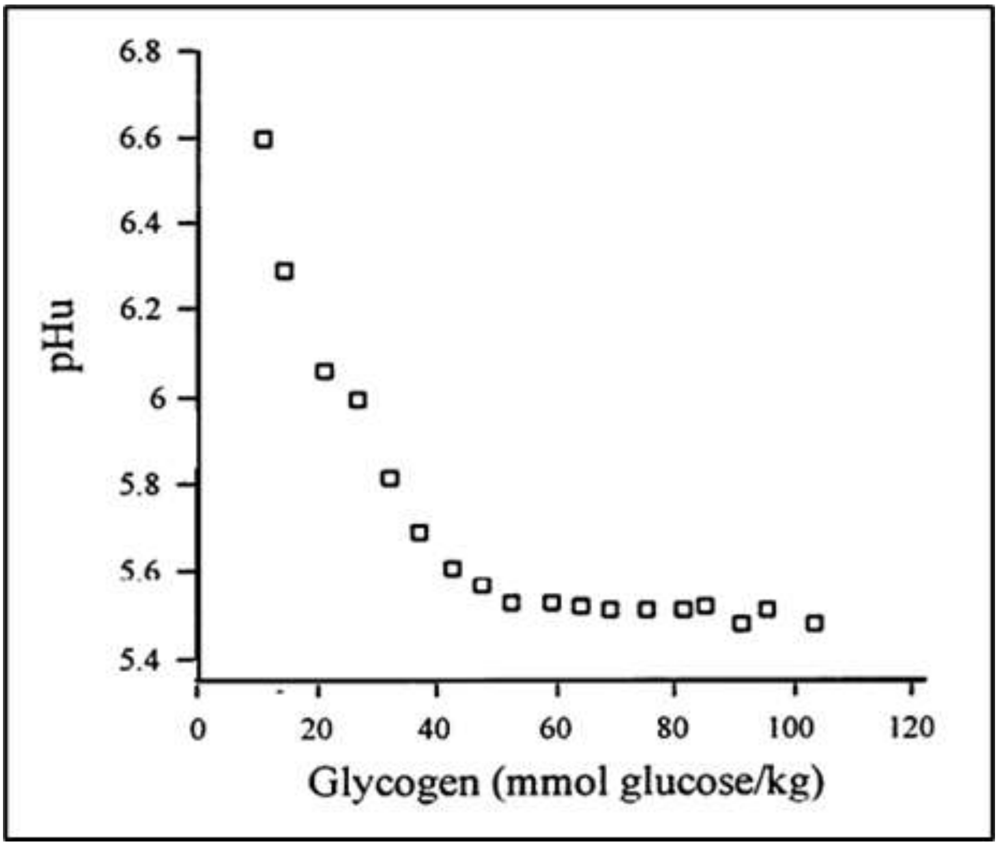


Figure 2.2: The relationship between ultimate pH (pH_u) of meat and the concentration of glycogen in muscle (LD) immediately post-slaughter (From Pethick *et al*., 1995).

In healthy and well-rested cattle, the glycogen concentration is approximately 40-50 mmol glucose/kg muscle, therefore at 48 hours post-slaughter the muscle pH will drop from 7.4 in the living muscle to a pH_u of about 5.5 (Figure 2.2) (Pethick *et al.*, 1995; Miller, 2007; Chuhan & England, 2018). However, there are variations in the pH_u value between and within species (Chuhan & England, 2018). Additionally, chronic pre-slaughter stress results in glycogen depletion, past 40-50 mmol glucose/kg muscle (Pethick *et al.*, 1995; Loredo-Osti *et al.*, 2019). Thus, there is a reduction in the formation of lactic acid and H^+ during post-mortem muscle metabolism, therefore the meat will not acidify correctly and result in high pH meat, also known as DFD meat (Figure 2.3) (Viljoen *et al.*, 2002; Neethling *et al.*, 2017; Ponnampalam *et al.*, 2017; Loudon *et al.*, 2018; Lu *et al.*, 2018; Gonzalez-Rivas *et al.*, 2019; Loredo-Osti *et al.*, 2019; Ijaz *et al.*, 2020).

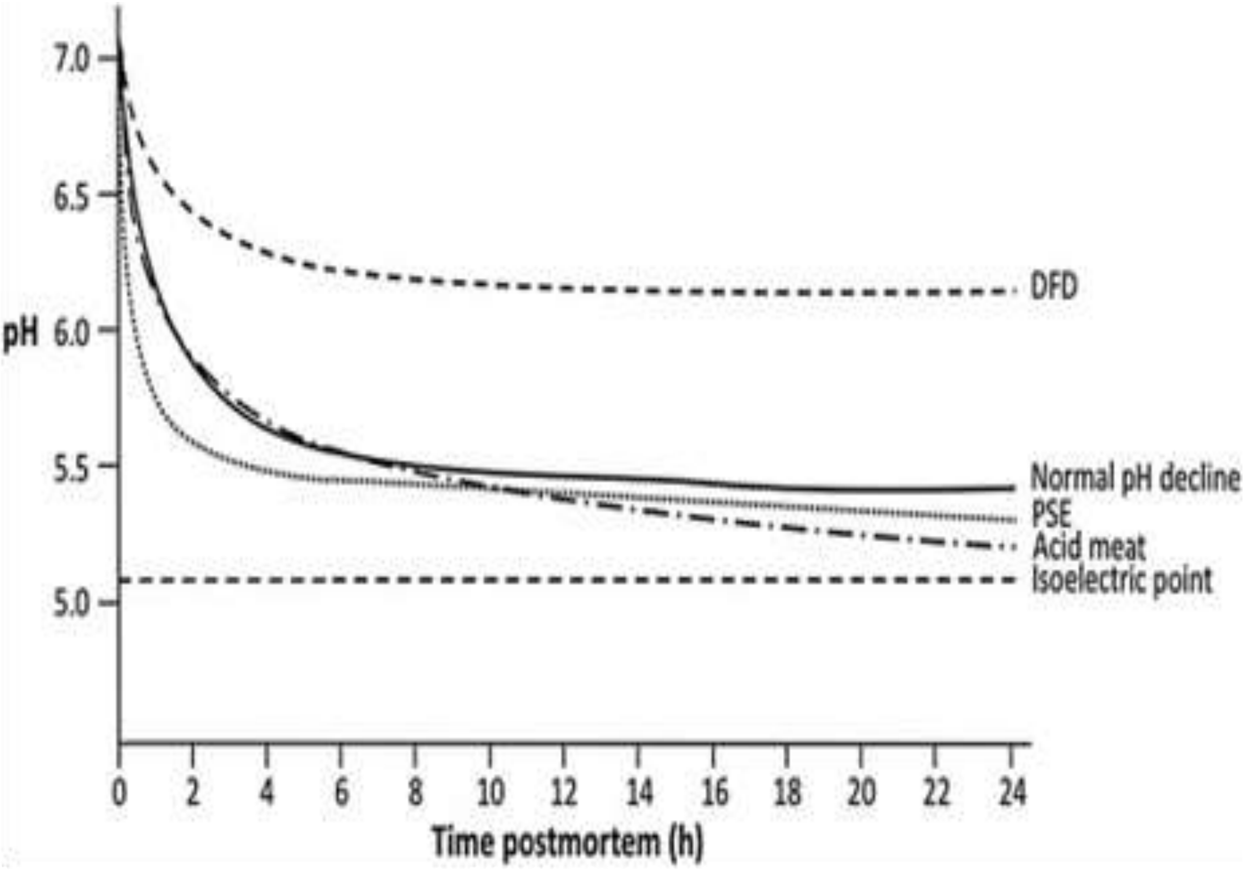


Figure 2.3: The relationship between ultimate pH and the time post mortem (h) (From Matarneh *et al.*, 2017).

2.1.3 What are the defects of DFD meat?

Much research has been carried out to identify the major quality defects that define DFD. DFD beef is characterised by an unattractive dark, purplish-red to almost black colour, often with a sticky lean surface. DFD meat has a higher water-holding capacity, therefore muscles reflect less light and lead to a darker appearance. The muscle is firm and dry because the water is firmly held within the muscle. The increased pH_u, limited formation of lactic acid and higher water-holding capacity create an ideal environment for microbial spoilage and lead to meat with a limited shelf-life, off-flavour and -odour (Viljoen, 2000; Ferguson *et al.*, 2001; Miller, 2007; Ijaz *et al.*, 2020).

Consumers prefer bright, cherry-red meat that is not too pale nor too dark (Frylinck *et al*., 2015). Furthermore, consumers purchase meat based on visual preferences (Viljoen *et al*., 2002), since it is the only meat quality attribute consumers can estimate at the point of sale (Neethling *et al*., 2017). Thus, the unattractive dark red colour of DFD meat (Viljoen *et al*., 2002; Miller, 2007; Neethling *et al*., 2017; Ponnampalam *et al*., 2017), continues to be the main cause of consumers' rejection of the meat (Miller, 2007; Neethling *et al*., 2017; Apaoblaza *et al*., 2020). According to Smith *et al*. (2000), consumer perception of meat colour determines approximately 15% variation in consumer acceptance of meat, even though meat colour is not strongly correlated with the eating quality (Miller, 2007; Webb & Erasmus, 2013; Frylinck *et al*., 2015). Therefore, the significance of meat colour as an indication of the freshness and flavour of meat may be overvalued by consumers (Viljoen *et al*., 2002; Troy & Kerry, 2010). Moreover, retailers discriminate against the limited shelf-life of DFD meat (Mahmood *et al*., 2016). When retailers identify DFD meat in a shipment, the whole batch is often returned to the abattoir. This may consequently increase the financial loss to the abattoirs by up to 10-fold, and add reputation damage to the suppliers (Viljoen, 2000). Table 2.3 compares consumers' acceptance of DFD and "normal" pH meat in both uncooked and cooked form. Nevertheless, it is an example to indicate that although DFD beef has meat quality defects, it is safe and nutritious to use for consumption (Miller, 2007).

Table 2.3: Differentiation between consumer acceptability of DFD and "normal" pH beef in both uncooked and cooked form (Viljoen *et al*., 2002).

Consumers evaluation	**Consumer Preference**		**Description**
	"Normal" pH beef	DFD beef	
Uncooked	X		Most consumers prefer raw normal beef steaks instead of DFD steaks because of the physiological attractiveness of uncooked bright-red normal steaks compared to raw darker DFD steaks. Furthermore, when meat is uncooked, DFD meat with a pH ranging from 5.8 to 6.2 has lower tenderness than normal pH meat
Cooked	No difference	No difference	There were no noticeable differences in consumer acceptability between cooked normal pH steaks and DFD steaks. Nevertheless, according to some female consumers, comparing cooked normal pH and DFD beef meat, normal pH meat has a better flavour and a more appealing colour than DFD cooked meat,

	whereas for male consumers there was no significant difference. As soon as DFD meat is cooked, morphological changes occur and meat with a higher pH tends to be more tender. This is because segmentation of myofibrils in DFD meat is greater in comparison with normal pH meat, which leads to fewer cooking losses in DFD meat followed by greater tenderness.

The price consumers are willing to pay for meat products is influenced by the quality and quantity of the meat (Adzitey & Nurul, 2011). Consequently, consumer, retailer and food services’ perceptions of meat and meat products have a direct effect on the profitability of the meat industry (Troy & Kerry, 2010). It would therefore be financially beneficial to identify dark-cutting cattle before slaughter (Mahmood *et al.*, 2016). Consumer preferences are complex, dynamic and difficult to describe (Troy & Kerry, 2010) and have changed significantly over the past two decades (Webb, 2006). Consumers are becoming more health-conscious, more aware of meat quality, the origin of the cattle, and production systems used for beef production (Muchenje *et al.*, 2008). Thus, in the meat industry producers are currently focused on producing products of good quality and consistent supply (Biraima *et al.,* 2019).

2.2 FACTORS AFFECTING THE OCCURRENCE OF DFD

It is evident that in all stages of beef production a combination of extrinsic and intrinsic factors contributes to pre-slaughter stress, consequently leading to DFD beef (Ferguson & Warner, 2008; Neethling *et al*., 2017; Ponnampalam *et al*., 2017; Loredo-Osti *et al*., 2019; Xing *et al*., 2019). Since some factors invoke different stress response mechanisms (acute- vs chronic stress), it is difficult to identify the underlying factors that cause a pre-slaughter factor-mediated change in meat quality (Ferguson & Warner, 2008). Although DFD is understood at the clinical level more research is required to understand the causative risk factors since the causation is not scientifically well quantified (Viljoen, 2000).

2.1.1 Extrinsic factors

i. The effect of climatic factors on DFD meat

Several authors noted a chemical and physical deviation in meat quality between different seasons (Kim *et al*., 2003; Wiklund *et al*., 2010; Neethling *et al*., 2014; Neetling *et al*., 2017). Adverse seasonal conditions can cause pre-slaughter stress in cattle and consequently negatively affect the carcass and meat quality traits (Kadim *et al*., 2004). Although stress negatively affects the meat quality (Grandin, 1996; Viljoen *et al.*, 2002; Adzitey & Nurul, 2011; Frylinck *et al.*, 2015; Neethling *et al.*, 2017; Ponnampalam *et al.*, 2017; Loudon *et al.*, 2018; Lu *et al.*, 2018; Gonzalez-Rivas *et al.*, 2019; Loredo-Osti *et al.*, 2019; Ijaz *et al.*, 2020), the direct influence of seasonal stressors have not adequately been taken into consideration (Kadim *et al.*, 2004; Warner & Tarr, 2018). According to Kadim *et al.* (2004), this could be because the major developments in the livestock industry are achieved in temperate regions where high ambient temperatures are not experienced. However, Warner and Tarr (2018) stated that the effect of weather terms on DFD was minimal and could be the reason that until now feedlots and processors have management systems in place to minimize the influence of heat stress and humidity on cattle, or it could indicate that the effect of weather conditions on dark-cutting are significant, but minor.

Previous studies that discovered the relationship between seasonal variability and dark-cutting carcasses were done in the United States (Amarillo, Texas, Kansas, Nebraska and Idaho) (Kreikemeier *et al.*, 1998; Mitlohner *et al.*, 2002; Boykin *et al.*, 2017), Southeastern coast of the Arabian Peninsula in Western Asia (Oman) (Kadim *et al.*, 2004) and Australia (Warner and Tarr, 2018) and concluded that the majority of dark-cutting carcasses occurred during the hot seasons compared to in cattle slaughtered in the cool seasons. Consequently, ruminants are more sensitive to hot temperatures compared to cold temperatures (Kadim *et al.*, 2004), because of their greater basal metabolic heat production, accelerated metabolic rate, fast growth and high level of production (Gonzalez-Rivas *et al.*, 2019). Thus, throughout the hot season cattle can experience heat stress and show signs of stressful behaviour and impaired physiological functions (increased respiration rate, increased heart rate, increased body temperature, decreased feed intake, increased water intake and ultimately a reduction in growth rate) (Warner & Tarr, 2018; Gonzalez-Rivas *et al.*, 2019) along with increased numbers of morbidity (Kadim *et al.*, 2004; Gregory, 2010). Additionally, as previously mentioned, glucose is the main energy source for ruminants and is used to decrease heat production from other biochemical processes (Warner & Tarr, 2018), thus chronic heat stress results in reduced muscle glycogen stores and ultimately increases the risk for DFD (Gonzalez-Rivas *et al.*, 2019).

Warner and Tarr (2018) analysed the relationship between weather conditions and dark-cutting carcasses over lag periods of 24 hours and 2, 3, 7, 14 and 28 days prior to slaughter. Furthermore, 3 to 28 days before slaughter higher maximum temperatures, humidity and temperature-humidity indexes (THI) increased the occurrence of DFD meat. However, 48 hours prior to slaughter these factors did not affect the incidence of DFD meat. The reason for this might be that throughout the first 3 to 4 days of exposure to excessive heat, there is a delay in cattle's acute body temperature responses, thus cattle will enter the chronic response stage after 3 days (Gaughan *et al.*, 2009). However, cattle's thermoregulatory responses will be determined by the intensity and length of exposure to hot conditions (Beatty *et al.*, 2006). The standard thermal zone of *Bos indicus* beef cattle is 16 to 27 °C and 15 to 25 °C for *Bos taurus* beef cattle. Additionally, for cattle to dissipate the heat that they gain throughout the day at night, the overnight temperature must fall below 21° for 3-6 hours (Warner & Tarr, 2018). Since

South Africa frequently has extreme temperatures during the hot seasons that are greater than these levels, cattle are particularly at risk for heat stress. Nevertheless, Warner and Tarr (2018) stated that more research is required to specify the exact period of exposure and the severity of heat stress that will lead to the depletion of glycogen stores and consequently DFD beef. Furthermore, low minimum temperatures and low humidity and THI 48 hours before slaughter also increased the occurrence of DFD meat. Additionally, hypothermia occurs when the body temperature of cattle is lower than the thermal neutral zone. Generally, this can be classified into mild (30-32°C), moderate (22-29°C) and severe (<20°C) hypothermia.

Warner and Tarr (2018) noted that increased variation in daily THI and temperatures 48 hours before slaughter increased the incidence of DFD meat. Moreover, DFD meat also increased when the standard deviation of temperature range and THI range increased 14 to 28 days before slaughter. However, DFD meat decreased with smaller minimum ranges in temperature 28 days before slaughter. According to Scanga *et al.* (1998) daily temperature alterations of more than 5.6 °C increase DFD meat. Viljoen (2000) proposed that the occurrence of DFD meat would be higher during the months with extreme hot and cold weather conditions or large daily temperature variations, whereas months with milder temperatures will have fewer incidences of DFD meat. These results suggest that both hot and cold conditions will have an effect on the muscle glycogen concentration and generally increase the risk of DFD meat.

Furthermore, rainfall 48 hours before slaughter reduced the incidence of DFD meat, Warner and Tarr (2018) speculated that rainfall increases the feed intake of cattle, which can amplify muscle glycogen storage prior to slaughter. Additionally, the wind speed had no significant effect on DFD meat.

Biological and adaptive responses will most likely be altered by management strategies, but the law of physics should still be taken into consideration in that the animal's heat production and losses should correspond within the restrictions of heat storage capacity (Gaughan *et al.*, 2009). Neethling *et al.* (2017) noted when estimating the seasonal effects of DFD there is a wide range of other factors that may have an effect on these observations, for instance the geographical locations, and breeds. Thus, when observing seasonal effects, it is necessary to consider these factors.

ii. The effect of hormone implants and growth promotants in cattle on DFD beef

Since the introduction of metabolic modifiers, there have been speculations that hormonal growth promotants promote carcass quality defects (Grandin, 1992). Metabolic modifiers modify growth curves, increase the rate of gain, improve feed conversion ratio (FCR), dressing percentage, carcass meat yield and meat palatability (Kerth *et al*., 1995; Dikeman, 2007; Johnson & Reinhardt, 2009; Gonzalez *et al*., 2017), through hormonal changes (Scanga *et al.*, 1998). These hormonal changes add additional sources of stress to hormonal shifts, which could result in a higher probability of DFD (Scanga *et al.*, 1998; Miller, 2007). Furthermore, the development of metabolic modifiers was mainly to improve feed efficiency and profitability of livestock production along with better carcass composition. However, few metabolic modifiers have been researched and developed specifically to enhance meat quality (Dikeman, 2007). According to Miller (2007) and Warner and Tarr (2018), it is critical to evaluate an implant strategy to minimise the risk for adverse effects, for instance DFD.

Warner and Tarr (2018) noted that the use of hormonal growth promotants in grain-fed cattle has no influence and no positive effect on the occurrence of DFD meat. Additionally, Dikeman (2007) and Lean *et al*. (2014) confirmed that beta-adrenergic agonists (ß-AA) will not have an effect on the final pH_u of the meat. However, these outcomes contradict the results of studies, which concluded that the use of metabolic modifiers will enhance the occurrence of DFD meat (Scanga *et al.*, 1998; Dikeman, 2003; Miller, 2007; Viljoen, 2000; Hunter, 2010). The effect of hormonal growth promotants depends primarily on the type of hormone used, the timing of its use, where there was incorrect use or over-dosing took place (Warner &Tarr, 2018).

iii. The effect of feeding systems and management on DFD meat

Although the genetic variation in beef quality is influenced by breed or type, nutrition is one of the main environmental factors (Pethick *et al.*, 1995; Webb, 2003). The glycolytic potential of muscles is affected by feeding and consequently the conversion of muscle to meat (Pethick *et al.*, 1995; Webb, 2006). Cattle that are undernourished, fasted or stressed have low pre-slaughter muscle energy reserves, which will increase the risk of DFD beef. Thus, beef quality can be enhanced through feeding, because both intrinsic- and extrinsic characteristics can be improved (Webb, 2006). However, more research is required to understand the metabolic basis of this control (Pethick *et al.*, 1995). In South Africa, production systems are determined by environmental and climatic conditions, and resources (Frylinck *et al.*, 2013; Webb & Erasmus, 2013; Ponnampalam *et al*., 2017), therefore pasture-based (extensive) systems frequently move to conventional or concentrate-based finishing (intensive) before marketing (Webb & Erasmus, 2013).

In South Africa, cattle are bred and kept on large grazing systems. To enhance the management of grazing systems and decrease stocking rates, weaned cattle are sold at live weights ranging from 160 kg to approximately 220 kg. A limited number of farmers produce standard carcasses for the South African market by keeping weaners to the age of 18 months or older on grazing systems with concentrate feeding. Consequently, 70% of all weaner calves are bought by feedlots. Feedlots fatten cattle with different proportions of concentrate diets, with or without feed additives and growth promotants, to slaughter cattle at target weights of approximately 400-450 kg live weight (± about 12-16 months of age) to yield carcasses of around 260-290 kg (Webb & Erasmus, 2013; Webb & Agbeniga, 2020). Thus, most beef cattle farmers focus on multiplying cattle numbers on pasture or natural grazing and feedlots buy weaned calves from grazing systems to fatten cattle until target weights are reached.

Breeds, feeding regimes, slaughter ages, handling and exercise conditions vary between production systems and may affect the physiology of the muscle (Frylinck *et al.*, 2015), leading to a variation in the quality of meat products produced from different production systems (Webb & Erasmus, 2013). There are, however, several advantages and disadvantages for each system (Webb & Erasmus, 2013).

