Culling-associated stress and meat quality in ungulates

by

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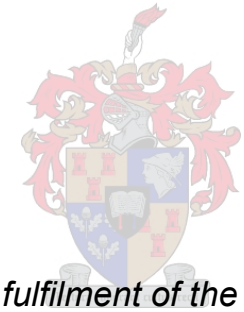

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in the Faculty of AgriSciences at Stellenbosch University

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DECLARATION

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SUMMARY

The purpose of this investigation was to evaluate the effects of helicopter-, day- and night-culling on the ante-mortem stress experienced by sub-adult impala (*Aepyceros melampus*) rams and mature blue wildebeest (*Connochaetes taurinus*) cows, by determining the effect of hunting method on the serum testosterone, cortisone and cortisol levels at death, and influence on meat quality parameters.

Blood samples were collected immediately post-mortem and analysed for the above-mentioned steroid hormones, and the serum levels assayed were compared to the expected diurnal secretion pattern of each hormone. During and up and to 24 hours post-mortem, the pH of the *Longissimus thoracis et lumborum* (LTL) was recorded at regular intervals to establish a post-mortem pH profile, which was then related to the respective meat quality parameters. The left LTL muscle was removed from the carcass for physical and proximate analysis. The physical parameters included pH, water-holding capacity, tenderness and colour, were subsequently correlated with the serum hormone levels.

The serum testosterone levels in the impala were not influenced by culling method, however, serum cortisone concentrations were higher in the night-culled impala, when compared to the helicopter- and day-culled animals. The serum cortisone and cortisol levels of night-culled impala were higher when compared to previously established baseline levels for impala. The serum glucocorticoid concentrations determined for the night-culled impala were similar to that of the helicopter- and day-culled animals, thus supporting the deviation from the established diurnal pattern in previous studies. Meat samples obtained from the night-culled impala had an increased water-holding capacity as well as were more tender than the meat samples from the impala of the other culling methods. Culling method did not influence the colour of the meat samples.

The serum concentrations of glucocorticoid hormones determined for the blue wildebeest appeared to conform to previously established diurnal patterns. Blood samples from the helicopter-culled animals were characterized by higher serum concentrations of glucocorticoid hormones than that of the day- and night-culled animals. However, the physical analysis of the meat samples indicated that helicopter-culling resulted in a high pH_U, although not statistically different from the other treatment groups, a decreased water-holding capacity and lower shear force values. The day- and night-culled blue wildebeest produced meat samples similar in quality, indicating that these culling methods had no influence on ante-mortem stress of blue wildebeest.

It was observed that all the treatments resulted in high ultimate pH values, characteristic of dry, firm and dark (DFD) meat that is typically caused by chronic ante-mortem stress.

The meat obtained from the helicopter-culled blue wildebeest exhibited DFD qualities which could be attributed to chronic stress. Therefore, correlations between pH parameters and meat quality parameters were analysed. From the correlations, it was determined that with an increasing muscle pH_U, there was lower L* values whilst an increasing rate of pH decline resulted in a decreased water-holding capacity.

This is the first study of its kind on impala and blue wildebeest and therefore further research is required to verify these results as all indications are that culling by helicopter, although expensive, has added advantages.

This thesis is dedicated to my family and friends who have loved and supported me through this journey.

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Chapter 1
General Introduction

The game farming industry is structured on four pillars, i.e. live sales, ecotourism, sport/trophy hunting, and game hunting for consumptive purposes. Of these four pillars, sport/trophy hunting and game hunting for consumptive purposes yield meat that can be made available to consumers.

According to a United Nations report, the world population is expected to reach 9.7 billion by the year 2050 (United Nations, 2019). It is expected that the use of conventional livestock as a protein source will be unable to support the ever-growing population demand for protein, which in turn creates a demand for other protein sources such as game meat (Bekker *et al.*, 2011; Cawthorn & Hoffman, 2014; Van Schalkwyk & Hoffman, 2016). Historically, game meat has not always been readily accessible to the general consumers, resulting in many still having reservations about the product (Hoffman *et al.*, 2005; Dokmanovik *et al.*, 2015).

South African consumer demand for game meat within the formal market has been considerably lower than for more conventional meat types such as beef, mutton and pork. The lower demand can potentially be attributed to limited availability, higher retail prices as well as the naturally darker colour of game meat (Hoffman *et al.*, 2005; Wassenaar *et al.*, 2019). A darker meat colour is usually associated with suboptimal meat quality, which results in a negative perception by consumers when they use meat colour as a tool to assess the quality of meat on display (Viljoen *et al.*, 2002; Hoffman *et al.*, 2005). Several studies have elucidated to the health benefits of game meat that appeals to consumers, and can be attributed to an improved fatty acid profile and increased protein content (Hoffman *et al.*, 2005; Hoffman & Wiklund, 2006; Bekker *et al.*, 2010; Wassenaar *et al.*, 2019).

As previously mentioned, most of the game meat being introduced to consumers is as a result of trophy hunting and hunting for consumptive purposes. The increase in game meat availability and research have contributed to a change in consumer associations and perceptions of game meat as a protein source (Bekker *et al.*, 2010; Erasmus & Hoffman, 2017). Consumers determine meat consumption trends and are more likely to purchase products that they enjoy and are less likely to accept meat of a darker appearance and poor quality (Viljoen *et al.*, 2002; Henchion *et al.*, 2014). Meat that is characterized by an unfavourable dark colour, is often the result of chronic or acute stress experienced by the animals prior to slaughter (Hart, 2012). Chronic stress can be defines as long-term stress often resulting from, amongst other factors, improper husbandry practices or inadequate feeding regimes, and can contribute to decreased fertility and increased mortality rates (Etim *et al.*, 2013). Acute stress occurs when animal experiences short-term stress, and is often induced by translocation and hunting activities. The

effects of these activities are typically associated with the “fight-or-flight” response (Stull, 1997; Etim *et al.*, 2013).

Worldwide, many animal species are farmed with and/or hunted for the primary purpose of meat production (Field, 2004). This is particularly true in Africa, which has the highest diversity of ungulate species (D’Amato *et al.*, 2013). In Africa, among the most hunted species are springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*), blesbok (*Damaliscus pygargus phillipsi*), kudu (*Tragelaphus strepsiceros*), blue wildebeest (*Connochaetes taurinus*) and red hartebeest (*Alcelaphus buselaphus caama*) (Jooste, 1983; Van Schalkwyk & Hoffman, 2016). Impala carcasses are characterized by one of the highest protein contents of ungulate species after the common duiker and red hartebeest (Hoffman & Cawthorn, 2012). The overall carcass yields are higher than that of kudu (Hoffman *et al.*, 2009) and due to husbandry practices can also be considered an organic meat source, with a suitable chemical and molecular profile for export to international markets (Hoffman, 2000b). From the limited research on blue wildebeest, it has been noted that the meat obtained is also high in protein and low in lipid content (Hoffman *et al.*, 2011; Van Heerden, 2018). Blue wildebeest are usually hunted for the purpose of population control, however meat quality analysis indicated that blue wildebeest meat has a favourable profile and is also considered low in fat (Van Heerden, 2018).

Culling/harvesting operations can be conducted at night or during the day. At night, culling is performed from a vehicle using spotlights for visibility. The use of spotlights is most effective at startling the animal on moonless nights (Lewis *et al.*, 1997; La Grange, 2006), also making a head or neck shot possible more often due to the animal being stationary, with its head up (Bothma, 2002). This method usually has a culling rate of approximately ten animals per hour (Veary, 1991) however, it is limited to areas with vehicle-access and species with distinct gender morphologies (Bothma, 2002). Culling that is conducted during the day can be done via vehicle (day-culling) or via helicopter (helicopter-culling). Day-culling is considered a more traditional hunting method where culling can occur at a rate of six animals per hour (Veary, 1991; Bothma, 2002; Hoffman & Laubscher, 2009a). The animals are more aware of their surroundings and more prone to being stressed (Kritzinger *et al.*, 2003). Day-culls are more efficient for more timid species and animals that occur in smaller groups (Bothma, 2002). Alternatively, hunting from a helicopter is more efficient when culling large numbers of animals at a time. Approximately 29 animals can be culled per hour (Veary, 1991). However, helicopter hunting does involve substantial expenses, and the efficacy of this method is limited by what can be observed from the air. Helicopter-culling will thus be more suitable for use in open grassland or savanna areas, but animals are normally chased considerably before being shot (Van der Waal & Dekker, 2000; Bothma, 2002; Hoffman & Wiklund, 2006; La Grange, 2006; Bothma *et al.*, 2010).

Consumers are often concerned about the welfare and sustainability of meat sources and many consumers are not comfortable with culling procedures used in the wildlife industry (Kristensen *et al.*, 2014; Van Schalkwyk & Hoffman, 2016). Therefore, in order to improve

consumer acceptability of game meat, hunting and culling methodologies need to be refined and if necessary, modified to reduce the extent of stress that will be experienced by the animals prior to hunting, which in turn will impact positively on meat quality and ensure that the welfare and sustainability of the animal is accommodated in the best possible way in the process.

Various studies have been conducted on the impact of culling methods on the meat quality of ungulate species. These studies however, only compared day- and night-culling procedures. Hoffman and Laubscher (2009a) and (2010) found that day-culled impala and gemsbok were more stressed, however meat quality was not severely affected. Kritzinger *et al.* (2003) was in agreement but found that night-culled impala yielded meat with better quality characteristics. In the case of red hartebeest, Hoffman and Laubscher (2011) found no significant differences in meat quality of day- and night-culled red hartebeest. Culling methods have an influence on water-holding capacity which can potentially be ascribed to effects of stress rather than the method itself as stress is known to increase the water-holding capacity (Hoffman, 2000a; Hoffman & Laubscher, 2009a). These two studies have suggested that stress resulting from the different culling methods, contributes to the differences in meat quality, however, very few studies have physically measured and quantified this assumption.

Stress is a difficult parameter to quantify as there are many factors that contribute to the stress experienced by the animal. Many of these factors are beyond control, especially in the case of game animals that are maintained *in situ* (Hoffman & Laubscher, 2010). Physiologically chronic and acute stress manifest differently in an animal, and with each type of stress it is important to determine the best sampling approach to be able to quantify the stress response as best possible. An example of one such method is the analysis of blood plasma for glucocorticoid and androgenic steroid hormones. Cockram *et al.* (2011) used this to determine the effects of culling method on red deer (*Cervus elaphus*) where helicopter-culling was found to be the most stressful. Some of the previously mentioned studies also made use of this analysis however it is unclear if any of the mentioned studies (Hoffman & Laubscher, 2009b, 2010, 2011) accounted for the diurnal rhythms of the glucocorticoids.

The purpose of this study is therefore to determine the influence of culling method on ante-mortem stress experienced by impala and blue wildebeest, by determining the blood glucocorticoid concentrations immediately after an animal was shot. Results obtained will be related to meat quality characteristics to verify the impact of ante-mortem stress on the meat quality traits of these two species. Findings from this study will assist in addressing consumer concerns about the origin and manner in which game meat is obtained.

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Chapter 2
Literature Review

It is expected that by the year 2050 the world population will be approximately 9.7 billion (Barnett & Patterson, 2006; Kristensen *et al.*, 2014; United Nations, 2019). Therefore, livestock producers are under increasing pressure to produce food products that are safe, and that can also meet consumer demands. In southern Africa, some areas that are too arid and not suitable for livestock production, holds the potential to allow for the farming of several game species that have adapted to these environmental conditions (Jooste, 1983; SADAFF, 2010). As a consequence of areas being unsuitable for livestock production, game ranching is becoming increasingly important in terms of food security (Barnett & Patterson, 2006; Van Schalkwyk & Hoffman, 2016). The game industry has been expanding significantly since the 1960s. In a parliamentary presentation it was noted that wildlife ranching had expanded to occupy a total of 18.7 million ha in South Africa (Munzhedzi, 2018).

2.1 The game industry

The game industry is structured on four pillars that are either non-consumptive or consumptive in nature. The non-consumptive pillar includes all eco-tourism and associated activities, whereas the three consumptive pillars include live game sales, recreational and trophy hunting as well as harvesting animals for meat production (Van Schalkwyk & Hoffman, 2016). It is common for a game ranch operation to consist of one or more of these pillars, with some of the larger ranches incorporating all the pillars in a more integrated approach to game farming.

2.1.1 Ecotourism

This pillar provides tourists with the opportunity to enter game parks/ranches with the primary intention of observing the animals in their natural habitats. Ecotourism has played a vital role in the promotion of sustaining and protecting wildlife in their natural habitats, as it requires the upkeep of the natural land (Barnett & Patterson, 2006). Game drives and general safari-type trips are classified among the most important tourism activities in southern Africa, and it creates many jobs for local communities in close proximity to such operations (Van Schalkwyk & Hoffman, 2016).

2.1.2 Live game sales

In 2018, live game sales contributed approximately 1,1 billion ZAR to the South African economy (Munzhedzi, 2018). Game animals can either be sold to a specific buyer or sent to an auction. There are two types of auctions available, the first type is a live auction, where the animals are kept in a boma and presented to the potential buyers in person. The second type is a catalogue auction; these animals will only be captured and transported once they are purchased (Bothma,

2002; Van Hoving, 2011). The latter is becoming the preferred option as it is less costly and also less stressful to the animals. Game animals are also sold live between ranchers without going through the formal process of an auction. However, the size of this industry is unknown although anecdotal information would seem to indicate it as being significant.

2.1.3 Sport / trophy hunting

Sport hunting is a controversial topic and historically, has been temporarily banned in certain southern African countries such as Tanzania, Kenya and Zambia. However, trophy hunting alone has been declared “the most economically important” pillar of the wildlife industry, contributing in excess of 5 billion ZAR to the economy, and also providing several job opportunities (Field, 2004; Barnett & Patterson, 2006; Saayman *et al.*, 2018).

Sport hunting is often not a suitable management tool for a game rancher but rather a recreational activity, as too few animals are hunted at a time to assist in the maintenance of herd sizes or produce enough meat to be sold commercially. Usually performed by more wealthy hunters and international tourists, animals in prime condition are hunted with the intent of the deceased animal being processed using taxidermy (Barnett & Patterson, 2006; Van Schalkwyk & Hoffman, 2016). However, there is also a huge local market, the so-called ‘biltong’ hunter who hunts to provide meat for own consumption. Data on the value and numbers of animals hunted indicates that this form of hunting is substantial; Saayman *et al.* (2011) calculated the contribution of ‘biltong hunting’ to real GDP to be in excess of 6 billion ZAR.

2.1.4 Game harvesting / culling:

Harvesting and culling differ in that the former is focused on removing a certain number of animals, whilst the latter is the selective removal of animals. An example of the latter would be the culling (killing) of a certain percentage of males, leaving selected animals that can be hunted as trophy animals. This pillar is aimed at culling of usually large numbers of animals for consumptive purposes locally and internationally.

Game meat is gaining commercial value and is highly sustainable especially in South Africa (Van Schalkwyk & Hoffman, 2016). According to worldwide trends in 2008, South Africa was ranked 20th in the production of game meat (SADAFF, 2010) produced in the form of biltong or steaks (Jooste, 1983; Field, 2004).

2.2 The contribution of the wildlife industry to the South African economy

In southern Africa there has been a noticeable decline of financial security in the economy. Ecotourism and other wildlife-associated income generating activities have been a valuable income source of foreign currency, allowing the industry and thus the country to grow on a global scale (Van der Waal & Dekker, 2000; Field, 2004; Barnett & Patterson, 2006; Van Schalkwyk & Hoffman, 2016). The communities in the more rural areas of southern Africa are also benefiting

from this increase in size and sustainability of the wildlife industry (Barnett & Patterson, 2006; Carruthers, 2008).

To maximise the income generated by the wildlife industry, it is important that animals are used for multiple purposes, and not limited by farmers to a single category of income. For example, a single animal on a wildlife farm has the ability to generate several opportunities for income. Firstly, the animal can be used for viewing purposes where tourists pay to view and photograph the animal, secondly the animal can be sold to a hunter who will take the trophy and lastly, the remaining hide and meat (including the offal) that is left after the trophy has been taken, can be sold for decorative and consumptive purposes, respectively (Barnett & Patterson, 2006). Alternatively, animals could be auctioned off and sold as breeding stock and thereafter enter the breeding cycle to produce offspring that will be used for the abovementioned process.

Saayman *et al.* (2011) investigated the effect of hunting for the purpose of biltong production on the South African economy and found that there was a largely positive economic impact. In the same study, they found that biltong hunting had a contribution of over 6 billion ZAR to the Gross Domestic Profit (GDP) of the country along with job creation. Van der Waal & Dekker (2000) found approximately 13 700 permanent jobs created as well as extra people being hired temporarily during hunting season. In a 2018 report, the same authors found that trophy hunting contributed significantly to the national economy and supplied over 17 000 jobs, which could result in areas of lower income becoming more economically stable (Saayman *et al.*, 2018).

Hunting generates a significant income whether it is due to the direct monetary contribution from hunters or the corresponding multiplier effects that come into play. Hunters spend huge sums of money on licenses and equipment required before they even consider the costs of the actual hunting expedition (Field, 2004). In 2010 in the Limpopo Province, the sectors that benefitted the most from both trophy and biltong hunting include trade and accommodation, transport and communication, manufacturing, as well as financial and business services. Overall the income generated was in excess of 1.2 billion ZAR, based on 2006 prices (Van Der Merwe *et al.*, 2014).

2.3 Wildlife species are a contributor to food security

There are a vast number of wildlife species hunted worldwide for the purpose of meat production, including antelope, various predators, rodents as well as many avian species (Field, 2004). In South Africa, springbok (*Antidorcas marsupialis*) is considered the most important of the antelope species in game farming, along with gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*), mountain reedbuck (*Redunca fulvorufula*), blesbok (*Damaliscus pygargus phillipsi*), kudu (*Tragelaphus strepsiceros*), Hartmann's zebra (*Equus zebra hartmannae*), red hartebeest (*Alcelaphus buselaphus caama*), and blue- (*Connochaetes taurinus*) and black wildebeest (*Connochaetes gnou*), depending on the area and terrain (Jooste, 1983; Van Schalkwyk & Hoffman, 2016). When deciding on which species to harvest it is important that the species has

an acceptable population size (i.e. warrants culling), reproduce efficiently, and the species is accessible and easy to harvest (Van Schalkwyk & Hoffman, 2016). Lewis *et al.* (1997) did however caution hunters to avoid hunting from herds that were too large, as the animals not hunted are left highly stressed, posing possible welfare problems. Other requirements for responsible hunting include being able to easily distinguish ages and sexes of animals.

For the purpose of this study, only two of these species will be considered, namely impala and blue wildebeest. Within South Africa, these two species have shown potential for the production of quality game meat and are considered as surplus animals in the game breeding industry. To understand the potential of the use of these two species for production of quality meat, more information is required regarding the influence of hunting activity on their behaviour, and how this relates to carcass characteristics and meat quality parameters.

2.3.1 Impala *(Aepyceros melampus)*

Impala are considered as a small ungulate species, with mature males weighing approximately 50 kg and females 40 kg. Impala are one of the more commonly hunted and traded species in southern Africa (Hitchins, 1966; Hoffman, 2000b; Field, 2004; La Grange, 2006; Hoffman *et al.*, 2009; Selier *et al.*, 2016). Impala are known to be an appropriate species that can be harvested and sold within the formal meat trade and/or exported as the meat that results from hunting or culling activities, is considered a healthy meat, with a desirable fatty acid profile and high protein content (Hoffman, 2000b).

Within free-roaming populations, impala typically cluster into two types of herds, i.e. breeding herds and bachelor herds. Breeding herds consist of a dominant ram with up to 100 ewes and their lambs (Schenkel, 1966; Apps, 2014). Lewis *et al*. (1997) noted that within these breeding herds, the rams are more susceptible to hunting-associated stress. Bachelor herds are normally smaller herds that consist of up to 60 male antelope of all ages (Schenkel, 1966; Lewis *et al.*, 1997). Breeding herds are known to have closer associations, and are characterized by more social interactions than bachelor herds, which can be ascribed to the herding by the dominant ram that ensures none of his ewes stray too far or get lost as well as helping ensure the herd's safety (Schenkel, 1966). During rutting season, rams are highly active due to courting, mating and defending their position within their herd, which makes them more alert and thus susceptible to stress (Schenkel, 1966; Hoffman, 2000a).

Impala tend to prefer savannah or dense bushveld terrains and avoid open grasslands and floodplains with limited hiding space. They require a stable water supply and will remain within a territory unless the herd is threatened and chased away (Schenkel, 1966; Selier *et al*., 2016).

Naturally, impala are skittish animals and are constantly aware of their surroundings (Schenkel, 1966; Matson *et al.*, 2005; Apps, 2014). Matson *et al.* (2005) noted that there are various factors that influence a herds' awareness to predation. For example, when an animal is stationed towards the outside of the herd, they spend more time scouting their surroundings to

ensure the herd is safe. Herds exposed to hunting frequently will be more skittish, run further distances and remain nervous for several hours when threatened (Schenkel, 1966; Matson *et al.*, 2005). When threatened at close range, the herd will disperse in all directions, leaping and galloping at full speed to confuse the predator (Schenkel, 1966; Apps, 2014). If the threat is sudden, such as the sound of a rifle, the impala often freeze facing the threat with their head held high (Lewis *et al.*, 1997).

Impala are considered suitable for commercial culling as they are abundant in numbers, easily identifiable, and the sex of the animal is easily identified. According to the National Red List status, impala are classified as being of least concern and can be found almost anywhere in the country. It is speculated that impala can become the most important wildlife species in providing a sustainable and environmentally friendly protein source. This could be due to the species' ability to adapt to most environments and terrains. It is possible that impala could outcompete other local species as they have a high fecundity and reproductive ability, thus it is important that the population numbers are not allowed to grow out of control (Fairall, 1985; Selier *et al.*, 2016).

2.3.2 Blue Wildebeest (*Connochaetes taurinus*)

Also known as the Brindled Gnu, blue wildebeest are a larger ungulate species. Mature cows have a body weight of approximately 170 - 200 kg, and bulls weigh between 200 - 250 kg (Hitchins, 1966; Field, 2004; La Grange, 2006; Hoffman *et al.*, 2011; Furstenburg, 2013). Hoffman *et al.* (2011) also noted blue wildebeest meat to be high in protein and low in fat with a desirable fatty acid profile, therefore comparable to the meat of other ungulate species.

In free-range populations, the herd dynamics are fairly similar to that of the impala. Blue wildebeest live in sparse bushveld areas with a subtropical or semi-arid climate, and require large quantities of water and palatable, sweet grass (Furstenburg, 2013). Blue wildebeest live in herds, with a bull surrounded by multiple cows. Bulls that are cast out from a herd during the rutting season, will form bachelor herds (La Grange, 2006; Apps, 2014). In contrast to impala herds, blue wildebeest do not have a hierarchy but rather form family groups (Furstenburg, 2013).