Several researchers observed a higher percentage of DFD meat in pasture-fed cattle or poorly conditioned cattle compared to other systems (Frylinck *et al.,* 2013; Webb & Erasmus, 2013; Ponnampalam *et al*., 2017). Possible reasons that may explain these findings may be the reduced dietary energy density in pasture diets (Frylinck *et al.*, 2013; Webb & Erasmus, 2013; Neethling *et al.*, 2017; Ponnampalam *et al*., 2017), compared to feedlot diets, along with the increased animal homeostatic maintenance requirements involved with grazing (Ponnampalam *et al*., 2017). Vestergaard *et al.* (2000) noticed that the differences in physical activity, feeding level and to a lesser extent the diet between production systems have an effect on muscle fibre types in cattle. Cattle reared on pasture have higher levels of physical activity compared to feedlot cattle, therefore pasture-fed cattle have more slow-contacting fibres with a higher oxidative metabolic potential. Additionally, oxidative muscle fibre types contain less glycogen, which makes them more prone to DFD meat (Adzitey & Nurul, 2011; O'Neill *et al.,* 2018). Another possible reason for more DFD beef in pasture reared cattle compared to feedlot cattle might be the minimal human contact and handling that cattle receive in extensive systems. Therefore, pasture-fed cattle could be more susceptible to pre-slaughter stress compared to feedlot cattle, which may contribute to more dark-cutting carcasses in cattle raised on pasture (Neethling *et al.*, 2017). From a nutritional point of view, a high energy diet is an essential factor affecting post-mortem glycolysis (Frylinck *et al.*, 2013; Ponnampalam *et al*., 2017). Ponnampalam *et al*. (2017) suggested from reviewed data that throughout the finishing phase, ruminants need a high-energy diet to decrease the incidence of DFD meat. However, cattle with chronic acidosis will be at risk of DFD because of the reduced feed intake relative to the expected intake. According to Webb (2006), DFD meat can be reduced by better feeding and feed supplements, such as antioxidants.

iv. The influence of the fasting period on DFD meat

During the pre-slaughter period, meat-producing animals are deprived of food and water to decrease stomach contents and prevent the release and spread of microbial contamination of carcasses (O'Neil *et al*., 2018; Xing *et al*, 2019). Although feed withdrawal reduces feed consumption, and morbidity and mortality rates during transport, it has a significant effect on muscle glycogen depletion (Tarrant, 1989; Viljoen, 2000; Ferguson & Warner, 2008; Adzitey, 2011; O'Neil *et al*., 2018) and weight loss in animals (Adzitey, 2011; Xing *et al*, 2019). Ultimately, pre-slaughter stress in fasted cattle induced more DFD meat compared to fed cattle (Viljoen, 2000). Despite the adverse effects of feed withdrawal, it is commonly practised (Xing *et al*, 2019).

The fasting period is mainly determined by lairage time of the cattle (Adzitey, 2011; Xing *et al*, 2019). In general, the fasting period should not be more than 24 hours (Ferguson & Warner, 2008; Adzitey, 2011). However, in some circumstances, it may increase up to 36-48 hours (Ferguson & Warner, 2008). Throughout marketing and if animals are transported or kept in lairage for longer hours than recommended, it is essential to provide feed and water to prevent starvation and dehydration (Adzitey, 2011). Moreover, it is advised that water should be made available for animals at the abattoir to rehydrate. However, not all animals will drink because of the unfamiliar and limited access to watering facilities (Ferguson & Warner, 2008).

Viljoen (2000) noted that a shorter fasting period had a significant effect on the pH_u. Results of this study also agree with Frylinck *et al*. (2015) who compared two feed withdrawal periods, namely 24 hours prior to slaughter (FW-24) and 3 hours to prior slaughter (FW-3) (Figure 2.4). FW-3 had a minimal incidence of dark-cutting compared to FW-24, indicating that the energy of the muscle can be controlled before slaughter and DFD meat can be prevented to a great extent. Thus, beef quality can be manipulated through feeding. Consequently, it presents new possibilities to benefit the beef industry. However, it will only remain sustainable if it is practical, economical and produces beef in a manner that is acceptable for the South African market (Webb, 2006).

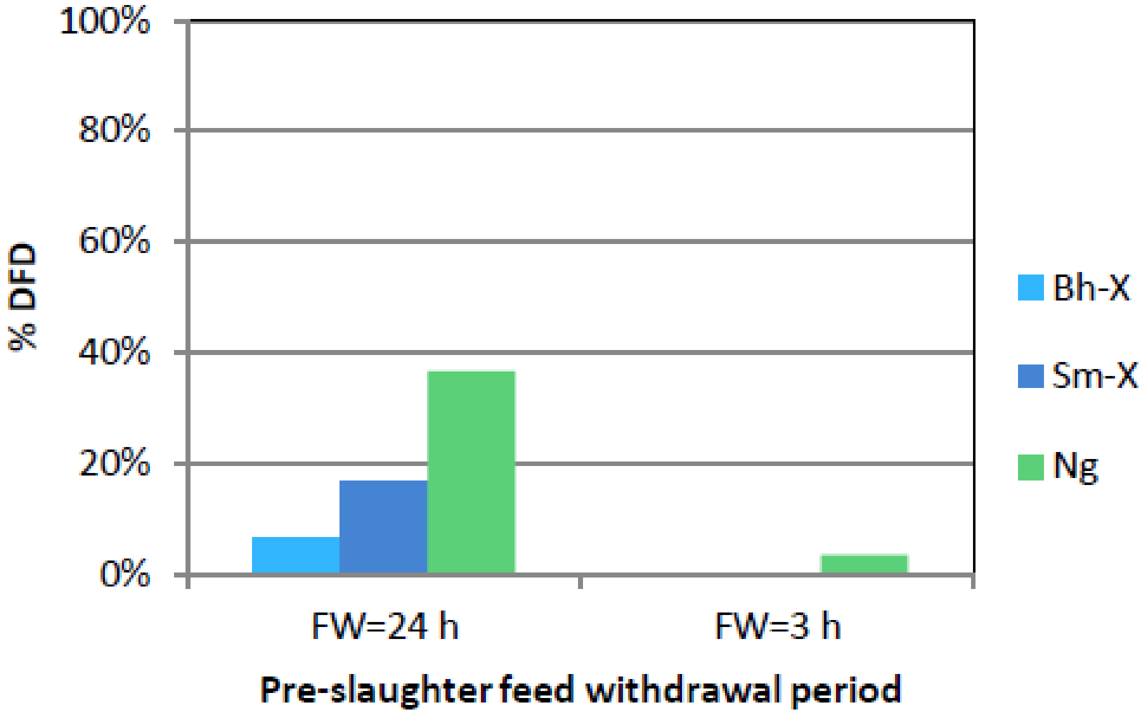


Figure 2.4: Incidence of Dark, Firm and Dry (DFD; pH 24 ≥ 5.9) after two feed withdrawal periods, 24 hours pre-slaughter (FW-24) and 3 hours pre-slaughter (FW-3); n = 180 animals. Breed: Bh-X = Brahman crosses; Sm-X = Simmentaler crosses; Ng = Nguni (From Frylinck *et al.*, 2015).

v. The effect of pre-slaughter animal handling on DFD meat

Pre-slaughter handling includes all human-animal interactions that cattle endure until slaughter, such as on-farm activities, transportation, marketing and lairage (Adzitey, 2011; Birhanu, 2020). In many countries throughout the world pre-slaughter animal handling has become a major issue of concern (Adzitey, 2011). Human-animal interaction results in physiological stress due to physical stresses (extreme temperatures, humidity, vehicle vibration and rapid acceleration, noise, confinement, crowding etc.), psychological stresses (handling, mixing unfamiliar cattle, social regrouping, unfamiliar smells and surroundings etc.) and physiological activities (increased heart rate, respiratory rate, body enzyme activity, body temperature and stress hormones) depleting muscle glycogen and ultimately leading to dark-cutting (Adzitey, 2011; Ponnampalam *et al*., 2017; Birhanu, 2020). According to Ponnampalam *et al*. (2017), humans play a vital part in the occurrence of dark-cutting beef in cattle.

Loudon *et al.* (2018) specified that habituating cattle to humans can reduce pre-slaughter stress experienced during transport and lairage handling, although more research is required to specify the optimal preparation type and level. Furthermore, stress during handling can also be minimised by training personnel responsible for pre-slaughter handling and frequently upgrading or improving facilities and equipment used for livestock (Adzitey & Nurul, 2011; Ponnampalam *et al.,* 2017; Loredo-Osti *et al*., 2019; Birhanu, 2020). However, age, breed and gender will influence how cattle respond to different handling activities and the degree of meat quality will be affected (Adzitey, 2011).

a. The effect of marketing and transportation conditions on DFD meat

Animals are transported over distances from farms to markets and abattoirs (Adzitey, 2011; Birhanu, 2020). During transportation and auction marketing animals are exposed to numerous psychological and physical stressors. Additionally, animals are removed from familiar environments to unfamiliar surroundings, loaded and unloaded onto vehicles and frequently endure long journeys (Adzitey, 2011; Birhanu, 2020). The effect of transport and marketing stress on the muscle glycogen concentration in cattle will vary according to the cattle’s body condition, type, nutritional history and onboard transport conditions (Ferguson & Warner, 2008).

Cattle are either auctioned at markets or transported directly from farms to abattoirs. Throughout markets, cattle are exposed to various stressors such as unfamiliar environments, noise and cattle (Adzitey, 2011). Loredo-Osti *et al.* (2019) noted that the cattle transported from an auction are more prone to DFD than cattle transported from a farm as they are exposed to much greater stressors, which cause exhaustion of muscle glycogen by the time they are slaughtered. Warriss (2000) suggested that a computer auction marketing system should rather be used to avoid unnecessary pre-slaughter stress.

Jones and Tong (1989) stated that as the transportation distance changes from less than 100 km to more than 300 km, the incidence of DFD meat increases. Chulayo *et al.* (2016) noticed that cattle that travelled less than 200 km had a higher glucose concentration compared to a transport duration between 400-800 km. Thus, a negative correlation between transport duration and pH_u was observed. However, Brown *et al.* (1990) noticed increased DFD meat in beef cattle in both short (≤ 30 km) and long (≥ 240 km) transport distances.

Birhanu (2020) reviewed that loading cattle is more stressful than unloading because of the close human-animal interactions. Furthermore, loading density is considered a major issue, because a maximum number of cattle are loaded onto the vehicles to decrease transportation costs, which will lead to increased incidences in DFD meat (Barton, 2014). Njisane and Muchenje (2017) reviewed that longer transportation time along with a high stocking density has a significant negative effect on meat quality. Moreover, Mounier *et al*. (2006) noted that as the transport distance increases between locations cattle became more difficult to manage during unloading. Additionally, increased transport distance is negatively associated with body condition, therefore increasing the risk of DFD carcasses (Adzitey & Nurul, 2011; Adzitey, 2011).

Njisane and Muchenje (2017) suggested that feedlot- or fattening facilities should be brought closer to the abattoir to eliminate pre-slaughter transportation stress (Figure 2.5). Cattle can be transported to these facilities two to three months prior to slaughter and on the day of slaughter herded by foot to the abattoir. Accordingly, cattle are given the chance to adapt to the new surroundings and workers, and in the long run profits may increase. This suggestion has the potential to improve animal adaptation to the abattoir workers, surrounding conditions as well as increase profits in the long run.

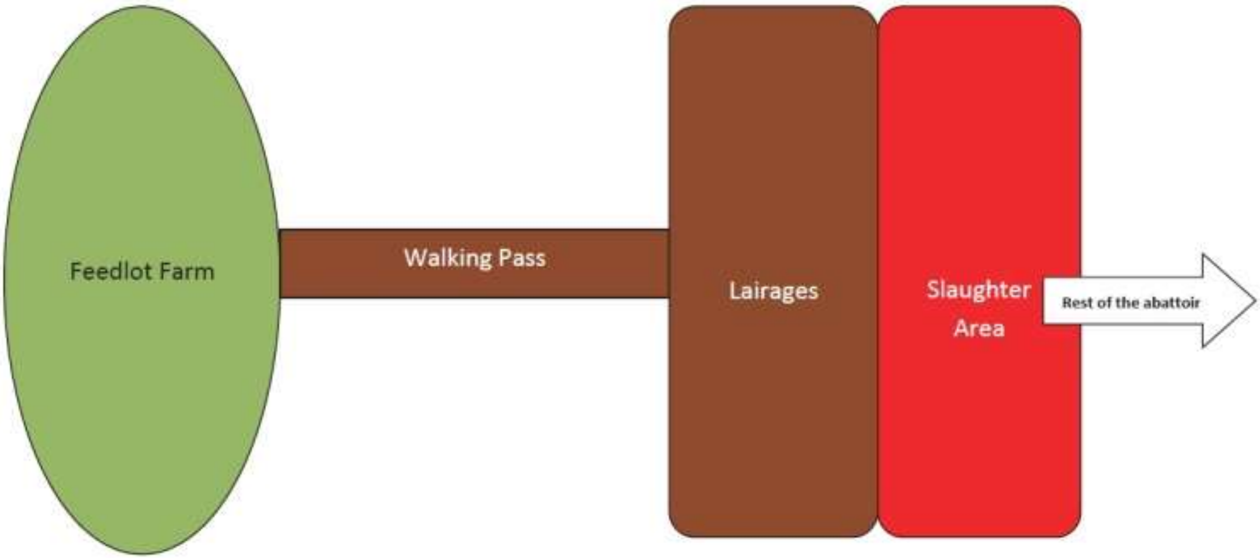


Figure 2.5: A proposed model for bringing the feedlot farms closer to the abattoirs in trying to minimize transportation stress (Njisane & Muchenje, 2017).

Nevertheless, ensuring good transport does not only benefit animal welfare but also contributes to producing high meat quality products, which is of economic importance (Njisane & Muchenje, 2017).

b. The effect of lairage conditions and times on DFD meat

The lairage period serves as a temporary collection point for cattle prior to slaughter and provides recovery from the stressful effects during transportation (Adzitey, 2011; Adzitey & Nurul 2011; Xing *et al*., 2019; Birhanu, 2020). Nevertheless, the unfamiliar surroundings, handling procedures, overcrowding, pre-slaughter mixing, feed restriction and sudden events may contribute to pre-slaughter stress (Ferguson & Warner, 2008; Loredo-Osti *et al*., 2019). Throughout lairage, careless handling, high stocking density and mixing of unfamiliar cattle should be avoided (Adzitey, 2011; Terlouw *et al.*, 2012; Ponnampalam *et al*., 2017).

The lairage period is unquantifiable and uncontrollable, as it mainly depends on the schedule of the abattoirs (Xing *et al.*, 2019). Several researchers noted that as the lairage period increases, there is an increase of DFD carcasses in beef cattle, and suggested that the lairage period should be kept as short as possible (Ferguson & Warner, 2008; Adzitey & Nurul, 2011; Loredo-Osti *et al*., 2019). These results, however, contradicted previous findings by Brown *et al.* (1990), who noted increased DFD beef in cattle slaughtered on the day of arrival rather than the day after. Furthermore, when the duration of transportation is 4 hours, a resting time of more than 3 hours is recommended, depending on the lairage conditions (Del Campo *et al.*, 2010). Teke *et al.* (2014) recommend a resting period of 72 hours for transportation up to 30 hours. Since the duration of transportation has a significant influence on livestock, and the lairage times are directly correlated to the transportation hours, it is common to observe different lairage periods in literature (Loredo-Osti *et al*., 2019).

vi. The effect of electrical stimulation on DFD meat

The use of electrical stimulation will increase the rate of post-mortem muscle metabolism and prevents the occurrence of cold shortening (Fjelkner-Modig & Ruderus, 1983a; Fabiansson *et al.*, 1985; Frylinck *et al*., 2013; Frylinck *et al.*, 2015; Xing *et al*., 2019). Thus, the time to reach pH_u will be shortened (Fjelkner-Modig & Ruderus, 1983a). Furthermore, several researchers reported that electrical stimulation has a tenderizing effect by causing a mechanical disturbance of cytoskeletal proteins, thus improving meat quality (Dutson *et al.*, 1981; Nortje *et al.*, 1985; Frylinck *et al*., 2015; Webb & Agbeniga, 2020).

Electrical stimulation should be monitored correctly, as it has a major effect on the sarcomere length and negatively affects the water-holding capacity. For instance, overstimulation can result in tougher and less juicy meat. Therefore, applying a shorter electrical stimulation time might be essential in commercial abattoirs, such as at 500 V for 15-120 seconds rather than 2 minutes (Frylinck *et al*., 2015; Xing *et al*., 2019). Furthermore, Agbeniga and Webb (2019) recommend the use of low voltage electrical stimulation as it decreases the irregularity in meat quality as a result of variability in carcass weight.

Fabiansson *et al.*, (1985) demonstrated that in non-stimulated and stimulated DFD beef carcasses, ultrastructural and biochemical modifications were monitored very early in the post-mortem period. In electrically stimulated DFD beef carcasses, the depletion of ATP is extremely rapid (Figure 2.6) (Fjelkner-Modig & Ruderus, 1983a; Fabiansson *et al.*, 1985). Additionally, Fabiansson *et al.* (1985) noted ultrastructural modifications in DFD beef samples through strong contractions and complete disruption of tissues. Modifications in DFD beef samples could be due to a cumulative effect of super-contractions and proteolytic activity. Nevertheless, there was no improvement in the tenderness of electrically stimulated dark-cutting carcasses. These results were also concluded by Dutson *et al.* (1981) and Fjelkner-Modig and Ruderus (1983a).

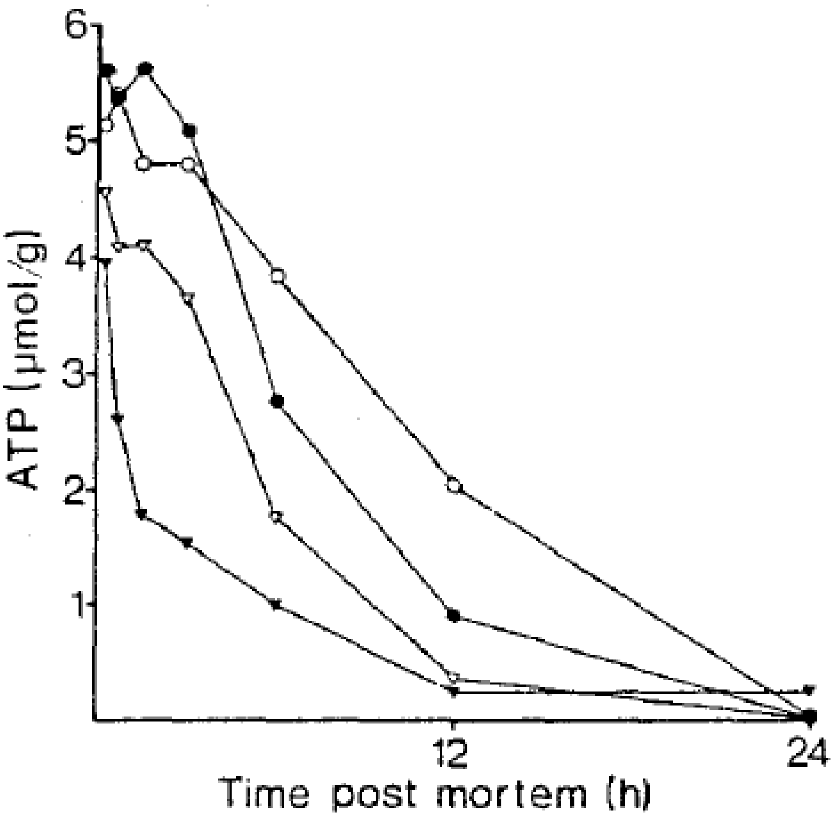


Figure 2.6: The influence of electrical stimulation on post-mortem ATP turnover rate in M. longissimus dorsi of normal and DFD carcasses. ○, pH ≤ 5.8, not stimulated (NS): ●, pH ≥6.2; Δ, pH ≤5.8, electrically stimulated (ES):▲, pH ≥6.2 (Fabiansson *et al.*, 1985).

According to Fjelkner-Modig and Ruderus (1983b) at one-day post-slaughter, electrically stimulated DFD beef carcasses have a higher tenderness than non-stimulated DFD beef carcasses, however after 14 days of aging it is no longer significant. Nevertheless, electrical stimulation of carcasses from stressed animals that are not completely exhausted (slight DFD meat) may produce a somewhat unpredictable effect, resulting in PSE-like meat (Fjelkner-Modig & Ruderus, 1983a).

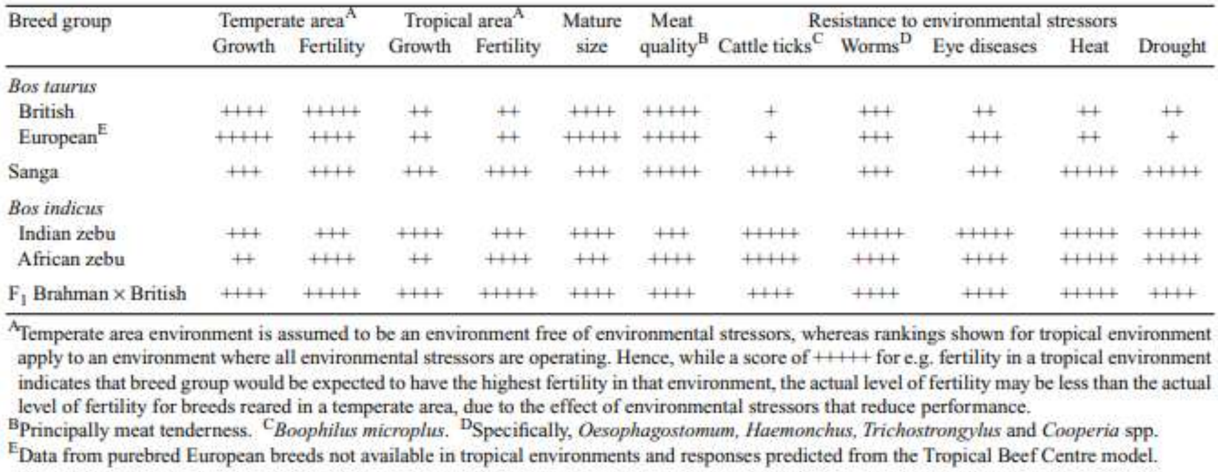
2.2.2 Intrinsic factors

i. The effect of genetic diversity consisting of breed and temperament on DFD meat

For most carcass and beef quality attributes there are significantly large differences between breeds (Burrow *et al.*, 2001). Cattle breeds are divided into distinct geographical groupings. *Bos taurus* breeds are mostly adapted to temperate environments and do well in feedlot situations (e.g. Angus, Simmentaler, Hereford etc.), whereas *Bos indicus* or Zebu breeds (e.g. Brahman etc.) and *Bos taurus africanus* or African Sanga breeds (e.g. Africander, Nguni, Tuli) are more adapted to tropical environments rather than feedlots (Burrow, 2014; Frylinck *et al.*, 2015). However, Frylinck *et al.* (2015) did not experience the phenomenon where *Bos indicus* cattle are not adapted to feedlots. Moreover, Sanga breeds are smaller in carcass size and are not common in commercial feedlots however, their adaptability and meat quality are acceptable to feedlots (Frylinck & Heinze, 2003; Strydom *et al.*, 2008). No single cattle breed can produce beef efficiently in all environments and meet the specific meat quality attributes required (Burrow *et al.*, 2001). Thus, the genotype-environmental interactions have a large effect on the breed and breed type rankings for some traits (Table 2.4) (Burrow *et al.*, 2001), and contribute to the occurrence of dark-cutting meat (Ponnampalam *et al*., 2017). Table 2.4 indicates the variation in the performance for both productive and adaptive traits between breeds.