Blue wildebeest can be extremely difficult to capture due to their flight behaviour. Upon identification of a threat, blue wildebeest will maintain a distance of 40 – 150m from the perceived threat (Furstenburg, 2013). The herd will remain in large open areas, so they have space to run, if they are chased. Typically, they will run in a single file line, using speed and agility to confuse predators (La Grange, 2006; Furstenburg, 2013). However, when they stop running or are cornered, the blue wildebeest seek comfort and protection from their fellow herd members, forming a close huddle until they break out into a run again (Apps, 2014).

Being more resilient to stress and their ability to survive stressful environmental conditions, blue wildebeest can be considered a sustainable and more durable meat source (La Grange, 2006; Hoffman *et al.*, 2011). However, sex identification is more difficult than with impala as both bulls and cows have well-developed horns.

2.4 Population management through the use of culling

Culling is a necessary management tool in the protection and prevention of overpopulation of wildlife (Kritzinger *et al.*, 2003; Field, 2004), which was supported by a study in the Limpopo Province of South Africa. Evidence indicated that an average of 20.3 % of the game species in the area were subjected to annual culling to maintain appropriate population sizes (Van der Waal & Dekker, 2000). The culling method used should be determined by the species being hunted as well as the terrain of the area in which the hunt will take place (Hoffman & Laubscher, 2010). The use of culling methods is not limited to the killing of animals, as the methods used frequently also form part of the capture of animals for the purpose of veterinary care or translocation (Field, 2004).

It is important to understand the difference between the terms hunting, harvesting and culling. Hunting is the term used when describing the pursuit of an animal and usually incorporates the concept of “fair chase”. Harvesting or cropping involves the killing of a large number of animals regardless of sex and/or age. Culling is similar to harvesting or cropping but involves a degree of selection of the animal prior to the animal being removed from the herd.

The process of culling animals, if used as an effective management tool, should not result in the depletion of entire populations or the disruption of population stability. Instead, it should reduce the population and by the next cull, the population should have sufficiently recovered, and the genetic structure maintained or improved (Van Schalkwyk & Hoffman, 2016). For example, it is important that culling is not limited to a sex but rather aimed at reducing population numbers without affecting the animals that are at their peak production. This requirement often results in more males being culled than females due to a single, dominant male being able to mate with multiple females. However, it is also important that the number of males is not depleted too much as this will disrupt social behaviour and create limitations in the genetic pool of the population. Other requirements include that the process be humane, economical and efficient, have a low wounding percentage, result in minimal damage to the meat, and be conducted on an appropriate terrain that allows for carcass bleeding (Young, 1992; La Grange, 2006).

Culling procedures can be carried out during the night- or daytime. If culling takes place during the day, the marksman could be in a vehicle, on foot or in a helicopter. For the purpose of this study, three culling methods (i.e. day-culling, night-culling and helicopter-culling) were considered in terms of the effect of stress, behaviour, and meat quality of impala and blue wildebeest.

2.4.1 Day-culling

This method could be conducted on foot however, hunting on foot is not suitable for large-scale culling/harvesting, as it is a time-consuming process (Hoffman & Laubscher, 2009a). For the purpose of this study, it was conducted from a vehicle.

Animals are highly active and aware of their surroundings during the day, therefore they are more prone to being stressed when chased (Kritzinger *et al.*, 2003). There is also a tendency

for increased ambient temperatures, when compared to the temperatures at night. If chased intensely, this could lead to additional thermal stress, which can affect meat quality (Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009a). Another problem frequently encountered with day-culling is the occurrence of flies in the presence of blood, which in turn, may pose health concerns if not managed.

Although this approach has disadvantages, day-culling also has its benefits. The daylight allows for increased visibility for the marksmen, allowing them to shoot from further distances (up to 300m), which could result in the animals being less aware of them, and body recovery is quicker (Hoffman & Laubscher, 2009a). Hoffman *et al.* (2011) noted that the use of two vehicles when culling blue wildebeest, approaching from opposite ends, in an effort to corner them, was efficient in terms of the time required to execute a successful culling activity.

2.4.2 Night-culling

Night-culls are best conducted on moonless nights to ensure that it is as dark as possible while using spotlights to scan the area for the animals being targeted (Lewis *et al.*, 1997; La Grange, 2006). This requirement limits night culling operations to only 14-20 days per month and terrains that are vehicle-accessible with minimal debris and contours on the ground (Van Schalkwyk & Hoffman, 2016).

Spotlights are important in temporarily blinding the animals to ensure they remain still whilst the marksman takes his aim (Lewis *et al.*, 1997; Hoffman & Laubscher, 2009a). Evidence provided by Lewis *et al.* (1997) and Hoffman and Laubscher (2009a) suggests this method is less stressful due to the relative unawareness of the animals to the hunter. Nevertheless, the animals tend to become fairly aware of the vehicles when they approach the herd and due to decreased visibility, the vehicle needs to approach within 150 m to increase and ensure shot accuracy (Hoffman & Laubscher, 2009a).

This method has been highly effective in culling impala and springbok as they are startled by the spotlights, assuming a stiff posture and staring straight into the spotlights. Sex of these species are also easily determined (La Grange, 2006; Hoffman & Laubscher, 2009a). However, in the case of animals with dark or black coloured heads such as wildebeest species and black impala, night culling is not as efficient because it is difficult to see the head silhouette.

2.4.3 Helicopter-culling

Helicopters are of great use on game farms whether for hunting, capturing, or counting of animals (Van der Waal & Dekker, 2000). The helicopter used in hunting or capture, usually consists of two seats, for a pilot and a hunter/shooter that flies steadily approximately six meters above the animal being targeted (Hoffman, 2000a). The marksman takes aim at the head or upper neck, while the helicopter moves at the same speed as the animal (Hoffman, 2000a; La Grange, 2006). This method is most commonly utilized for bushveld species such as impala, kudu, blue wildebeest, zebra and eland (Hoffman & Wiklund, 2006).

The use of helicopters is costly, resulting in a need to crop more than 100 blue wildebeest or 500 impala in a single session spanning a number of days to be financially viable (Van der Waal & Dekker, 2000; Hoffman & Wiklund, 2006; La Grange, 2006). Although this method is quick, the team often remain in the field for longer periods in order to locate the shot animals. The longer the time period that carcasses are exposed to ambient conditions, the more the carcasses are predisposed to a faster rate of decay (La Grange, 2006).

Where the use of helicopter-culling of impala and blue wildebeest is concerned, there is limited information available regarding the influence of this technique on animal behaviour and associated stress, and the ultimate effect on meat quality.

2.4.4 Shot placement during a culling operation

Shot placement during hunting or culling is of much debate and each hunter has his/her own preferences on where to place a shot. On average, a hunter loses 13.9% of the carcass weight due to bullet damage when a chest shot is used (Von La Chevallerie & Van Zyl, 1971). According to Lewis *et al.* (1997), a headshot is considered the most ethical approach, as the animal is immediately rendered unconscious and has no sense of awareness. A neck shot is also useful as the animal will generally be paralysed but will still be conscious. These two types of shots have shown to result in minimal meat wastage when compared to body shots. Animals that were culled by means of a head shot, however, cannot be used to make a trophy (Hoffman, 2000a). In some countries head shots are illegal, as a near miss could end in a jaw shot which results in welfare concerns. Such an animal cannot drink or graze and, unless followed and killed, will die a slow death.

Animals shot in more traditional body locations such as the flank, also known as a fore-flank or body shot and including bullets to the lower neck and ribs, could suffer more from being wounded, for they are not rendered unconscious instantaneously (Von La Chevallerie & Van Zyl, 1971; Lewis *et al.*, 1997; Hoffman & Laubscher, 2009a). The benefit of body shot placement involves a larger target area, allowing for some margin of error that will still result in death of the animal, although slower and many animals might still be breathing when retrieved (Hoffman & Laubscher, 2009b). Most trophy hunters will take the chest shot as a headshot will destroy the trophy. The effect of such chest shots on the meat quality has not yet been elucidated, although work in Europe and Canada has shown that these shots also cause copper and lead fragments to be distributed throughout the carcass (Knott *et al.*, 2010; Fachehoun *et al.*, 2015).

2.5 The influence of hunting-associated stress on animal behaviour and wellbeing

Irrespective of the species being culled, most animals experience the same physiological response to stress, and to understand the impact of stress on meat quality, an overview of these responses is required.

Animal welfare is a difficult concept to define and definitions found in dictionaries are considered inadequate (Moberg, 1985a; Curtis, 1985). It is imperative that animals are maintained by tending to all their basic needs. Curtis (1985) summarised these needs based on Maslow's Scheme, which indicates that the three most important requirements of animals are their physiological, safety and behavioural needs. Physiological needs include all aspects vital for normal physiological functioning such as the absence of stressors, appropriate feeding regime, suitable environment and health care. Safety needs include protection from weather and predation as well as equipment and facilities that should not cause any harm to the animals. The behavioural needs of the animal are considered satisfied if the animal can exhibit all natural behavioural traits without hindrance through abuse, neglect or deprivation (Ewbank, 1985).

There has always been debate over the use of animals, in any way, for the purpose of meat production and research (Moberg, 1985a). Many people believe that experiments conducted using animals are cruel and unnecessary (Festing & Wilkinson, 2007). Throughout the animal production industry, the most pressing issue is stress, however, this is a broad concept that accounts for a large variety of situations that cause animals discomfort and threaten their well-being to varying degrees. In light of this, stress is considered the appropriate measure of animal welfare (Moberg, 1985a).

The concept of stress is a perception, based on past experiences of the animal, the immediate physiological and psychological state of the animal as well as the current environmental condition the animal is subjected to and not only the duration and intensity of the stressor (Curtis, 1985). It is human nature to assume that what people perceive as stressful is what would also be stressful to animals, however this is not necessarily true. Some situations may appear to be uncomfortable to an animal but if there are no signs of stress, it is questionable if there is actually a stressor present (Moberg, 1985a). Physical stress often involves a panic-associated fleeing response that generally results in injury to the animals, in varying degrees (Ferguson & Warner, 2008).

In meat production systems, most of the ethical concerns involve breeding programs, husbandry practices and abattoir processes (Warris, 2010). Various factors need to be accounted for when slaughtering animals to ensure they experience the least amount of stress possible. These factors however, are species-specific and each animal's behaviour and history needs to be accounted for (Lewis *et al.*, 1997).

Ante-mortem stress is often as a response of excessive exercise or an alteration to the animal's immediate environment which could lead to fatigue or injury of the animal. Evidence suggests that ante-mortem stress could have negative effects on meat quality and should be monitored for the benefit of the consumer as well as to ensure ethical practices (Ferguson & Warner, 2008). However, game farming is different to the farming of other livestock species; in the former there is minimal handling of the animals, therefore it is not as easy to control pre-slaughter effects on meat quality (Hoffman & Laubscher, 2010).

2.5.1 Types of stressors

A stressor is explained as an external stimulus that has the potential to alter the physiological or psychological homeostasis of an animal. Stressors result in various stress responses, which stimulate the appropriate physiological and chemical responses in the animal in an attempt to maintain the initial state of homeostasis (Moberg, 1985a; Dantzer & Mormede, 1985).

The most common types of stress experienced by animals include thermal, environmental, disease and/or inflammation, social, and human induced/management stress (Klasing, 1985; Stull, 1997). When hunted, it is possible that the degree of ante-mortem stress experienced is amplified by prolonged chasing, wounding, and poor placement of the shot by an inexperienced marksman (Hoffman *et al.*, 2011).

There are many sources that represent a form of environmental stress, such as access to enough water, appropriate vegetation and nutrition, population density and the presence of predators, to name a few (Young, 1992). Environmental stress is largely inclusive of whether there is sufficient space within the camp for the animal to carry out all natural activities (Stull, 1997). For example, blue wildebeest are a migratory species and therefore require large areas of land to migrate. If a camp is too small and they cannot move around freely, then this could be considered a form of environmental stress (Furstenburg, 2013).

Pain is also a common source of stress however, the degree at which an individual experiences pain differs from other individuals according to each individual animal's pain threshold. There is no physical measure of pain as it is one of the most adapted sensory processes in the body, according to Kitchell and Johnson (1985). When animals are hunted, pain from poor shot placement could be a significant stressor. A prerequisite for humane culling procedures is immediate death, normally achieved with a headshot (Van Schalkwyk & Hoffman, 2010; Van Schalkwyk & Hoffman, 2016).

Management and human-induced stress encompasses all human activities conducted on the farm, including hunting activities and methodologies. There are many studies on various hunting methods and their effects on stress and meat quality, this will be discussed further later.

2.5.2 Acute vs. chronic stress

Stress can be classed as either acute or chronic, based on the length of exposure to the stressor, regardless of the source. Acute stress is a sudden, short-term stress, typically characterised by the "fight-or-flight" response, which is a response of animals to a threat where they will either flee or try and fight back to defend themselves (Stull, 1997). A general physiological response that the animal will experience includes a rapid secretion of certain hormones like cortisol, which will result in an elevated heart rate and vasoconstriction of certain blood vessels. If the threat remains present for longer than a minute, various other responses occur, which will include, amongst others, an elevated respiration rate, digestive upset and reduced feed intake (Moberg, 1985a; Ewbank, 1985; Stull, 1997; Ferguson & Warner, 2008).

Chronic stress is usually a long-term stress, in excess of 24 hours, with effects that could last long after the threat has been removed. Chronic stress is considered a pathological stage of stress, and once it has reached this stage, the welfare of an animal has been compromised (Moberg, 1985a). The consequences of chronic stress have been thoroughly studied, and the results show that chronic stress can result in hormonal imbalances. Such hormonal imbalances can have a negative effect on reproduction, growth, metabolism, and the ability of the animal to offer resistance to infection and disease (Moberg, 1985a; Golub & Gershwin, 1985; Roth, 1985; Moberg, 1985b; Stull, 1997). If chronic stress is not managed properly it could lead to an increased mortality percentage (Etim *et al.*, 2013). However, in most instances chronic stress should not be a factor during wildlife culling procedures if culling activities are managed and carried out properly.

Stress experienced by an animal is a complex concept, and in order to fully understand it, it is necessary to understand the physiology and biological processes that ultimately lead to the stress response.

2.5.3 Physiology of the stress response

Various hormones are involved in and contribute to the stress response, with the most important hormones being catecholamines and glucocorticoids (Withers, 1992). The catecholamines include hormones such as epinephrine and norepinephrine, which are released from the adrenal medulla and sympathetic nerves, respectively. These hormones contribute to the stimulation for the secretion of adrenocorticotropin from the anterior pituitary, and act on the liver to stimulate glycogenolysis and lipolysis (Axelrod & Reisine, 1984; Withers, 1992). Glucocorticoids on the other hand, are a class of steroid hormones that includes, amongst others, cortisol and cortisone released from the adrenal cortex (Withers, 1992).

2.5.4 The response of the central nervous system to stress

The nervous system is the initial detector of a stressor (Moberg, 1985a), therefore understanding the structure of the nervous system is vital in understanding the initiation of a stress response. The nervous system is made up of the central nervous system (CNS) and peripheral nervous system (PNS) (Withers, 1992; Sherwood, 2010).

The CNS is composed of the brain and spinal cord. It is responsible for determining whether a stressor is a significant threat. If significant enough, the CNS initiates a response which may be behavioural, autonomic or neuro-endocrine in nature, or that may comprise a combination of the three (Moberg, 1985a; Withers, 1992). Within the PNS is the autonomic nervous system (ANS) and somatic nervous system (Withers, 1992; Sherwood, 2010).

The ANS is very important during an acute stress response and comprises of the parasympathetic, sympathetic and enteric nervous systems (Moberg, 1985a; Withers, 1992; Sherwood, 2010). The parasympathetic nervous system, usually activated during chronic stress responses, uses acetylcholine as a neurotransmitter, while norepinephrine is the neurotransmitter

used by the sympathetic system. Both these neurotransmitters can act on the adrenal glands (Withers, 1992; Warris, 2010).

2.5.5 The adrenal glands

The adrenal glands are located on the anterior side of the kidneys, and each gland consists of the adrenal cortex, which is continuous with the adrenal medulla (Withers, 1992; Warris, 2010).

The adrenal cortex is the outer region of the gland, and is responsible for the synthesis of cortisol, and the adrenal medulla is the inner region of the gland and is responsible for the production of glucocorticoid hormones that result from the stress response (Axelrod & Reisine, 1984; Withers, 1992).

2.5.6 The stress response

When an animal is exposed to a stressor, a series of reactions and processes are activated within that animal to ensure that the animal can respond to the stressor in the most appropriate way, thus ensuring the animal's safety and survival (Axelrod & Reisine, 1984).

Initially the response is a behavioural response, where the animal will attempt to remove itself from the situation. If this is not possible, the sympatho-adrenal response will be initiated (Moberg, 1985a). This response is a stimulation of the sympathetic nervous system and the adrenal medulla to produce epinephrine and/or norepinephrine to initiate the "fight-or-flight" response (Axelrod & Reisine, 1984; Warris, 2010). The latter is a defence mechanism that results in various behavioural and physiological changes to help maintain a homeostatic state as well as assist in the survival of threatening situations (Etim *et al.*, 2013).

A typical response includes an increased heart rate and increased concentrations of glucose and free fatty acids available in the blood. This ensures that the blood is highly nutritious and oxygen-rich for optimal organ and muscle functioning, although it may also have a negative influence on meat quality parameters (Moberg, 1985a; Ewbank, 1985). Additionally, the spleen, a red blood cell reservoir, contracts to release more red blood cells into the circulation to increase the oxygen carrying capacity of the blood. Blood is also redirected to more essential organs such as the skeletal muscles and heart, while minor changes such as pupil dilation, decreased salivation, pilo-erection and increased sweat production, can occur (Warris, 2010).

The next stage of a stress response is an adaptive response coordinated by and referred to as the hypothalamic-pituitary-adrenal axis (HPA Axis). Once the PNS senses a stressor, the signals generated by the afferent nerves are collated in the hypothalamus in the brain (Hart, 2012). The hypothalamus secretes corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), and these hormones are transported in the hypophyseal portal blood to ultimately act on the anterior pituitary. The anterior pituitary, in reaction to CRH stimulation, produces endorphins to reduce pain perception. Both the CRH and AVP act on the corticotropic cells within the anterior pituitary and stimulate the transcription of pro-opiomelanocortin (POMC), a precursor protein, which in turn, results in the synthesis and release of adrenocorticotropin

hormone (ACTH) (Warris, 2010). Adrenocorticotropin hormone acts on the adrenal cortex, stimulating the latter to synthesise and secrete glucocorticoids (Axelrod & Reisine, 1984; Roth, 1985; Withers, 1992; Bornstein *et al.*, 2008; Hart, 2012).

In most mammals, a glucocorticoid that plays a key role in the stress response is cortisol. Cortisol is commonly known as the stress hormone, however, circulatory levels are not solely dependent on the amount of stress an animal is experiencing, and can be influenced by the species, type and source of stress, as well as post-stress disturbances (Hart, 2012). The duration of the stress also has an influence although, only for the first hour, thereafter blood cortisol concentrations stabilise, and only minor fluctuations can be observed. Cortisol secreted as a response to a chronic stressor contributes to the regulation of catecholamine biosynthetic enzymes, and the inhibition of adrenocorticotropin secretion (Hart, 2012; Gentsch *et al.*, 2018).

Cortisol is released in response to most stressors and in most mammalian species, therefore it provides an unbiased measurement for the possible quantification of stress. However, it is often misinterpreted due to HPA axis physiology which is often not taken into account. It is important to understand that cortisol secretion also varies with age, and depends on ultradian-, diurnal- and seasonal rhythms. It is vital that all of these considerations are accounted for before analysing and interpreting the relation of cortisol concentrations to an animal's stress experience (Hart, 2012).

In response to cortisol and the catecholamines acting on the liver, glycogen catabolism is stimulated and thus increases blood glucose levels, which ensures that there is energy available for the animal to respond by using the "fight-or-flight" response (Moberg, 1985a). In cases where glycogen reserves in the skeletal muscle and liver are depleted, the body can make use of gluconeogenesis, which allows for the use of non-carbohydrate precursors such as fatty acids or amino acids to synthesize glucose (Moberg, 1985a).

Under normal, non-stressed circumstances, glucocorticoids are secreted by the adrenal glands in rhythmic pulses of cortisol, followed by a period of inhibition. During this time of inhibition, it has been shown that animals do not respond to mild stressors, therefore it has been speculated that the magnitude of a stress response is dependent on the stage of the secretory cycle at the time of stress exposure (Lightman, 2008). In the same study, it was noted that when an animal is exposed to chronic stressors, there is a significant increase of frequency of the glucocorticoid pulsations flattening the circadian rhythm. However, there was also an increased time of inhibition leading to a condition known as stress hypo-responsiveness, where the animals are seemingly unresponsive to stress stimuli (Windle *et al.*, 1998; Lightman, 2008).

Figure 2.1 indicates the respective pathways that are involved in a stress response. The mineralocorticoid pathway is indirectly involved in the response by ensuring water balance, and within the context of the current study, only the glucocorticoid and androgen pathway will be considered (Bloem *et al.*, 2013; Pretorius *et al.*, 2016).

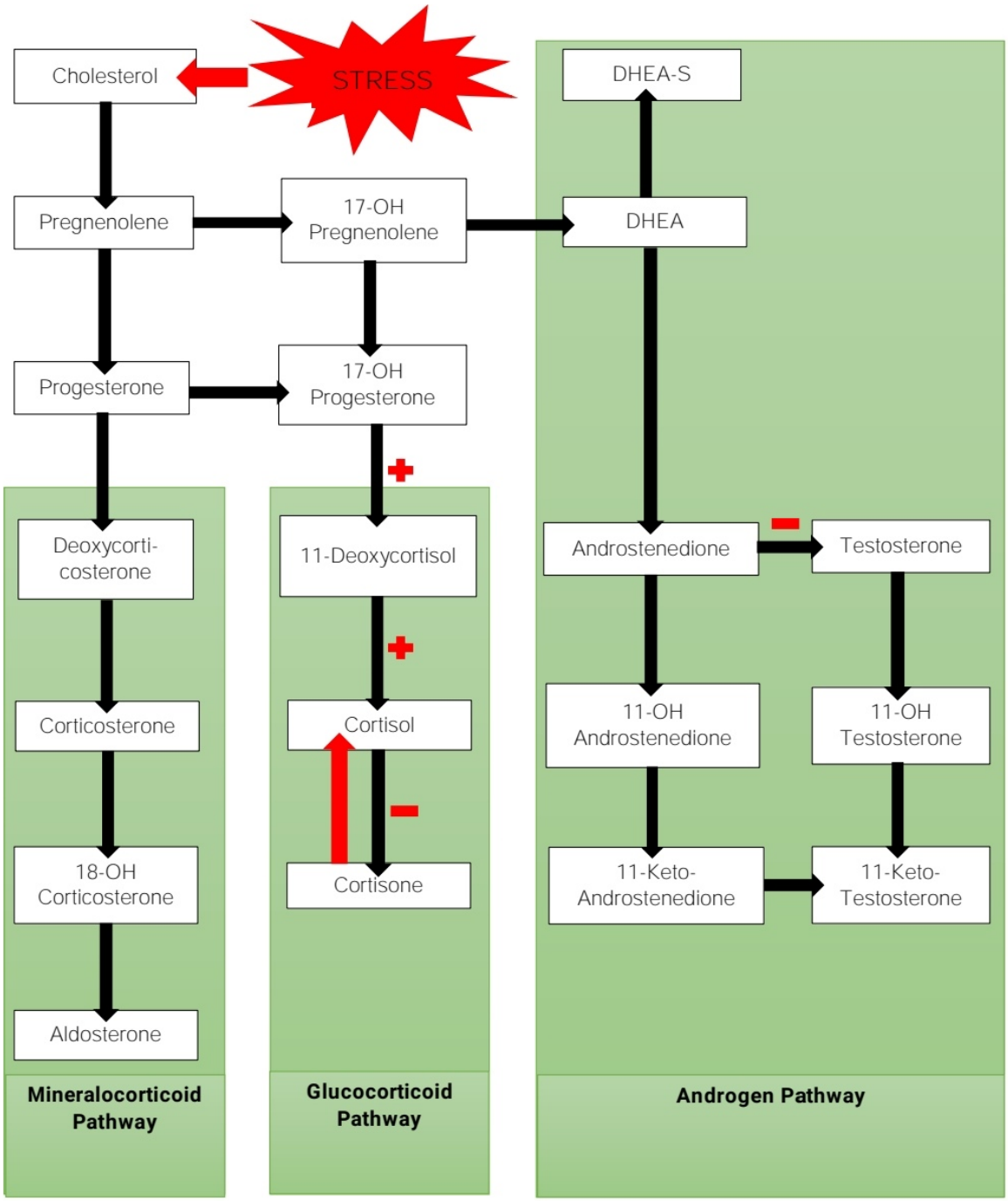

Figure 2.1 The mineralocorticoid, glucocorticoid and androgen pathways involved in a stress response in mammals, with the + and – and red arrows indicating the respective influences in the different pathways, adapted from Bloem *et al.* (2013) and Pretorius *et al.* (2016).