Table 2.4: Comparative rankings of different breed groups for productive traits in temperate and tropical environments and adaptation to stressors of tropical environments (adapted from Frisch 1997 and MRC 1997) (From Burrow *et al*., 2001).

The higher the number of +s, the higher the value for the trait



Breed group	Temperate area^{A}		Tropical area^{A}		Mature	Meat	Resistance to environmental stressors				
	Growth	Fertility	Growth	Fertility	size	quality^{B}	Cattle ticks^{C}	Worms^{D}	Eye diseases	Heat	Drought
Bos taurus											
British	++++	+++++	++	++	++++	+++++	+	+++	++	++	++
European^{E}	+++++	++++	++	++	+++++	+++++	+	+++	+++	++	+
Sanga	+++	++++	+++	++++	+++	+++++	++++	+++	+++	+++++	+++++
Bos indicus											
Indian zebu	+++	+++	++++	+++	++++	+++	+++++	+++++	+++++	+++++	+++++
African zebu	++	++++	++	++++	+++	++++	+++++	++++	++++	+++++	+++++
F_1 Brahman × British	++++	+++++	++++	+++++	++++	++++	++++	++++	++++	+++++	++++

[A] Temperate area environment is assumed to be an environment free of environmental stressors, whereas rankings shown for tropical environment apply to an environment where all environmental stressors are operating. Hence, while a score of +++++ for e.g. fertility in a tropical environment indicates that breed group would be expected to have the highest fertility in that environment, the actual level of fertility may be less than the actual level of fertility for breeds reared in a temperate area, due to the effect of environmental stressors that reduce performance.
[B] Principally meat tenderness. [C] *Boophilus microplus*. [D] Specifically, *Oesophagostomum, Haemonchus, Trichostrongylus* and *Cooperia* spp.
[E] Data from purebred European breeds not available in tropical environments and responses predicted from the Tropical Beef Centre model.

Although the adaptability of *Bos indicus* cattle to hot climates is the main trait that distinguishes *Bos indicus* from *Bos taurus* subspecies (Table 2.4), it is well known that *Bos indicus* cattle have higher temperament scores, which can be a genotype characteristic, compared to *Bos taurus* and Sanga types (O'Neill *et al.*, 2012; Frylinck *et al.*, 2015). Some previous research indicated that other physiological measures of stress, such as the levels of cortisol and catecholamines, are correlated with temperament

(King *et al.*, 2006; Bozzo *et al*., 2018). Thus cattle with a more excitable temperament, e.g. *Bos indicus*, are more susceptible to stress, which results in a higher prevalence of borderline dark-cutters (Viljoen, 2000; Frylinck *et al.*, 2015; Ponnampalam *et al.*, 2017). However, these results contradicted previous findings where Sanga breeds were more susceptible to stress compared to *Bos indicus* and *Bos taurus* cattle (Frylinck *et al.*, 2015). Nevertheless, temperament has a relatively high heritability (h_2 =0.45) (Lanier *et al.*, 2000) and Ferguson and Warner (2008) suggested that in meat-producing ruminants temperament should be considered in genetic selection programs.

As previously mentioned Frylinck *et al.* (2015) compared two feed withdrawal periods, FW-24 and FW-3 between typically processed breed types in South Africa (Figure 2.4). Brahman crosses represent *Bos indicus*, Simmentaler crosses represent *Bos taurus* and Nguni represents Sanga types. Consequently, *Bos indicus* had the lowest frequency of DFD beef in both feed withdrawal periods, followed by *Bos taurus* and Sanga type. According to O'Neill *et al.* (2018), breed type has a relatively large effect on glycogen and glucose-6-phosphate concentration at slaughter, which might be due to the different muscle fibre types between breeds and may contribute to the variation of DFD beef in different breed types.

From the aforementioned results, it can be concluded that dark-cutting carcasses in Bos *indicus* cattle are mostly because of the more excitable temperament that predisposes stress (O'Neill *et al.*, 2012), whereas DFD beef in Sanga and *Bos taurus* cattle is primarily caused by the fasting period that results in depletion of muscle glycogen (Frylinck *et al.*, 2015; O'Neill *et al.*, 2018). Adzitey and Nurul (2011) suggested that there is a need to develop stress-resistant breeds, therefore a combination of both temperate and tropical genotypes might be beneficial to decrease the occurrence of dark-cutting.

ii. The effect of gender on DFD meat

Although gender determines various management practices that influence the perception of dark-cutting, it is also a contributing factor to the occurrence of DFD meat (Scanga *et al*., 1998; Tarrant, 2012). Nevertheless, there are various contradicting results in opinions regarding dark-cutting in different gender categories.

Generally, bulls are thought to be more affected by DFD meat compared to other gender categories (Viljoen, 2000; Węglarz, 2010; Tarrant, 2012; Ponnampalam *et al*., 2017; Loredo-Osti *et al*., 2019). Consequently, muscle glycogen depletion and dark-cutting in bulls are associated with excitable temperaments and sexual activity culminating in significantly more fighting and mounting behaviour, especially in mixed penning (Tarrant 1989; Lanier *et al.*, 2000; Viljoen, 2000; Tarrant, 2012). These results, however, contradicted the previous findings by Warner and Tarr (2018), where male lots had 2% less dark-cutting carcasses compared to females and mixed-gender lots.

Various researchers considered that steers are not significantly affected by dark-cutting, compared to bulls and heifers (Viljoen, 2000; Tarrant, 2012). Contradictorily Jones and Tong (1989) and Bass *et al.*

(2010) recorded more DFD meat in steers compared to heifers. Additionally, social regrouping in steers has been shown to increase the incidence of DFD beef (Tarrant, 1989; Viljoen, 2000).

There is a significant association between the likelihood of heifers producing DCB and the presence of oestrus (Tarrant 1989; Pethick & Tudor, 1995; Viljoen, 2000; Mahmood *et al.*, 2016), reduced carcass weights (Mahmood *et al.*, 2016) and temperament (Voisinet *et al.*, 1997). During oestrus, heifers exhibit mounting activity that causes a substantial decrease in muscle glycogen reserves and leads to muscle pH_u variation (Tarrant, 1989; Pethick *et al.*, 1995; Viljoen, 2000; Mahmood *et al.*, 2016). Furthermore, as the live- and carcass weights increase in heifers, the risk of dark-cutting decreases (Murray, 1989; Mahmood *et al.*, 2016). According to Mahmood *et al.* (2016), carcass weight in heifers is the most important indicator of DFD meat, therefore growth performance, live animal phenotype and carcass measurements can be used to predict DFD beef in heifers. Additionally, previous studies reported a more excitable temperament in heifers compared to steers (Voisinet *et al.*, 1997). Although excitable temperaments in bulls and heifers play a significant role in glycogen depletion, temperament is driven by *Bos indicus* content and not gender (Hearnshaw & Morris, 1984).

Węglarz (2010) observed a statistically significant effect between gender, slaughter season and meat pH. Bulls had higher pH_u values (> 5.8) in the summer season, whereas heifers had pH_u values above 5.8 independent of the slaughter season. Thus, bulls might be more susceptible to different stressors, such as air temperature.

Mounting activity has conclusively been shown to be the behaviour mostly correlated to muscle glycogen depletion in bulls, heifers and steers (Tarrant 1989; Loudon *et al.*, 2018). Mounting behaviour is particularly stimulated by social regrouping, consequently mixing unfamiliar cattle during transport and holding periods in bulls and steers, and during oestrus in heifers (Tarrant 1989; Viljoen, 2000). However, these assumptions contradict previous findings of Mach *et al*. (2008), who noted that mixing unfamiliar cattle and genders during transport did not affect the pH_u.

iii. The effect of cattle weight on DFD beef

According to Mahmood *et al.* (2016), carcass weight seems to be an important indicator of cattle producing DFD carcasses. Several researchers indicated that the probability of DFD may decrease as the live weight increases (Kreikemeier *et al.*, 1998; Mahmood *et al.*, 2016). For instance, live weights greater than 550kg (Mahmood *et al.*, 2016) or carcasses weighing more than 150kg to 220kg (McGilchrist *et al.*, 2012) and 275kg (Jones & Tong, 1989), seem to have lower incidences of DFD meat. However, these findings are in contrast to previous studies where increased muscle area was related to increased DFD (McGilchrist *et al.*, 2011; Loredo-Osti *et al.*, 2019). Subsequently, selection for muscling may increase the glycolytic and glycogenolytic capacity, which increases the adrenaline responsiveness of muscle tissues and ultimately results in reduced muscle glycogen storage (McGilchrist *et al.*, 2011). Nevertheless, McGilchrist *et al.* (2016) confirmed that cattle selected for increased muscling did not have increased glycolytic potential, but had a larger oxidative capacity compared to cattle with reduced muscling. Thus, during energy expenditure cattle with increased muscling might completely oxidize glycogen or use lipids as a source of energy, consequently sparing muscle glycogen (Mahmood *et al.,* 2016). Results of this research were supported by Gardner *et al.* (2005), which specified that selection for muscling will result in more muscle glycogen at slaughter because the stress sensitivity in muscle tissue will theoretically decrease and dark-cutting in cattle will decline.

In several countries, including South Africa, abattoir pricing favours heavier carcasses. Furthermore, in developing countries, where malnutrition and hunger are common, the demand for good quality animal protein is rising (Agbeniga & Webb, 2018). Although heavier carcasses have to be processed in the same facilities that were designed to process smaller carcasses, Agbeniga and Webb (2018) suggested that the production of heavier carcasses in South Africa should be encouraged, along with more research to mitigate the adverse effects on meat quality attributes.

iv. The effect of animal age on DFD meat

According to Ponnampalam *et al.* (2017), when considering the age at slaughter as a factor contributing to dark-cutting meat, it is necessary to note that age at slaughter is frequently confounded with other extrinsic and intrinsic factors such as breed characteristic, growth rate, production systems and diet. Therefore, segregating cattle age at slaughter as an effect in animal studies is complex.

It is well established that as ruminants mature, the myoglobin (Mb) concentration increases (Humanda *et al.,* 2014; Cho *et al.,* 2015), which leads to meat that is darker and has lower colour stability (Figure 2.7) (Cho *et al.,* 2015; Ponnampalam *et al.,* 2017). Furthermore, the elevated intramuscular Mb concentration has been affiliated with greater redness (a* values) and a decline in lightness (L* values) (Cho *et al.,* 2015; Ponnampalam *et al.,* 2017). Such an effect indicates that the oxidative muscle capacity increases as the ruminant matures. Theoretically, cattle with a higher oxidative muscle capacity might be more susceptible to DFD, because they will possibly have lower muscle glycogen content (Ponnampalam *et al.,* 2017).

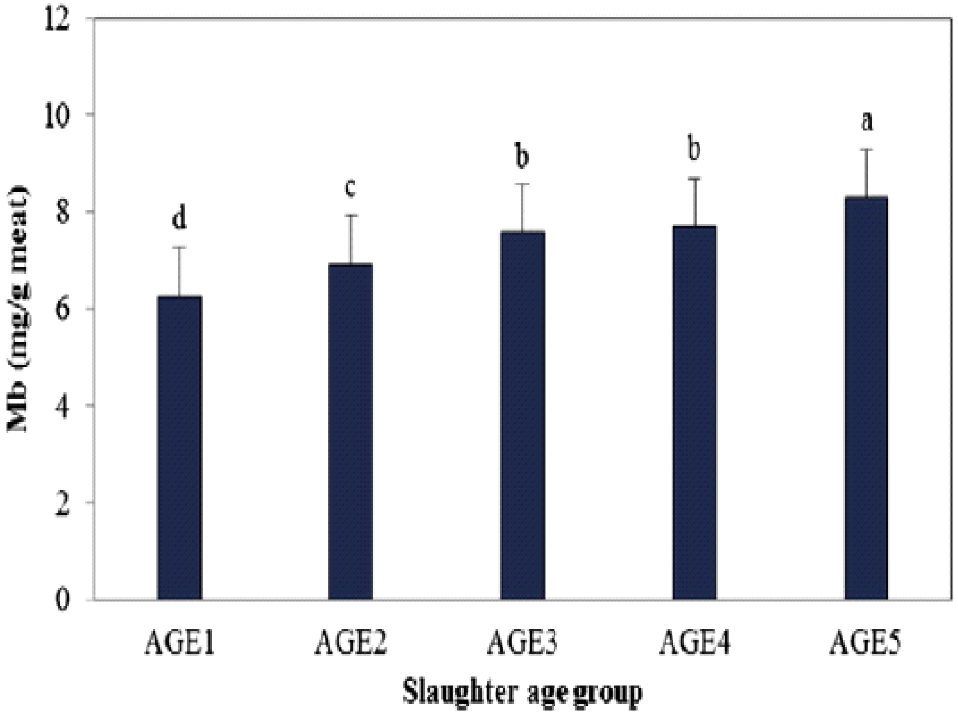


Figure 2.7: Effect of slaughter age on the myoglobin (Mb) content (mg/g meat) in M. longissimus lumborum from Hanwoo cows. These data are presented as the mean ± standard error. Different letters indicate significant differences among slaughter age groups ($P < 0.05$). AGE1: 1.9 to 3.7 yr; AGE2: 4.0 to 4.8 yr; AGE3: 5.0 to 5.7 yr; AGE4: 6.0 to 6.9 yr; AGE5: 7.5 to 11.5 yr (From Cho *et al.*, 2015).

Furthermore, Kelava *et al.*, (2008) compared 10-14- months old to 15-18- and 19-24 months old Simmental cattle that were slaughtered in Croatia and noticed a slight increase in pH_u as the cattle matured. Moreover, Gardner *et al.*, (2005) reported an increased adrenaline sensitivity in Angus and Piedmontese (heavily muscled genotype), sires from 15-36 months of age. Thus, the rise in adrenaline sensitivity as the animal matures leads to an increase in glycogen depletion. Therefore, it could be attributable to the increase in pH_u as the cattle mature (Ponnampalam *et al.*, 2017). Thus, as the age at slaughter increases, the risk of DFD meat rises.

South African consumers prefer carcasses of younger cattle compared to those of older cattle. Therefore, in South Africa mainly all cattle are slaughtered at the age of 12-16 months before permanent incisors appear (Frylinck *et al*. 2013). Thus, the age of the animal is not a major factor contributing to DFD meat in South Africa and meat quality is influenced more by production systems, diets and slaughter conditions than age (Webb & Erasmus, 2013).

v. The effect of muscle-specific variation and muscle fibre types on DFD meat

The different relative proportion of fibre types present in the muscle, along with the oxidative and reductive capacities of the post-mortem muscle could influence the muscle-specific colour phenomena (McGilchrist *et al.*, 2011; Neethling *et al.*, 2017). Furthermore, the muscle fibre type affects the concentration of Mb, oxidative capacity and the rate of post-mortem pH decline in the muscle. Altogether these factors will have a major effect on the colour and colour consistency of the meat, which is of major importance for consumer acceptability (Kim *et al.*, 2014).

Fibre types are classified into different categories, such as Type I (slow-twitch oxidative fibre), Type IIA (fast-twitch oxidative fibres), IIX and IIB (fast-twitch glycolytic fibres). These categories differ according to the energy source utilized, contraction rate and metabolic pathways (Lawrie & Ledward, 2006; Neethling *et al.*, 2017). Thus, slow-twitch and fast-twitch fibres respond differently to stress (Lacourt & Tarrant, 1985) leading to a variation in glycogen concentration between muscle fibre types (Hopkins, 2006). Therefore, the pH_u will differ between muscles (Samuelsson, 2020). Furthermore, Neethling *et al.* (2017) stated that different muscle fibre types primarily affect the susceptibility of muscles to dark-cutting. Table 2.5 summarizes the relationship between muscle fibre types, muscle types and DFD meat.

Table 2.5: Summary of the relationship between muscle fibre types, muscle types and DFD

Fibre type	Description	Reference	Muscles most liable to be affected by DFD
Type I fibres/ red or dark fibres (slow-twitch oxidative fibres)	- Produce ATP **aerobically** - Slow contracting - Small in size - Contain a large number of mitochondria - Extremely resilient to fatigue	Neethling *et al.*, 2017; Ponnampalam *et al.*, 2017	Tarrant (1989) and McGilchrist *et al*. (2011) indicated that muscles with the largest quantity of fast-twitch glycolytic fibres are most likely to be dark-cutting meat due to physical stress, for instance the *longissimus thoracis et lumborum* and *semitendinosus.* Neethling *et al.* (2017) reviewed that cattle more prone to pre-slaughter stress had a larger proportion of white, anaerobic, fibre types. Furthermore, Hunt and Hedrick (1977) stated that depending on the nature of pre-slaughter stress, fast-twitch oxidative fibres are the predictor of DFD beef. However, Zerouala and Stickland (1991) specified that in DFD beef there is an increased concentration of both slow-twitch oxidative and fast-twitch oxidative fibres.
Type IIA/ Intermediate fibres (fast-twitch oxidative fibres)	- Produce ATP **aerobically** and **anaerobically** - Fast contracting - Contain moderately large numbers of mitochondria - Resistant to fatigue		
Type IIX/ White or pale fibres (fast-twitch	- Produce ATP **anaerobically** - Fast contracting (faster than type IIA)		

glycolytic fibres)	- Contain a small number of mitochondria - Fatigue rapidly	Furthermore, according to Tarrant (1981) muscles most likely to be affected by DFD meat in descending order is the *M. logissimus dorsi* > *M. semimemraosus* > *M. biceps femoris* > *M. semitediosus* > *M. adductor* > *M. gluteus medius* > *M. trapezius* > *M. biceps brachii* > *M. psoas major* > *M. infraspinatus and M. supraspiatus*
Type IIB/ White or pale fibres (fast-twitch glycolytic fibres)	- Produce ATP **anaerobically** - Low levels of mitochondria - Highly receptive to fatigue - Lower-levels oxidative capacity in comparison with type IIX fibres	

CHAPTER 3

MATERIAL AND METHODS

This project was approved by the Ethics Committee (NAS303/2020).

3.1 THE OBJECTIVES OF THIS STUDY

Aim:

To review and determine the incidence of dark, firm and dry (DFD) beef carcasses in South African beef abattoirs.

To identify and study the causative risk factors of DFD beef carcasses in South African beef abattoirs from feedlot and pasture systems.

Modelling of the causative risk factors associated with DFD beef in South African beef abattoirs and identification of mitigation strategies to better manage the risks.

Objectives:

To analyse possible causative risk factors associated with DFD beef in South African conditions, through a comprehensive retrospective review of production data from feeding systems, transportation, lairage, slaughter and cooling to retail.

To identify basic strategies in South Africa to decrease the risk of DFD meat.

3.2 GENERAL OVERVIEW

The proposed research consists of a retrospective data analysis to predict the risk of all possible factors associated with DFD beef in South Africa. Data were collected from a specialized abattoir in the Gauteng province, where approximately 300 cattle are slaughtered per day.

The studied material consisted of 29,787 cattle from 52 suppliers, distributed over Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West and Western Cape provinces in South Africa (Figure 3.1) and different production systems (Angus = 2,116; Commercial = 10,580; Free-range = 12,976).

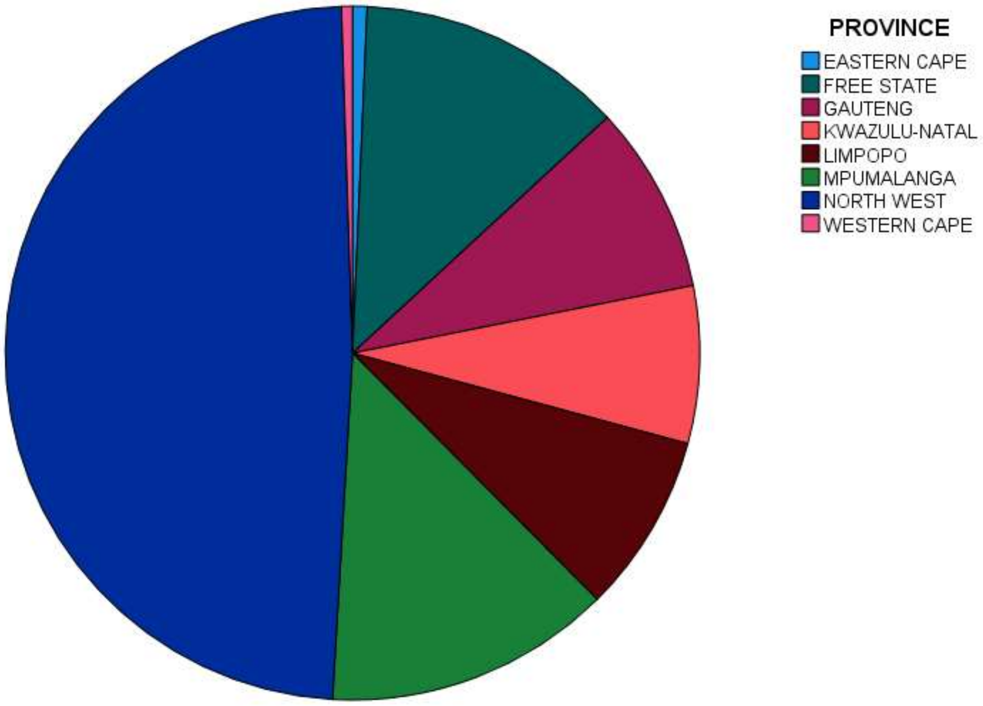


Figure 3.1: A summary of Suppliers from different provinces in South Africa included in the data collected.

Data were obtained and processed for the period from January 2020 to December 2020. The large data set included data per individual animal to the carcass, and for all four seasons of 2020, to quantify the effects of seasonal changes on the DFD condition in beef with seasons defined as follow: Summer (November-January), Autumn (February- April), Winter (May- July), and Spring (August-October). Added to the database were the climatic factors (minimum and maximum daily temperature and humidity recorded at the farm and abattoir), farm production systems, estimated standing time, breed type, age class, gender, cattle conformation, live weight, carcass weight and fat content that may contribute to the occurrence of DFD meat (Table 3.1).

Climatological data were obtained from the closest meteorological station from the loading location of cattle at the farms and the unloading location at the slaughter plant. The daily minimum and maximum temperatures and humidity differences and their interactions were evaluated to determine which had an effect on the percentage of DFD per individual animal.

There was a large variation between geographical locations and from previous studies in pH values at which DFD is identified. Therefore in this study the 24-hour pH values post-mortem were divided into risk categories: Normal ($pH_u < 5.8$), Intermediate (pH_u $5.8 \leq X < 6.2$) and High ($pH_u \geq 6.2$) (Węglarz, 2010; Chulayo *et al.*, 2015).

Table 3.1: Model Summary

The Analysis Factor	**Factors**	**Levels**	**Description**
Dependent Variable	CARCASS PH 24H POST-MORTEM (PH$_U$) (LOIN)		Post-mortem carcass pH
Fixed Effects	SLAUGHTER SEASON	AUTUMN	February-April
		SPRING	August-October
		SUMMER	November-January
		WINTER	May-July
	AGE CLASS	A	0 Teeth
		AB	1-2 Tooth
		B	3-6 Tooth
		C	More than 6 teeth
	FAT CONTENT	0	No fat
		1	Very lean
		2	Lean
		3	Medium
		4	Fat
		5	Overfat
		6	Excessively fat
	CONFORMATION	1	Very flat
		2	Flat
		3	Medium
		4	Fat
		5	Overfat
		6	Excessively fat
	GENDER	C	Cow
		H	Heifer
		O	Steer
		B	Bull
	BREED	BOS INDICUS	Purebred cattle more adapted to tropical environments rather than feedlots
		BOS INDICUS X	Composite breed, but more adapted to tropical environments rather than feedlots
		BOS TAURUS	Purebred cattle more adapted to temperate conditions and do well in feedlot situations

		BOS TAURUS X	Composite breed, but more adapted to temperate conditions and do well in feedlot situations
		COMPOSITE BREED	Bos indicus and Bos taurus crossbred
	PRODUCTION SYSTEM	A	Angus
		C	Commercial feedlot
		FR	Free-range
	STANDING PERIOD	1	Slaughtered same day as received at the abattoir
		2	Slaughtered next day after received at the abattoir
		3	Slaughtered 3/ more days after received at the abattoir
Covariates	LIVE MASS		Live weight of cattle
	WARM MASS		Hot standard carcass weight
	CARCASS TEMPERATURE 1H POST-MORTEM (LOIN)		Post-mortem carcass temperature
	CARCASS TEMPERATURE 24H POST-MORTEM (LOIN)		
	CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE)		
	CARCASS PH 1H POST-MORTEM (LOIN)		Post-mortem carcass pH
	CARCASS PH 16H POST-MORTEM (LOIN)		
	Tx_FARM - Tx_ABATTOIR		Daily maximum temperature differences between the farm and abattoir

	Tn_FARM - Tn_ABATTOIR		Daily minimum temperature differences between the farm and abattoir	
	RHx_FARM - RHx_ABATTOIR		Daily maximum humidity differences between the farm and abattoir	
	RHn_FARM - RHn_ABATTOIR		Daily minimum humidity differences between the farm and abattoir	
	LOADING DENSITY		Number of cattle slaughtered in a batch	
	TRANSPORT DISTANCE		The distance the cattle travelled from the farm to the abattoir	
Random Effects	SUPPLIER:		The origin of the cattle	
			Farm Production Systems:	Province:
		Supplier A	Commercial & Free-Range	Gauteng
		Supplier B	Angus	Free State
		Supplier C	Angus, Commercial & Free-range	Limpopo
		Supplier D	Angus, Commercial & Free-range	Free State
		Supplier E	Commercial	Eastern Cape
		Supplier F	Free-range	KwaZulu-Natal
		Supplier G	Free-range	KwaZulu-Natal
		Supplier H	Free-range	KwaZulu-Natal
		Supplier I	Angus	KwaZulu-Natal
		Supplier J	Free-range	KwaZulu-Natal
		Supplier K	Free-range	Free State
		Supplier L	Free-range	Free State
		Supplier M	Free-range	KwaZulu-Natal
		Supplier N	Free-range	Free State
		Supplier O	Free-range	KwaZulu-Natal
		Supplier P	Free-range	Free State
		Supplier Q	Commercial & Free-range	Free State
		Supplier R	Free-range	Eastern Cape
		Supplier S	Angus	Eastern Cape
		Supplier T	Commercial & Free-range	Free State
		Supplier U	Free-range	KwaZulu-Natal
		Supplier V	Free-range	Free State
		Supplier W	Angus & Free-range	Free State
		Supplier X	Free-range	KwaZulu-Natal
		Supplier Y	Free-range	KwaZulu-Natal

Supplier Z	Free-range	Free State
Supplier AA	Commercial	Limpopo
Supplier BB	Commercial	Gauteng
Supplier CC	Free-range	Free State
Supplier DD	Free-range	Western Cape
Supplier EE	Free-range	Free State
Supplier FF	Free-range	KwaZulu-Natal
Supplier GG	Free-range	KwaZulu-Natal
Supplier HH	Commercial	North West
Supplier II	Free-range	KwaZulu-Natal
Supplier JJ	Free-range	Free State
Supplier KK	Angus, Commercial & Free-range	North West
Supplier LL	Angus	North West
Supplier MM	Free-range	Eastern Cape
Supplier NN	Free-range	KwaZulu-Natal
Supplier OO	Angus	Free State
Supplier PP	Angus	Eastern Cape
Supplier QQ	Free-range	KwaZulu-Natal
Supplier RR	Commercial & Free-range	Mpumalanga
Supplier SS	Free-range	KwaZulu-Natal
Supplier TT	Free-range	KwaZulu-Natal
Supplier UU	Commercial	Mpumalanga
Supplier VV	Commercial	Gauteng
Supplier WW	Free-range	Free State
Supplier XX	Angus & Free-range	KwaZulu-Natal
Supplier YY	Free-range	Western Cape
Supplier ZZ	Free-range	KwaZulu-Natal

3.3 CARCASS PH AND TEMPERATURE MEASUREMENTS AT THE ABATTOIR

The abattoir measured the post-mortem carcass pH in the loin and temperature in the loin and silverside. The primary muscles in the loin and silverside are the *M. longissimus dorsi* and *M. biceps femoris* (Figure 3.2). pH was measured in the abattoir at 1 h and 16 h post-mortem with an ETI 8000/8100 pH meter, AD111 Standard Professional pH-ORP-TEMP portable meter or AD1230B pH electrode, whereas the 1 h and 16 h post-mortem temperatures were measured with a Hanna Checktemp 4 HI 151-00 meter. In the defatting section of the abattoir, the 24 h post-mortem pH was measured with a Hanna HI 98163 meat pH meter or Hanna FC2323 pH electrode, whereas the 24 h post-mortem temperature was measured with a Thermapen Classic Thermometer.

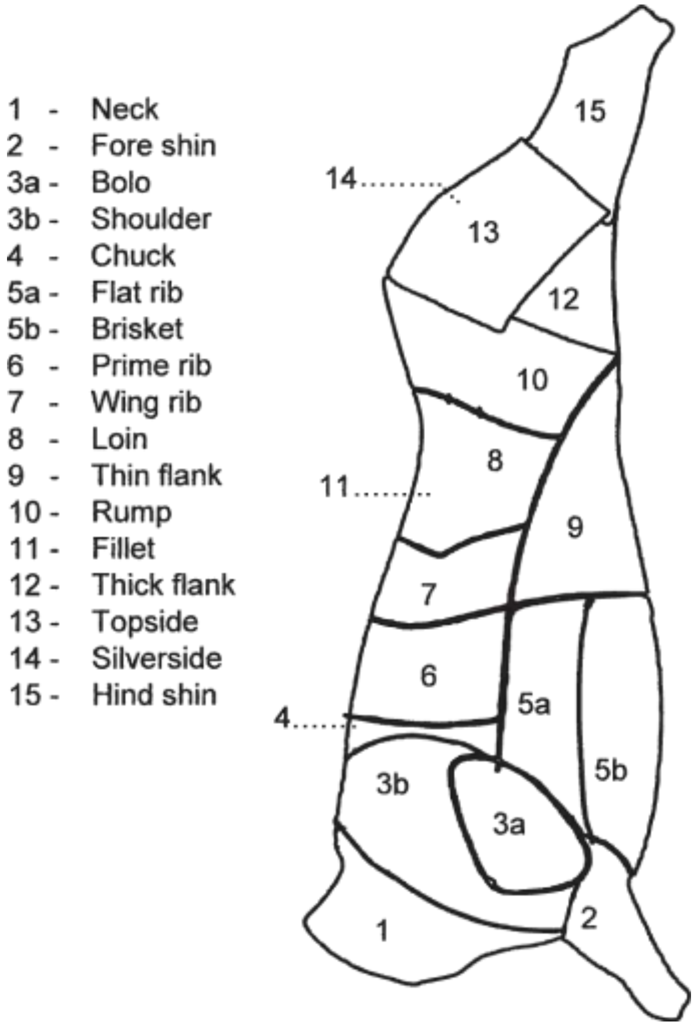


Figure 3.2: Wholesale cuts of the South African beef carcass (from Strydom & Smith, 2010).

3.4 STATISTICAL ANALYSIS

The statistical analysis was conducted using SPSS IBM Statistics version 27 (2089, 2020). The General linear mixed model was performed on the unbalanced data, with Supplier as a random effect, and with other factors modelled as fixed effects and covariates. The Restricted Likelihood Method (REML) was used to estimate the model. For post-hoc testing, LSD (Least Significant Difference) was used. Influential observations with residuals > 2 were identified and omitted, a normal probability plot was compiled for residuals to determine any deviation from the normal distribution and outliers were identified and excluded. The statistical differences were considered significant at a probability level of 5% ($p \leq 0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 A GENERAL OVERVIEW OF THE INCIDENCE OF DFD BEEF BASED ON THE 24 H PH POST-MORTEM

Table 4.1: An estimate of the incidence of DFD beef at a high throughput abattoir in South Africa for the period from January 2020 to December 2020.

DFD risk categories	**Carcass pH 24h post-mortem (pHu) (Loin)**			**Total**
	Normal **(pH_u < 5.8)**	Intermediate **(pH_u 5.8 ≤ X < 6.2)**	High **(pH_u > 6.2)**	
Frequency	15900	12176	121	28197
Percentage	56.4%	43.2%	0.40%	100%

The mean percentage of high-risk DFD beef for the period from January 2020 to December 2020 was reasonably low, i.e., 0.4%. Although the proportion of total normal carcasses for the period was 56.4%, there was a relatively high percentage of carcasses with an intermediate risk of DFD, i.e., 43.2%. Intermediate carcasses can be classified as borderline carcasses because certain muscles may produce DFD beef and it may not necessarily be the muscles where pH_u was measured post-mortem (*M. longissimus dorsi* and *M. biceps femoris*), therefore it is difficult to identify DFD in carcasses classified as an intermediate risk of DFD meat. Furthermore, 43.6% of carcasses (intermediate and high-risk carcasses) had a pH_u value of more or equal to 5.8, which increased the risk of DFD beef and financial losses for both the abattoir and retailers (Table 4.1).

Figure 4.1 indicates the average post-mortem pH-temperature decline over the period from January 2020 to December 2020. Interestingly, there was a minor increase in the pH from 16 h to 24 h post-mortem. However, this is not unusual as Agbeniga and Webb (2021) also noted a slight increase in the pH from 16 h to 24 h post-mortem. A possible reason for this may be that during rigor mortis cells rupture and release cytoplasm and lactate which dilute the H^+ and may cause a slight increase in pH from 16 h to 24 h post-mortem. Furthermore, in the abattoir the 16 h pH post-mortem was measured, whereas the 24 h pH was measured in the defatting section 24 hours post-mortem. However, different pH meters were used in the abattoir and the defatting section, which may also partly explain the minor increase in the 16 h to 24 h pH post-mortem.

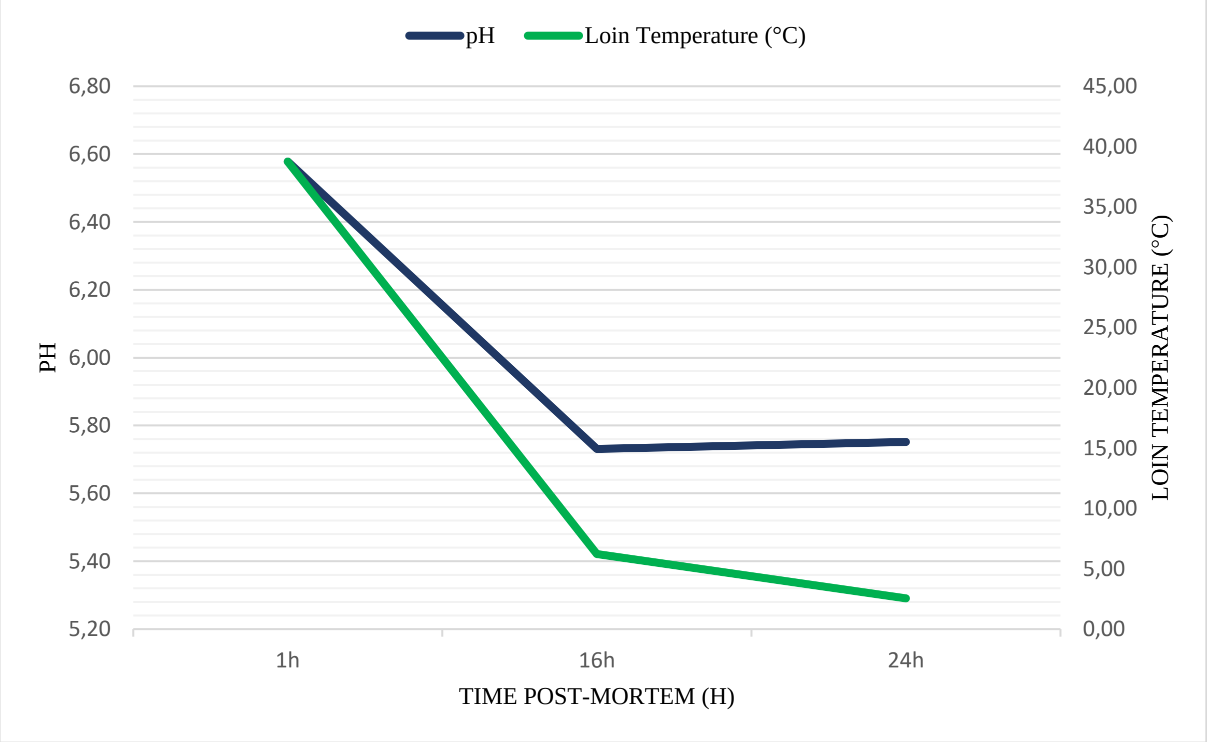


Figure 4.1: The average loin pH-temperature decline post-mortem (h).

4.2 FACTORS THAT INCREASED THE RISK OF DARK, FIRM AND DRY BEEF

4.2.1 Extrinsic factors

i. The effect of suppliers on the incidence of DFD beef

The supplier had a significant effect on the pH_u ($p < 0.05$) and slightly increased the pH_u post-mortem by 0.044% (Table 4.2). Furthermore, there was a large variation between suppliers and the pH_u. Supplier CC had the largest proportion of high-risk DFD meat (27.3%) followed by Supplier N (5.6%) and Supplier L (2.2%) (Appendix B). The variation between suppliers could be due to animals purchased, producers of cattle, breeds selected, nutritional and mineral deficiencies in cattle, human-animal handling techniques, facilities and machinery, pre-consignment management, stock transporter used, transportation distance, health and weather management strategies (Warner & Tarr, 2018). Interestingly, the suppliers with a higher proportion of high-risk DFD beef did not slaughter cattle throughout the year at the abattoir. A reasonable explanation might be due to new surroundings and management techniques used at the abattoir, which suppliers must familiarize themselves with. However, there is an endless list of factors or any combination of extrinsic or intrinsic factors that may contribute to the increased risk of dark-cutters (Warner & Tarr, 2018).

Table 4.2: The Mixed model analysis of the effects of extrinsic and intrinsic factors on DFD beef

Estimates of Fixed Effects[a]

Parameter	Estimate	Estimate %	Std. Error	df	t	Sig.	95% Confidence Interval		F	p-value
							Lower Bound	Upper Bound		
Intercept	5.545311		.054756	1198.663	101.274	**.000**	5.437883	5.652738	14918.127	**.000**
[SLAUGHTER_SEASON=AUTUMN]	.067557	1.176132	.002524	26267.869	26.762	**.000**	.062609	.072505	1999.969	**.000**
[SLAUGHTER_SEASON=SPRING]	-.055683	-0.969412	.002303	26523.207	-24.174	**.000**	-.060198	-.051169		
[SLAUGHTER_SEASON=SUMMER]	-.087792	-1.528412	.002666	26391.809	-32.927	**.000**	-.093018	-.082566		
[SLAUGHTER_SEASON=WINTER]	0^b	0	0	.	.	.	.	.		
[AGE_CLASSr=A]	-.046556	-0.810515	.009093	26492.060	-5.120	**.000**	-.064379	-.028734	9.797	**.000**
[AGE_CLASSr=AB]	-.045607	-0.793994	.009417	26433.813	-4.843	**.000**	-.064065	-.027148		
[AGE_CLASSr=B]	-.049287	-0.858061	.009420	26283.993	-5.232	**.000**	-.067751	-.030824		
[AGE_CLASSr=C]	0^b	0	0	.	.	.	.	.		
[FAT_CONTENT=0]	.118649	2.065616	.024840	26572.249	4.777	**.000**	.069963	.167336	14.247	**.000**
[FAT_CONTENT=1]	.001299	0.022615	.007749	26562.181	.168	.867	-.013889	.016486		
[FAT_CONTENT=2]	-.016781	-0.292148	.005864	26559.110	-2.862	**.004**	-.028275	-.005287		
[FAT_CONTENT=3]	-.021239	-0.369760	.006006	26551.445	-3.536	**.000**	-.033011	-.009467		
[FAT_CONTENT=4]	0^b	0	0	.	.	.	.	.		
[CONFORMATION=3]	-.032480	-0.565460	.022204	26533.789	-1.463	.144	-.076001	.011041	1.076	.341
[CONFORMATION=4]	-.033299	-0.579718	.023767	26533.539	-1.401	.161	-.079884	.013286		
[CONFORMATION=5]	0^b	0	0	.	.	.	.	.		
[GENDER=C]	-.001552	-0.027019	.007917	25911.703	-.196	.845	-.017069	.013965	50.739	**.000**
[GENDER=H]	-.027255	-0.474495	.002706	26179.636	-10.074	**.000**	-.032558	-.021952		
[GENDER =O]	0^b	0	0	.	.	.	.	.		
[BREEDr=1,00]	-.025236	-0.439345	.055087	34.871	-.458	.650	-.137083	.086611	.326	.859
[BREEDr=2,00]	-.003197	-0.0556580	.027955	34.925	-.114	.910	-.059953	.053559		
[BREEDr=3,00]	.005488	0.095543	.020104	37.826	.273	.786	-.035217	.046193		
[BREEDr=4,00]	-.017997	-0.313318	.023191	35.594	-.776	.443	-.065048	.029055		
[BREEDr=5,00]	0^b	0	0	.	.	.	.	.		
[PRODUCTION SYSTEM =A]	-.029198	-0.508322	.004920	19124.268	-5.934	**.000**	-.038842	-.019554	19.053	**.000**
[PRODUCTION SYSTEM =C]	.003075	0.053534	.006148	20743.216	.500	.617	-.008975	.015125		
[PRODUCTION SYSTEM =FR]	0^b	0	0	.	.	.	.	.		
[STANDING PERIOD 1same_day2next_day3gt2_days=1]	.152257	2.650714	.020477	26551.578	7.436	**.000**	.112121	.192393	53.696	**.000**
[STANDING PERIOD 1same_day2next_day3gt2_days=2]	.123338	2.147249	.020740	26550.978	5.947	**.000**	.082687	.163989		
[STANDING PERIOD 1same_day2next_day3gt2_days=3]	0^b	0	0	.	.	.	.	.		
LIVE_MASS	-4.433996E-6	-0.000077	1.758786E-5	26543.163	-.252	.801	-3.890713E-5	3.003914E-5	.064	.801
WARM_MASS	-.000172	-0.002994	3.222148E-5	26572.988	-5.346	**.000**	-.000235	-.000109	28.580	**.000**
CARCASS TEMPERATURE 1H_POST-MORTEM (LOIN)	.002361	0.041104	.000447	26545.469	5.285	**.000**	.001485	.003236	27.930	**.000**
CARCASS PH 1H POST-MORTEM (LOIN)	-.005509	-0.095909	.003099	26556.680	-1.777	.076	-.011584	.000566	3.159	.079

CARCASS PH 16H POST-MORTEM (LOIN)	.034370	0.598364	.004521	26564.054	7.602	**.000**	.025509	.043232	57.794	**.000**
CARCASS TEMPERATURE 24H POST-MORTEM (LOIN)	-.009013	-0.156912	.000750	26541.336	-12.011	**.000**	-.010484	-.007542	144.257	**.000**
CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE)	.002397	0.041731	.000258	26562.174	9.292	**.000**	.001891	.002903	86.339	**.000**
Tx_FARM - Tx_ABATTOIR	.001029	0.017914	.000230	25351.969	4.463	**.000**	.000577	.001480	19.917	**.000**
Tn_FARM - Tn_ABATTOIR	.000948	0.016504	.000281	26365.954	3.371	**.001**	.000397	.001499	11.364	**.001**
RHx_FARM - RHx_ABATTOIR	.000101	0.001758	6.617415E-5	25976.133	1.520	.129	-2.913189E-5	.000230	2.310	.129
RHn_FARM - RHn_ABATTOIR	-.000529	-0.009210	8.331509E-5	26419.027	-6.354	**.000**	-.000693	-.000366	40.378	**.000**
LOADING DENSITY	9.866460E-5	0.001718	4.944750E-5	26021.161	1.995	**.046**	1.744797E-6	.000196	3.981	**.046**
TRANSPORT DISTANCE	-5.443922E-5	-0.000948	3.913931E-5	37.159	-1.391	.173	-.000134	2.485311E-5	1.935	.173

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (PH_U) (LOIN).
b. This parameter is set to zero because it is redundant.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Estimate %	Std. Error	Wald Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Residual		.011756		.000102	115.181	.000	.011558	.011958
SUPPLIERXX	Variance	.002548	0.044359	.000638	3.995	**.000**	.001560	.004162

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (PH_U) (LOIN).