2.6 The influence of stress on meat quality

The negative effects of stress that animals are subjected to during a slaughter procedure and the effects thereof on meat quality has not been given the credit it deserves (Ferguson & Warner, 2008). There is uncertainty as to whether unexplained variances of meat quality can be attributed to variation in how an animal of a particular species responds to stress, however it has been shown that pre-slaughter stress has negative effects on the meat quality of numerous mammalian species (Braggins, 1996; Ferguson & Warner, 2008). Culling methods should, therefore be

efficient and aimed at minimizing the stress experienced by the animal prior to culling or slaughter (Veary, 1991; Hoffman & Laubscher, 2009a).

There are two main conditions that result from ante-mortem stress in animals. These are known as DFD (dark, firm and dry) and PSE (pale, soft and exudative) meat. In game animals, the occurrence of PSE meat is more apparent in warthogs and wild boar (Swanepoel *et al.*, 2016), in extreme chronic stress situations, a similar phenomenon known as white muscle capture myopathy could occur. Capture myopathy is a metabolic condition usually triggered by animals being chased, captured or restrained, and is characterised by metabolic acidosis resulting in the death of animals if not addressed properly to ensure recovery of the animal (Paterson, 2008). The occurrence of DFD can be ascribed to a depletion of glycogen reserves, as a result of the action of the hormones associated with the “fight-or-flight” response. Glycogen depletion in the liver and skeletal muscles results in the production of lactic acid in the anaerobic Krebs cycle, which in turn results in skeletal muscle pH increasing to be higher than 6.0 24 hours post-mortem (pH_U).

Muscles characterised by a high post-mortem pH 24 hours after slaughter have a strong water-binding capacity (WBC), resulting in more water being retained within the muscle as well as darker colour than muscles of a ‘normal’ pH (Shange *et al.*, 2019).

2.6.1 Skeletal muscle microstructure

To fully understand the mechanisms and reactions that occur causing the changes in meat quality, one needs to have an understanding of the microstructure of skeletal muscle. Various studies have reported on the complex microstructure of skeletal muscle (Offer *et al.*, 1989; Huff-Lonergan & Lonergan, 2005; Lawrie, 2006; Warris, 2010). Surrounding the entire muscle is a thick connective tissue called the epimysium. This holds bundles of fibres together, that are surrounded by another layer of connective tissue, the perimysium. Each fibre bundle is surrounded by another layer of connective tissue called the endomysium which encases the multinucleated muscle fibres; the cells that make up muscles (Offer *et al.*, 1989; Warris, 2010).

There are two types of muscle fibres that are of interest in this study, namely white muscle fibres and red muscle fibres. White muscle fibres are responsible for short-term activity, usually as a response to fear, while red muscle fibres are responsible for most of the endurance activity exhibited by animals (Warris, 2010). The muscle fibre types have an influence on the colour of the muscle as well as the metabolic activity of the muscle post-mortem and could therefore be influential to the ultimate quality of the meat (Lawrie, 2006).

Skeletal muscles have a striated appearance due to the microstructure of the myofibrils, which are the contractile elements of the muscles (Offer *et al.*, 1989; Warris, 2010). There are two main proteins in the microstructure of a fibre, namely, actin and myosin (Warris, 2010). Actin is a globular protein that forms the majority of the thin filaments within muscle cells, while myosin makes up the majority of the thick filaments and is able to interact with actin. The darker regions of the striations are the thick filaments and overlapping regions known as the A-band, whereas

the lighter regions are only the thin filaments known as the H-zone. Within the H-zone is a division called the Z-line, and the area between two Z-lines is referred to as a sarcomere (Huff-Lonergan & Lonergan, 2005; Warris, 2010). When a muscle contracts, there is an increased degree of interaction between the actin and myosin, whereby they form, break- and re-form cross-bridges. This results in an overlapping of the filaments, thus decreasing the size of the sarcomeres. The formation and breakage of cross-bridges includes the hydrolysis of ATP molecules by the myosin heads (Warris, 2010)

After an animal dies, the contraction of muscles continues. The ATP hydrolysis continues, and ATP levels are maintained by the anaerobic breakdown of glycogen into lactic acid. Once glycogen stores are depleted, ATP depletion soon follows, and the myosin heads cannot break their cross-bridges. This leads to the muscle becoming stiff and rigid, a condition or state referred to as *rigor mortis* (Warris, 2010).

2.6.2 pH

As mentioned before, ante-mortem stress can have an influence on the pH of meat. Changes in muscle pH have been shown to affect various sensory attributes of the meat (Braggins, 1996). More specifically, pH has an influence on water-holding capacity, tenderness and colour of the meat (Van Schalkwyk, 2004; Hoffman *et al.*, 2011). Factors that have been shown to influence post-mortem muscle pH in game species can include amongst others; calibre of weapon (Hoffman, 2000a), sex (Hoffman, 2000a; Van Schalkwyk, 2004; Hoffman & Laubscher, 2009b), age, season (van Schalkwyk, 2004) and rutting season (Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009b).

When an animal dies, there is a natural decline of muscle pH regardless of any temperature change and conditions due to anaerobic glycolysis taking place in the muscle (Farouk & Lovatt, 2000). However, temperature and pH have a complex relationship. Evidence has shown that changes in ambient temperature and decline of the post-mortem muscle temperature affect ultimate pH and more importantly, the rate of pH decline (Jeacocke, 1977; Farouk & Lovatt, 2000; Kritzinger *et al.*, 2003; Bekhit *et al.*, 2007; Hoffman *et al.*, 2011). Bekhit *et al*. (2007) showed that a lower ambient temperature results in a slower decline of pH. However, it was also found that muscles that entered *rigor mortis* at lower ambient temperatures had higher ultimate pH values than those that entered *rigor mortis* at higher ambient temperatures. Similarly, Hoffman *et al*. (2011) indicated that pH differences could also be attributed to the ambient temperature at the time of death, which may result in a difference in carcass chilling rates which in turn, influenced the activity of the enzymes in the Krebs cycle as well as the activity of the proteolytic enzymes which are partly responsible for the tenderness of the muscles.

Acute ante-mortem stress has deleterious effects on the rate of hydrogen ion production and therefore pH of meat, generally affecting the rate of pH decline post-mortem, and the pH_U values (Hoffman & Laubscher, 2009a). One of the major effects of acute ante-mortem stress is

metabolic acidosis. Hoffman (2000a) reported on meat samples obtained from a highly stressed animal that had the highest ultimate pH in their study. This finding was supported by a study on meat samples obtained from reindeer bulls, where it was shown that the animals least stressed and transported for five hours with no restraint, had a significantly lower ultimate pH than the other treatments (Wiklund *et al.*, 2001). The higher ultimate pH and faster rate of decline are potentially attributed to the effects of stress on the glycogen reserves of the muscles. However, it is also influenced by epinephrine (Braggins, 1996; Hoffman & Laubscher, 2009a). For example, an increase in cortisol results in the mobilisation of muscle glycogen stores causing increased concentrations of lactic acid, resulting in increased muscle acidification post-mortem (Hoffman & Laubscher, 2009a; Hoffman & Laubscher, 2010). Glycolysis rate is also affected by ambient temperature. Although an ambient temperature of between zero and ten degrees Celsius results in the rate of glycolysis being accelerated, the ultimate pH is not affected (Jeacocke, 1977; Farouk & Lovatt, 2000; Bekhit *et al*., 2007).

2.6.3 Water-holding capacity (WHC)

Water makes up approximately 75% of lean muscle (Offer *et al.,* 1989; Huff-Lonergan & Lonergan, 2005). The amount of water and its distribution determines how juicy or dry consumers perceive the meat to be, therefore, water-holding capacity is an important quality characteristic of meat (Offer *et al.,* 1989; Warris, 2010). The water-holding capacity of a muscle is determined by how much liquid is lost from the fresh meat as drip loss, and during cooking as cooking loss (Hoffman & Laubscher 2009a; Hoffman *et al.,* 2009).

Consumers perceive meat with a large amount of drip loss to be unattractive and indicative of a lower yield, however, it is impossible to prevent drip loss all together (Kritzinger *et al.*, 2003; Warris, 2010). Moisture can be lost via evaporation or as exudate from cut surfaces, with the degree of water-loss being regulated to a certain extent by controlling various factors including; amongst others, ante-mortem stress and ambient temperature (Offer *et al.*, 1989).

The effects of pH on water-holding capacity are well documented. Ultimately water-holding capacity is determined by the ultimate pH 24 hours post-mortem and the rate of pH decline such that a higher ultimate pH results in less water being lost via cooking loss (Bouton *et al.*, 1971; Purchas, 1990; Hoffman & Laubscher, 2009a; Hoffman & Laubscher, 2010). If the pH declines whilst the carcass temperature is still relatively high, this results in a decreased water-holding capacity, which can be ascribed to the denaturation of muscle proteins, which results in the muscle losing its ability to bind and retain water (Hoffman & Laubscher, 2009a). Therefore water-holding capacity is also influenced by ambient and rigor temperature as well as the rate of temperature decrease (Bekhit *et al.*, 2007; Warris, 2010; Hoffman *et al.,* 2011). Other factors such as carcass suspension method which is linked to the level of muscle contraction (Hutchison *et al.*, 2010), region of the country from which the animal is culled (Hoffman *et al.*, 2007), muscle type (Bouton *et al.*, 1971), muscle damage (Bouton *et al*., 1971; Warris, 2010) and sex of the animal

(Van Schalkwyk, 2004; Hoffman *et al.*, 2007) also influence water-binding capacity of muscle. Hoffman *et al.* (2009) provided evidence that suggested drip and cooking loss were not affected by species or age of the animal, while sex had an effect on drip loss only, contrasting to the findings by Hoffman & Laubscher (2009b) in another study thereby indicating the complexity of this relationship.

Evidence has shown that the temperature at which a carcass enters *rigor mortis* affects drip loss, although it was also noted that this could be species- and muscle-specific (Farouk & Swan, 1998; Bekhit *et al.*, 2007). Cooking loss is not affected by rigor temperature (Bekhit *et al.*, 2007), however, Farouk & Swan (1998) found that a lowered rigor temperature potentially caused a decreased drip loss and total moisture loss, which is indicative of an increased water-holding capacity.

Meat obtained from chronically stressed animals is characterised by an increased water-holding capacity due to a higher ultimate pH value (Hoffman & Laubscher, 2009a). This was confirmed by Hoffman (2000a), who reported that meat obtained from an extremely stressed animal was characterised by an increased water-holding capacity as evident in no drip loss reported, which resulted from a high ultimate pH recorded post-mortem. Therefore, an increased ultimate pH value is indicative of a higher water-holding capacity (Bouton *et al.*, 1971; Hoffman *et al.*, 2009).

Many factors can influence the loss of moisture from meat, however, there are limitations as to how much liquid can be lost (Farouk & Swan, 1998). Water-holding capacity was found to be at a minimum when the muscle had reached its isoelectric point, typically at a pH of 5.6 (Hoffman *et al.*, 2009). The isoelectric point of meat is important to note as the proteins have a minimal or zero charge and therefore a low water binding capacity. Also, at a lower pH, protein denaturation proceeds and the water binding capacity is reduced, thus leading to meat that is less juicy (Huff-Lonergan & Lonergan, 2005). Hoffman *et al.* (2007) also noted that the juiciness of meat is correlated with the tenderness of the meat.

2.6.4 Tenderness

Shear force is used to determine meat tenderness, and values are inversely correlated to sensory tenderness ratings. With higher shear force values, meat is expected to be tougher and less juicy (Hoffman *et al.*, 2007; Warris, 2010). As with water-holding capacity, there is also an association between the ultimate pH and shear force (Marsh *et al.*, 1981; Hoffman & Laubscher, 2009a; Hoffman *et al.*, 2011). Other influential factors include ambient temperatures (Hoffman & Laubscher, 2009a), cooling rates (Hoffman & Laubscher, 2009a; Hoffman *et al.*, 2011), amount of fat on the carcass (Hutchison *et al.*, 2010), carcass suspension method (Hutchison *et al.*, 2010), post-mortem storage (Bekhit *et al.*, 2007), ante-mortem stress (Veary, 1991), region of the country (Hoffman *et al.*, 2007) as well as the season of culling (Van Schalkwyk, 2004). Conflicting results have been found regarding the influence of sex, species and age on the tenderness of meat

samples (Van Schalkwyk, 2004; Hoffman *et al.*, 2007; Hoffman *et al.*, 2009; Hutchison *et al.*, 2010)

It has been reported that ultimate pH values have a major influence on tenderness ratings of meat. Hoffman *et al.* (2007) noted a significant toughening of meat as the ultimate pH increased from 5.4 to 5.8 however, beyond a pH of 6.0 the tenderness showed improvement again. Thus, the suggestion that the relationship between pH and tenderness in game species is not linear but rather curvilinear (Purchas, 1990). This study also noted correlations between water-holding capacity and tenderness, such that decreased cooking loss indicated increased tenderness ratings.

Cooling rate affects tenderness of meat. When the carcass is chilled too rapidly while the pH is still high, this could result in a condition known as cold shortening (Van Schalkwyk, 2004; Hoffman & Laubscher, 2009a). Cold shortening occurs when extremely strong cross-links are formed at the onset of *rigor mortis*, causing the sarcomeres to be extremely short and thereby decreasing the tenderness (Swatland, 2004; Hoffman & Laubscher, 2009a). In cooler months and at night, there is an increased cooling rate observed due to lower ambient temperatures. It is possible that this leads to tougher meat due to an increased occurrence of cold shortening (Van Schalkwyk, 2004).

If cold shortening does not occur, glycolysis is slowed by the cool temperatures, which in turn can result in improved tenderness (Marsh *et al.*, 1981) as seen in night-cropped impala (Kritzinger *et al.*, 2003). However, Hoffman and Laubscher (2009a; 2009b) found the opposite for kudu due to higher ultimate pH values of those cropped at night. Since, this has been confirmed by further studies (Hoffman & Laubscher, 2010), it was speculated that these conflicting results were as a result of differing levels of stress experienced by the animals.

2.6.5 Colour

Consumers are very particular when choosing meat and it has been shown that there is a high correlation between the colour of meat and their willingness to purchase it (Stevenson *et al.*, 1989; Warris, 2010).

Game meat is darker in colour than meat produced by livestock species (Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009a). In most cases this can be attributed to increased amounts of pigment called myoglobin, in the muscles (Field, 2004; Hoffman *et al.*, 2009) however, it could also be due to their more active lifestyle that stimulates the production of myoglobin (Hoffman, 2000a). Darker coloured meat has also been attributed to decreased muscle glycogen content and thus a higher ultimate pH (Purchas, 1990; Shange *et al.*, 2019). Various other factors have also shown to influence the colour of meat, including species (Von La Chevallerie, 1972; Warris, 2010), muscle type (Neethling *et al.*, 2018; Neethling *et al.*, 2019), ultimate pH 24 hours post-mortem (Hoffman & Laubscher, 2009a; Hoffman *et al.*, 2009; Hoffman & Laubscher, 2010), sex (Hoffman *et al.*, 2009; Hutchison *et al.*, 2010), ante-mortem stress (Hoffman, 2000a; Hoffman &

Laubscher, 2010), age (Hoffman *et al.*, 2009), carcass cooling rate, as well as rigor temperature (Swatland, 2004; Farouk & Swan, 1998) and season (Van Schalkwyk, 2004). Von La Chevallerie (1972) also found that smaller game species produced meat of darker colour than larger game species, with the latter being paler in colour.

Meat with a higher ultimate pH is associated with a darker colour (Hoffman *et al.*, 2009; Shange *et al.*, 2019). At higher pH, myoglobin oxidation is reduced, therefore this darker colour can be attributed to a reduced degree of myoglobin oxidation (Neethling *et al.*, 2019) and greater degree of light absorption into the meat. Thus explaining the reason a highly stressed individual, mentioned in previous sections, produced very dark meat (Hoffman, 2000a).

The colour and pH relationship could also be due to water-holding capacity. As the ultimate pH decreases, so the amount of exudate lost may increase due to a loosened protein structure from proteolysis. Often myoglobin is lost through this exudate therefore making the meat appear lighter (Farouk & Swan, 1998).

2.6.6 Hunting associated stress and the impact thereof on ungulate meat quality

Based on the information provided above, it is evident that animals experience stress and can retain a memory thereof (McLean, 2003). Stress has an impact on meat quality and thus could influence the consumer's perception of the product (Wassenaar *et al.*, 2019). This study will focus on the determination of the influence of ante-mortem stress on meat quality characteristics of impala and blue wildebeest. These species were specifically chosen because of their desirable meat quality characteristics, and because they differ vastly in their responses to culling techniques for example; when culled from a helicopter, impala tend to seek cover under shrubs and bushes whist blue wildebeest will flee in all directions until the threat has passed.

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Chapter 3
The influence of culling method on ante-mortem stress in impala (*Aepyceros melampus*) and blue wildebeest (*Connochaetes taurinus*)

3.1 Abstract

This study evaluated the effects of helicopter-, day- and night-culling on the ante-mortem stress experienced by sub-adult impala rams (*Aepyceros melampus*) and mature blue wildebeest cows (*Connochaetes taurinus*). Blood was collected immediately after death, and analysed for testosterone, cortisone and cortisol. Cortisol and cortisone blood concentrations were related to the expected diurnal rhythm of the hormones. Cortisol and testosterone concentrations recorded for impala was not influenced by culling method, i.e. helicopter-, day- or night-culling. Serum cortisone levels were higher in the night-culled animals than the helicopter- and day-culled impala. When related to the expected diurnal rhythm, the serum cortisol levels for night-culled impala was also higher than expected. Serum cortisol concentrations of the night-culled animals exhibited a similar pattern as the serum cortisone levels, i.e. measured cortisol levels were higher than expected for the night-culled impala. The blue wildebeest serum concentration of testosterone was not influenced by culling method. Serum cortisone concentrations were higher for helicopter- and day-culled blue wildebeest than those culled at night. Blood cortisol concentrations only differed between helicopter- and night-culled animals. The results of this study indicate that the culling method influenced the ante-mortem stress experienced by both impala and blue wildebeest, with impala experiencing more stress during the night-cull, and the blue wildebeest more stress during the helicopter-culling method.

3.2 Introduction

Game farms occupy approximately 15.6 % of South Africa's total surface area (Munzhedzi, 2018), and due to the hardiness of most game species, game farming allows for the utilization of environments that are considered unsuitable for livestock farming (Jooste, 1983; SADAFF, 2010).

According to population growth analysis, by the year 2050, the world human population is expected to be 9.7 billion (United Nations, 2019). With a growing population comes a growing demand for ethically produced sustainable meat sources. Game meat presents a healthy alternative to conventional animal protein sources, although some consumers have their concerns regarding the quality and acceptability of meat originating from game species (Kristensen *et al.*,

2014; Van Schalkwyk & Hoffman, 2016). Most concerns can be attributed to the darker colour of game meat, as this is commonly associated with meat that is dry and with an unfavourable sensory profile (Viljoen *et al.*, 2002; Hoffman *et al.*, 2005).

Impala (*Aepyceros melampus*) and blue wildebeest (*Connochaetes taurinus*) are of the most commonly hunted ungulate species in South Africa. These species are abundant and produce meat that is high in protein and low in fat (Hoffman, 2000b; Hoffman *et al.*, 2011; Selier *et al*., 2016). Studies on the influence of culling method on the quality of meat obtained from impala (Lewis *et al.*, 1997; Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009a), kudu (Hoffman & Laubscher, 2009b), springbok (Veary, 1991) and gemsbok (Hoffman & Laubscher, 2010) made inferences to the potential impact of ante-mortem stress on meat quality, but only a few of these studies have measured cortisol concentrations (Hoffman & Laubscher, 2009b; Hoffman & Laubscher, 2010; Cockram *et al.*, 2011). In most of these studies, however, stress was not directly measured or quantified using cortisol as an indicator hormone.

Stress is a multi-dimensional concept and can be classified as for example, thermal stress, environmental stress, disease-related stress and management-induced stress (Stull, 1997). Stress is also multi-factorial, with one or more stressors that can contribute to the exhibition of a stress response in animals. The “fight-or-flight” response is a general term used to illustrate how an animal reacts to a stressor to maintain homeostasis within physiological homeostasis values (Warris, 2010; Etim *et al.*, 2013). The extent and degree of response to stress is highly dependent on the species, exposure to stress and the type of stressor, with acute and chronic stress manifesting differently in animals (Klasing, 1985; Etim *et al.*, 2013; Gentsch *et al.*, 2018).

It is known that stress has an effect on various hormone concentrations. Cortisol, known as the stress hormone, is the most commonly measured hormone, with values then related to the degree of stress experienced by an animal (Hart, 2012). Cortisol has been demonstrated to influence serum testosterone concentrations. It has been found that stress and increased cortisol concentrations could potentially be related to a decrease of testosterone levels (Bubenik & Reyes-Toledo, 1994; Retana-Ma´rquez *et al.*, 2003; Deviche *et al.*, 2010; Davies *et al.*, 2016). In addition to cortisol it is important to consider cortisone, which is the inactive form of biologically active cortisol. The ratio of cortisol:cortisone is important to keep in mind, for during a stressful situation, adrenocorticotropic hormone (ACTH) secreted by the anterior pituitary, results in a shift of the ratio favouring cortisol production (Vogeser *et al.*, 2001). When an animal’s response to stress is considered, two biological pathways play a fundamental role in the stress response. The first pathway is known as the glucocorticoid pathway that involves the production of glucocorticoid hormones such as cortisol and cortisone, and the second pathway is the androgenic pathway that involves the production of reproduction-related hormones such as testosterone (see Figure 2.1).