Management systems used by suppliers play a vital role in the occurrence of DFD (Ponnampalam *et al.*, 2017), therefore pre-slaughter management techniques have become a major issue of concern. Although the overall effect of suppliers in this study was minor, management practices from suppliers influence the majority of extrinsic and intrinsic pre-slaughter stress factors on the occurrence of DFD. According to Warner and Tarr (2018), management techniques used by suppliers are one of the main factors contributing to the occurrence of dark-cutting meat. Furthermore, pre-slaughter stress could be minimised by training personnel responsible for pre-slaughter handling and, facilities used for livestock, which should be upgraded or improved frequently (Adzitey & Nurul, 2011; Ponnampalam *et al.*, 2017; Loredo-Osti *et al.*, 2019; Birhanu, 2020).

One of the biggest concerns is that suppliers are not aware of the effect they have on the meat quality, especially during the finishing phase of beef production, and ultimately their profit. Therefore, suppliers need to be more informed about the importance of management techniques which they can use to decrease the pre-slaughter stress that cattle endure and minimize the risk of dark-cutting meat.

ii. The effect of farm production systems on the incidence of DFD beef

Table 4.3: The effects of farm production systems on the incidence of dark-cutting beef (Pairwise Comparison).

Dependent Variable	**Farm Production System**		
	(a) **Angus**	**(b)** **Commercial**	**(c)** **Free Range C**
CARCASS PH 24H POST-MORTEM (PH$_U$) (LOIN)	5.724 ± 0.020^{abc}	5.756 ± 0.020^{ab}	5.753 ± 0.019^{ac}

The mean difference was significant at the .05 level

$^{1\ abcd}$- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)
b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

Farm production systems had a significant effect on the pH_u post-mortem ($p < 0.05$) (Table 4.2). Furthermore, commercial feedlots pH_u was 0.054% higher than free-range production systems, whereas Angus production systems pH_u was 0.508% lower than free-range production systems ($p < 0.05$) (Table 4.2; Table 4.3). However, the difference between production systems throughout the year was extremely small (Figure 4.2). Nevertheless, these results do not agree with some previous findings reported in the literature, that a higher percentage of DFD beef are observed in pasture-fed cattle compared to commercial feedlots (Frylinck *et al.*, 2013; Webb & Erasmus, 2013; Ponnampalam *et al*., 2017).

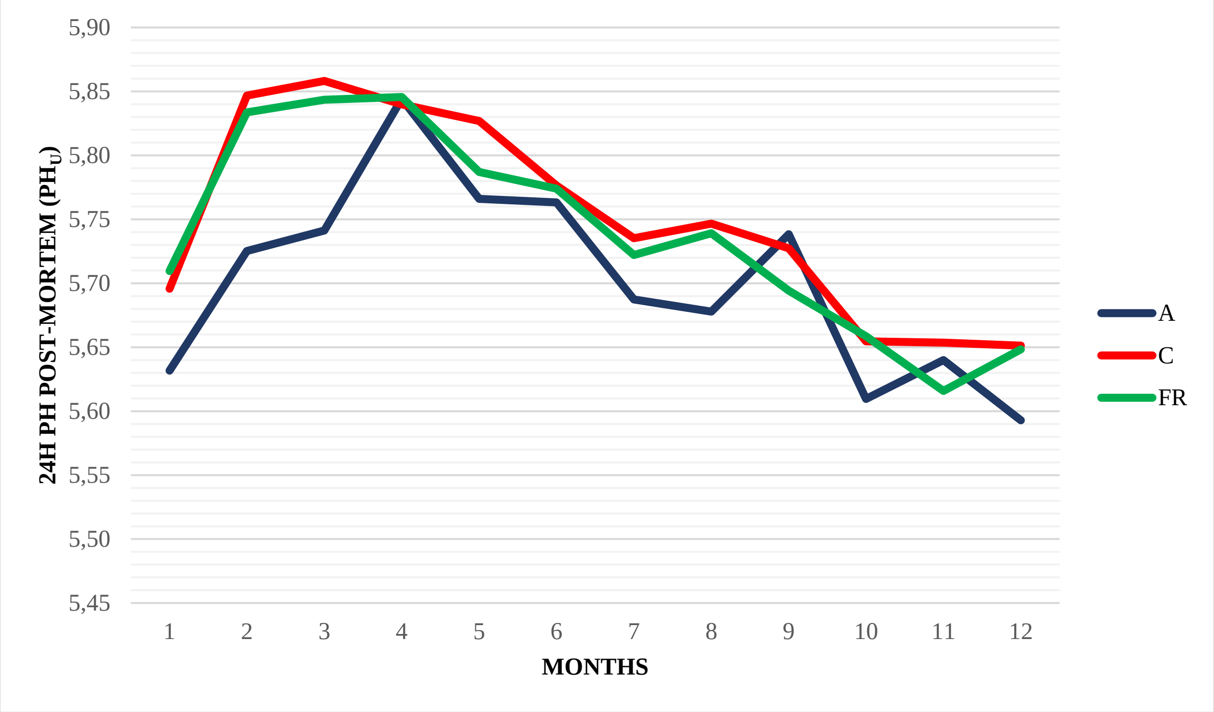


Figure 4.2: The relationship between the ultimate pH and different production systems throughout the year. A, Angus production system; C, commercial feedlots; FR, free-range production system.

Additionally, Angus production systems had the lowest average pH_u estimate compared to commercial and free-range production systems (Table 4.3). A possible reason for the lower average pH_u in Angus production systems compared to commercial feedlots and free-range production systems in this study could be due to different management techniques and a variation in the diets that cattle receive between different production systems. Moreover, cattle slaughtered from Angus production systems were transported in much smaller batches compared to commercial feedlots and free-range systems, which could contribute to a lower muscle glycogen depletion and ultimately a lower risk of DFD. Additionally, as previously mentioned production systems have a large influence on the diets cattle receive and therefore will influence the glycolytic potential of muscles (Pethick *et al.*, 1995; Webb, 2006). Thus, beef quality can be enhanced through feeding (Webb, 2006). According to Ponnampalam *et al.* (2017), these diets influence the carcass weight, fat thickness, muscle glycogen, the rate of muscle glycogen depletion during transportation and slaughter and the rate of carcass cooling, which are all contributing factors to DFD. However, further research is needed to understand the metabolic basis of this control (Pethick *et al.*, 1995). Nevertheless, there is an infinite list of factors that could contribute to the difference in the pH_u post-mortem between production systems, such as difference between breeds, feeding regimes, slaughter ages, handling and exercise conditions that may influence the physiology of the muscle (Frylinck *et al.*, 2015). However, although feeding systems had a significant effect on the pH_u, the difference between the production systems, especially commercial and free-range systems, and the effect on the pH_u was very small.

iii. The effect of climatic factors on DFD beef

Table 4.4: The effect of season on the incidence of dark-cutting beef (Pairwise Comparison).

Dependent Variable	**Seasons**			
	(a) Summer	**(b) Autumn**	**(c) Winter**	**(d) Spring**
CARCASS PH 24H POST-MORTEM (PH_U) (LOIN)	5.676 ± 0.019^{abcd}	5.831 ± 0.019^{abcd}	5.763 ± 0.019^{abcd}	5.708 ± 0.019^{abcd}

The mean difference was significant at the .05 level

[1] ^{abcd}- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)
b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

The average pH_u varied significantly between seasons ($p < 0.05$) (Table 4.2). These findings agree with the results of Kim *et al*. (2003), Wiklund *et al*. (2010), Neethling *et al*. (2014) and Neetling *et al*. (2017) who indicated that there is a chemical and physical deviation in meat quality between different seasons. Additionally, a variation in the occurrence of DFD meat between different seasons was reported (Kreikemeier *et al.*, 1998; Mitlohner *et al.*, 2002; Kadim *et al.*, 2004; Boykin *et al*., 2017; Warner and Tarr, 2018).

Furthermore, in South African conditions during autumn (February-April) the average pH_u was 1.176% higher compared to winter (May-July), whereas spring (August-October) and summer (November-January) had a pH_u of 0.969% and 1.528% lower than throughout winter ($p < 0.05$) (Table 4.2; Table 4.4). Whereas during summer (November-January) the average pH_u was the lowest, compared to the other seasons (Table 4.4). The variation in average pH_u between seasons may be caused by adverse seasonal conditions, which result in pre-slaughter stress and ultimately affect meat quality (Kadim *et al.*, 2004). Another possible reason for the difference in average pH_u might be the variation in dry matter (DM) yield, crude protein (CP) and digestible organic matter (DOM) of the natural veld grazing between seasons, which may affect the body mass of cattle and consequently influence meat quality (De Waal *et al.*, 2000). However, it is important to note that the seasonal trends in DM yield, CP and DOM of the natural veld vary between years (De Waal *et al.*, 2000). Nevertheless, the effect of seasonal effects on the occurrence of DFD was confounded with a wide range of other factors, for instance, geographical locations and breeds. Therefore, it is essential to consider these factors while observing seasonal effects (Neethling *et al*., 2017).

The differences in maximum and minimum temperatures between the farm and abattoir had a significant effect on the pH_u ($p < 0.05$) (Table 4.2). Once the daily maximum temperature between the farm and abattoir increased by 1°C the pH_u increase by 0.018% ($p < 0.05$). Furthermore, an increase in daily minimum temperatures between the farm and the abattoir by 1°C, resulted in an increase in pH_u by 0.017% ($p < 0.05$; Table 4.2). The minimum and maximum average temperatures and average pH_u for each month are shown in Figure 4.3.

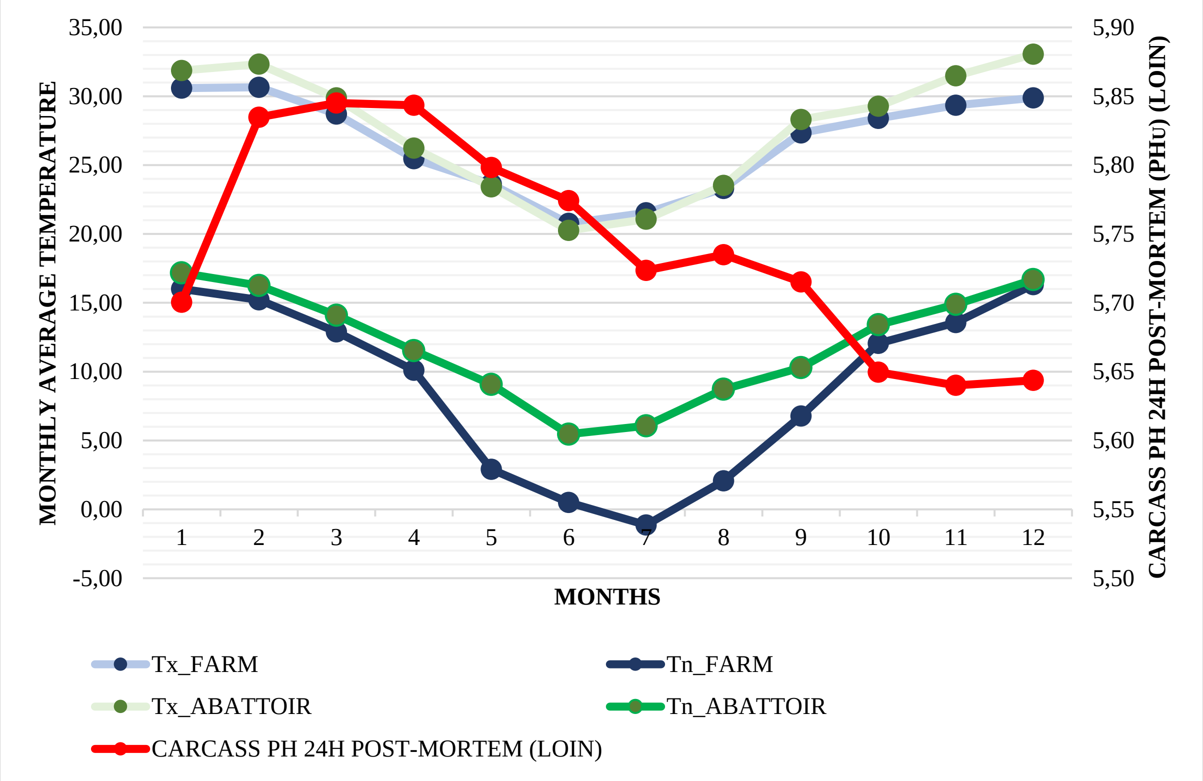


Figure 4.3: The monthly average temperature (°C) and the monthly average 24h carcass pH post-mortem for the period from January 2020 to December 2020.

The difference in maximum humidity between the farm and abattoir did not have a significant effect on the pH_u ($p > 0.05$). However, the difference between the minimum humidity between the farm and abattoir had a significant impact on the pH_u ($p < 0.05$). Once the minimum humidity increases by 1%, the pH_u decreased by 0.009% ($p < 0.05$; Table 4.2). The minimum and maximum average humidity and average carcass pH_u over the year are shown in Figure 4.4.

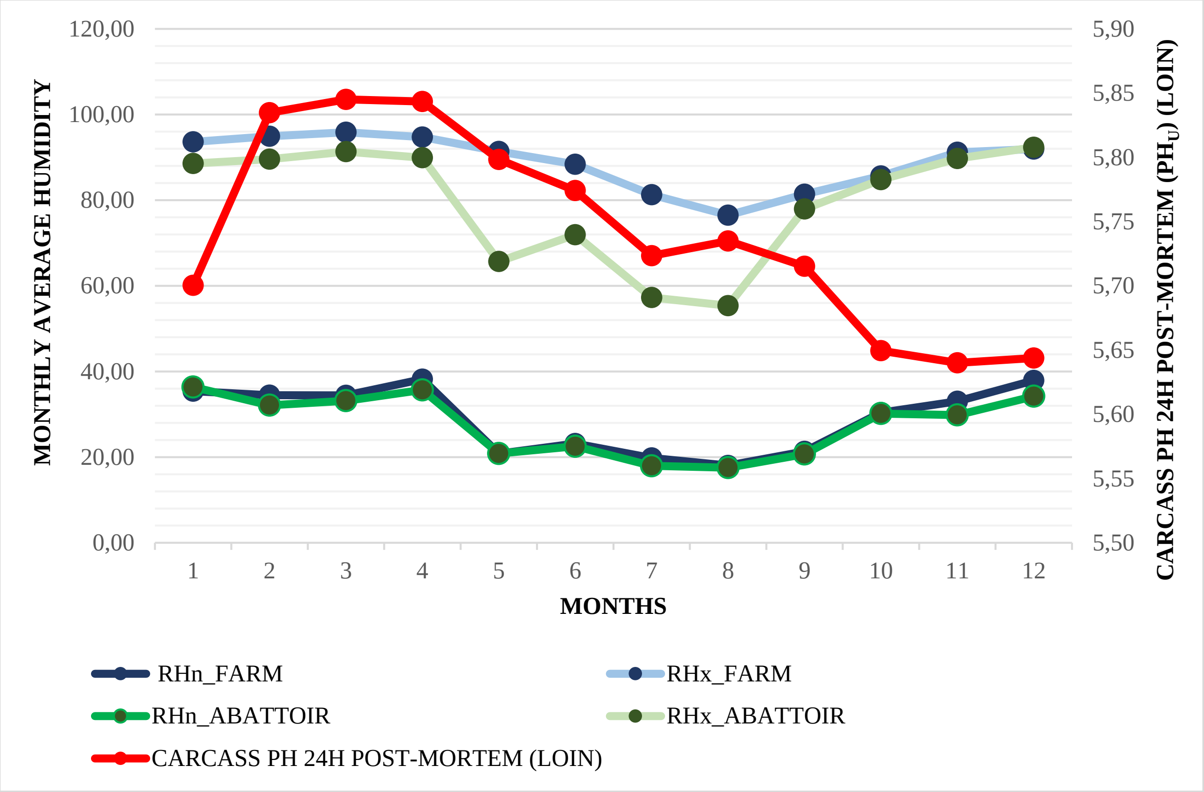


Figure 4.4: The monthly average humidity (%) and the monthly average carcass pH$_u$ post-mortem for the period from January 2020 to December 2020.

Although environmental conditions had a significant effect on the 24h pH post-mortem, Figure 4.3 and Figure 4.4 indicates that the difference in average temperature and humidity between farms and abattoirs throughout seasons had a small effect in terms of increasing the risk of DFD beef. The small environmental effect on the average pH$_u$ may also indicate that the cattle in this study were adapted to the extreme climatic conditions between seasons. However, these results agree with the findings of Warner and Tarr (2018), who noted a small but meaningful effect of environmental conditions on the incidence of DFD meat compared to other factors. According to De Waal *et al.* (2000), the climatic effect on livestock production cannot be controlled, therefore skilful manipulation of the animal production system should be used to minimize the effect of adverse climatic conditions on meat quality.

iv. The effect of transportation conditions on DFD beef

The distance that cattle were transported from farm to abattoir did not have a significant effect on the pH$_u$ ($p > 0.05$) (Table 4.2). Furthermore, the transportation distances between the farms and abattoir varied between 4 km to 897 km, but there was no correlation between transportation distances and DFD risk categories (Appendix A). This is in agreement with a study by Viljoen (2000), who found that transportation distances did not affect the incidence of DFD meat. In contrast to the findings in the current study previous research indicated that increased transportation distances increased the risk of dark-cutting meat (Mounier *et al*., 2006; Adzitey & Nurul, 2011; Adzitey, 2011; Chulayo *et al.*, 2016). However, Brown *et al.* (1990) did note that dark-cutting carcasses increased in both short and long transport distances.

Furthermore, Njisane and Muchenje (2017) found that longer transportation distances along with an increased loading density increased the risk of DFD meat. Although the transportation distance between the farms and abattoir did not have a significant influence on the pH_u post-mortem, the loading density significantly increased the pH_u by 0.002 % ($p < 0.05$; Table 4.2). Furthermore, cattle transported in batches of ca. 72±82 had more high-risk DFD beef compared to cattle transported in groups of ca. 60 (Appendix A). These results support the findings of Batron (2014), who indicated that a higher loading density increases the risk of DFD meat. According to Batron (2014), suppliers load a maximum number of cattle onto the vehicles to decrease transportation costs without knowing that it will increase the incidence of dark-cutting beef.

Thus, from this study, it follows that loading density has a larger effect on muscle glycogen depletion compared to transportation distance.

v. The effect of the estimated standing period on the occurrence of DFD beef

Table 4.5: The effect of the standing period on the incidence of dark-cutting beef (Pairwise Comparison).

Dependent Variable	**STANDING PERIOD (DAYS)**		
	(a) **1 Day**	**(b)** **2 Days**	**(c)** **3 Days**
CARCASS PH 24H POST-MORTEM (PH$_U$) (LOIN)	5.805 ± 0.018^{abc}	5.776 ± 0.018^{abc}	5.653 ± 0.027^{abc}

The mean difference was significant at the .05 level

$^{1\ abcd}$- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)

b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

Cattle slaughtered on the day of arrival at the abattoir had a 2.651% higher pH_u value compared to cattle slaughtered 3 days after arrival, whereas cattle slaughtered a day after arrival only had a 2.147% higher pH_u value compared to cattle slaughtered 3 days after arrival (Table 4.2; Table 4.5). Thus, as the standing period increased the risk for the occurrence of DFD significantly decreased ($p < 0.05$) (Table 4.2).

Additionally, 96.4% of the cattle were slaughtered on the day of arrival, 3.5% of cattle were slaughtered one day after arrival and only 0.1% of cattle were slaughtered two or more days after arrival. Furthermore, 0.4% of the cattle slaughtered on the same day of arrival, 0.1% of the cattle slaughtered a day after arrival and 0.0% of the cattle slaughtered more than two days after arrival were high-risk DFD beef (Appendix B). Thus, as the estimated standing time increased, the risk of dark-cutting decreased. These findings suggest that cattle should be rested after transportation. The results of this study also agree with the findings of Brown *et al.* (1990), who noted that cattle slaughtered on the day of arrival rather than overnight had increased incidences of DFD beef. However, these findings contradict the findings suggesting that the transportation (Mounier *et al.*, 2006; Adzitey & Nurul, 2011; Adzitey, 2011; Chulayo *et al.,* 2016) and lairage (Ferguson & Warner, 2008; Adzitey & Nurul, 2011; Loredo-Osti *et al*., 2019) periods should be kept as short as possible. Furthermore, meat-producing cattle are deprived of feed and water prior to slaughter to reduce the stomach contents and avoid the release and spread of microbial contamination of carcasses (O’Neil *et al*., 2018; Xing *et al*, 2019). Generally, it is suggested that the fasting period should not be more than 24 hours (Ferguson & Warner, 2008; Adzitey, 2011) but in some circumstances, it may increase up to 36-48 hours (Ferguson & Warner, 2008). For fasting periods exceeding 24 hours, it is essential to provide feed and water to prevent starvation and dehydration (Adzitey, 2011). The reason that the cattle slaughtered on the day of arrival had a higher pH_u compared to cattle slaughtered more than 24 hours after arrival may be because they were not given a resting period to rehydrate and regain muscle glycogen that was depleted during stressful pre-slaughter factors contributing to the occurrence of DFD.

According to Del Campo *et al.* (2010), depending on the lairage conditions, a resting period of more than 3 hours is recommended if the duration of transportation is more than 4 hours. However, Teke *et al.* (2014) recommend a resting period of 72 hours for transportation periods more than 30 hours. In this present study the cattle that were slaughtered at the abattoir come from all over South Africa, thus the duration of transportation, transportation circumstances, environmental effects, fasting periods, feeding systems and management varied between cattle, which could all contribute to the difference in muscle glycogen depletion before slaughter and will influence the estimated resting time cattle should receive after transportation. Thus, more research is required to quantify a general estimated resting time according to the pre-slaughter stress factors cattle experience prior to slaughter.

4.2.2 Intrinsic factors

i. The influence of breed on the occurrence of DFD beef

Breed did not have a significant effect on the post-mortem carcass pH_u ($p>0.05$) (Table 4.2). Although cattle breeds did not have a significant effect on the pH_u, there was a small difference in the pH_u between breeds. Purebred *Bos indicus* cattle had no high-risk DFD beef within the breed, whereas purebred *Bos taurus* cattle had 0.12% high-risk DFD beef throughout the year. Furthermore, crossbred *Bos indicus* and *Bos taurus* cattle had 0.64% and 0.12% high-risk DFD beef throughout the year. Interestingly, composite breeds had 0.70% high-risk DFD carcasses (Figure 4.5) and generally it is suggested that a combination of both temperate and tropical genotypes might be favourable to decrease the occurrence of dark-cutting (Adzitey & Nurul, 2011).