Culling of ungulates such as impala and blue wildebeest can be carried out by either culling from a vehicle or culling from a helicopter. When using the former approach, culling can be carried out during daytime or night-time hours. Each of the culling approaches can directly or

indirectly influence meat quality through the potential effect on the degree of ante-mortem stress that the animal experience.

Typically impala and blue wildebeest are skittish animals and when frequently exposed to hunting, the impala become even more nervous and will flee further distances when chased whereas the blue wildebeest will maintain a further distance from potential threats (Schenkel, 1966; Apps, 2014; Matson *et al.*, 2005; Furstenburg, 2013). When chased during culling procedures, blue wildebeest in a herd will typically run in single file, while impala herds will disperse to create confusion for the predator in an effort to escape being caught by the predator (Schenkel, 1966; Apps, 2014; La Grange, 2006; Furstenburg, 2013). Increased activity levels of animals prior to death, as typically seen when hunted from helicopters, can result in a depletion of glycogen levels in the muscles and liver of the animals, which in turn will be reflected in the ultimate pH of the meat, meat colour and tenderness (Shange *et al.*, 2019).

There is little information available regarding the potential of plasma cortisol, cortisone and testosterone concentrations to be used as an indicator of ante-mortem stress in impala and blue wildebeest.

The aim of this trial was therefore to evaluate the influence of three culling methods i.e. culling from a vehicle during the day- or night-time, and culling from a helicopter, on the ante-mortem stress experienced by impala and blue wildebeest, respectively. The serum testosterone, cortisol and cortisone levels will be related to the expected diurnal rhythm of each respective hormone to determine whether the respective hormones can be used individually or in combination to quantify the degree of ante-mortem stress in ungulates during culling operations.

3.3 Materials and methods

Ethical approval for the trail was obtained from the Stellenbosch University Animal Care and Use Ethical Committee (ACU-2018-6598).

3.3.1 Experimental location and animals

The culling of the animals was carried out over a period of 21 days on the ROMACO Ranch located approximately 220km north of Johannesburg, on the outskirts of Modimolle and Mookgophong in the Limpopo Province of South Africa. During the trial, ambient temperatures ranged from 23 to 32°C during the day, and between 9 and 14°C during the night. The ranch is characterized by vegetation that varies from dense bush to grasslands, as well as some mountainous areas that are home to many plains and exotic game species.

A total of 24 sub-adult impala rams and 24 mature blue wildebeest cows were culled for the purpose of this study; eight animals per treatment and per species. The impala were all between 18 months or 30 months old, and the blue wildebeest were all older than 8 years and at the end of their reproductive prime. Age determination for the impala was done by correlating ear tags with birth records while the blue wildebeest were bought in as older cows and had been on

the farm for five years. The animals were maintained under free-range conditions in large camps (>150 ha). Although wild, the animals were somewhat habituated to the presence of the hunting vehicle, as the same type of vehicle would enter and drive through each camp regularly while conducting routine fence and stock inspections. The helicopter was used regularly for conducting stock counts, which was not as frequent as the routine fence and stock inspections.

3.3.2 Experimental treatments

The influence of three different culling methods, i.e. day-, night- and helicopter-culling, on the ante-mortem stress of the animals was investigated.

- *Day-culling* involved shooting animals from the back of a vehicle that was equipped with a bench to ensure stability for the marksman. Culling operations for this method were conducted from 11:00 to 18:00 by a team of four to eight people. Once the animals had been sighted by the vehicle driver and marksman, the animals were approached cautiously. Once the animals had settled, i.e. in cases where they were skittish of the vehicle, the marksman took aim, and to standardize, aimed for the head/high neck. Once the shot was taken and the animal went down, there was a short waiting period for the rest of the herd to move off before the culled animal was approached.
- *Night-culling* was conducted using the same approach protocol as for the day-culling. The night-culling was carried out between the hours of 21:00 and 02:00, and a spotlight (one million candlelight strength) was used to identify the animals to be shot.
- *Helicopter-culling* involved the use of a small, four-seater helicopter, crewed by only the pilot and a single marksman. Helicopter-culling was carried out between the hours of 06:00 and 12:00. The marksman inside the helicopter would spot the animals and then fly to within four to six meters above the target animal, which was running at full speed, before taking a headshot. Vehicles on the ground were crewed with four to twelve people and used as pick-up vehicles only. The helicopter team would communicate the position of the animal, allowing the vehicle team to locate the animal as soon as possible after the culled animal went down.

3.3.3 Blood sampling and hormone analysis

Blood samples were collected according to the method used by Laubscher (2009), within five minutes after the animal went down from the jugular vein. Samples were collected using lithium heparin vacutainer tubes and gently everted to allow for blood to mix with the tube content. Samples were then placed on ice and transported to the field laboratory, where the samples were kept at 5 - 7 °C for up to 12 hours after collection before processing. The cooled blood was centrifuged at 4000 rpm for five minutes and allowed to settle for two minutes. The serum was then transferred to cryotubes and stored at -20 °C until further processing.

Standards were made up in duplicate for each of the following steroid hormones:

- Testosterone,
- Androstenedione,

- 11β-hydroxyandrostenedione (11OHA4),
- Cortisol,
- 11-ketotestosterone (11KT),
- Cortisone,
- Progesterone,
- 11-ketoandrostenedione (11KA4),
- Dehydroepiandrosterone (DHEA),
- 11β-hydroxytestosterone (11OHT), and
- 17α-hydroxyprogesterone (17OHP4)

The following methodology was used to prepare the standards and samples for analysis:

- Serum (400 μL) was transferred into a labelled test tube, (In the case of the standards for each of the abovementioned hormones, 400 μL of a known single-hormone sample, was used instead of the serum.)
- Internal solution (100 μL), consisting of testosterone, androstenedione, 11OHA4, cortisol, 11KT and progesterone was added to the test tube.
- Then 300 μL of distilled water was added to the test tube.
- Samples were then pipetted into tabled solid-phase liquid extraction columns (SLE columns), and a vacuum was applied for five seconds in order to load the sample into the column.
- The sample was left under gravity for a further five minutes
- Methyl tert-butyl ether (MTBE) (2 mL) was added to the sample which was left under gravity for another 5 minutes before the vacuum was applied for 15 seconds.
- Another 2 ml of MTBE was added, and the sample was left under gravity for 5 minutes and the vacuum was applied for 15 seconds.
- Samples were then dried in a sample concentrator for approximately 30 minutes,
- Then re-suspended in 80 μL of 50 % methanol
- The re-suspended sample was then transferred into new glass cryovials
- Samples were stored at -20 °C for further steroid hormone analysis.

Prior to the analysis of the samples using Ultra High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS/MS) was carried out using an Acquity UPLC HSS column (2.1 mm x 50 mm, 1.8 µm particle size) coupled to an Acquity UPLC system (Waters Corporation, Milford, USA). Two blank samples were run through the UHPLC-MS/MS whcih was followed by one of each of the standard samples being run in order to calibrate the machine and two more blank samples. All the impala samples were analysed by the UHPLC-MS/MS in duplicate before

recalibrating the device. Thereafter the blue wildebeest samples were also analysed in duplicate. From the standard sample analysis, an accuracy of approximately 89 % was achieved.

3.3.4 Data Recorded

3.3.4.1 Time category of hunt

Culling operations were conducted throughout the day and due to the natural diurnal nature of the hormones analysed, it was important that the exact time of death was noted. The animals were then divided into three different time category groups, i.e. 'morning', 'afternoon' and 'night', according to the time of death. The time categories were applied to both impala and blue wildebeest.

- 'Morning': Time of day: 05:00 – 12:00
  The animals allocated to this group were mainly helicopter-culled impala and blue wildebeest as well as two blue wildebeest cows that were culled during a day-cull.
- 'Afternoon': Time of day: 12:00 – 18:00
  The animals that had been allocated to this group were only impala and blue wildebeest that had been culled during the day-cull.
- 'Night': Time of day: 18:00 – 05:00
  The animals that were allocated to this group included only night-culled impala and blue wildebeest.

3.3.4.2 Firearms used

Research suggests that the rifle calibre used during hunting activities has an effect on the meat quality as well as the well-being of the remaining herd members (Hoffman, 2000a). All rifles were fitted with sound suppressors and marksmen were all experienced and licensed. The culled animals were thus classed according to which firearm was used, i.e. 'Heavy calibre', 'Light calibre' and 'Shotgun'.

- 'Heavy calibre': Includes the .306 and .308 rifles.
  The .306 rifle was used on the impala while the .308 rifle was used on the blue wildebeest, during the day- and night-culling operations.
- 'Light calibre': Includes a .243 rifle.
  This rifle was used when culling some of the impala during the day- and night-culls.
- 'Shotgun': Loaded with SSG lead pellets.
  This firearm was only used during helicopter-culls where rifles were not suitable.

3.3.4.3 Exposure to hunting activity

Prior to this trial, the camps from which the animals used in this study were culled, had not been hunted in a year. Throughout the trial, the camps were revisited, often multiple times, which could have caused animals to become more aware or skittish as the trial progressed. Animals would have also been able to identify the hunting vehicle as a threat before any culling had taken place, therefore it was a necessary to account for exposure to prior hunting activities in order to determine the influence of repeated hunting on the blood hormone levels and meat quality of the animals culled. Based on the above, culled animals were divided into three groups i.e. 'No prior', 'Some' and 'Recurrent'.

- 'No prior': No previous exposure
  These animals were last exposed to any hunting or culling activity for a minimum of one year.
- 'Some': Recent exposure
  These animals were in camps with herds that, for the purpose of this 21-day trial, had been chased by the hunting vehicle or helicopter, a maximum of twice, for culling operations, regardless of whether an animal was actually shot, with at least a 24-hour break between exposures.
- 'Recurrent': Recent and repeated exposure
  The animals included in this category were culled from camps where herds had been chased more than three times throughout the 21 days as well as herds that had been chased more than once within 24 hours.

3.3.4.4 Chase time

When hunting, animals were chased for various lengths of time prior to being shot, the exact time period of the chase was recorded in order to determine whether this had an influence on the concentrations of stress-associated hormones in the blood. Chase time started from the moment the animal became aware of the vehicle or helicopter and ended when the animal dropped after being shot. The times were then grouped and labelled as 'short', 'medium' or 'long'.

- 'Short': < 5 minutes
- 'Medium': 5 – 20 minutes
- 'Long': > 20 minutes

3.3.4.5 Number of shots required

In the event of a shot being off target resulting in injury, but not immediate death of the animal, it is expected that the animal will become extremely stressed and thus needs to be recorded. Number of shots was classified as 'single' or 'multiple' depending on the number of shots fired

that hit the targeted animal. All animals that were injured due to off-target shots were immediately pursued and killed humanely.

- 'Single': One shot
  This group was allocated to animals that were shot and killed using one bullet as well as the animals that were culled from a herd that was fired at but completely missed all animals.
- 'Multiple': Injured animals
  The animals in this group were shot and injured by a bullet that was off target. These animals were not rendered unconscious immediately and required a second shot to humanely kill the animals. The time it took render the animal completely unconscious was recorded.

3.3.5 Statistical analysis

Statistical analysis was performed using XLStat version 19.2. The following main effects of prior exposure to culling activity, chase time, time of day category, number of shots, as well as culling methods and their interactions, where appropriate, was explored by means of descriptive statistics. A multi-factor ANOVA was used to determine differences between the various treatment groups of the main effects, and Fishers' least significant difference *post-hoc* tests were calculated where an alpha of 5 % was considered significant.

3.4 Results

For the purpose of this study, only the hormone concentrations of testosterone, cortisone and cortisol steroid hormones will be considered. Even though the hormone assay produced data on androstenedione, 11OHA4, 11KT, progesterone, 11KA4, DHEA, 11OHT or 17OHP4 levels, these hormones are not involved in the ante-mortem stress response and were thus, not considered in this study although are presented in Addendum I.

Many of the main effects such as exposure, chase time and number of shots could not be considered in the statistical analysis due to insufficient sample size and the unbalanced nature of the data and are thus reported as descriptive statistics.

3.4.1 The influence of culling method on serum testosterone, cortisone and cortisol concentrations of impala at death

The influence of culling method on impala blood serum concentrations of testosterone, cortisone and cortisol is presented in Table 3.1. Culling method did not affect the serum testosterone and cortisol concentrations at death ($p > 0.05$; Table 3.1). Culling method did have a significant effect on serum cortisone concentrations, with the highest levels reported for night-culled animals when compared to the helicopter- and day-culled animals, respectively (Night: 13.71 nmol/L vs. Helicopter: 6.44 nmol/L and Day: 7.31 nmol/L; $p \leq 0.05$). Large variances were noted for serum

testosterone, cortisone and cortisol concentrations of animals culled at night, when compared to the other culling methods.

Table 3.1 The serum steroid hormone concentrations (mean ± SE) of impala, as influenced by helicopter-culling, day-culling or night-culling, and measured in blood samples collected immediately after death.

Hormones	Helicopter	Day	Night	Range	p-value	CV
Testosterone (nmol/L)	2.69±0.78	1.86±0.82	1.87±0.47	0.34 – 5.20	0.63	0.80
Cortisone (nmol/L)	6.44[b]±0.83	7.31[b]±1.88	13.71[a]±2.71	2.58 – 23.73	0.03	0.68
Cortisol (nmol/L)	75.72±19.32	68.47±26.61	110.88±25.35	14.72 – 223.22	0.42	0.79

[ab] Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

3.4.2 The influence of culling time and method on serum testosterone, cortisone and cortisol concentrations of blue wildebeest at death

The influence of culling time and culling method on the serum hormone concentrations of blue wildebeest are presented in Table 3.2 and Table 3.3, respectively.

Culling time did not influence serum testosterone levels ($p = 0.63$; Table 3.2). Culling time did influence the serum cortisone and cortisol concentrations. Cortisone concentrations were higher for animals culled in the morning, compared to the serum levels of the animals culled at night (24.15 nmol/L vs. 5.45 nmol/L; $p \leq 0.05$). There was a tendency for the serum cortisone and cortisol levels to be higher in animals culled during the afternoon, when compared to the animals culled during the night ($p = 0.06$; Table 3.2). Morning-culled animals had an average serum cortisol level of 353.68 nmol/L, which was higher than that reported for the night-culled animals (134.84 nmol/L; $p = 0.02$).

Table 3.2 The serum steroid hormone concentrations (mean ± SE) of blue wildebeest, as influenced by morning-culling, afternoon-culling or night-culling, and measured in blood samples collected immediately after death.

Hormones	Morning	Afternoon	Night	Range	p-value	CV
Testosterone (nmol/L)	0.30±0.04	0.37±0.03	0.35±0.02	0.10-0.48	0.49	0.29
Cortisone (nmol/L)	24.15[a]±4.21	19.38[ab]±6.26	5.45[b]±1.00	2.20-49.65	0.03	0.79
Cortisol (nmol/L)	353.68[a]±50.98	288.69[ab]±101.03	134.84[b]±40.79	17.87-606.49	0.05	0.73

[ab] Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

The analysis of culling method as a main effect on steroid hormone concentrations, indicated that serum testosterone levels were not influenced by either helicopter-, day- or night-culling (Table 3.3). The serum cortisone level for the animals culled by helicopter and day methods, respectively, were higher than the levels reported for the animals culled at night (27.88 nmol/L and 16.85 nmol/L vs. 5.45 nmol/L; $p = 0.01$). Similar differences were observed for the cortisol concentrations.

Table 3.3 The serum steroid hormone concentrations (mean ± SE) of blue wildebeest, as influenced by helicopter-culling, day-culling or night-culling, and measured in blood samples collected immediately after death.

Hormones	Helicopter	Day	Night	Range	p-value	CV
Testosterone (nmol/L)	0.34±0.01	0.31±0.01	0.35±0.01	0.10-0.48	0.76	0.29
Cortisone (nmol/L)	27.88[a]±4.30	16.85[ab]±4.88	5.45[b]±1.00	2.20-49.65	0.01	0.79
Cortisol (nmol/L)	387.45[a]±75.17	271.16[ab]±57.55	134.84[b]±40.79	17.87-606.49	0.02	0.73

[ab] Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

3.5 Discussion

3.5.1 The influence of culling method on serum testosterone, cortisone and cortisol concentrations of impala at death

3.5.1.1 Background

Culling method determined the type of reaction that the impala had. The expected reaction of impala to helicopter-culling procedures was for them to flee at full pace, seeking cover under

shrubs and bushes (Schenkle, 1966), this reaction is also associated with states of panic. The impala in the current investigation reacted as expected. When they hid under the bushes the draft from the helicopter blades was used to coax them out from under cover, an action that caused bruising (not quantified) in some of the carcasses.

During the daytime, animals are able to see further distances, therefore becoming aware of activity a lot sooner than they would at night. This means that the impala were aware of the hunting vehicles as soon as they entered the camp. Lewis *et al.* (1997) found that animals were able to associate specific vehicles with danger and hunting activities, providing an explanation why many of the impala culled in this study began to flee the moment they became aware of the hunting vehicles. It is possible that this behaviour increased with animals that had been recently hunted, however, due to a too small number of animals used in this study, this could not be analysed although there was a tendency for this phenomenon to occur. The learned fear of the vehicles was expected to result in an increased concentration of stress hormone. This was not the case in the current investigation, these results will be discussed later.

Spotlights were used during the night-cull by the hunting party to scan the area for animals. Due to decreased visibility at night, the sudden exposure to a bright light resulted in the impala standing upright with their heads held high, ideal for a headshot. Veary (1991) noted that during a night-cull, impala herds stood still when in the spotlight and would not move unless the dominant male moved first. Night-culling was therefore expected to be a quick and stress-free activity resulting in impala with minimal increased stress hormone concentrations although, this was not the case and will be discussed further.

Impala are diurnal animals, and hormone concentrations will typically follow a diurnal rhythm. Glucocorticoid hormone concentrations in the blood typically increase until maximum concentration in the blood is reached at approximately 09:00 according to typical diurnal rhythms. Thereafter the blood glucocorticoid levels gradually decrease throughout the remainder of the afternoon and night, until approximately 03:00, at this time the blood glucocorticoid concentrations typically start increasing again (Bubenik *et al.*, 1983; Mormede *et al.*, 2007; Ramamoorthy & Cidlowski, 2016; Keselman *et al.*, 2017). Modification of typical behaviour is however common in ungulate species that become aware of a recurrent threat (Makin *et al.*, 2017). Meyer *et al.* (2008a) found that impala can be habituated to human interaction therefore if interactions are positive, the impala should remain calmer however if interactions are negative in nature, the animals are likely to become more anxious in the presence of humans. The same study also found that regardless of the animals being habituated, they all showed a stress response when restrained.

To establish to what extent the serum hormone levels of testosterone, cortisone and cortisol measured in this study can be related to potential ante-mortem stress, the measured hormone levels were compared to a typical diurnal variation of these hormones in impala. Hormone values reported in previous studies (Hattingh, 1988; Meyer *et al.*, 2008a; Meyer *et al.*,

2008b; Mezzullo *et al.*, 2017) were used to depict a typical diurnal pattern for testosterone (Figure 3.1), cortisone (Figure 3.2), and cortisol (Figure 3.3). It is important to note that the expected trend lines were not indicative of exact values and are for the purpose of establishing a typical trend for each hormone during a 24-hour cycle only.

3.5.1.2 Testosterone

There are indications that stress can affect sexual behaviour and reproduction, although how this happens exactly is unclear (Tilbrook *et al.*, 2002). When a stressful situation arises, the body experiences a state of emergency and diverts specific resources required as well as energy to focus on maintaining homeostasis. This commonly takes place during the “fight-or-flight” reflex and is also known as the *Resource Reallocation Hypothesis* (Braude *et al.*, 1999).

In a stress situation, the body needs to produce a large concentration of glucocorticoids. Glucocorticoids are made from cholesterol which is used in the androgenic pathway to produce testosterone and other androgens (Bloem *et al.*, 2013; Pretorius *et al.*, 2016). Therefore, in response to stress, it is expected that the body would decrease the rate of the androgenic pathway and increase the rate of the glucocorticoid pathway. The results from the current investigation, however, do not support this expectation. No significant influence of culling method was observed for the serum testosterone levels, which suggests that the stress experienced by the impala was acute in nature, and not long enough in duration to result in a shift from the androgenic to the glucocorticoid pathway (Tsuchiya & Horii, 1995). Some studies have suggested that serum testosterone levels could influence the degree of stress experienced, rather than stress influencing serum testosterone concentrations (Cizauskas *et al.*, 2015; Kutlikova *et al.*, 2019).

The impala blood samples showed a few animals that did not conform to the expected diurnal trends, however the deviations are possibly due to some of the impala rams being at a more advanced stage of puberty, with some of the rams approaching sexual maturity slightly earlier than others (as indicated by their thicker necks, etc. – data not shown).

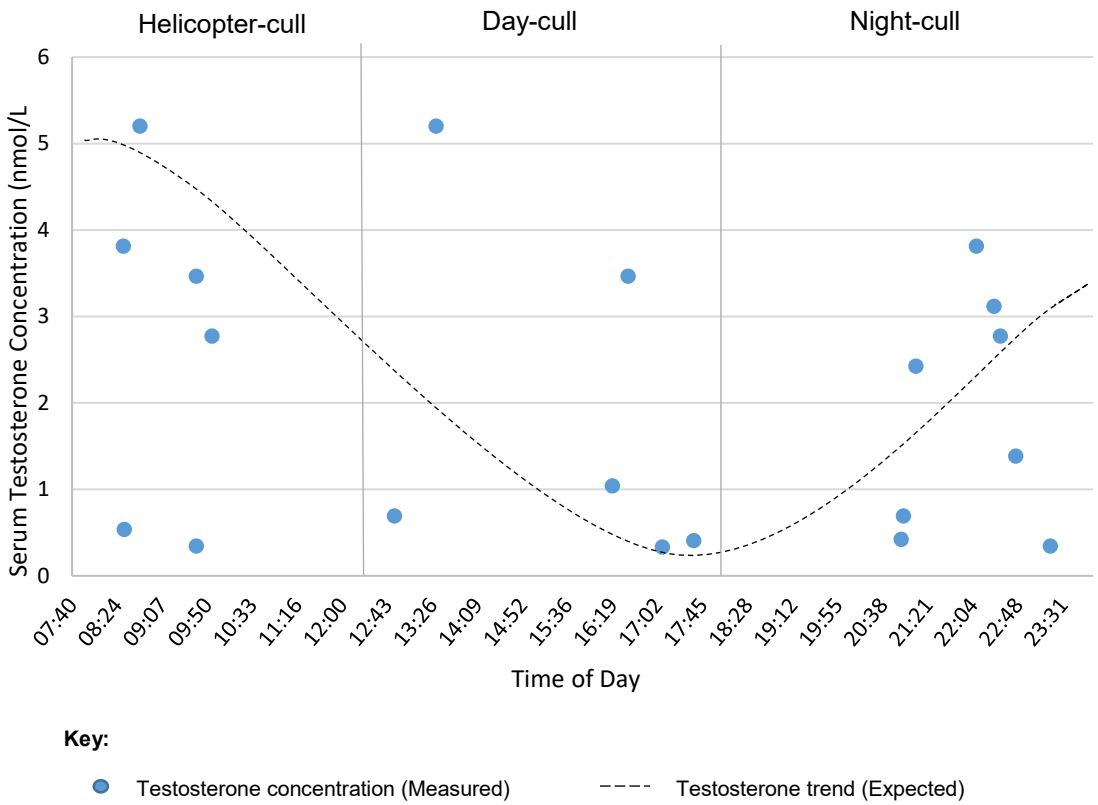

Figure 3.1 Measured serum testosterone concentrations of various impala culled using different methods (i.e. helicopter-, day-, or night-culling) at different times of the day with indication of treatment groups. Deviation of measured testosterone concentrations from the expected testosterone concentration trend line indicates treatment influences.