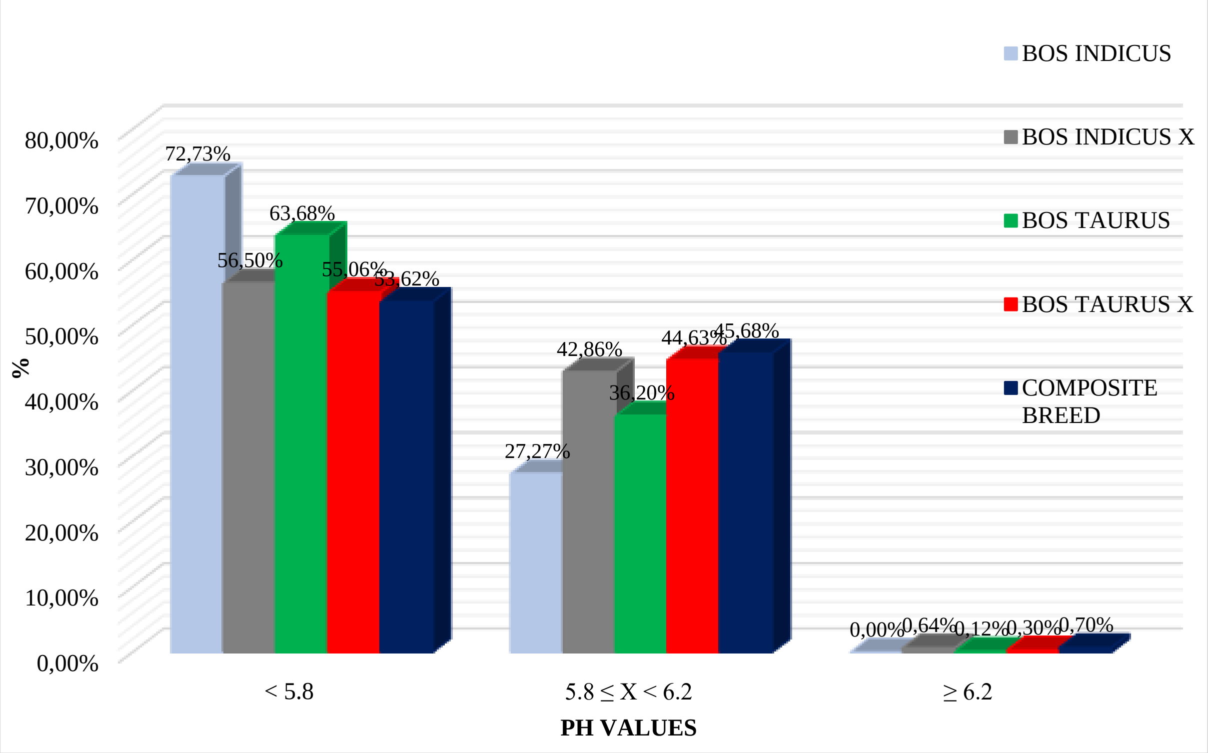


Figure 4.5: Percentage distribution of 24h carcass pH values in different cattle breeds.

However, in this study, mostly composite breeds were slaughtered at the abattoir which explains the higher proportion of high-risk DFD beef in composite breeds compared to the other breeds. Therefore, the variation in the pH_u post-mortem between breeds is not a clear indication of the breed effect on muscle glycogen depletion. Furthermore, the breed effect on muscle glycogen depletion is a compound effect. Cattle slaughtered at the abattoir were distributed throughout South Africa, from various suppliers, different production systems, management techniques, diets, gender and age. According to Burrow *et al.* (2001), for some traits in the breed and breed type rankings, the effect of genotype-environmental interactions is significant, which may contribute to the incidence of DFD (Ponnampalam *et al.*, 2017). Thus, to get a clear indication of the breed effect on muscle glycogen depletion and the occurrence of dark-cutting, it is necessary to do more research with cattle of the same gender and age under similar environmental conditions and management techniques. Nevertheless, from previous research, the assumption can be made that *Bos indicus* cattle are more sensitive to human-animal interactions because of their more excitable temperament that inclines stress and they are better adapted to extreme environmental conditions (O'Neill *et al.*, 2012), whereas *Bos taurus* cattle are primarily more sensitive to extended fasting periods and major environmental conditions that result in the depletion of muscle glycogen (Frylinck *et al.*, 2015; O'Neill *et al.*, 2018).

ii. The effect of gender on the incidence of DFD beef

Table 4.6: The effect of gender on the incidence of dark-cutting beef (Pairwise Comparison).

Dependent Variable	**Gender**		
	(a) Cow	**(b) Heifer**	**(c) Steer**
CARCASS PH 24H POST-MORTEM (PH_U) (LOIN)	5.752 ± 0.020^{ab}	5.727 ± 0.020^{abc}	5.754 ± 0.019^{bc}

The mean difference was significant at the .05 level

$^{1\ abcd}$- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)

b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

Gender had a significant effect on the pH_u post-mortem ($p < 0.05$) (Table 4.2). Cows and heifers had a 0.027% and 0.474% lower pH_u value compared to steers (Table 4.2; Table 4.6). However, the difference between steers and cows was very small. Furthermore, 89.4% of the cattle that were slaughtered were steers, whereas only 2.6% and 8.0% were cows and heifers. Additionally, 0.54% of the cows, 0.46% of the steers and 0.09% of the heifers that were slaughtered throughout the year were high-risk DFD carcasses (Figure 4.6).

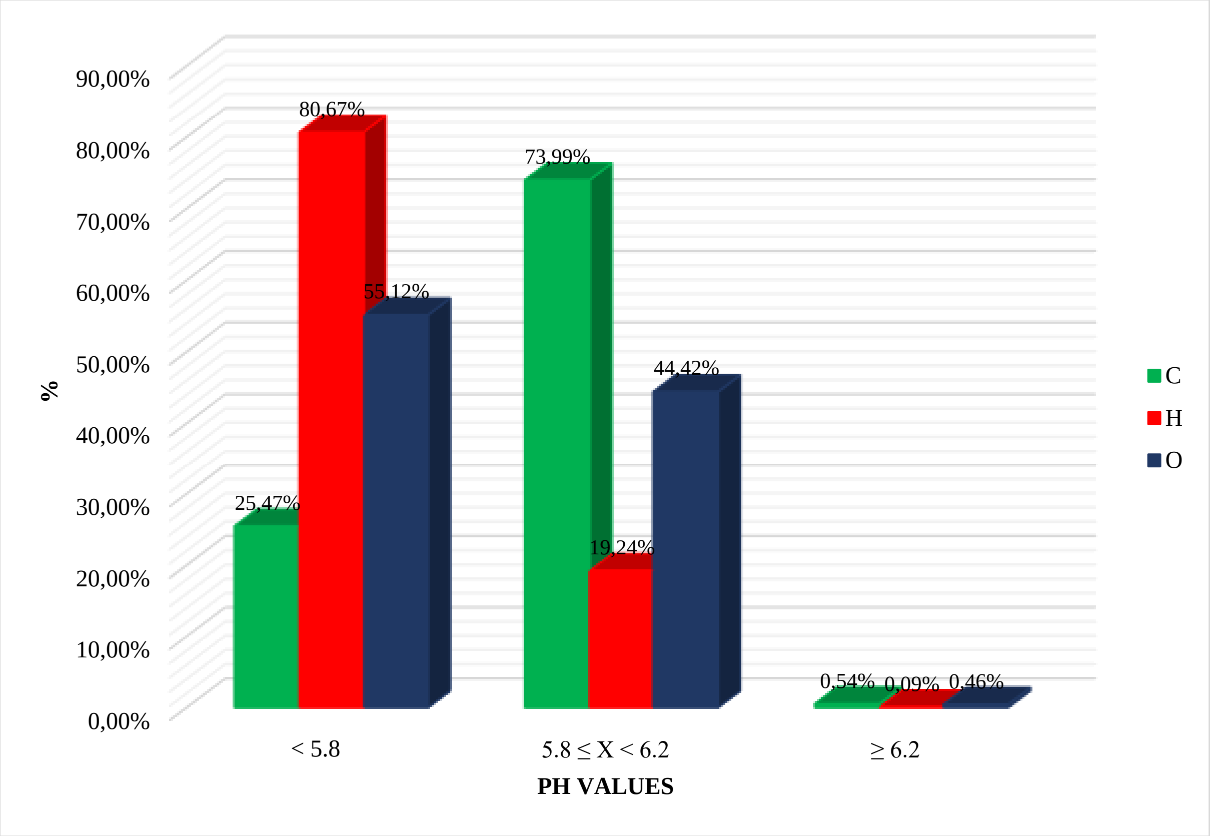


Figure 4.6: Percentage distribution of carcass pH_u values in different gender categories of cattle.

From previous research, the results and opinions regarding DFD beef in different gender categories are extremely contradicting. However, as aforementioned generally most cattle that are slaughtered are steers and bulls. Thus, the precise gender effect on muscle glycogen depletion and ultimately the pH_u post-mortem is unclear and will vary.

Furthermore, cattle age plays a very important role in the effect of gender on muscle glycogen depletion. Hormonal changes occur when cattle reach maturity and lead to behavioural changes, such as excitable temperaments, fighting and mounting activity, which cause muscle glycogen depletion and increase the risk of DFD. However, in South Africa cattle are slaughtered at the age of 12 to 16 months, before permanent incisors appear (Frylinck *et al.* 2013). Thus, the effect of excitable temperaments and sexual activity culminating in excessive muscle glycogen depletion is low. Therefore in South Africa, the effect of gender on muscle glycogen depletion can be assumed to be mostly due to the mixing of unfamiliar cattle and genders during transport and holding periods (Tarrant 1989; Viljoen, 2000), social regrouping (Tarrant 1989; Viljoen, 2000) and a variety in live- and carcass weights between genders (Murray, 1989; Mahmood *et al.*, 2016).

iii. The effect of age at slaughter on the incidence of DFD beef

Table 4.7: The effect of age at slaughter on the incidence of dark-cutting beef carcasses (Pairwise Comparison).

Dependent Variable	**Age at Slaughter**			
	(a) **A**	**(b)** **AB**	**(c)** **B**	**(d)** **C**
CARCASS PH 24H POST-MORTEM (PH_U) (LOIN)	5.733±0.019^{ad}	5.734±0.020^{bd}	5.731±0.020^{cd}	5.780±0.020^{abcd}

The mean difference was significant at the .05 level

$^{1\ abcd}$- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)

b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

Older cattle have a significantly higher pH_u post-mortem compared to younger cattle ($p < 0.05$) (Table 4.2). A-class carcasses had a pH_u value of 0.811% lower than C-class carcasses, whereas AB- and B-class carcasses had a pH_u value of 0.794% and 0.858% lower than C-class carcasses (Table 4.2; Table 4.7). Thus, pH_u tends to increase with animal age, which may increase the risk for dark-cutting carcasses (Table 4.7).

Furthermore, 86.9% of the cattle that were slaughtered were A-class, 6.0% were AB-class, 5.3% were B-class, and only 1.8% were C-class carcasses. Additionally, 0.45% of the A-class, 0.18% of the AB-class, 0.27% of the B-class and 0.78% of the C-class cattle that were slaughtered throughout the year were high-risk DFD carcasses (Figure 4.7). These results correspond with the findings of Kelava *et al.* (2008), Gardner *et al.* (2005), Frylinck *et al.* (2013) and Hughes *et al.* (2014), where increased muscle pH_u was associated with increased animal age.

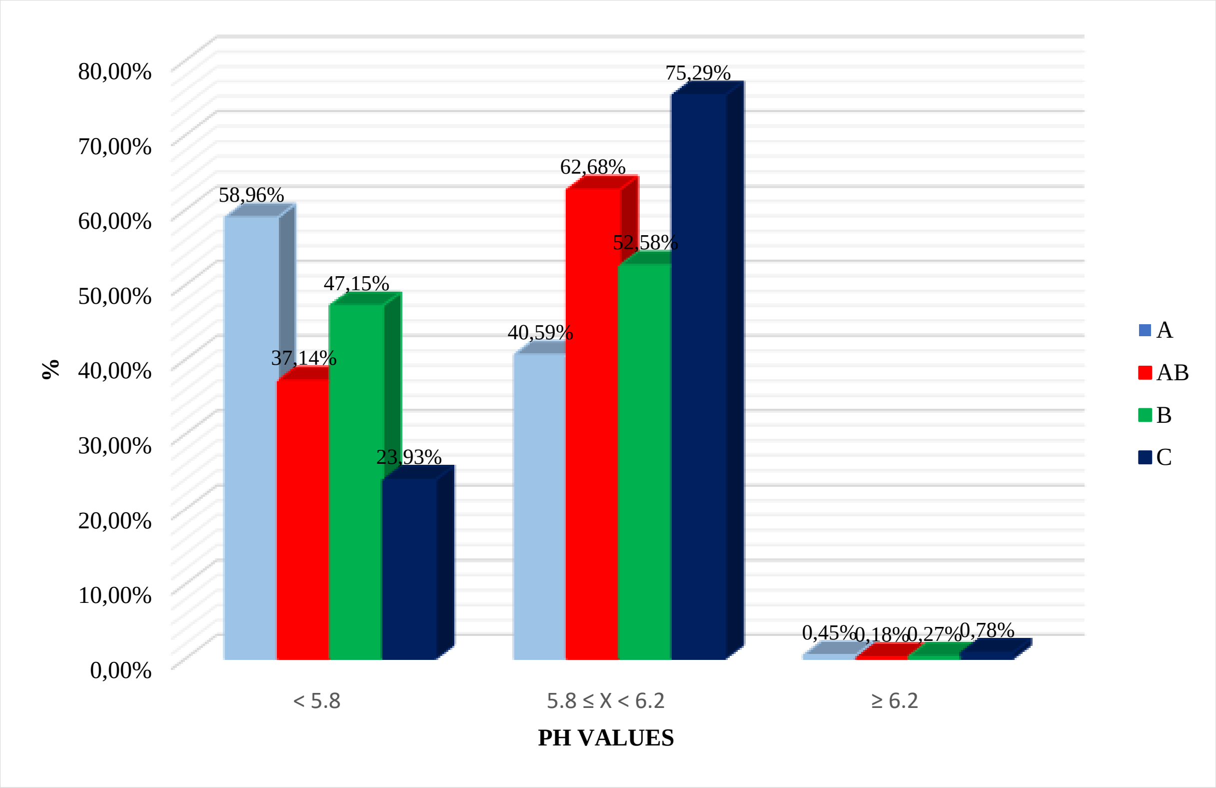


Figure 4.7: Percentage distribution of 24h carcass pH values in different age classes of cattle.

Gardner *et al.* (2005) suggested that an increased pH_u with age could be due to increased adrenaline sensitivity as cattle mature since it enhances muscle glycogen depletion. However, in South Africa consumers prefer meat from younger cattle compared to older cattle, therefore, most cattle are slaughtered before permanent incisors, at the age of 12 to 16 months. Furthermore, the age effect on DFD beef can be compounded by feeding regime, because younger cattle normally come from feedlots and older cattle usually come from pasture finished systems. However, Webb and Erasmus (2013) suggested that production systems, diets and slaughter conditions have a larger effect on muscle glycogen depletion compared to age, suggesting that cattle age is not a major factor contributing to dark-cutting meat in South Africa.

iv. The effect of carcass conformation on the incidence of DFD beef

The conformation of the cattle did not have a significant effect on the pH_u post-mortem ($p > 0.05$) (Table 4.2). However, carcasses with a lower carcass conformation seem to have a higher pH_u post-mortem (Appendix B). According to Irshad *et al.* (2013), genetics, age, gender, nutrition and environmental effects are contributing factors influencing the variation in carcass conformation. Furthermore, carcasses with a better conformation had an increased dressing percentage, higher carcass lean meat content and lower bone to fat and fat ratios (Moloney & McGee, 2017). Thus, in this study, the warm mass and fat content of the carcasses gave a good indication of carcass conformation.

v. The influence of live mass, carcass mass and fat content on DFD beef

a. Live mass and carcass mass

Live mass did not have a significant effect on the 24 h pH post-mortem ($p > 0.05$) (Table 4.2). However, cattle with a live mass lower than 458 kg tended to have a higher risk of DFD carcasses in South Africa (Figure 4.8).

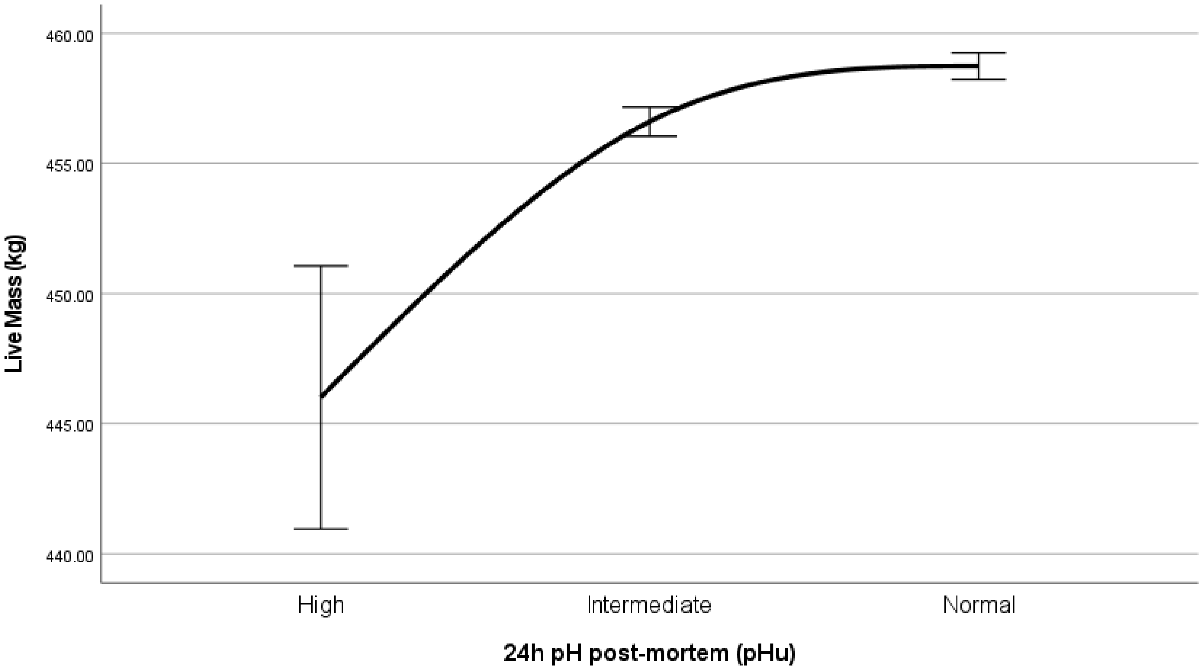


Figure 4.8: The relationship between live mass and the ultimate pH.
Normal ($pH_u < 5.8$), Intermediate (pH_u $5.8 \leq X < 6.2$) and High ($pH_u \geq 6.2$).

Warm carcass mass had a significant effect on the pH_u post-mortem ($p < 0.05$) (Table 4.2). Carcasses weighing less than 281 kg tended to have a higher risk of DFD (Figure 4.9). Previous research indicated that the risk of DFD beef was lower in carcasses weighing more than 150-220kg (McGilchrist *et al.*, 2012) or 275kg (Jones & Tong, 1989), which is in agreement with the observation in the present study. Furthermore, as the carcass weight increased by 1 kg, the pH_u decreased by about 0.003 %. Although the effect was very small, carcass weight seems to be one of the most important indicators to predict the risk of cattle producing DFD meat (Mahmood *et al.*, 2016).

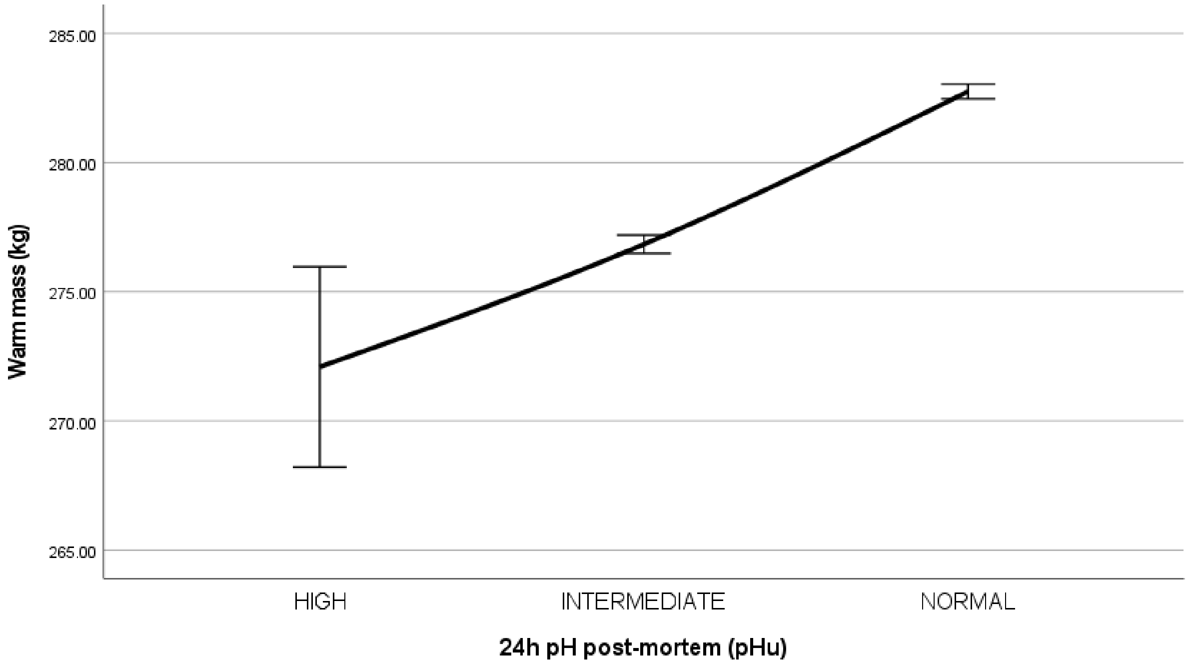


Figure 4.9: The relationship between warm mass and the ultimate pH
Normal ($pH_u < 5.8$), Intermediate (pH_u $5.8 \leq X < 6.2$) and High ($pH_u \geq 6.2$).

These results agree with the findings of Kreikemeier *et al.* (1998) and Mahmood *et al.* (2016) who noted that the risk of dark-cutting meat increases in cattle with reduced live weight and lower carcass weight.

Mahmood *et al.* (2016) observed that carcass weight is positively correlated to muscularity and feed intake. Therefore, increasing the amount and energy concentrations in feed intake for cattle will increase the muscle and liver glycogen, and reduce the likelihood of dark-cutting carcasses. According to McGilchrist *et al.* (2012), selection for increased muscling in cattle will increase the oxidative capacity of the muscle. Thus, cattle with increased muscling consequently spare muscle glycogen during energy expenditure, because they might use lipids as a source of energy or completely oxidise glycogen (Mahmood *et al.*, 2016). Furthermore, McGilchrist *et al.* (2012) stated the importance of this finding for the beef industry, as it establishes that selection for muscling can be encouraged in beef production without increasing the risk of dark-cutting meat.