3.5.1.3 Cortisone

Glucocorticoids are a general term for stress hormones; these hormones follow a diurnal rhythm whereby the hormones reach a peak, at approximately 09:00, and progressively decline throughout the day and the night, where-after levels start to increase again from approximately 03:00 onwards (Mormede *et al.*, 2007; Ramamoorthy & Cidlowski, 2016; Keselman *et al.*, 2017).

Cortisone is the inactive form of cortisol and is released in anticipation of stress (Vogeser *et al.*, 2001; Ramamoorthy & Cidlowski, 2016). Serum cortisone concentrations in this study showed that night-culled animals (13.71 nmol/L) had higher concentrations than those measured for animals culled using the helicopter (6.44 nmol/L) or day methods (7.31 nmol/L).

It can be seen in Figure 3.2 that the measured serum cortisone levels, comply with the expected diurnal rhythm until the early evening (17:00). However, after 17:00 the serum cortisone concentrations are expected to be extremely low, instead, a dramatic increase in serum cortisone levels can be seen for the animals culled during the night, resulting in serum cortisone levels exceeding that of the animals culled around 09:00 when the expected serum cortisone concentrations were at a maximum. Thus, it is possible that the night-culled animals were exposed to more and/or showed a greater response to ante-mortem stress.

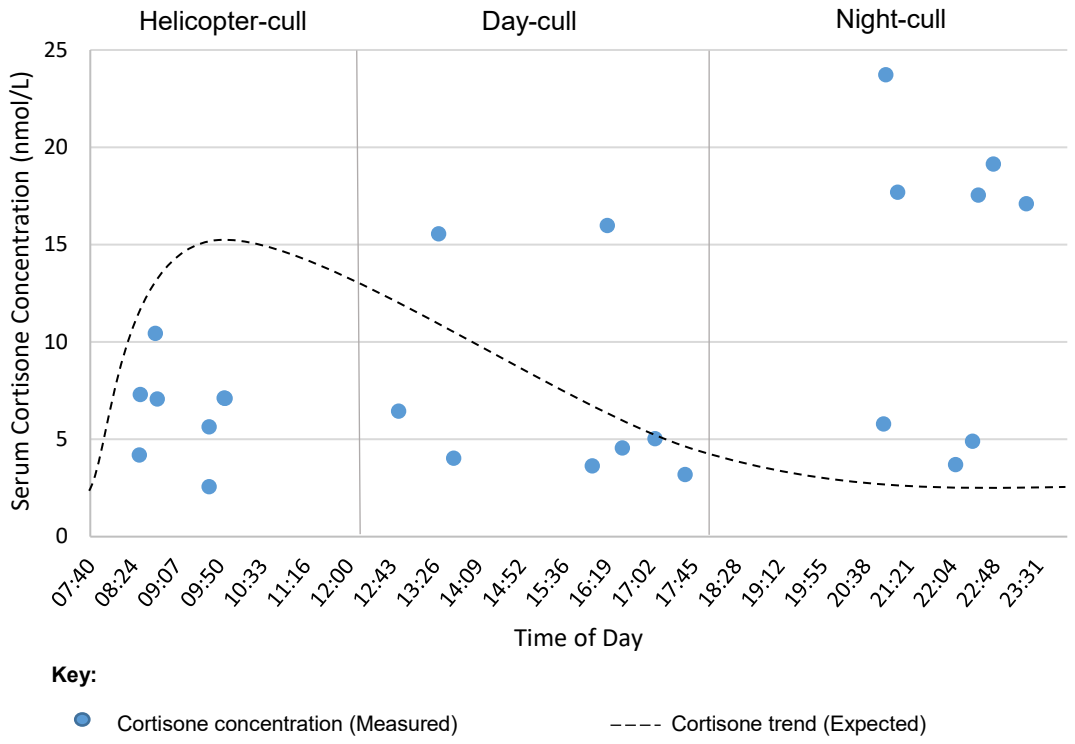

Figure 3.2 Measured serum cortisone concentrations of 24 impala culled at different times of the day. Expected cortisone concentrations are as follows: an increase in the morning, reaching a maximum at approximately 09:00 followed by a gradual decrease into the afternoon and throughout the night until 03:00 where serum concentrations of cortisone start to increase again. The expected trend line is not indicative of exact expected concentrations but rather an indication of fluctuations that would exist under stress-free circumstances

3.5.1.4 Cortisol

Cortisol, the stress hormone, is secreted into circulation in response to stress and plays a vital role in the "fight-or-flight" response. The statistical analysis of serum cortisol data revealed no significant differences between serum cortisol levels in the blood samples of helicopter-, day- and night-culled animals. Hattingh (1988) found similar serum concentrations of cortisol as was observed for the night-culled impala of this study. In the study of Hattingh (1988), impala that experienced acute stress, and had an average serum cortisol level of 114 nmol/L that ranged between 71 to 148 nmol/L.

The measured serum cortisol concentrations over 24 hours are displayed in Figure 3.3 with the expected trend of the diurnal rhythm, as was done for serum testosterone and cortisone concentrations. The serum cortisol concentrations (Figure 3.3) showed similar trends to that of the serum cortisone concentrations (Figure 3.2). The measured serum cortisol concentrations conform to expected trends from 08:30 until 17:07, thereafter serum cortisol concentrations are expected to remain low until 03:00 (Mormede *et al.*, 2007; Ramamoorthy & Cidlowski, 2016; Keselman *et al.*, 2017), instead the measured cortisol concentrations increased to levels exceeding those of the animals culled in the morning when the highest serum cortisol concentrations were expected. These results indicate that the night-culled animals experienced more ante-mortem stress than the helicopter- and day-culled animals. These results support that

of the serum cortisone concentrations of the current investigation thus suggesting the night-cull is the most stressful, this could possibly be due to the fact that the impala were regularly exposed to both vehicles and helicopter activities during routine management practises carried out during the day, whilst there was hardly any vehicle movements during night-time. Contradictory results were found by Laubscher (2009), where it was found that blood samples from day-culled impala had higher levels of serum cortisol and were thus, more stressed than night-culled animals. However it should be noted that in Laubscher's study the impala were not habituated to any hunting vehicles and thus it would be expected that the observation of any vehicle during the day-time would initiate the 'fight-or-flight' response more strongly than in this present study.

Vogeser *et al.* (2001) postulated that ACTH that is secreted by the anterior pituitary during a stress response stimulates the conversion of cortisone to its biologically active form, cortisol therefore a shift of the cortisone:cortisol ratio. These ratios could provide more insight into the stress response for similar studies however, further research is required to determine the baseline ratio values.

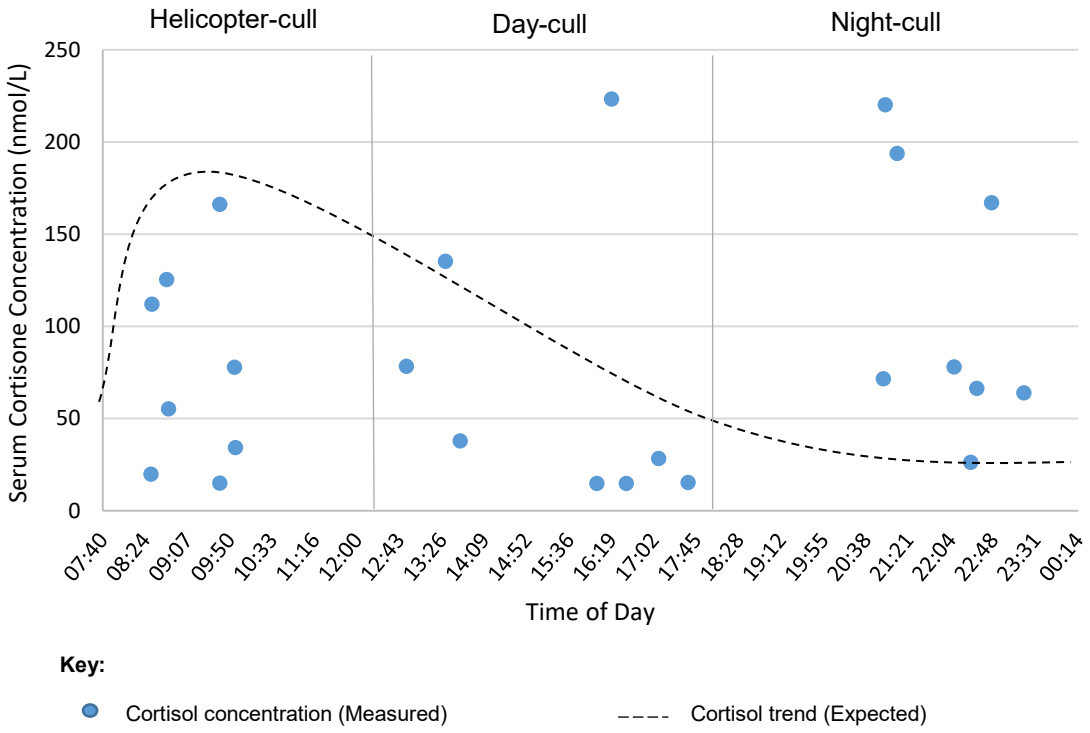

Figure 3.3 Measured serum cortisol concentrations of 24 impala culled at different times of the day. Expected cortisol concentrations are as follows: an increase in the morning, reaching a maximum at approximately 09:00 followed by a gradual decrease into the afternoon and throughout the night until 03:00 where serum concentrations of cortisol start to increase again. The expected trend line is not indicative of exact expected concentrations but rather an indication of fluctuations that would exist under stress-free circumstances

3.5.1.5 Outliers

All measured serum steroid hormone concentrations in this study had large variances therefore to interpret the results further, it is necessary to discuss individual animals. As noted previously,

the day-culled animals had an average serum cortisol concentration of 68.47 nmol/L. One ram culled at 17:37 had a higher serum cortisol level (223.22 nmol/L) than the average of the treatment group, and an increase of serum cortisol level at this time of the day was not expected. This ram was chased for a long time, approximately 34 minutes before being shot, potentially indicating that chase time is an important factor that needs to be considered in follow-up studies. It is worth nothing that this ram appears to also be an outlier for serum cortisone concentrations.

Of the night-culled animals, three rams had serum cortisol levels (220.23, 193.81 and 167.11 nmol/L respectively) that exceeded the average serum level for the group (110.88 nmol/L). A possible explanation for this result was that these three animals had previously been exposed to culling activity prior to death. Although prior exposure to culling could not be considered in the statistical analysis of the data in this study, Veary (1991) suggested that a minimum of two days should be allowed between hunting activities to reduce stress exposure. The helicopter-culled animals also showed large variances in serum cortisone and cortisol concentrations however, the measured serum levels did not exceed the expected serum concentrations for cortisone and cortisol therefore the variation was assumed to be due to population variation.

During this study, only one ram was injured during the culling procedures. This animal was culled at 16:04, and thus was allocated to the day-cull group. Contrary to expectation, this animal was not an outlier, and serum cortisol (14.73 nmol/L) and cortisone (3.65 nmol/L) levels recorded were within the expected pattern fluctuations. It is worth noting that the serum testosterone concentration for this animal was too low to be detected (< 0.001 nmol/L). Another three rams, two culled from the helicopter and one day-culled, were removed from the data set when analysing testosterone concentrations due to serum levels being too low to be detected. No interactions were observed between cortisol, cortisone and testosterone concentrations.

3.5.1.6 Influence on remaining herd members

The focus of this study has been on the influence of culling method on the ante-mortem stress of culled animals; however, it is important to consider the possible effects of these methods on the animals that remain within the herd. An advantage of the night-culling was that when an animal was shot and went down, the remaining herd members were sometimes unaware of what was happening, which allowed another animal within the same herd to be targeted. This suggests that stress levels for these animals should be relatively low, however, this was not reflected in the serum glucocorticoid levels reported for the animals that were shot after the initial animal went down. An observation made during the trial suggested that the herds that were culled from more regularly, with ‘recurrent’ exposure, were more skittish and more difficult to hunt. This proposes that the animals remaining in the herd had identified the culling vehicle as a threat. Since culling activity in the camps used for this study are limited to once per year, no long-term effects on the remaining herd members are expected.

3.5.2 The influence of culling time and method on serum testosterone, cortisone and cortisol concentrations of blue wildebeest at death

3.5.2.1 Background

All blue wildebeest in this study were mature cows in breeding herds. Therefore, caution was taken to avoid culling cows that had young calves and were still in their breeding prime. As the animals in this study were mature cows and the impala were rams of a younger age, the species could not be compared with each other.

The use of helicopters in the wildlife industry can be considered invasive, and thus can potentially raise some welfare concerns (Solberg *et al.*, 2006). When being followed and targeted by a helicopter, the blue wildebeest exhibited a flight response that was characterized by dramatic changes in the direction they were running in. In this study it was observed that once a blue wildebeest was shot, the animal rolled over a short distance, which could have resulted in some bruising of the carcass. De Haast (2016) noted that the use of helicopters during capture procedures only affected the animals being caught, and not the remainder of the herd. In De Haast's study, it was postulated that the stress experienced by the captured animals could be attributed to the restraint of the animal, rather than the helicopter presence. If restraint is the primary cause of stress and not the presence of a helicopter, it is possible that, since the animals are not restrained during culling, the helicopter-culling method could be less stressful than the day- and night-culling.

When hunting from a vehicle during the day, visibility is naturally far better than at night. Blue wildebeest are known to maintain a distance of 40 to 150 m from potential danger (Furstenburg, 2013). During the current investigation, the hunting vehicles struggled to approach within 100 m of the animals, therefore the use of an experienced marksman was important to ensure shot accuracy. Once startled, the animals fled in single file. Furstenburg (2013) reported that they can run up to 2 km before coming to a rest again.

Night-culling procedures still have the benefit of darkness, which limits the animal's vision. The reaction of blue wildebeest to a spotlight differs from that of impala. Impala's eyes are located towards the front of their head whereas the blue wildebeest's eyes are on the side of their head. When the spotlight is directed at the blue wildebeest, instead of being temporarily blinded, they appear to be able to see with the other eye not exposed to the spotlight and can therefore flee. During the night-culls, it proved to be beneficial to utilize multiple vehicles and herd the animals into a corner of the camp in order to cull efficiently, although this 'herding' could have increased the stress that the animals were exposed to. Also, blue wildebeest have been known to minimize night-time activity to avoid predators therefore the sudden need to be active during this time is possibly stressful to the animals (Selebatso *et al.*, 2016).

Similar figures have been designed for the blue wildebeest serum testosterone, cortisone and cortisol concentrations as was compiled for the impala (Figure 3.4, Figure 3.5 and Figure 3.6

respectively). The expected trends for cortisone and cortisol were based on bovine studies (Chantaraprateep & Thibier, 1978; Petty *et al.*, 1994) and testosterone trends were based on human male studies (Rose *et al.*, 1972) as none could be sourced in the scientific literature for this species, therefore values are approximations.

3.5.2.2 Testosterone

No significant differences were observed between the averages of serum testosterone concentrations of the helicopter-, day-, and night-culling methods. However, the reported serum testosterone levels did not conform to the expected diurnal pattern (Figure 3.4). At 11:09 the serum testosterone levels were expected to decrease until 20:00 however, the measured testosterone levels remained constant during this time. It is possible that the diurnal pattern of serum testosterone levels differs for female animals when compared to male animals although this requires further research.

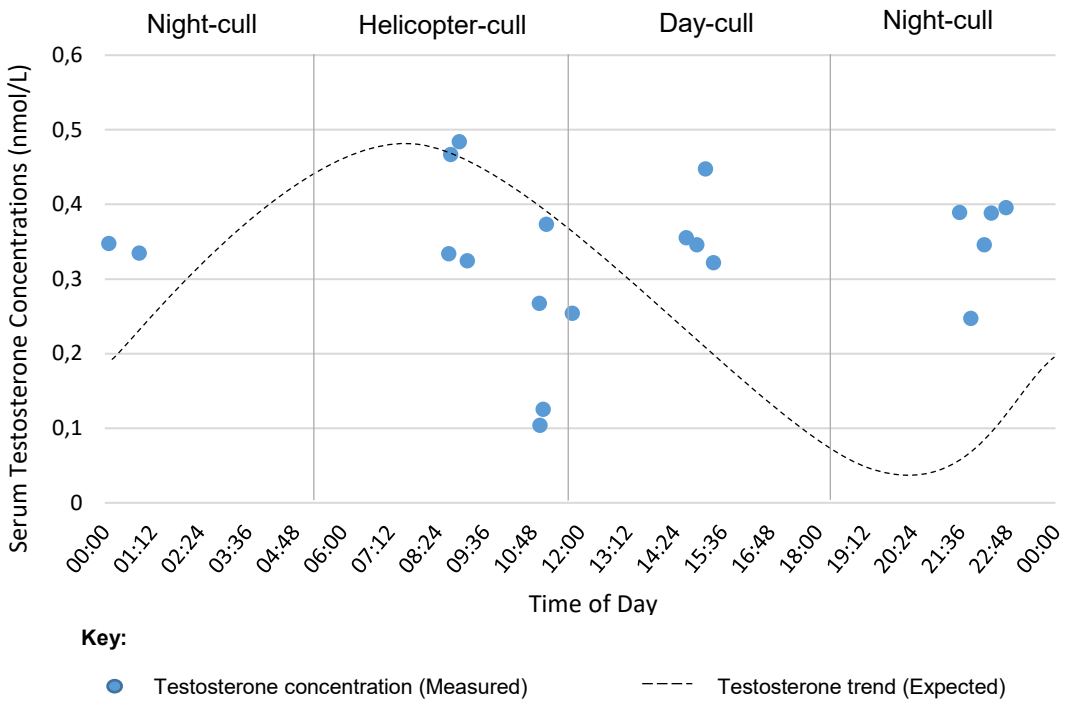

Figure 3.4 Measured serum testosterone concentrations of 24 mature blue wildebeest cows culled at different times of the day. Expected testosterone concentrations are as follows: an increase in the morning, reaching a maximum at approximately 08:00 followed by a gradual decrease into the afternoon reaching a minimum at approximately 20:00. The expected trend line is based on data retrieved from human males and not indicative of exact expected concentrations but rather an indication of fluctuations that would exist under stress-free circumstances

3.5.2.3 Cortisone

Results of the statistical analysis are reported in Table 3.2 and Table 3.3. The results from the statistical analysis revealed differences in the serum cortisone levels between the helicopter-, day- and night-culled animals however, these differences were expected as they were similar to that of the typical diurnal rhythm of cortisone. The blood samples from animals culled between

00:52 and 15:10 exhibited serum cortisone levels that complied with the expected trends of the diurnal rhythm for serum cortisone levels, however, measured cortisone levels from blood samples of a few outlier animals culled from 15:00 until late into the night deviate from the expectations (Figure 3.5). These outlier animals will be discussed further.

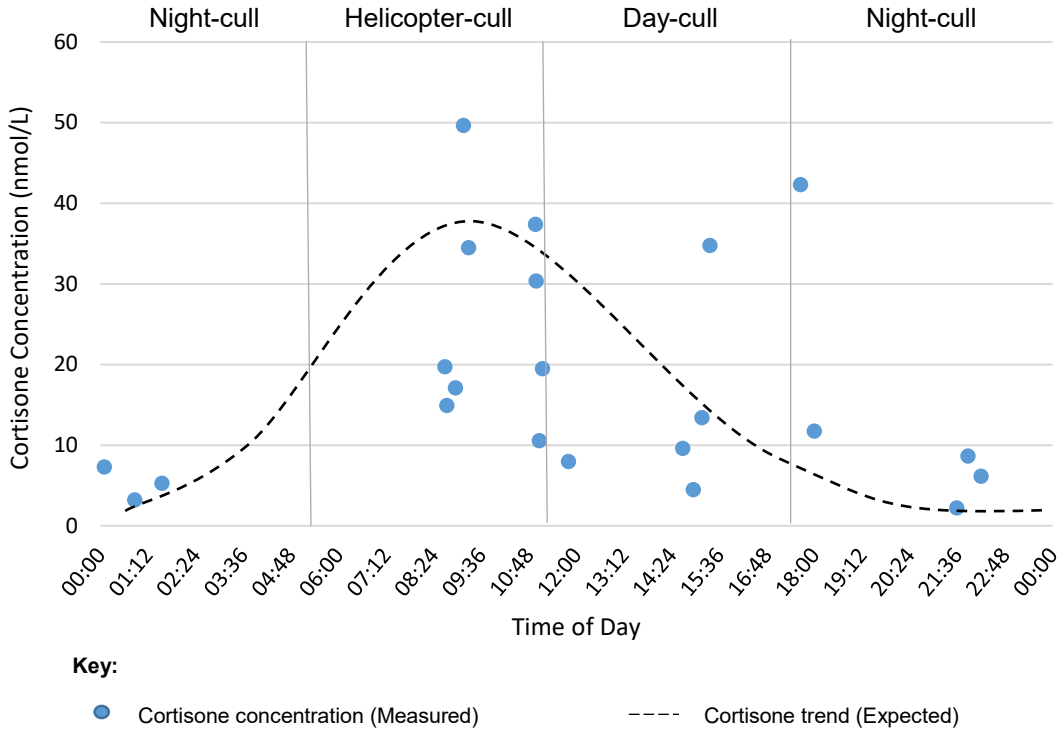

Figure 3.5 Measured serum cortisone concentrations of 24 blue wildebeest culled at different times of the day. Expected cortisone concentrations are as follows: an increase in the morning, reaching a maximum at approximately 09:00 followed by a gradual decrease into the afternoon and throughout the night until 03:00 where serum concentrations of cortisone start to increase again. The expected trend line is not indicative of exact expected concentrations but rather an indication of fluctuations that would exist under stress-free circumstances

3.5.2.4 Cortisol

The serum cortisol concentrations also differed between the helicopter-, day- and night-culled animals (Table 3.2 and Table 3.3), similar to the results recorded for serum cortisone levels. Figure 3.6 displays the measured serum cortisol concentrations against time of day and compares the measured serum cortisol concentrations with the serum levels expected throughout the day. The measured cortisol complied with expected trends from 00:06 until 14:57, thereafter, serum cortisol concentrations were expected to remain relatively stable but measured levels exhibited outlier animals, similar to that observed for serum cortisone concentrations.