Agbeniga and Webb (2018) noted that in South Africa slaughterhouse pricing favours heavier carcasses and there is an increasing demand for good quality protein in developing countries. However, heavier carcasses need to be processed in the same facilities used for smaller carcasses, which could cause problems with increased maintenance for machinery, hygienes, carcass cooling systems and packaging.

Nevertheless, from this study, it is suggested that the production of heavier carcasses should be encouraged in South Africa as it has several advantages and ultimately decreases the risk of DFD. However, more research should be done to mitigate the adverse effects of heavier and smaller carcasses on meat quality attributes, and facilities should be designed in a manner to process smaller and heavier carcasses (Agbeniga & Webb, 2018).

a. Fat content

Table 4.8: The effect of fat content on the incidence of dark-cutting beef (Pairwise Comparison).

Dependent Variable	**Fat Content**				
	(a) **0**	**(b)** **1**	**(c)** **2**	**(d)** **3**	**(e)** **4**
CARCASS PH 24H POST-MORTEM (PH_U) (LOIN)	5.847 ± 0.031^{abcde}	5.729 ± 0.019^{abcd}	5.711 ± 0.019^{abcde}	5.707 ± 0.019^{abcde}	5.728 ± 0.020^{acde}

The mean difference was significant at the .05 level

$^{1\ abcd}$- CARCASS PH 24H POST-MORTEM (LOIN) (%) with different superscripts differed significantly

a. Dependent Variable: CARCASS PH 24H POST-MORTEM (LOIN)

b. Covariates appearing in the model are evaluated at the following values: LIVE MASS = 457.874598202916400, WARM MASS = 280.65333394786820, CARCASS TEMPERATURE 1H POST-MORTEM (LOIN) = 38.76155, CARCASS PH 1H POST-MORTEM (LOIN) = 6.57853, CARCASS PH 16H POST-MORTEM (LOIN) = 5.73041, CARCASS TEMPERATURE 24H POST-MORTEM (LOIN) = 2.5649, CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE) = 10.7654, Tx_FARM-Tx_ABATTOIR = -1.1760, Tn_FARM-Tn_ABATTOIR = -2.9391, RHx_FARM-RHx_ABATTOIR = 8.2798, RHn_FARM-RHn_ABATTOIR = .9074, LOADING DENSITY = 59.28, TRANSPORT DISTANCE = 240.66.

Cattle with a lower fat content had a significantly higher pH_u compared to cattle with a higher fat content ($p < 0.05$) (Table 4.2; Table 4.8). These results correspond with the findings of McGilchrist *et al.* (2012), who noted that cattle with increased subcutaneous fat depth have a lower risk of DFD. McGilchrist *et al.* (2012) suggested that it may be due to improved nutrition and better nutrient availability increasing the accumulation of muscle glycogen and fat for both lipogenesis and glycogenesis. Figure 4.10 indicates that cattle with a lower fat content had a higher pH_u.

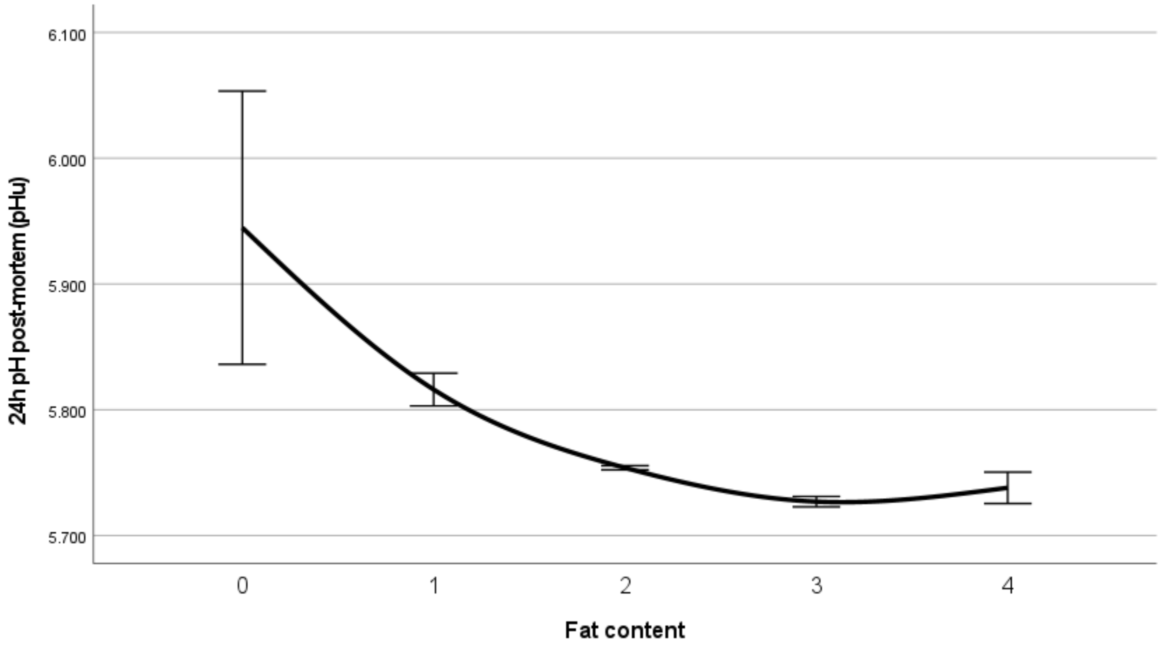
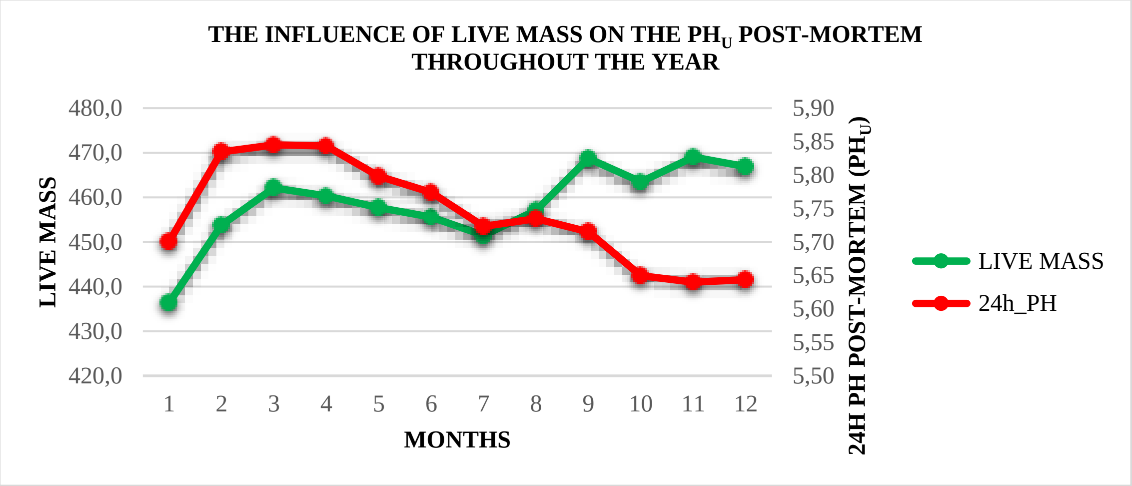


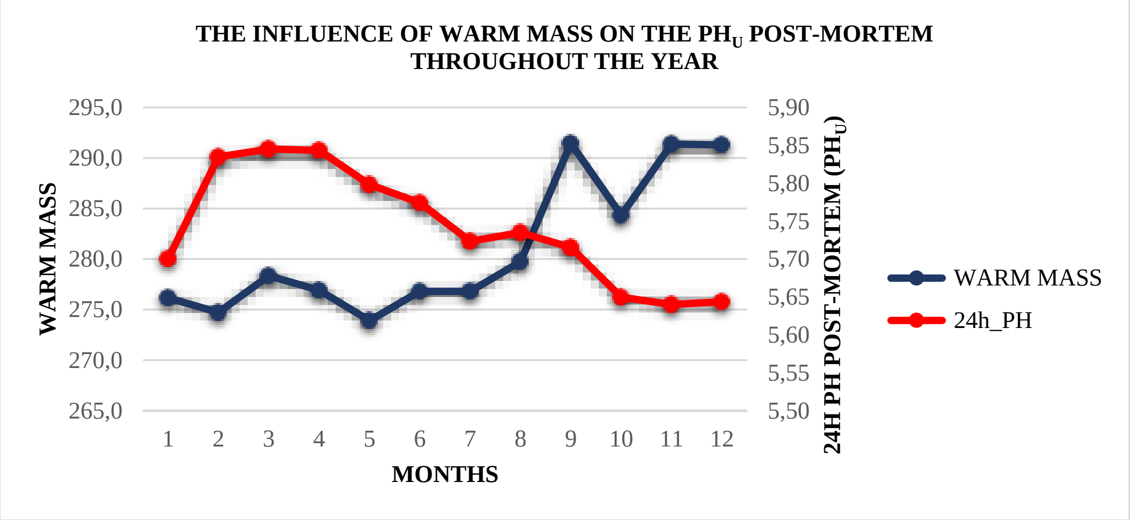
Figure 4.10: The relationship between fat content and the 24h pH post-mortem (pH_u).

c. The seasonal variation in live mass, warm mass and fat content

Figure 4.11 indicates the variation in pH_u over the year and the influence of live mass, warm carcass mass and fat content on the average pH_u. There is a clear indication that during autumn and winter the average pH_u was much higher compared to spring and summer. Moreover, during autumn and winter, the average live mass and warm carcass mass was significantly lower compared to spring and summer. Thus, Figure 4.11 evidently indicates that cattle with a lower live mass and warm carcass mass tend to have an increased risk of dark-cutting. Additionally, there was a higher proportion of carcasses with a fat content of 0, 1 and 2 during autumn and winter compared to spring and summer, which contributed to the increased average pH_u during the first quarter of the year. Thus, live mass, warm carcass mass and fat content are correlated to one another and collectively increase the risk of DFD.

These results agree with the findings of McGilchrist *et al.* (2012), who noted the collective influence of fat depth and muscling scores on the occurrence of DFD meat. Additionally, McGilchrist *et al.* (2012) stated that more muscular cattle have a higher muscle to fat ratio, therefore they have more adipose tissue that is stress-responsive to adrenaline. Thus, when high muscled cattle experience pre-slaughter stress they utilise more adipose tissue for energy production compared to low muscled cattle that utilise more stored glycogen for energy production. Furthermore, it is well known that the nutritional quality of pastures varies significantly between seasons and years. Therefore, it can be assumed that heavier cattle and cattle with a higher fat content received better nutrition in the months leading up to slaughter resulting in increased muscle glycogen concentrations and a variation in average pH_u between seasons (McGilchrist *et al.*, 2012).





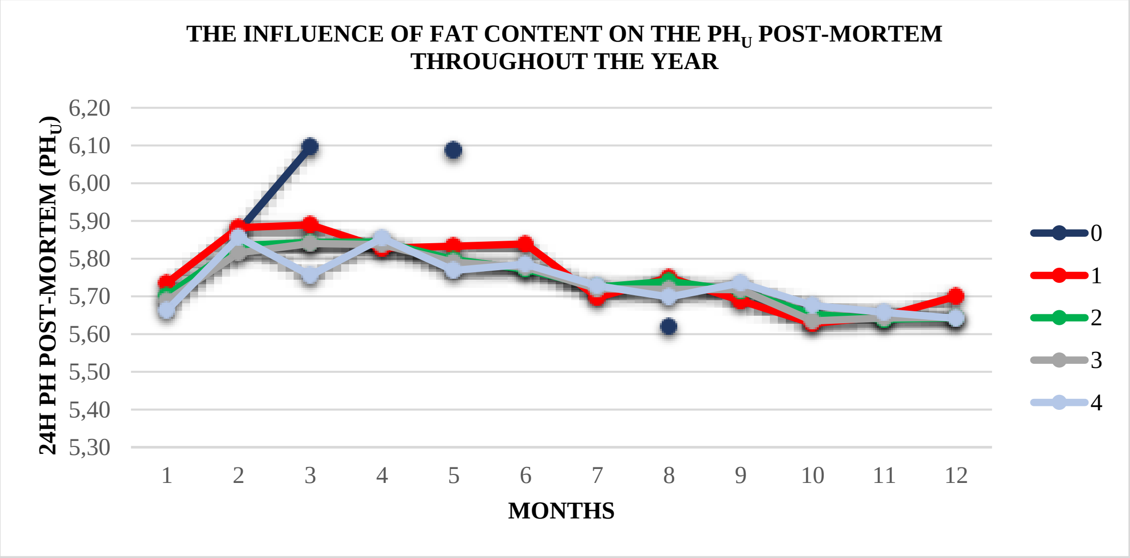


Figure 4.11: The relationship between the carcass pH$_u$ post-mortem and cattle live mass, warm mass and fat content throughout the year.

4.2.3 Carcass measurements

Table 4.9: Average carcass pH-temperature values post-mortem.

Carcass Measurements	Average
CARCASS TEMPERATURE 1H POST-MORTEM (LOIN)	38.762 °C
CARCASS TEMPERATURE 24H POST-MORTEM (LOIN)	2.565 °C
CARCASS TEMPERATURE 24H POST-MORTEM (SILVERSIDE)	10.765 °C
CARCASS PH 1H POST-MORTEM (LOIN)	6.579
CARCASS PH 16H POST-MORTEM (LOIN)	5.730

A carcass with a higher loin temperature 1 h after slaughter, had a significantly higher pH_u ($p < 0.05$) (Table 4.2). The average loin temperature 1 h after slaughter was ca. 39°C (Table 4.9), thus with every 1°C increase in loin temperature 1 h post-slaughter, the pH_u will rise by 0.041 % (Table 4.2). Furthermore, a carcass with a higher 24 h loin temperature, had a significantly lower pH_u ($p < 0.05$). The average 24 h loin temperature was ca. 3°C (Table 4.9), thus with every 1°C increase in loin temperature 24h post-slaughter, the pH_u will rise by 0.157 % (Table 4.2). Moreover, a carcass with a higher 24 h silverside temperature had a significantly higher 24 h pH ($p<0.05$). The average silverside temperature 24 h after slaughter was ca. 11°C (Table 4.9), thus with every 1°C increase in loin temperature 1 h post-slaughter, the pH_u will rise by 0.042 %. Thus, post-mortem carcass temperatures gave a good indication if the carcass is at risk to produce dark-cutting meat.

Furthermore, the carcass pH 1 h post-mortem was not a significant indication if the carcass will have a higher pH_u and an increased risk of dark-cutting meat ($p > 0.05$) (Table 4.2). Whereas the carcass pH 16 h post-mortem significantly indicated if the carcass will have a higher pH_u and an increased risk of dark-cutting meat ($p < 0.05$) (Table 4.2). The average carcass pH 16 h post-mortem was ca. 5.73 (Table 4.9), thus as the 16 h pH post-mortem increases above 5.73, the pH_u will increase by 0.598 % (Table 4.2). Thus, the 16 h pH post-mortem gave a good indication if the carcass is at risk to produce dark-cutting meat.

4.3 THE INTEGRATING EFFECT OF ALL PRE-SLAUGHTER STRESS FACTORS THAT MAY INCREASE THE RISK OF DFD BEEF

DFD beef is such a complex problem to be resolved because there is an endless list of factors contributing to pre-slaughter stress and all the factors are confounded by one another. Nevertheless, by minimising the effect of one factor, the overall impact of another factor may be reduced.

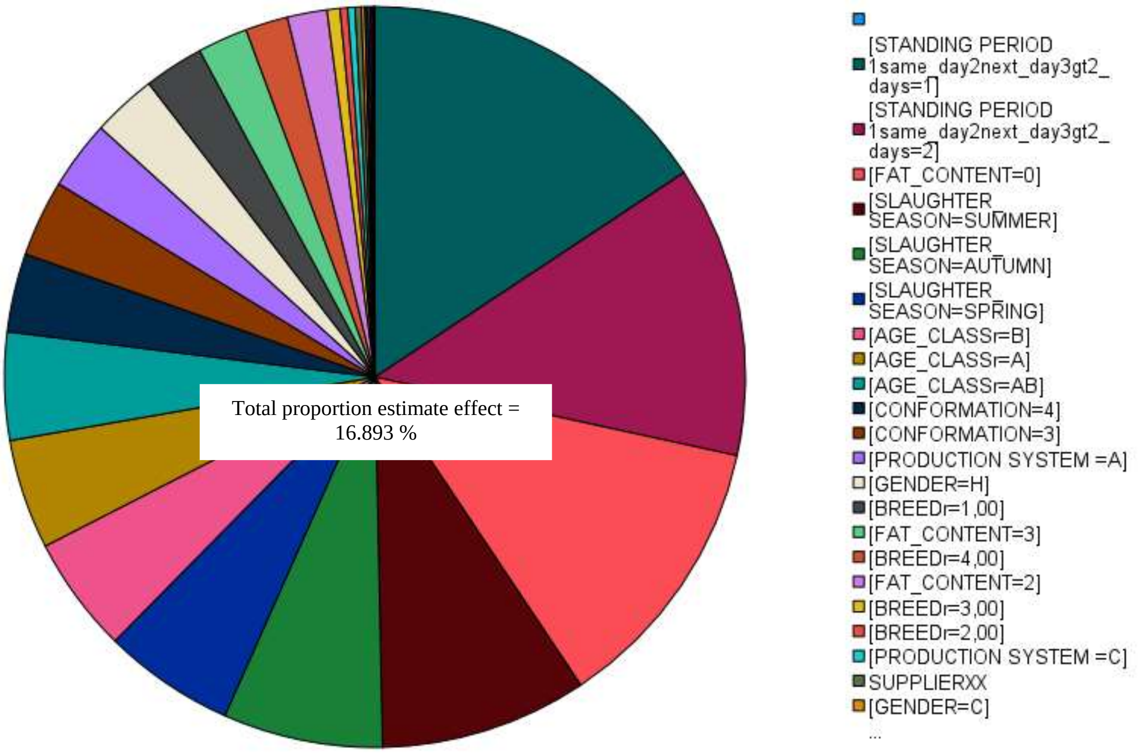


Figure 4.12: Integrating effect of all pre-slaughter stress factors that may increase the risk for DFD beef in South Africa

The proportion estimate effect of each factor on the pH_u was very small (Table 4.2). However, combining the proportion estimate effects of all factors on individual cattle, substantially increased the risk of DFD with c.a 17% (Figure 4.12).

Figure 4.13 indicates that the average pH_u values throughout February-June, specified as the DFD beef window, were much higher compared to the rest of the year. Furthermore, cattle slaughtered throughout the DFD beef window in South Africa had notably lower live mass, warm carcass mass and fat contents compared to cattle slaughtered the rest of the year (Figure 4.11). Therefore, the results of this study indicate that cattle's body condition can be used as the main determining factor to indicate if an individual animal is at risk of DFD. However, it is important to note that all the other extrinsic and intrinsic factors still have a small proportional effect throughout the year, but cattle with a lower body condition will respond more severely to these pre-slaughter stress factors compared to cattle with a good body condition.

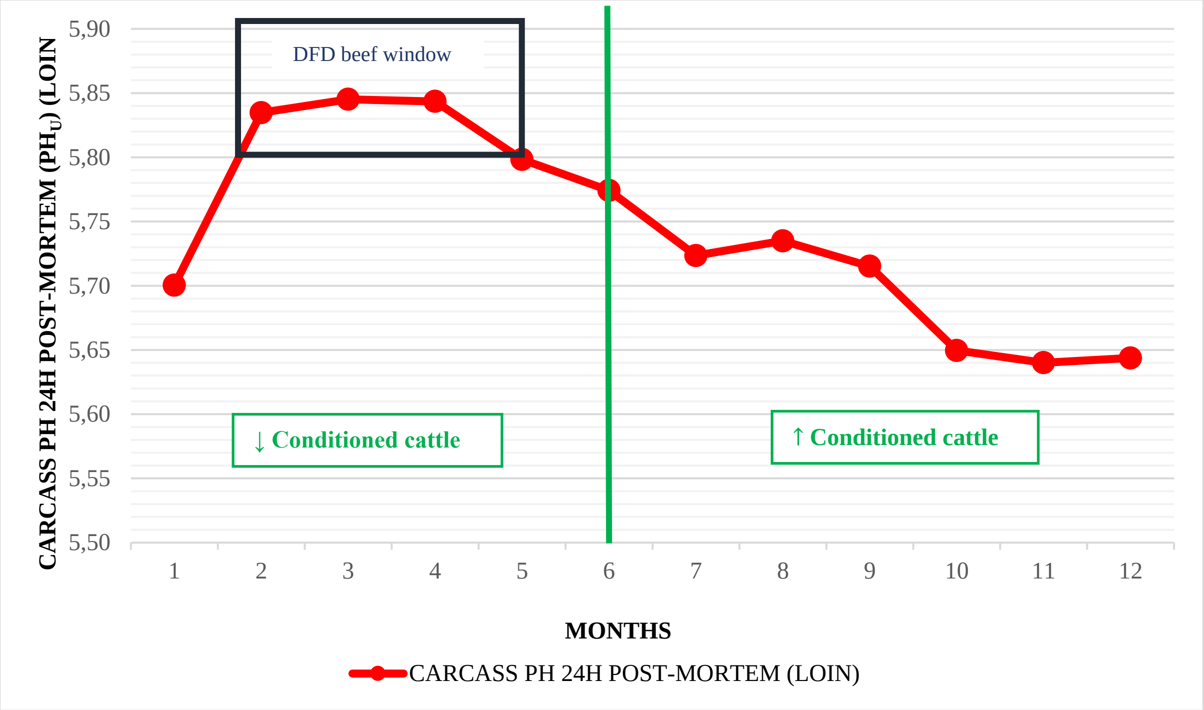


Figure 4.13: The monthly average pH_u post-mortem for the period from January 2020 to December 2020.