From the statistical analysis performed, there was evidence suggesting that there were higher serum concentrations of stress hormones of the animals culled from a helicopter than the other treatment groups. This could indicate that blue wildebeest experience more ante-mortem stress when culled from a helicopter than during the day- and night-culling procedures. Behavioural observations also suggested that the helicopter chased animals were more startled as they were seen to flee at faster speeds than when exposed to the hunting vehicles. However, as the serum

concentrations of hormones measured fluctuated as depicted by the diurnal pattern, there remains the possibility that the differences between treatment groups were due to the diurnal rhythm and not stress-induced.

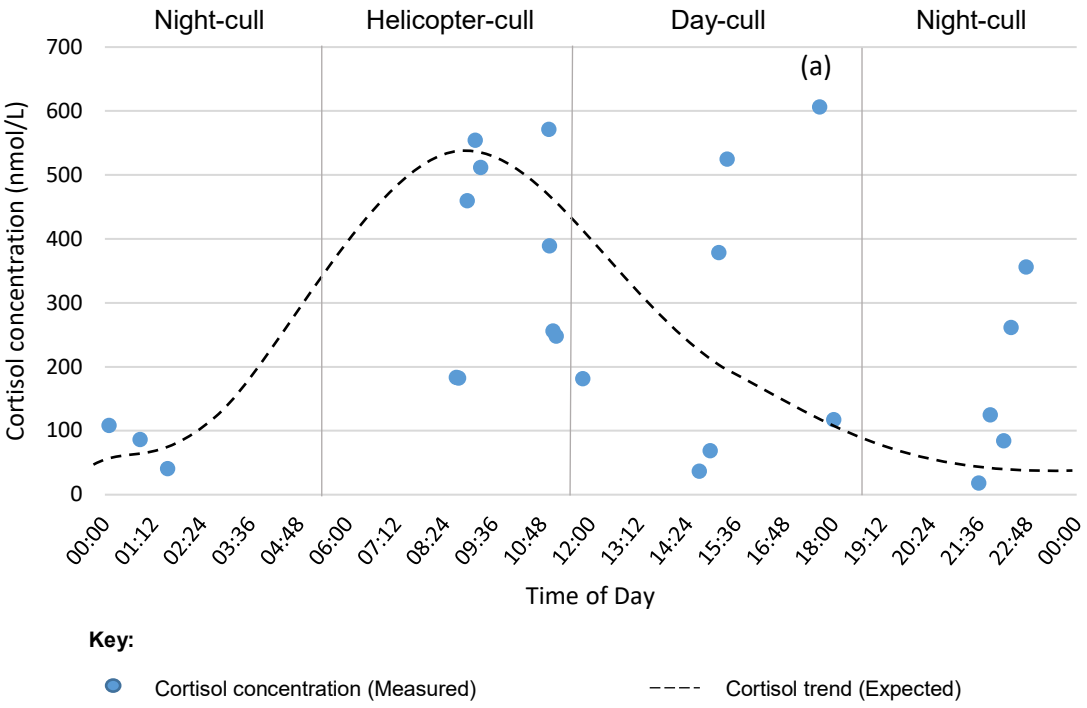

Figure 3.6 Measured serum cortisol concentrations of 24 blue wildebeest culled at different times of the day. Expected cortisol concentrations are as follows: an increase in the morning, reaching a maximum at approximately 09:00 followed by a gradual decrease into the afternoon and throughout the night until 03:00 where serum concentrations of cortisol start to increase again. The expected trend line is not indicative of exact expected concentrations but rather an indication of fluctuations that would exist under stress-free circumstances

3.5.2.5 Outliers

At 17:39 in the afternoon, serum cortisone and cortisol levels should be stabilizing at a relatively low concentration (Mormede *et al.*, 2007; Ramamoorthy & Cidlowski, 2016; Keselman *et al.*, 2017), however, from the blood samples in this study, serum cortisone and cortisol were at their highest concentration, illustrated by the notation (a) in Figure 3.6. This is possibly indicative of extreme stress experienced at this time. The highest point of the peak represented by notation (a), was representative of an animal that had been chased for over 45 minutes in total. After 17 minutes of being chased, this animal (a) was shot and wounded and the marksman was only able to fire the next shot after another 28 minutes of chasing the animal. Animals that are chased for longer periods of time are expected to have higher serum cortisol concentrations, which is also expected for animals that are injured during culling procedures (Gentsch *et al.*, 2018).

After 22:12 another deviation from the expected serum cortisone and cortisol pattern can be observed in Figure 3.5 and Figure 3.6, respectively. This deviation was representative of two animals that were exposed to recurrent culling activity, chased for longer than 34 minutes and

culled immediately after each other. During the chasing of these two animals, two vehicles were used to herd the animals into a corner of the camp and the animals had been running excessively. It is possible that the intense chase using multiple vehicles resulted in more ante-mortem stress.

3.5.2.6 Influence on remaining herd members

Animals that remain in the herds were chased each time an animal from that herd was targeted therefore subjecting them to repetitive chasing which could lead to increased amounts of stress experienced by the animals. This could lead to welfare concerns, especially on farms where culling and hunting activity is a regular occurrence, animals exposed to excessive chasing on a regular basis, could develop metabolic acidosis and thereafter conditions that occur with capture myopathy (Montané *et al.*, 2002).

It has been shown that some species have limitations regarding memory (McLean, 2003), thus making it plausible that the remaining herd members, if in a herd that is not regularly exposed to hunting and/or culling activity, could have minimal memory regarding the events. McLean's study, however, was based on equine memory, blue wildebeest could have a different result if exposed to a similar trial therefore further research would be required.

3.6 Conclusion

The results for impala in this study indicated that the animals culled at night have higher serum levels of cortisone and cortisol than what was expected, when compared to the expected diurnal pattern. This potentially indicates that the night-culling procedure was more stressful than the helicopter- and day- culling protocols.

The results from the blue wildebeest investigation indicated that the blood samples collected from the helicopter-culled cows had higher levels of serum cortisone and cortisol than the day- and night-culled animals. The differences reported between the serum cortisone and cortisol levels of the helicopter-, day- and night-culled animals were similar to the expected diurnal pattern of these hormones. Since the exact baseline serum cortisone and cortisol concentrations were unknown, it is possible that the helicopter-culled blue wildebeest experienced more ante-mortem stress than the animals culled using the day- and night-culling methods

Further research on the diurnal rhythms of these steroid hormones within these species is required in order to obtain verifiable baseline values for these species. This will be beneficial in assessing to what extent hormone concentrations reported in this study vary from baseline data for these hormones in impala and blue wildebeest.

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Chapter 4

The influence of culling-associated stress on impala (*Aepyceros melampus*) meat quality

4.1 Abstract

The purpose of this study was to investigate the influence of culling methods and shot placement (i.e. head/neck vs. body) on the quality of meat obtained from sub-adult impala rams (*Aepyceros melampus*; N = 66). The rams were culled using three different methods, i.e. a daytime, night-time and helicopter culling method. Meat samples were characterised in terms of physical and proximate meat parameters, with pH profiles recorded at set time intervals over the first 24 hours post-mortem. Meat quality characteristics were related to serum testosterone, cortisone and cortisol levels. The initial pH (pH_I) values indicated that helicopter-culling had an influence on the meat quality obtained from these animals, although the exact reasoning for this is unknown. There was a tendency for the ultimate pH (pH_U) of meat obtained from night-culled impala to be lowest (5.63±0.04), when compared to that recorded for the daytime (5.73±0.03) and helicopter-culled animals (5.68±0.02). Meat obtained from the night-culled animals had the highest water-holding capacity, and a tendency to result in the most tender meat. The aforementioned results were not statistically significant although this could have been attributed to the relatively small sample size. The colour profile of the meat obtained from the night-culled animals was not negatively influenced by the culling method and was within an acceptable range for consumer expectations of game meat. Based on the findings from this study, it is postulated that even though the night-culled animals experienced acute ante-mortem stress, meat quality parameters were not affected negatively. The helicopter- and day-culling methods did not exhibit signs of ante-mortem stress. The shot placement had an influence on initial pH and water-holding capacity of the meat, and it was concluded that the meat obtained from head/neck shot impala was of a better quality in terms of these characteristics, than body shot impala.

4.2 Introduction

According to a presentation by the Department of Environmental Affairs in 2018, South Africa is the third most biodiverse country in the world (Munzhedzi, 2018). Part of this biodiversity includes game farms that occupy 15.3 % of the country's total surface area, with the industry generating a revenue of approximately 10.1 billion ZAR per year. Impala (*Aepyceros melampus*), the second most hunted game species after springbok (*Antidorcas marsupialis*), represented 24.15 % of all the animals hunted for meat and trophy purposes in 2018 (Munzhedzi, 2018). According to Hoffman *et al.* (2009), impala carcasses are characterised by a higher dressing percentage when

compared to other plains species such as kudu and the meat from impala is considered a healthy source of protein.

Culling and hunting are often a much-debated topic when the welfare of the animals being targeted is considered. Traditionally hunting takes place during the day and usually on foot; alternatively hunting can also be conducted from a vehicle or helicopter (Hoffman & Laubscher, 2009a). When hunting is conducted on a larger scale with a degree of selection, usually for the purpose of population control, it is classified as culling. Culling can be conducted during the day or night, with each method eliciting different responses from animals, which, in most cases, include a stress response. The type and degree of stress response will depend on the species and the animal's prior exposure to specific hunting scenarios (Grandin & Shivley, 2015). Studies have compared the meat quality of impala hunted during both the day and at night (Kritzinger *et al.*, 2003, Hoffman & Laubscher, 2009a), as well as the influence of being hunted during the night only (Lewis *et al.*, 1997; Hoffman, 2000a). According to these studies, night-culling is considered as an appropriate method to use when culling impala. However, where literature is considered, limited information is available regarding the influence of helicopter-culling on the ante-mortem stress experienced by impala.

There is also limited research on the potential influence of shot placement on stress experienced by and the resulting welfare of impala. Traditionally, body shots are more common (Hoffman & Laubscher, 2009a) but these bullet wounds result in more wastage of meat, when compared to neck or head shots. Body shots are considered less humane, due to the increased suffering from the delayed collapse of the animal (Von La Chevallerie & Van Zyl, 1971). Neck shots are considered a good alternative to head shots, as there is minimal wastage of meat and the animals are usually rendered unconscious immediately.

Stress experienced by animals during a hunt could have a detrimental effect on the quality of the meat. Often stress leads to dark, firm and dry (DFD) meat (Von La Chevallerie & Van Zyl, 1971; Van Schalkwyk, 2013), which is perceived as being of a lower quality by consumers (Viljoen *et al.*, 2002; Wassenaar *et al.*, 2019). It is of vital importance thus to minimize the stress associated with culling procedures, to potentially produce the best quality product. By ensuring the welfare of the animals during a hunt, consumers would also be more willing to buy game meat products that have been harvested in a humane as possible manner, which in turn will have a positive effect on the game meat market.

The purpose of this investigation is thus to determine the influence of various commercially-used culling methods on the meat quality parameters of sub-adult impala rams. The influence of method will be determined comparing the effects of day-, night- and helicopter-associated methodologies as well as location of the shot (during day-time culling) on the animals' body on the meat quality from the culled animal. Associations with stress hormones will also be discussed.

4.3 Materials and methods

Ethical approval for the trial was obtained from the Stellenbosch University Animal Care and Use Ethical Committee (ACU-2018-6598).

4.3.1 Experimental location and animals

The harvesting of the animals for this study took place during two different culling trips at the ROMACO Ranch in the Limpopo Province. A total of 84 animals were culled, i.e. 24 sub-adult impala rams during the first culling trip, and 60 impala during the second trip. The impala culled during the second trip consisted of sub-adult rams as well as some four-year-old ewes.

4.3.2 Experimental treatments

Figure 4.1 presents the respective treatments, and the information of the relevant animals that were culled using the respective methods.

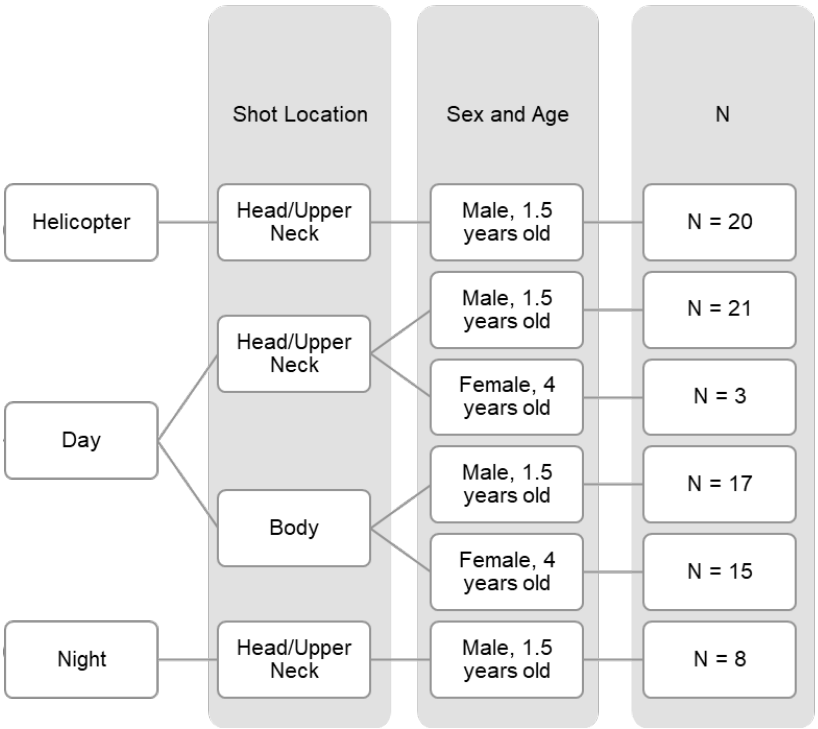

Figure 4.1 The comparison of the different culling methods, in terms of shot placement and animals that were culled within each method.

The three culling methods used were *via* helicopter and using a vehicle during the day and at night, respectively.

- *Helicopter-culling* was conducted between 06:00 and 12:00 and involved the use of a small helicopter to chase the animals being targeted by a marksman seated in the helicopter. Animals were shot in the head/high neck region with a shotgun loaded with SSG lead bullets. Animals were exsanguinated by a second field team

in the field and loaded onto pick-up vehicles which transport them to the abattoir within 30 minutes of death.

- *Day-culling* involved the culling of impala from the back of a vehicle between 06:00 and 18:00. Animals were shot in the head/high neck or body region by a marksman on the back of the vehicle using a .306 or .243 rifle. The body shots were aimed at the shoulder of the animals with the primary aim of hitting the heart and/or lungs. The animals were exsanguinated in the field and loaded onto the vehicle and transported to the abattoir within two hours of death.
- *Night-culling* was similar to the day-culling, however, was conducted between 21:00 and 02:00 using spotlights (one million candlelight strength) to locate and temporarily blind the animals so that a headshot could be taken. The animals were exsanguinated in the field and loaded onto the vehicles and transported to the abattoir within three hours of death

4.3.3 Blood sampling and hormone analysis

Blood samples were collected in the field as soon as possible after the animal went down, within five minutes, with blood collected from the jugular vein using lithium heparin vacutainer tubes. Blood samples were centrifuged at 4000 rpm for five minutes and allowed to settle for two minutes. The serum was then transferred to clearly marked cryotubes and stored at -20 °C until further processing.

For the hormone assays, standards and samples were prepared in duplicate and analysed using Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS), with an accuracy of 89 %. For more details, please refer to Chapter 3.

4.3.4 Processing of carcasses

Upon arrival at the registered on-farm abattoir (certificate number: 2/4G), all intact carcasses were weighed, and this weight was recorded as the *dead weight*. Each carcass was then skinned and eviscerated, and all organs, trotters, heads and skin were transferred to a crate for weighing later. After removal of the skin, organs, head and trotters, each carcass was weighed again to obtain the *warm carcass* weight, before being tagged and transferred to a cold room. These carcasses were stored at 4 °C, for 24 hours, until processing.

4.3.5 pH profiles

Throughout the first 24 hours post-mortem, pH and temperature readings were taken at regular intervals for all the animals, using a Crison pH 25 pH meter (Crison Instruments, Barcelona, Spain), measured at the last rib, in the LTL (*Longissimus thoracis et lumborum*) muscle. Readings were taken hourly for the first four hours post-mortem, thereafter every two hours until 12 hours post-mortem and then every four hours until processing at 24 hours post-mortem. The pH meter

was calibrated before every session of readings according to the manufacturer's guidelines. The electrode was rinsed with distilled water and dried between each carcass reading.

The first pH reading was taken within ten minutes of death (pH_I) and the last pH reading was taken 24 hours post-mortem, at carcass processing (pH_U). The measured pH values were not suitable for use due to varying muscle temperature conditions. Therefore, adjusted pH (pH_{adj}) values were calculated and used for analysis purposes using the following equation (Bruce *et al.*, 2001):

$$pH_{adj} = \text{measured } pH_t + \{(T_t - T_{adj}) * 0.01\}$$

Where the pH_t is the actual pH measured at the measured muscle temperature (T_t) at time (t). T_{adj} is the temperature (4 °C) to which the data was adjusted.

4.3.6 Meat sampling and physical analysis

The *cold carcass* weight was obtained after the 24-hour cold storage period and prior to the carcass being processed. For physical and proximate analysis, only the left LTL muscle was removed from the carcass and weighed. The muscle was then de-membraned, and physical analysis was conducted immediately after the dissection of the muscle from the carcass.

Half of the LTL was vacuum-packed and stored at -20 °C for proximate analysis at a later stage. From the other half, two steaks approximately two centimetres thick, were cut for physical analysis (Honikel, 1998). One steak was weighed and suspended in an inflated polythene bag and placed in a refrigeration unit at 4 °C. Care was taken to ensure the sample did not touch the sides of the bag. After 24 hours the meat was patted dry with absorbent paper and weighed to determine the *drip loss*, which was calculated and expressed as a percentage of the initial weight.

The second steak was allowed to bloom for 30 minutes before five colour readings were taken randomly per steak, using a Color-guide 45°/0° (BYK-Gardner GmbH, Gerestried, Germany) colorimeter to measure the three colour parameters, i.e. L*, a* and b*, which were used to calculate the chroma and hue angle values using the following equations:

$$\text{Hue angle:} \quad h_{ab} = \tan^{-1}(b^*/a^*)$$

$$\text{Chroma value:} \quad C^* = [(a^*)^2 + (b^*)^2]^{1/2}$$

The same steak that was used to obtain the colour parameters was then weighed and placed into a cooking bag that was sealed and suspended in the water bath (80 °C) for one hour. The bag containing the cooked sample was subsequently removed and allowed to cool at 4 °C before the meat sample was patted dry and weighed to determine the cooking loss. The cooking loss was also calculated as a percentage of weight lost per sample.

The chilled, cooked, samples were cored using a 1.27 cm diameter cylindrical hand corer. Cores were cut parallel to the muscle fibre direction of each cooked meat sample at random places, care was taken to avoid visible connective tissue in the sample. A total of six shear force

readings (kg/1.27 cm Ø) were taken per meat sample using a mobile Instron machine (Emerson Electrics, S44EXTJ-988, ST. Louis, USA). The mobile Instron machine was fitted with a Warner-Bratzler shear blade (1.2 mm). The samples were sliced by the blade perpendicular to the fibre direction and a shear force value (kg/1.27cm Ø) was determined at cross head speed of 33.3 mm/s. The shear force readings were converted into Newton before an average value was calculated. The conversion to Newton was done by using the following equation:

$$\text{Shear Force (N)} = (SF*9.81) / \pi*(1.27/2)^2$$

Where 'SF' is the measured shear force value (kg/1.27cm Ø).

4.3.7 Data recorded

4.3.7.1 Firearms

In total four weapons were used in this trial: a shotgun, .243, .306 and .308 rifles. These were classified as 'Heavy calibre', 'Light calibre' or 'Shotgun', respectively.

- 'Heavy calibre': Includes the .306 and .308 rifles only.
  These weapons were used during the day- and night-culling procedures. These rifles were also used during the shot placement trial.
- 'Light calibre': Includes the .243 rifle only.
  This rifle was used during the day- and night-culling procedures as well as during the shot placement trial.
- 'Shotgun': Loaded with SSG lead pellets.
  This firearm was only used during helicopter-culling procedures where rifles were not suitable. This firearm was not used during the shot placement trial.

4.3.7.2 Exposure to culling activity

Prior to both culling excursions, the impala within the camps which were used in this trial, had no prior exposure to any form of hunting or culling procedures for a minimum of a year. Throughout both culling excursions however, camps were revisited, often multiple times therefore exposure was categorized as 'No prior', 'Some' and 'Recurrent', as in Chapter 3.

- 'No prior': No previous exposure.
  Prior to being culled, the camp was not hunted in a year.
- 'Some': Recent exposure.
  Prior to culling, the animals in this group had been exposed to minimal hunting and culling activity, whereby the herd had been chased once or twice but had been allowed to rest for at least 24 hours between each exposure.
- 'Recurrent': Recent and repeated exposure.

The animals included in this category were culled from camps where herds had been chased several times throughout the trial as well as herds that had been exposed to culling-related activities more than once within 24 hours.

4.3.7.3 Chase time

The chase time was divided into two categories: 'short' and 'medium'. No animals were pursued for longer than 20 minutes in this trial to avoid the possible effects of chronic stress and fatigue. The timer was started the moment the herd became aware of the hunting party and stopped when the animal went down, intervals and times were taken note of if multiple shots were fired.

- 'Short': < 5 minutes.
- 'Medium': 5 – 20 minutes.

4.3.7.4 Number of shots required

The number of shots were classed into two groups: 'single' and 'multiple', as in Chapter 3. All animals that were injured due to off-target shots were immediately pursued and killed humanely.

- 'Single': One shot.
  This was assigned when animals were shot and killed using only one bullet, as well as animals killed with a single bullet after a missed shot.
- 'Multiple': Injured animals.
  This was allocated when the first bullet fired hit but did not kill the animal, requiring further intervention to humanely kill the animal.

4.3.7.5 Distance travelled after fatal shot

The distance travelled by the animals after they were shot was noted as animals shot using a body-shot tend to go down less quickly than animals shot in the head. This was not done using an accurate distance recording method but rather as an estimation. The distances were categorized as '1', '2' and '3'.

- '1': 0 meters travelled after being shot.
  Animals allocated to this group were mostly head/neck shot animals and a few body-shot animals that went down immediately after being shot.
- '2': 1 – 50 meters travelled after being shot.
  This group included only body-shot animals that ran up to 50 meters before they went down.
- '3': >50 meters travelled after being shot.
  This group was only body-shot animals that ran further than 50 meters before they went down.

4.3.8 Proximate analysis

Proximate analysis was only carried out on the samples collected from the 24 animals culled during the first culling trip. Frozen samples were thawed at 4 °C over-night, and the LTL samples were then trimmed of excess fat, if present, as only the intramuscular fat was of interest, and cut into smaller pieces before being placed into a bowl, fitted with a sharp blade for homogenisation. Each sample was placed into individual bags and vacuum-sealed before being immediately frozen again at -20 °C until proximate analysis could be performed. Proximate analysis was performed in duplicate for each sample. If an error percentage of more than 10 % was noted between duplicates, the analysis was repeated.

The moisture content (g/100 g) per sample was determined according to the AOAC Official method 934.01 (AOAC International, 2002c). Exactly 2.5 g of each homogenised muscle sample was dried for a period of 48 hours in an oven set at 100 °C. Once moisture content was determined, the moisture-free samples were placed into a furnace at 500 °C for six hours to determine the ash content (g/100 g meat) of each sample as per the AOAC Official Method 942.05 (AOAC International, 2002a).

Total fat content (g/100 g meat) was determined using a rapid solvent extraction method as described by Lee *et al.* (1966) and using a 5.0 g sample of the homogenized muscle. A mixture of chloroform/methanol was used in a 1:2 ratio (v/v) as the solvent as recommended for game animals due to the expected low lipid content of game meat.

Once the fat was extracted, the remaining filtrate was collected and dried at 60 °C. Once dried, the sample was ground into a fine powder, which was used in the crude protein content (g/100 g meat) determination. For each ground sample, one gram was enclosed into a LecoTM tinfoil sheet and analysed in a Leco Nitrogen/Protein Determinator (FP528 – Leco Corporation, USA) according to the Dumas combustion method stipulated by the AOAC Official Method 992.15 (AOAC International, 2002b). The Leco Nitrogen/Protein Determinator was calibrated using a 0.15 g sample of EDTA (Leco Corporation, USA). To ensure accuracy, the Leco Nitrogen/Protein Determinator was calibrated after every eight samples. The results were obtained as a nitrogen percentage (% N) which was multiplied by a 6.25 conversion factor to obtain a crude protein content (g/100 g) per sample. It was assumed that protein consists of 16 % nitrogen (100/16 = 6.25) to obtain the conversion factor.

4.3.9 Statistical analysis

Animals that were overly stressed due to severe injury and/or suspected disease were analysed individually although it was observed that these animals were not influential outliers. Due to the effects of age and sex on meat quality, the four-year-old impala ewes could not be used in comparisons with the sub-adult impala rams. The data was analysed using XLStat version 19.2 where descriptive statistics were utilised to explore the data, and multi-factorial ANOVA analysis was performed to compare the main effects and interactions. The influence of the main effects

(culling method and shot placement) were analysed along with the effects of firearm, prior exposure to culling activity, chase time, number of shots and the distance travelled after being shot.

However, due to a too small sample size and the unbalanced nature of the data, only culling method and shot placement was included in the statistical comparisons/analysis and the other observations such as; firearm, prior exposure to culling activity, chase time, number of shots and the distance travelled after being shot, were used in explaining outliers, if any, and unexpected findings of the data. Non-linear regression was used to determine the pH decay model. Fisher's least significant difference post-hoc tests were used to compare culling method and shot placement differences and calculated LSMeans. A p-value of less than 0.05 was considered significant.

4.4 Results

For the purpose of this investigation, only the rams will be considered even through ewes were culled as well. This is due to a too large difference in age between the sexes, this will be discussed in the last chapter.

4.4.1 The influence of culling method / shot placement on carcass weights and organ yields of impala

In this study, the influence of culling method / shot placement on organ weights, as a percentage of the dead weight are presented in Table 4.1 and Table 4.2 respectively.

There was a tendency (Table 4.1) for culling method to affect the carcass weight of the impala, with the helicopter-cull group being characterized by the heaviest animals when compared to the head-shot day- and night-cull, respectively (Helicopter: 40.40 kg vs. Day: 36.83 kg and Night: 35.75 kg; $p = 0.07$). Warm carcasses were also heavier for the helicopter-culled impala rams, when compared, respectively, to the day- and night-culled animals (Helicopter: 24.84 kg vs. Day: 21.42 kg and Night: 20.98 kg; $p = 0.02$). Similar results were observed for the cold carcass weight (Helicopter: 24.07 kg vs. Day 20.90 kg and Night: 20.65 kg; $p = 0.02$).

When the percentage contribution of the offal items to the carcass weight was considered, interesting observations were recorded. Day-culled animals tended to have a heavier gastro-intestinal tract (GIT) weight, compared to that of the helicopter-culled animals (Day: 20.49 % vs. Helicopter: 19.51 %; $p = 0.07$). The percentage contribution of the tongue was higher for the night-culled animals than the day-culled animals (Night: 0.29 % vs. Day: 0.25 %; $p = 0.03$). The heart of the night-culled animals contributed more than that of the day- and helicopter-culled animals to the overall carcass weight (Night: 0.79 % vs. Day: 0.66 % and Helicopter: 0.69 %; $p \leq 0.05$). The proportion of the lungs did not differ between treatment methods. The contribution of the kidneys to overall carcass weight was higher in the helicopter-culled animals, when compared to the day-culled animals (Helicopter: 0.29 % vs. Day: 0.27 %; $p = 0.04$). The total organ mass when

considered as contributing to the overall carcass weight, was heavier for the night-culled impala when compared to that recorded for the helicopter-culled impala (Night: 25.81 % vs. Helicopter: 24.22 %; p = 0.02).

Table 4.1 A comparison on the carcass weight (mean ± SE) and percentage organ contribution to carcass weight (mean % ± SE) of head-shot impala, as influenced by helicopter-culling, day-culling or night-culling.

	Helicopter	Day	Night	Range	p-value	CV
Dead Weight (kg)	40.40±1.64	36.83±0.97	35.75±1.33	25.80-57.50	0.07	0.16
Warm Carcass (kg)	24.84^{a}±1.25	21.42^{b}±0.61	20.98^{b}±0.80	14.80-38.10	0.02	0.19
Cold Carcass (kg)	24.07^{a}±1.16	20.90^{b}±0.52	20.65^{b}±0.83	16.20-36.50	0.02	0.18
Head (+tongue, %)	6.67±0.14	6.56±0.18	6.79±0.15	4.87-9.01	0.70	0.10
Legs (%)	3.07±0.09	3.04±0.07	3.10±0.04	2.35-3.91	0.81	0.07
Skin (%)	5.40±0.10	5.32±0.13	5.38±0.12	3.82-6.53	0.87	0.09
GIT (%)	19.51±0.26	20.49±0.49	20.47±0.39	15.92-26.36	0.15	0.08
Tongue (%)	0.27^{ab}±0.01	0.25^{b}±0.01	0.29^{a}±0.02	0.17-0.37	0.08	0.15
Heart (%)	0.69^{b}±0.03	0.66^{b}±0.02	0.79^{a}±0.03	0.44-1.00	0.01	0.15
Lungs (%)	1.42±0.04	1.55±0.05	1.58±0.08	1.06-2.03	0.11	0.15
Liver (%)	1.61±0.03	1.55±0.03	1.61±0.04	1.26-1.92	0.29	0.09
Kidneys (%)	0.29^{a}±0.01	0.27^{b}±0.01	0.28^{ab}±0.01	0.23-0.37	0.13	0.10
Spleen (%)	0.47±0.03	0.53±0.03	0.51±0.02	0.29-0.75	0.25	0.24
Organ Total (%)	24.22^{b}±0.31	24.89^{ab}±0.45	25.81^{a}±0.43	20.13-27.99	0.07	0.07

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation
%: a percentage of dead weight values

Table 4.2 A comparison on the carcass weight (mean ± SE) and percentage organ contribution to carcass weight (mean % ± SE) of day-culled impala, as influenced by head/neck or body shot placement.

	Head/Neck	Body	Range	p-value	CV
Dead Weight (kg)	36.83±0.97	38.48±1.80	25.40-58.50	0.41	0.16
Warm Carcass (kg)	21.42±0.61	21.38±0.90	13.6-27.10	0.97	0.14
Cold Carcass (kg)	20.90±0.52	20.80±0.89	13.10-26.30	0.92	0.14
Head (+tongue, %)	6.56±0.18	6.32±0.08	4.87-9.01	0.27	0.10
Legs (%)	3.04±0.07	3.13±0.09	2.31-3.91	0.44	0.11
Skin (%)	5.32±0.13	5.33±0.10	3.82-6.46	0.94	0.09
GIT (%)	20.49±0.49	19.94±0.44	15.92-26.36	0.42	0.10
Tongue (%)	0.25^{a}±0.01	0.22^{b}±0.01	0.16-0.34	0.03	0.15
Heart (%)	0.66±0.02	0.63±0.02	0.44-0.82	0.26	0.11
Lungs (%)	1.55±0.05	1.65±0.07	1.06-2.31	0.22	0.16
Liver (%)	1.55±0.03	1.49±0.03	1.30-1.92	0.19	0.09
Kidneys (%)	0.27±0.01	0.27±0.01	0.22-0.31	0.36	0.08
Spleen (%)	0.53±0.03	0.52±0.03	0.31-0.78	0.85	0.24
Organ Total (%)	24.89±0.45	24.39±0.42	20.13-27.99	0.43	0.08

[ab] Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation
%: a percentage of dead weight values

The only difference observed when analysing the effects of shot placement on carcass characteristics and yields of impala was in terms of tongue weight (Table 4.2). Animals culled by using a head/neck shot had heavier tongues than the animals culled by using a body shot (Head/neck: 0.25 % vs. Body: 0.22 %; $p = 0.03$).

4.4.2 The influence of culling method and shot placement on the pH profile of impala meat

For the purpose of this study, the pH values were adjusted for temperature to standardize the temperature effect on pH. An exponential decay model ($y = a + be^{-ct}$) was fitted to the adjusted pH data and the a-, b-, and c-constants were determined. The R^2 values of all the exponential decay models from the adjusted pH values ranged from 0.71 to 0.99. For statistical comparisons, the constants derived from the decay model were compared as were the actual initial (pH_I) and ultimate pH (pH_U) values, after adjustment for temperature.

The pH_I values measured at death, differed between culling methods. The carcasses of the animals culled using a helicopter had a higher average pH_I, when compared to that recorded for the day- and night-culled animals (Helicopter: 7.45 vs. Day: 7.18 and Night: 7.10; $p \leq 0.06$). The helicopter-culled animals also had the highest average values for the b-constant (indicating rate of change) when compared to that of the day- and night-culled animals (Helicopter: 2.03 vs. Day: 1.59 and Night: 1.65; $p \leq 0.01$). None of the other pH parameters were significantly affected by culling method ($p > 0.05$), although a tendency was observed for the adjusted pH_U values between the day- and night-culled animals (Day: 5.73 vs. Night: 5.63; $p = 0.06$) to differ. The pH parameters as influenced by culling method are presented in Table 4.3.

Table 4.3 A comparison on the pH parameters (mean ± SE) of the impala carcass, as influenced by helicopter-culling, day-culling or night-culling, and measured at the last rib in the LTL muscle.

	Helicopter	Day	Night	Range	p-value	CV
pH_I (Adjusted)	7.45^{a}±0.10	7.18^{b}±0.07	7.10^{b}±0.13	6.53-7.80	0.06	0.05
pH_U (Adjusted)	5.68±0.02	5.73±0.03	5.63±0.04	5.50-6.01	0.13	0.02
a	5.54±0.06	5.64±0.04	5.59±0.07	4.91-5.90	0.31	0.04
b	2.03^{a}±0.10	1.59^{b}±0.08	1.65^{b}±0.11	0.67-3.00	< 0.01	0.24
c	0.34±0.06	0.35±0.03	0.24±0.05	0.06-0.96	0.39	0.60

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation
$y = a + be^{-ct}$ where $a = pH_U$ and b and c indicative the rate of decay

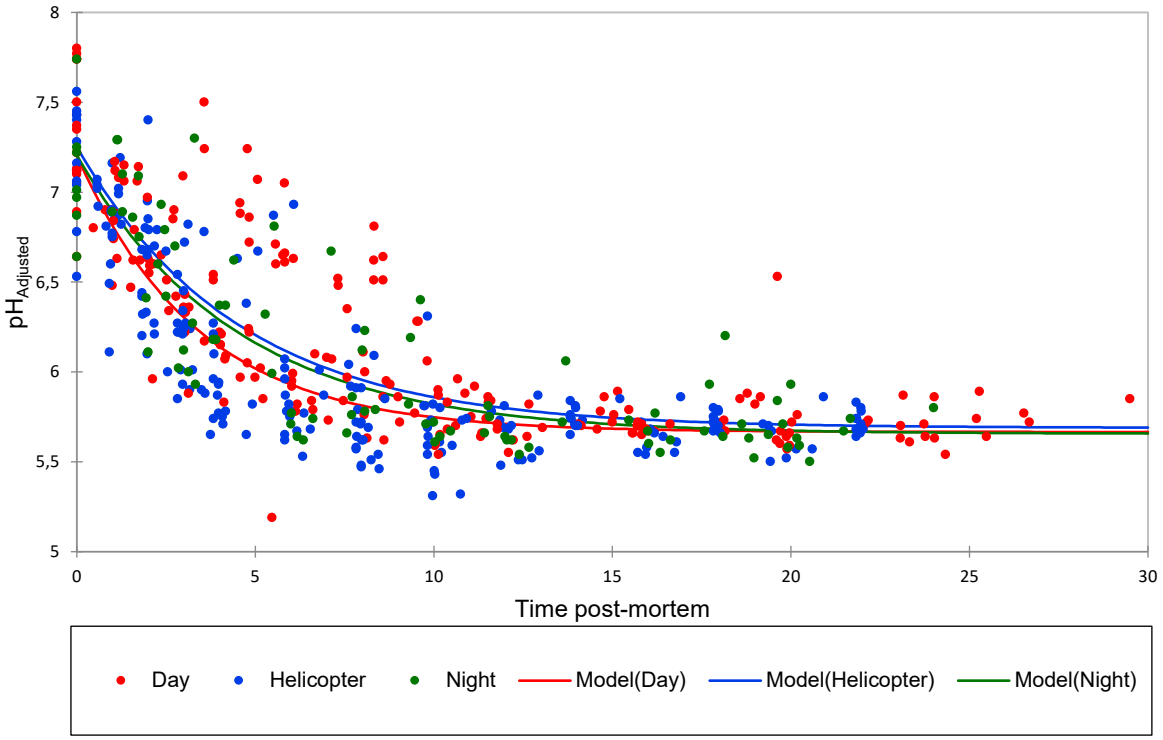

Figure 4.2 Non-linear regression showing the influence of helicopter- (R^2 = 0.63), day- (R^2 = 0.83) and night-culling (R^2 = 0.75) on the pH profiles of the LTL muscles of head-shot impala (values for the constants of the decay model are depicted in Table 4.3).

Results presented in Table 4.4 indicated that the adjusted pH_I values were influenced by shot placement whereby the LTL muscles of the head/neck shot animals had higher values than that of the body-shot animals (Head/Neck: 7.18 vs. Body: 6.94; p = 0.01). The b-constant values in the exponential decay models of the pH values obtained from the head/neck shot animals was also higher than that of the body shot animals (Head/Neck: 1.59 vs. Body: 1.32; p = 0.01).

Table 4.4 The comparison pH parameters (mean ± SE) of the impala carcass, as influenced by head/neck or body shot placement and measured at the last rib from the LTL muscle.

	Head/Neck	Body	Range	p-value	CV
pH_I (Adjusted)	7.18^{a}±0.07	6.94^{b}±0.05	6.43-7.80	0.01	0.04
pH_U (Adjusted)	5.73±0.03	5.72±0.03	5.48-6.01	0.88	0.02
a	5.64±0.04	5.70±0.03	5.37-5.92	0.25	0.03
b	1.59^{a}±0.08	1.32^{b}±0.06	0.67-2.23	0.01	0.23
c	0.35±0.03	0.31±0.02	0.11-0.79	0.41	0.40

^{ab} Means with different superscripts within the same row differ significantly (p ≤ 0.05)
CV - coefficient of variation
$y = a + be^{-ct}$ where a = pH_U and b and c indicative the rate of decay

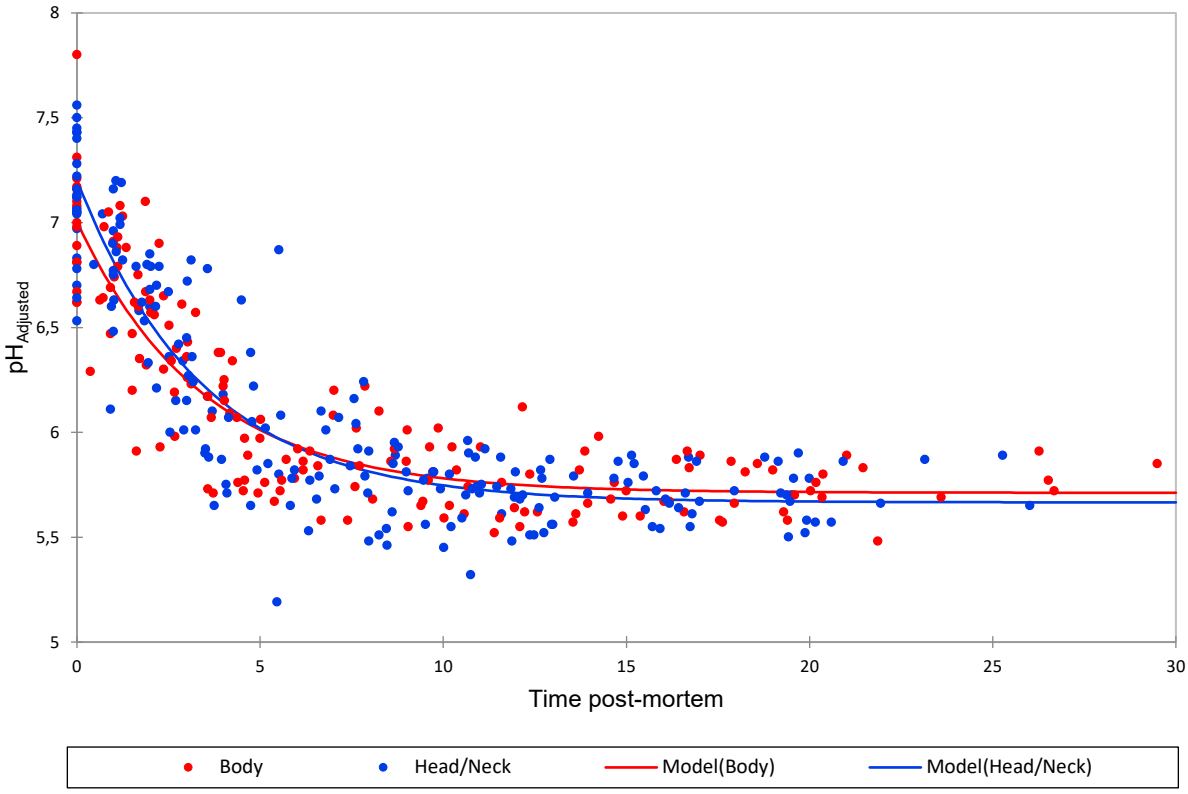

Figure 4.3 Non-linear regression ($y = a + be^{-ct}$) showing the influence head/neck shots ($R^2 = 0.83$) and body shots ($R^2 = 0.81$) on the pH profiles of the LTL muscles of day-culled impala.

4.4.3 The influence of culling method and shot placement on the water-holding capacity of impala meat

Water-holding capacity was determined from the analysis of the drip and cooking loss, represented as a percentage of the initial muscle sample weight, and the results are presented in Table 4.5. The drip loss in meat obtained from the helicopter-culled animals was higher, when compared to that recorded for the night-culled animals (Helicopter: 2.79 % vs. Night 1.42 %; $p = 0.04$). The meat obtained from the helicopter-culled animals also had a higher cooking loss percentage than that from the night-culled animals (Helicopter: 37.88 % vs. Night: 35.87 %; $p \leq 0.05$). This resulted in the combined moisture loss from meat samples obtained from the helicopter-culled animals being higher than that from the night-culled animals (Helicopter: 41.96 % vs Night: 37.29 %; $p = 0.02$) and tendencies indicated that the values recorded from meat sampled of the helicopter-culled animals were also higher than that from the day-culled animals (Helicopter: 41.96 % vs. Day: 39.15 %; $p = 0.07$).

Table 4.5 The comparison of water-holding capacity parameters (mean ± SE) measured from the LTL muscle samples obtained from impala carcasses, as influenced by helicopter-culling, day-culling or night-culling.

	Helicopter	Day	Night	Range	p-value	CV
Drip Loss (%)	2.79^{a}±0.54	2.07^{ab}±0.17	1.42^{b}±0.08	0.16-10.28	0.10	0.70
Cooking Loss (%)	37.88±0.68	36.74±0.38	35.87±0.80	32.34-41.61	0.11	0.07
Total moisture loss (%)	41.96^{a}±1.54	39.15^{ab}±0.52	37.29^{b}±0.81	33.71-61.95	0.05	0.13

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

Shot placement, results presented in Table 4.6, only had an influence on cooking-loss percentage. The muscle samples from the body-shot animals were characterised by a higher cooking loss, when compared to that recorded for animals culled using a head/neck shot (Body: 38.17 % vs. Head/neck: 36.74 %; $p \leq 0.01$).

Table 4.6 The comparison of water-holding capacity parameters (mean ± SE) measured from the LTL muscle samples obtained from impala carcasses, as influenced head/neck or body shot placement.

	Head/Neck	Body	Range	p-value	CV
Drip Loss (%)	2.07±0.17	1.80±0.28	0.08-4.08	0.38	0.45
Cooking Loss (%)	36.74^{b}±0.38	38.17^{a}±0.15	33.15-39.17	< 0.01	0.04
Total moisture loss (%)	39.15±0.52	40.43±0.88	34.95-48.40	0.19	0.07

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

4.4.4 The influence of culling method and shot placement on the tenderness of impala meat

Shear force values (N) were used as an indication of tenderness, i.e. a higher shear force value indicates that the meat would be perceived as tougher, and *vice versa*.

The analysis of shear force as influenced by culling method are presented in Table 4.7. Tendencies indicate that the meat samples from the night-culled animals had a lower shear force value when compared to that of the day-culled animals (Night: 26.09 N vs. Day: 32.04 N; $p = 0.06$). High variation was seen throughout these results.

Shot placement did not influence shear force values (Table 4.8; $p > 0.05$).

Table 4.7 The comparison of shear force and colour parameters (mean ± SE) measured from LTL muscle samples obtained from impala carcasses, as influenced by helicopter-culling, day-culling or night-culling.

	Helicopter	Day	Night	Range	p-value	CV
Shear Force (N)	31.58±1.21	32.04±1.63	26.09±3.99	13.94-48.26	0.15	0.25
L*	30.58±0.36	30.34±0.53	30.24±0.37	26.11-36.57	0.89	0.06
a*	10.57±0.18	10.23±0.28	10.11±0.30	8.47-12.93	0.44	0.10
b*	8.16±0.17	8.13±0.37	7.32±0.22	5.97-12.55	0.24	0.16
Chroma	13.37±0.23	12.95±0.39	12.49±0.35	10.71-17.12	0.30	0.11
Hue	37.61±0.43	37.60±0.58	35.86±0.51	32.67-42.63	0.13	0.06

CV - coefficient of variation

Table 4.8 The comparison of shear force and colour parameters (mean ± SE) measured in LTL muscle samples from impala carcasses, as influenced head/neck or body shot placement.