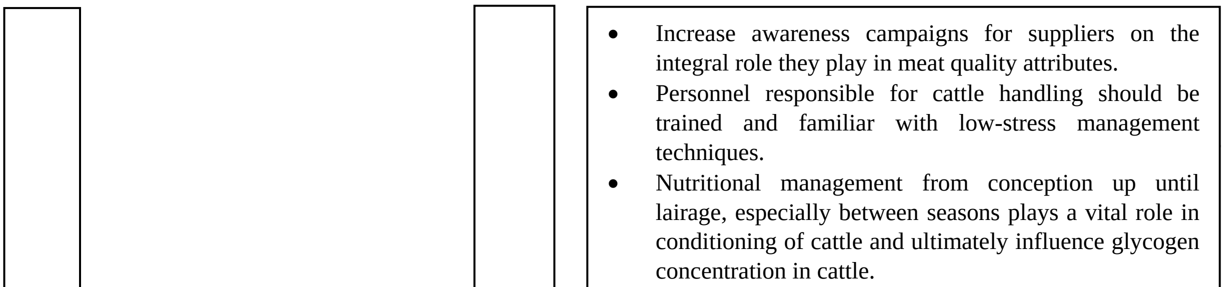
There are a few speculations about why the body condition during the DFD beef window during the South African autumn was much lower compared to the rest of the year. Firstly, it can be a supply-demand effect. Before and during Christmas and new year celebrations, which are usually from November to January, the demand for beef in South Africa is much higher compared to the rest of the year. Furthermore, more cattle are produced by suppliers to be slaughtered during the festive time of the year. Therefore, abattoirs now have the option to only slaughter good-conditioned cattle and not accept cattle that are poorly conditioned. Thus, cattle slaughtered during this time of the year are generally well-conditioned and therefore a pose lower risk of DFD. However, after the festive time of the year (January to April), the demand for meat and the number of cattle produced by suppliers decreases. Therefore, it can be that because of the lower number of cattle produced by suppliers after the festive season, cattle of all body condition types are slaughtered at the abattoir, including cattle with a lower body condition. Thus, the proportion of cattle with a lower body condition are much higher during the DFD beef window, therefore increasing the risk of DFD.

Secondly, the condition of cattle from conception up until lairage may influence the risk of DFD meat. Breeding (and calving) season management plays an important role in enhancing the reproductive performance of a breeding herd and the pre-wean growth rate of calves, and ultimately influences the profit margin of beef cattle enterprise (Bergh, 2004). Furthermore, nutrition is the main factor influencing an ideal breeding season (Bergh, 2004). According to De Waal *et al.* (2000), the pasture content between seasons varies in dry matter (DM) yield, crude protein (CP) and digestible organic matter (DOM), which affect the body mass of cattle. Furthermore, Bergh (2004) stated that calves that are born too late in the calving season have lower weaning weights because they are too small to utilize their dams' high milk production from high-quality summer pastures. Thus, breeding (and calving) season management and the variation in pasture content between seasons may also be another speculated theory why the body condition of cattle is much lower during the DFD beef window.

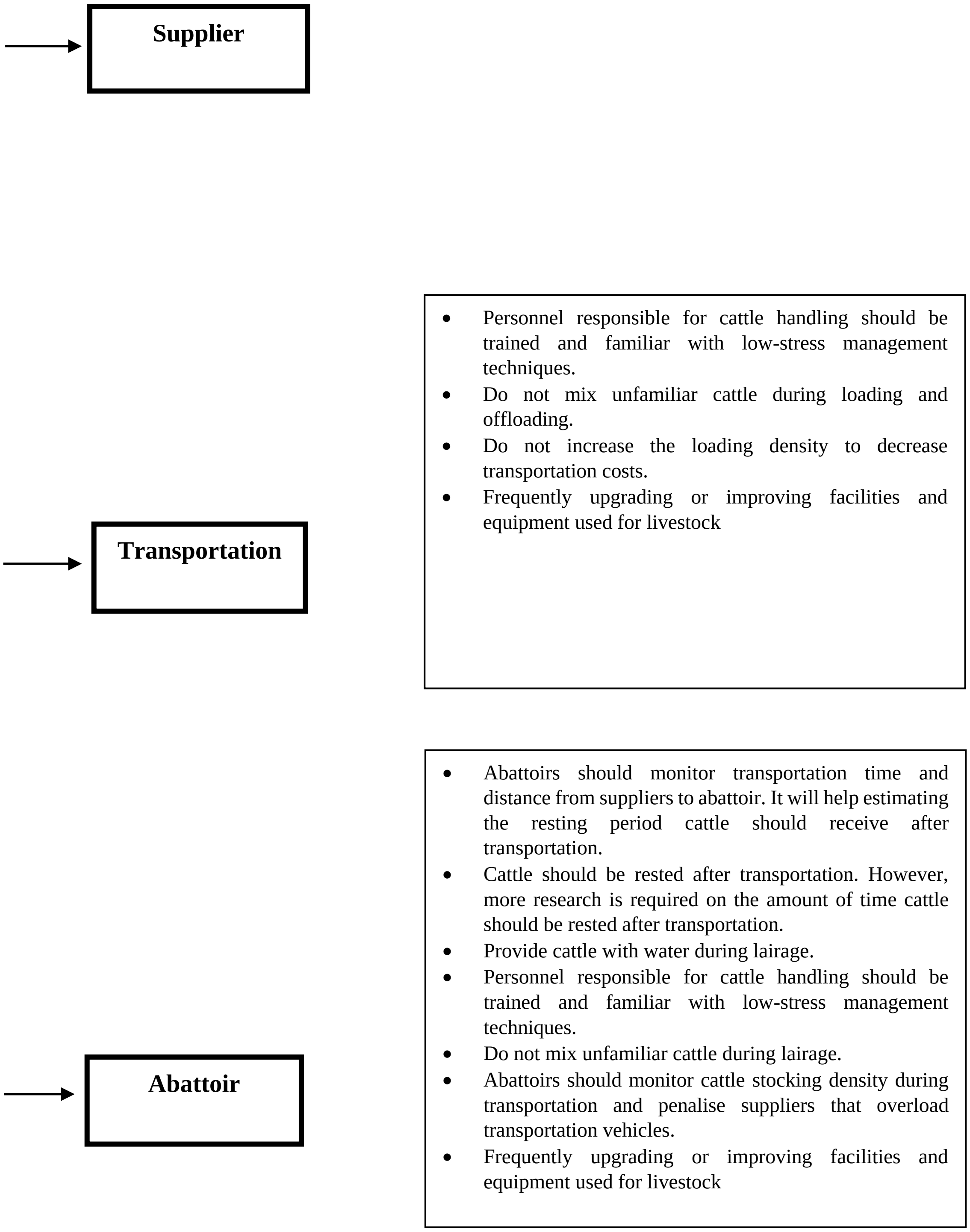
4.4 BASIC STRATEGIES TO DECREASE THE RISK OF DARK-CUTTING MEAT IN SOUTH AFRICA

The main areas where pre-slaughter stress should be minimised by skilful management techniques include from the supplier throughout transportation and to the abattoir.

Figure 4.14 suggests simple practical management techniques that can be applied at these main areas to minimize the pre-slaughter stress cattle experience during these crucial areas in beef production (the latter aspects can be in a Hazard Analysis Critical Control Points (HACCP) system). By applying these basic management techniques, the risk of DFD beef can be better managed and decreased, especially in poorly conditioned cattle.



- Increase awareness campaigns for suppliers on the integral role they play in meat quality attributes.
- Personnel responsible for cattle handling should be trained and familiar with low-stress management techniques.
- Nutritional management from conception up until lairage, especially between seasons plays a vital role in conditioning of cattle and ultimately influence glycogen concentration in cattle.



- Personnel responsible for cattle handling should be trained and familiar with low-stress management techniques.
- Do not mix unfamiliar cattle during loading and offloading.
- Do not increase the loading density to decrease transportation costs.
- Frequently upgrading or improving facilities and equipment used for livestock

- Abattoirs should monitor transportation time and distance from suppliers to abattoir. It will help estimating the resting period cattle should receive after transportation.
- Cattle should be rested after transportation. However, more research is required on the amount of time cattle should be rested after transportation.
- Provide cattle with water during lairage.
- Personnel responsible for cattle handling should be trained and familiar with low-stress management techniques.
- Do not mix unfamiliar cattle during lairage.
- Abattoirs should monitor cattle stocking density during transportation and penalise suppliers that overload transportation vehicles.
- Frequently upgrading or improving facilities and equipment used for livestock

Figure 4.14: Suggested steps to minimize pre-slaughter stress at the supplier, during transportation and at the abattoir and decrease the risk of dark-cutting beef.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

There is an endless list of factors contributing to pre-slaughter stress and all the factors are confounded by one another. Furthermore, it is important to keep in mind that the effect of pre-slaughter stress factors in South African conditions may not have the same effect in other countries, because of different genotype-environmental interactions which is another contributing factor to the long list of reasons why DFD beef is such a complex problem to solve.

The Mixed model analysis took into consideration the confounding between factors and provided a good method to indicate the influence of pre-slaughter stress factors onDFD beef. However, it is important to remember that this statistical procedure provides an estimate and not a definite indication of the effects of all the factors.

The overall estimated incidence of DFD beef at a South African abattoir was 0.40% high-risk ($pH_u > 6.2$), 43.2% intermediate risk (pH_u $5.8 \leq X < 6.2$) and 56.4 % ($pH_u < 5.8$) low-risk DFD carcasses. The proportion estimate effect of the pre-slaughter stress factors on the pH_u was significant but small. However, combining the proportion estimate effects of all factors on individual cattle substantially increased the risk of DFD beef by c.a 17%. But because there is confounding between some of the factors associated with DFD beef, which may decrease the overall effects of individual factors, there may be a reduced overall effect of other related factors.

In summary, the following extrinsic factors had a significant effect on the pH_u post-mortem namely: Suppliers, production systems, climatic factors, loading density and the standing period. Interestingly, transportation distances did not affect pH_u, but rather an increase in loading density of cattle during transportation increased pre-slaughter stress and ultimately increased the risk of DFD beef. The study also indicated that cattle should be rested after transportation because as the standing period increased the risk of DFD beef decreased. However, more research is required to quantify a general estimated resting time according to the pre-slaughter stress factors cattle experience before slaughter.

Moreover, the following intrinsic factors significantly influenced the pH_u post-mortem: Gender, age and carcass conformation which is determined by the live mass, carcass warm mass and fat content. Cattle breeds did not have a significant influence on DFD beef. However, mostly composite breeds were slaughtered at the abattoir, therefore, the variation in the pH_u post-mortem between breeds was not a clear indication of the breed effect on muscle glycogen depletion. Thus, it is necessary to do more research with cattle of the same gender and age under similar environmental conditions and management techniques to get a clear indication of the breed effect on muscle glycogen depletion.

The outcome of this study indicated that the seasonal variation in pH_u was mainly determined by the variation in cattle body condition (live mass, warm carcass mass and fat content) throughout the year. During the DFD beef window in South African conditions, cattle had a lower body condition (live weight (< 458 kg) and warm carcass mass (< 281 kg)) compared to the rest of the year and therefore responded more severely to pre-slaughter stress factors, increasing the risk of DFD beef. Therefore, body condition can be used as the main determining factor to identify individual cattle at risk to produce DFD beef prior to slaughter. All the pre-slaughter stress factors still play a role in the occurrence of DFD, but the body condition will determine the effect of pre-slaughter stress factors on individual cattle.

This study indicated that it is possible to decrease the risk of DFD beef by focussing on the factors that can be controlled, such as nutritional management, low-stress management techniques especially during transportation, avoiding the mixing of unfamiliar cattle, lower loading density and standing periods, and higher carcass weights. One of the biggest concerns, however, is that suppliers are not aware of the integral role they play in meat quality attributes, especially during the finishing phase of beef cattle production. Therefore, it is important that more awareness campaigns and training of suppliers, advisers and policymakers should occur to improve livestock production. In conclusion, the risk of DFD beef can be minimised to a great extent through skilful manipulation of cattle management techniques.

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APPENDIX

APPENDIX A

DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)

Descriptives Statistic

	DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)		95% Confidence Interval for Mean							
		Mean	Lower Bound	Upper Bound	Median	Std. Deviation	Minimum	Maximum	Skewness	Kurtosis
LIVE_MASS	HIGH	446.0141	437.1734	454.8547	439.9000	49.11636	341.09	564.88	.251	-.325
	INTERMED	456.6059	455.6360	457.5758	456.5100	54.59809	.00	711.65	-.734	7.029
	NORMAL	458.7464	457.8684	459.6245	459.4200	56.48339	.00	707.09	-2.053	16.392
WARM_MASS	HIGH	272.0942	265.3039	278.8845	270.0000	37.72507	180.60	353.40	.146	-.331
	INTERMED	276.8418	276.2247	277.4589	276.8000	34.73775	165.60	402.80	-.150	.004
	NORMAL	282.7597	282.2671	283.2523	282.4000	31.68626	165.20	375.20	-.098	.047
LOADING DENSITY	HIGH	66.87	60.85	72.88	60.00	33.428	4	148	1.529	1.700
	INTERMED	58.49	58.11	58.86	59.00	21.118	4	150	1.632	6.068
	NORMAL	59.78	59.46	60.09	59.00	20.540	2	150	1.774	6.481
TRANSPORT DISTANCE	HIGH	195.21	176.20	214.23	221.00	105.626	14	532	.342	1.372
	INTERMED	251.36	248.45	254.28	230.00	164.014	4	897	1.638	3.137
	NORMAL	242.48	240.08	244.88	230.00	154.291	4	897	1.735	4.319

APPENDIX B

Crosstabs

*SUPPLIER XX * DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH) Crosstabulation*

			DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE HIGH)			Total
			HIGH	INTERMEDIATE	NORMAL	
SUPPLIER XX	SUPPLIER A	Count	0	42	63	105
		% within SUPPLIER XX	0.0%	40.0%	60.0%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.3%	0.4%	0.4%
	SUPPLIER AA	Count	30	752	1056	1838
		% within SUPPLIER XX	1.6%	40.9%	57.5%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	24.8%	6.2%	6.6%	6.5%
	SUPPLIER B	Count	1	331	793	1125
		% within SUPPLIER XX	0.1%	29.4%	70.5%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	2.7%	5.0%	4.0%
	SUPPLIER BB	Count	18	973	1301	2292
		% within SUPPLIER XX	0.8%	42.5%	56.8%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	14.9%	8.0%	8.2%	8.1%
	SUPPLIER C	Count	3	270	212	485
		% within SUPPLIER XX	0.6%	55.7%	43.7%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	2.5%	2.2%	1.3%	1.7%
	SUPPLIER CC	Count	3	8	0	11
		% within SUPPLIER XX	27.3%	72.7%	0.0%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	2.5%	0.1%	0.0%	0.0%
	SUPPLIER D	Count	0	115	446	561
		% within SUPPLIER XX	0.0%	20.5%	79.5%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.9%	2.8%	2.0%
	SUPPLIER DD	Count	0	46	24	70
		% within SUPPLIER XX	0.0%	65.7%	34.3%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.2%	0.2%
	SUPPLIER E	Count	0	0	22	22
		% within SUPPLIER XX	0.0%	0.0%	100.0%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.0%	0.1%	0.1%
	SUPPLIER EE	Count	0	24	21	45
		% within SUPPLIER XX	0.0%	53.3%	46.7%	100.0%

	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.2%	0.1%	0.2%
SUPPLIER F	Count	0	138	5	143
	% within SUPPLIER XX	0.0%	96.5%	3.5%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	1.1%	0.0%	0.5%
SUPPLIER FF	Count	0	94	25	119
	% within SUPPLIER XX	0.0%	79.0%	21.0%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.8%	0.2%	0.4%
SUPPLIER G	Count	0	36	6	42
	% within SUPPLIER XX	0.0%	85.7%	14.3%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.3%	0.0%	0.1%
SUPPLIER GG	Count	1	31	34	66
	% within SUPPLIER XX	1.5%	47.0%	51.5%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.3%	0.2%	0.2%
SUPPLIER H	Count	0	35	13	48
	% within SUPPLIER XX	0.0%	72.9%	27.1%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.3%	0.1%	0.2%
SUPPLIER HH	Count	7	1197	1511	2715
	% within SUPPLIER XX	0.3%	44.1%	55.7%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	5.8%	9.8%	9.5%	9.6%
SUPPLIER I	Count	0	0	14	14
	% within SUPPLIER XX	0.0%	0.0%	100.0%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.0%	0.1%	0.0%
SUPPLIER II	Count	2	169	18	189
	% within SUPPLIER XX	1.1%	89.4%	9.5%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	1.7%	1.4%	0.1%	0.7%
SUPPLIER J	Count	0	49	0	49
	% within SUPPLIER XX	0.0%	100.0%	0.0%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.0%	0.2%
SUPPLIER JJ	Count	1	69	21	91
	% within SUPPLIER XX	1.1%	75.8%	23.1%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.6%	0.1%	0.3%
SUPPLIER K	Count	0	53	2	55
	% within SUPPLIER XX	0.0%	96.4%	3.6%	100.0%

	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.0%	0.2%
SUPPLIER KK	Count	28	4365	6167	10560
	% within SUPPLIER XX	0.3%	41.3%	58.4%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	23.1%	35.8%	38.8%	37.4%
SUPPLIER L	Count	2	79	11	92
	% within SUPPLIER XX	2.2%	85.9%	12.0%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	1.7%	0.6%	0.1%	0.3%
SUPPLIER LL	Count	0	96	412	508
	% within SUPPLIER XX	0.0%	18.9%	81.1%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.8%	2.6%	1.8%
SUPPLIER M	Count	0	99	108	207
	% within SUPPLIER XX	0.0%	47.8%	52.2%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.8%	0.7%	0.7%
SUPPLIER MM	Count	0	3	21	24
	% within SUPPLIER XX	0.0%	12.5%	87.5%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.0%	0.1%	0.1%
SUPPLIER N	Count	2	31	3	36
	% within SUPPLIER XX	5.6%	86.1%	8.3%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	1.7%	0.3%	0.0%	0.1%
SUPPLIER NN	Count	1	47	4	52
	% within SUPPLIER XX	1.9%	90.4%	7.7%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.4%	0.0%	0.2%
SUPPLIER O	Count	0	56	1	57
	% within SUPPLIER XX	0.0%	98.2%	1.8%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.5%	0.0%	0.2%
SUPPLIER OO	Count	1	40	19	60
	% within SUPPLIER XX	1.7%	66.7%	31.7%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.3%	0.1%	0.2%
SUPPLIER P	Count	0	70	45	115
	% within SUPPLIER XX	0.0%	60.9%	39.1%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.6%	0.3%	0.4%
SUPPLIER PP	Count	0	6	39	45
	% within SUPPLIER XX	0.0%	13.3%	86.7%	100.0%

	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.0%	0.2%	0.2%
SUPPLIER Q	Count	0	30	102	132
	% within SUPPLIER XX	0.0%	22.7%	77.3%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.2%	0.6%	0.5%
SUPPLIER QQ	Count	0	22	32	54
	% within SUPPLIER XX	0.0%	40.7%	59.3%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.2%	0.2%	0.2%
SUPPLIER R	Count	0	38	39	77
	% within SUPPLIER XX	0.0%	49.4%	50.6%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.3%	0.2%	0.3%
SUPPLIER RR	Count	15	1167	1827	3009
	% within SUPPLIER XX	0.5%	38.8%	60.7%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	12.4%	9.6%	11.5%	10.7%
SUPPLIER SS	Count	1	40	6	47
	% within SUPPLIER XX	2.1%	85.1%	12.8%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.3%	0.0%	0.2%
SUPPLIER T	Count	2	491	119	612
	% within SUPPLIER XX	0.3%	80.2%	19.4%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	1.7%	4.0%	0.7%	2.2%
SUPPLIER TT	Count	0	28	0	28
	% within SUPPLIER XX	0.0%	100.0%	0.0%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.2%	0.0%	0.1%
SUPPLIER U	Count	0	94	3	97
	% within SUPPLIER XX	0.0%	96.9%	3.1%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.8%	0.0%	0.3%
SUPPLIER UU	Count	1	328	246	575
	% within SUPPLIER XX	0.2%	57.0%	42.8%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	2.7%	1.5%	2.0%
SUPPLIER V	Count	0	204	225	429
	% within SUPPLIER XX	0.0%	47.6%	52.4%	100.0%
	% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	1.7%	1.4%	1.5%
SUPPLIER VV	Count	0	17	101	118
	% within SUPPLIER XX	0.0%	14.4%	85.6%	100.0%

		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.1%	0.6%	0.4%
	SUPPLIER W	Count	0	46	109	155
		% within SUPPLIER XX	0.0%	29.7%	70.3%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.7%	0.5%
	SUPPLIER WW	Count	0	47	9	56
		% within SUPPLIER XX	0.0%	83.9%	16.1%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.1%	0.2%
	SUPPLIER X	Count	0	106	257	363
		% within SUPPLIER XX	0.0%	29.2%	70.8%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.9%	1.6%	1.3%
	SUPPLIER XX	Count	0	48	128	176
		% within SUPPLIER XX	0.0%	27.3%	72.7%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.4%	0.8%	0.6%
	SUPPLIER Y	Count	0	30	10	40
		% within SUPPLIER XX	0.0%	75.0%	25.0%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.2%	0.1%	0.1%
	SUPPLIER YY	Count	0	56	23	79
		% within SUPPLIER XX	0.0%	70.9%	29.1%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.5%	0.1%	0.3%
	SUPPLIER Z	Count	0	1	25	26
		% within SUPPLIER XX	0.0%	3.8%	96.2%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.0%	0.2%	0.1%
	SUPPLIER ZZ	Count	2	59	223	284
		% within SUPPLIER XX	0.7%	20.8%	78.5%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	1.7%	0.5%	1.4%	1.0%
Total		Count	121	12176	15901	28198
		% within SUPPLIER XX	0.4%	43.2%	56.4%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	100.0%	100.0%	100.0%	100.0%
CONFORMATION	3	Count	120	12116	15764	28000
		% within CONFORMATION	0.4%	43.3%	56.3%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	99.2%	99.5%	99.2%	99.3%
	4	Count	1	47	122	170
		% within CONFORMATION	0.6%	27.6%	71.8%	100.0%

		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	0.4%	0.8%	0.6%
	5	Count	0	12	12	24
		% within CONFORMATION	0.0%	50.0%	50.0%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.1%	0.1%	0.1%
Total		Count	121	12175	15898	28194
		% within CONFORMATION	0.4%	43.2%	56.4%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	100.0%	100.0%	100.0%	100.0%
STANDING PERIOD (1- same_day, 2- next_day, 3- >2_days)	1	Count	120	11728	15323	27171
		% within *STANDING PERIOD* (1- same_day, 2- next_day, 3- >2_days)	0.4%	43.2%	56.4%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	99.2%	96.3%	96.4%	96.4%
	2	Count	1	432	564	997
		% within *STANDING PERIOD* (1- same_day, 2- next_day, 3- >2_days)	0.1%	43.3%	56.6%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.8%	3.5%	3.5%	3.5%
	3	Count	0	16	14	30
		% within *STANDING PERIOD* (1- same_day, 2- next_day, 3- >2_days)	0.0%	53.3%	46.7%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	0.0%	0.1%	0.1%	0.1%
Total		Count	121	12176	15901	28198
		% within *STANDING PERIOD* (1- same_day, 2- next_day, 3- >2_days)	0.4%	43.2%	56.4%	100.0%
		% within DFD RISK_24h (5.4-5.79 NORMAL, 5.8-6.19 INTERMEDIATE, ≥6.2 HIGH)	100.0%	100.0%	100.0%	100.0%