	Head/Neck	Body	Range Min-Max	p-value	CV
Shear Force (N)	32.04±1.63	33.14±2.12	13.94-49.62	0.68	0.24
L*	30.34±0.53	29.76±0.51	26.11-36.57	0.45	0.08
a*	10.23±0.28	10.37±0.37	7.78-13.69	0.77	0.13
b*	8.13±0.37	8.30±0.46	5.60-12.77	0.76	0.21
Chroma	12.95±0.39	13.63±0.69	9.59-21.36	0.38	0.17
Hue	37.60±0.58	37.51±0.58	32.67-42.68	0.91	0.07

CV - coefficient of variation

4.4.5 The influence of culling method and shot placement on the colour of impala meat

The colour of meat is analysed by means of five parameters; L*, a*, b*, chroma value and hue angle and presented in Table 4.7 and Table 4.8. Tendencies indicate that the hue angle values were affected by culling method (Table 4.7) whereby the meat samples obtained from night-culled

impala had a lower value than that from animals culled using the helicopter-culling method (Night: 35.86 vs. Helicopter: 37.61; p = 0.06) as well as that of the day-culling method (Night: 35.86 vs. Day: 37.60; p = 0.06).

It is apparent that shot placement does not influence colour parameters (Table 4.8; p > 0.05).

4.4.6 The influence of culling method and shot placement on the proximate composition of impala meat

The proximate analysis of LTL muscle samples from impala carcasses as influenced by culling method will be discussed (Table 4.9) however, proximate analysis was not conducted on LTL muscle samples of the impala used for the shot placement comparison.

Tendencies indicate that LTL muscle samples from the night-culled animals were higher in moisture content than that of the samples from the day-culled animals (Night: 75.44 % vs. Day: 74.83 %; p = 0.07). Tendencies also indicated that meat protein percentage was higher in meat obtained from the day-culled animals than that from the helicopter-culled animals (Day: 23.49 % vs. Helicopter: 22.83 %; p = 0.06). Culling method had an influence on the fat content whereby the meat samples obtained from the night-culled animals had a lower fat content than that from the helicopter- and day-culled animals (Night: 1.35 % vs. Helicopter: 1.71 % and Day: 1.66 %; p < 0.01).

Table 4.9 The comparison of proximate composition (mean ± SE) of LTL muscle samples obtained from impala carcasses, as influenced helicopter-culling, day-culling or night-culling.

	Helicopter	Day	Night	Range	p-value	CV
Moisture %	75.83±0.19	74.83±0.18	75.44±0.30	74.17-76.71	0.17	< 0.01
Protein % 'g/100g'	22.83±0.20	23.49±0.19	23.16±0.32	21.82-24.34	0.17	0.03
Fat %	1.71^{a}±0.06	1.66^{a}±0.04	1.35^{b}±0.03	1.23-2.00	< 0.01	0.13
Ash %	1.22±0.05	1.22±0.03	1.25±0.02	1.09-1.47	0.75	0.07
Total	101.09±0.07	101.20±0.07	101.23±0.11	100.75-101.67	0.54	< 0.01

^{ab} Means with different superscripts within the same row differ significantly (p ≤ 0.05)
CV - coefficient of variation

4.5 Discussion

Some studies have found culling-related stress and shot placement can influence the meat quality and yield of game species (Von La Chevallerie & Van Zyl, 1971; Laubscher, 2009; Hoffman &

Laubscher, 2010). Previous research has shown that animals exposed to acute stress ante-mortem have increased serum concentrations of glucocorticoid hormones, show rapid muscle pH decrease post-mortem which leads to a lowered water-holding capacity and in turn, meat that is of a lighter colour profile (Kritzinger *et al.*, 2003; Swatland, 2004; Laubscher, 2009; Warris, 2010; Gentsch *et al.*, 2018).

The hormone concentrations analysed in Chapter 3 did not show significant differences between the concentrations of glucocorticoids in blood samples from the helicopter-, day- and night-culled animals. Although, when compared to expected trends, it was suggested that the night-culled animals had experienced higher levels of stress as the measured glucocorticoid concentrations from the obtained blood samples deviated from the diurnal rhythm that was expected. It has been shown that cortisol has the potential to influence meat quality (Dokmanovic *et al.*, 2015). From the results seen in Chapter 3, it was expected that the meat samples obtained from the night-culled animals would show signs of increased ante-mortem stress. The meat obtained from the night-culled impala showed pH and water-holding capacity variations however, due to a small sample size, further investigation is required. It is worth noting that, although some of the impala investigated in Chapter 3 were seen to be influential outliers when considering serum glucocorticoid concentrations, the meat samples obtained from these same animals did not exhibit any meat quality parameters that were outliers in this chapter.

Meat samples from all animals that were injured during the culling procedures were analysed individually, contrary to expectation, the meat samples from the injured animals did not differ from that of the uninjured animals.

4.5.1 The influence of culling method and shot placement on the carcass and organ yields of impala

In the current investigation it was not expected for culling-related stress to influence the carcass yields of the animals culled in this trial. However, shot placement may have resulted in a degree of meat loss and/or organ loss. Von La Chevallerie and Van Zyl (1971) showed that body shots produced the most meat damage and wastage when compared to that of head or neck shots.

From the results, it was shown that the helicopter-culled animals were heavier, in terms of dead, warm and cold carcass weight (40.40 kg, 24.84 kg and 24.07 kg respectively), than that of both the day- and night-culled animals. Although statistically significant, the differences noted between culling methods for the carcass weights were not considered to be meaningful and could be attributed to biological variation within the population or to the fact that the slightly larger animals were better targets for the marksman in the helicopter.

The shot placement did not affect carcass characteristics, which is contrary to the findings of Von La Chevallerie & Van Zyl (1971). However, in the latter study the carcasses were trimmed of bloody meat and bone chips before the carcass weights were recorded, whilst in the present study this was not done.

It is known that age has an influence on carcass size, providing explanation for the smaller carcass size reported in the sub-adult impala rams of the current investigation compared to that found for mature impala rams in the study of Hoffman (2000b). The average impala carcass weight reported in the current investigation was similar to that found for sub-adult impala rams by Hoffman *et al.* (2009), and it was therefore assumed that all animas were healthy and in good body condition.

4.5.2 The influence of culling method and shot placement on the pH parameters of impala meat

When an animal dies, there is a natural pH decline in skeletal muscles due to a decrease in available oxygen as a result of no blood circulating after the animal has been exsanguinated. Under anaerobic conditions, glycogen stores in the muscles are converted into lactic acid, resulting in the muscles becoming more acidic (Lawrie, 2006). Acute culling-related stress has been known to result in low ultimate pH values, which can cause the meat to become pale and exhibit a reduced water-holding capacity (Swatland, 2004), although it is acknowledged that this phenomenon is more prevalent in porcine species.

Culling method influenced initial pH values - the LTL muscles of the animals culled from a helicopter showed higher values than the LTL muscle from those that were culled using the day- and night methods. The higher initial pH reported for the LTL muscles of the helicopter-culled animals may be an indication of acute stress experienced by these animals. During the day- and night-culls, the animals were chased for a longer period of time suggesting that the decreased initial pH values in the LTL muscles of those animals could have been due to increased amounts of lactic acid produced during the chase. Also, Cockram *et al.* (2011) showed that blood plasma concentration of lactate, which is formed during the anaerobic glycolysis of lactic acid, was higher in animals shot from a helicopter than those shot using a method similar to the day method used in this study. It is theorised that there was sufficient time before death for the lactate formed in the muscle (due to the anaerobic glycolysis that was enhanced by the stress) to be moved from the muscle into the blood steam; this would also account for the higher pH_l readings in the muscle of the helicopter-culled animals measured immediately post-mortem. Lactic acid concentrations were not measured in the current investigation and therefore remains speculation however, it has been suggested that lactic acid concentration could be used as an indicator of meat quality, as well as of ante-mortem stress (Dokmanovic *et al.*, 2015). There was no difference between the LTL initial pH values of the muscles from day- and night-culled animals. This agrees with Hoffman and Laubscher (2009a), however, Kritzinger *et al.* (2003) found that the initial pH of the LTL was significantly higher in night-culled animals than that of the day-culled animals.

The ultimate pH values of the LTL muscles were all within a normal range and no significant differences were observed. However, tendencies indicate that the muscle samples from the day-culled animals had a higher ultimate pH than that measured for the night-culled,

which is in agreement with the findings of Veary (1991) and Kritzinger *et al*. (2003). In contrast, Hoffman and Laubscher (2009a; 2010) reported that, for kudu and gemsbok, muscle samples from night-culled animals had a higher ultimate pH than those seen in muscle samples from day-culled animals. Cockram *et al.* (2011) also noted lower ultimate pH values in meat samples from night-culled animals however, the study also included a helicopter-cull comparison, which was not included in many of the other studies. Lower ultimate pH value of the meat samples from the night-culled animals was not expected from the results reported in Chapter 3.

It has been found that animals who have experienced ante-mortem stress typically had higher pH_U than animals who have not been stressed. Table 4.10 was therefore designed to interpret the relationship between serum cortisone and cortisol levels measured in the blood samples from the animals in this study and various physical properties found in the meat of the animals. In this study, it was apparent that increased concentrations of glucocorticoid hormones had a weak positive correlation to ultimate pH values. Therefore, with an increasing serum cortisone and cortisol concentration, there is a slight increase of muscle ultimate pH accompanied by a minor decrease in the water-holding capacity of the muscle sample.

Table 4.10 The correlation coefficient values indicating the relationship of serum cortisone and cortisol levels from culled impala with pH_I and pH_U values as well as drip and cooking loss percentages as measured from samples from the LTL muscle.

	Cortisone	**Cortisol**
pH_I (Adjusted)	-0.19 (p = 0.39)	0.09 (p = 0.70)
pH_U (Adjusted)	0.40 (p = 0.06)	0.30 (p = 0.26)
Drip Loss (%)	0.01 (p = 0.97)	0.09 (p = 0.67)
Cooking Loss (%)	0.21 (p = 0.34)	0.09 (p = 0.69)

The a-, b- and c-constants from the pH exponential decay rate models provide an indication as to how rapidly the pH of the meat decreased to the ultimate pH value. The night-culled animals have shown increased concentrations of glucocorticoid hormones, and thus ante-mortem stress, therefore it was expected that the muscles would exhibit an increased pH decay rate. This was not the case, the b-constant values were higher in the exponential decay models of the helicopter-culled animals indicating a faster rate of decline of pH. The faster rate of pH decay of the muscles of the helicopter-culled animals could be attributed to the increased amount of physical activity ante-mortem. The intense running would have resulted in increased body temperatures as well as the activation and functioning of the enzymes required to make ATP during glycolysis ante-

mortem. Therefore, anaerobic glycolysis occurred at a faster rate post-mortem due to both higher carcass temperature as well as more active enzymes.

Of the day-culled impala, the LTL muscle of one ram exhibited a pH_U of 6.01. This ram had been exposed to some culling activity prior to being culled and was chased for 20 minutes before being shot and killed. Although the pH_U of this animal was considered high, this animal was not considered an outlier for any of the other meat quality traits.

Animals culled using a head/neck shot are known to go down immediately provided the shot is accurate, whilst body-shot animals can run some distance before they go down from the bullet wound (Von La Chevallerie & Van Zyl, 1971). A body shot was aimed at the flank of the animals, just below the shoulder, with the intention of hitting the heart and/or lungs. It was seen that when the heart was hit, the animals went down relatively quickly however, if only the lungs were hit, the animal was able to run for longer before it went down. The shot placement only significantly influenced the initial adjusted pH values of the muscles and the b-constant of the exponential decay model. The animals shot using a head/neck shot had higher muscle pH adjusted values at death (7.18) than the muscles of those culled using a body shot (6.94) indicating that body shot animals were already in a state of anaerobic glycolysis (having produced higher levels of muscular lactic acid) at the time of death. These findings were similar to those of Von La Chevallerie and Van Zyl (1971) who also noted that the head/neck shot animals were the least stressed. There were no differences seen in the pH_U values of the muscles which indicates that neither the head/neck nor the body shots were excessively stressful. Although, it has been noted that deleterious effects on meat quality from stress can occur in the absence of pH variation (Ferguson & Warner, 2008).

4.5.3 The influence of culling method and shot placement on the water-holding capacity of impala meat

Muscles are made up of myofibrillar proteins, which retain water when the muscle becomes meat. These proteins are sensitive to pH variations as well as the rate of pH decline, which affect the processes of proteolysis (Huff-Lonergan & Lonergan, 2005; Hughes *et al.*, 2014a). As discussed previously, when an animal dies, there is a natural decline in pH within the muscles due to anaerobic respiration, which results in a decrease of muscle water-holding capacity (WHC) (Kritzinger *et al.*, 2003). The loss of moisture from meat is unavoidable however, the volume of moisture loss can be manipulated. When acute ante-mortem stress is experienced, an accelerated rate of pH decline is experienced, which results in an increased amount proteolysis that decreases the ability of the muscle to retain water (Hoffman & Laubscher, 2009b)

Water-holding capacity was determined by the amount of liquid that was lost during both drip-loss and cooking loss tests. Drip loss tests determined the moisture lost while the meat was raw while cooking loss was the amount of moisture lost from the meat during the cooking process (Hoffman & Laubscher 2009a; Hoffman *et al.*, 2009). In the current investigation water-holding

capacity was analysed as drip loss, cooking loss and combined moisture loss (Drip Loss + Cooking Loss).

The culling method affected the pH values. Since there was a significant difference in the initial pH values of the muscles and a tendency for the muscles of the night-culled animals to have a lower ultimate pH, there was an expectation for the night-culled animals to have a reduced water-holding capacity. This was not supported by the results of this study.

The results from the current investigation showed no significant differences for the water-holding capacity of meat samples between the day- and night-culled animals. Hoffman and Laubscher (2009b) and Kritzinger *et al.* (2003) both found contrary results whereby the meat samples from night-culled animals had an increased water-holding capacity compared to meat samples from the day-culled animals. A possible explanation could be that in the two reported studies, the animals were not habituated to any vehicle and were stressed, particularly the impala shot in the daytime who had a faster pH decline post-mortem resulting in a higher level of protein denaturation and thus a decreased water binding capacity, whilst the impala in the present study were habituated to the hunting vehicle and were thus stressed on the same level. According to Bekhit *et al.* (2007), the percentage of moisture lost from the meat is positively correlated to increasing rigor temperatures of the carcass. Therefore, the reasons for the difference in water-holding capacity of the meat samples between the helicopter- and night-culled groups could have been due to ambient temperatures. During the morning helicopter-culls the ambient temperatures averaged at 18 °C which increased to 26 °C by noon, when the animals were being processed at the abattoir in ambient temperatures and were yet to be placed in the cold room, and at night temperatures averaged at 8 °C. These higher carcass temperatures would also have caused a faster rate of anaerobic glycolysis and thus faster decrease in pH (as indicated by the b-values; Table 4.3) causing a higher level of protein denaturation and thus decreased water binding capacity in the helicopter-culled animals.

As discussed previously, body shot animals were expected to be exposed to more stress than the head/neck shot animals, therefore there was an expectation for the meat obtained from the body shot animals to have a higher water-holding capacity. The data of the current investigation exhibited no significant difference in total moisture loss of the meat samples between the shot placement treatments or for drip loss from the meat samples, however, a difference was observed in the percentage cooking loss. The cooking loss of the meat samples from the animals shot using a head/neck shot was less than that of the meat samples from animals shot using body shots. These results were indicative of an increased water-holding capacity of the meat from those animals culled using headshots, suggesting that the animals shot using headshots might have juicier meat than the body shot animals, with the latter group that may have experienced higher levels of stress ante-mortem. The values seen in this study for drip loss were higher than those found by Hoffman *et al.* (2009), but lower than those found by Kritzinger *et al.* (2003). The cooking loss values reported in the current investigation were higher than the values found by Hoffman *et*

al. (2009), Hoffman and Laubscher (2009a) and Kritzinger *et al.* (2003) during their research on impala. The reason for this is unknown and warrants further investigation.

4.5.4 The influence of culling method and shot placement on the tenderness of impala meat

It is well known that there is a curvilinear relationship between muscle pH values and the tenderness of meat (Guignot *et al.*, 1994; Hoffman & Laubscher, 2010). Purchas (1990) indicated that shear force reaches a minimum when pH_U of the muscle is > 6.0 and thereafter increases with a decreasing ultimate pH value. Water-holding capacity of meat has also been known to influence the tenderness of the meat whereby, the higher the water-holding capacity, the more tender it would be perceived (Hughes *et al.*, 2014a).

As mentioned previously there was a tendency for day-culled animals to have a higher pH_U than the night-culled animals, all ultimate pH values of the muscles were below 6.0 therefore it was expected that the day-culled animals would have tougher meat than that from the night-culled animals. Considering that the meat from the night-culled animals measured highest for water-holding capacity and the meat from the helicopter-culled animals measured lowest, it was also expected that the night-culled animals would have the more tender meat than the meat from the helicopter-culled animals. The data supported these expectations to some degree. There was a tendency observed for the meat samples from night-culled animals to have a lower shear force than meat samples from day-culled animals, indicating that the night-culled animals had the most tender meat. No difference in shear force was detected between the meat samples from the helicopter- and day-culled animals. The ambient temperatures at night were approximately 8 °C, provided cold shortening does not occur, it has been shown that a cooler ambient temperature slows the rate of glycolysis in the muscle which could result in improved tenderness (Marsh *et al.*, 1981) as observed for the meat samples from night-culled impala from the study of Kritzinger *et al.* (2003).

There were no significant differences between the muscle pH_U values of the head/neck and body shot animals; based on the pH_U results, there was an expectation that there would be no difference between the tenderness of the meat samples from the two treatment groups. The meat samples obtained from the body shot animals had a higher percentage of cooking loss. Thus, there was an expectation that the head/neck shot animals would have more tender meat than the body shot animals. The data was in agreement with the expectations based on pH_U values of the muscles as there were no differences between the tenderness of these two treatment groups ($p = 0.68$). The lack of conformation to the expectations based on cooking loss values suggests that the tenderness of meat was not influenced by an isolated component of water-holding capacity but rather the combined effects of all contributing components.

4.5.5 The influence of culling method and shot placement on the colour of impala meat

It has been speculated that the darker colour of game meat is due to culling-related stress (Von La Chevallerie & Van Zyl, 1971; Von La Chevallerie, 1972; Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009b; Van Schalkwyk, 2013). Other studies have indicated that the colour of game meat could also be attributed to the increased myoglobin content in the muscles of game meat species that accompanies the increased activity levels of game animals when compared to that of other meat species (Hoffman, 2000a; Kritzinger *et al.*, 2003).

The structure of muscle is known to influence the water-holding capacity and perceived colour of meat (Hughes *et al.*, 2014a). When the muscle acidifies there is a reduction of water-holding capacity with increased rate of proteolysis that influences the internal structure of the muscle (Huff-Lonergan & Lonergan, 2005). Water loss has been found to influence light scattering and therefore results in an alteration of meat colour (Hughes *et al.*, 2014a).

The data in the current study showed that the colour variables of the meat samples did not differ between culling methods although a tendency was reported for the hue angles. The hue angle value for the meat samples obtained from the night-culled animals was lower than that of the meat samples from the day- and helicopter-culled animals. Hue angle provides an indication of colour definition and a lower hue angle value is indicative of meat of a more yellow-brown colour rather than a bright red colour (Van Heerden, 2018). The hue angle values did not differ enough to produce a colour change that was visible to the human eye and therefore were considered minor. The findings from this investigation were not consistent with the findings of previous studies, the reasons for which are unclear.

There was no expected influence of shot placement on the colour parameters of the meat samples consequent to the similarity of the ultimate pH values and cooking loss results between the two treatments discussed above. The results of this investigation exhibited no differences between the shot placements for colour parameters of the meat samples. The animals of this investigation were unstressed ante-mortem and, although they were shot in the shoulder, death was timely, therefore no differences in meat quality were exhibited. It is possible that, in a situation where the animal had been wounded and/or run for a long time, differences in meat quality would be evident.

4.5.6 The influence of culling method on the proximate composition of impala meat

Overall, the proximate composition of the meat samples from the impala rams was similar to that found by Hoffman *et al.* (2009) indicating that these animals were comparable to those from similar studies. Some differences were noted regarding the fat content of the meat samples between the culling methods although could be attributed to coincidence. All animals had typical lipid contents, comparable to that from other studies, which was an indication that the animals were not malnourished and had enough glycogen reserves in their muscles therefore, it was considered unlikely that the animals were subjected to nutritional stress.

4.6 Conclusion

The ultimate pH was lowest in the muscles of the night-culled animals, however, meat obtained from these animals did not have a decreased water-holding capacity. The water-holding capacity of the meat samples from the night- and day-culled animals were similar but, meat samples from the helicopter-culled animals had a lowered water-holding capacity, which can potentially be ascribed to the ambient temperature at which the helicopter-culled animals entered into *rigor mortis*. The meat samples obtained from the night-culled animals also were the most tender when compared to that from the day- and helicopter-culled animals.

Overall the ultimate pH values did not differ between treatment groups, and there were minimal differences noted between treatment groups for the other meat quality characteristics. Therefore, it could be concluded that helicopter-, day- and night-culling did not have any detrimental effects on the meat quality of sub-adult impala rams. This provides evidence that all considered culling methods were appropriate for commercial culling procedures.

Further research regarding the correlations of glucocorticoid hormone concentrations and meat quality parameters would be beneficial in understanding the full effects of these hormones, and thus the stress response, on meat quality post-mortem.

Shot placement had an influence on the initial pH of the muscles and water-holding capacity of the meat, however, it did not affect tenderness or the colour of meat samples. Therefore, it was concluded that shot placement had minimal influence on the meat quality of sub-adult impala rams.

It would be beneficial for similar studies to compare the effects of broadside, quartering-to and quartering-away body shots on the meat yields and quality as opposed to classifying all these shot types as body shots. These different shot types target the same vital organs however often other organs are compromised in the process. Due to a too small sample size of the current investigation, this comparison was not possible in this study.

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Chapter 5

The influence of culling-associated stress on blue wildebeest (*Connochaetes taurinus*) meat quality

5.1 Abstract

The purpose of this study was to investigate the influence of helicopter-, day- and night-culling on the meat quality and welfare of blue wildebeest (*Connochaetes taurinus*). In total, 25 mature blue wildebeest cows were culled using three different methods, i.e. helicopter-cull, day-cull and night-cull. A pH profile of the LTL (*Longissimus thoracis et lumborum*) muscle was established for the first 24 hours post-mortem per animal and muscle samples were characterised in terms of physical and proximate meat parameters. Meat quality characteristics were related to serum cortisone and cortisol levels. Ultimate pH (pH_U) values of the muscle samples from many of the animals were considered high, some within a range that suggested a dry, firm and dark (DFD) condition. The meat from the helicopter-culled animals had a decreased water-holding capacity and the most tender meat while the night-culled animals had the toughest meat, as determined by the shear force values. Findings of this study indicate that animals had possibly been subjected to chronic stress prior to culling, as evident from the DFD meat obtained from these animals. This requires validation in future studies. Correlations indicated relationships between ultimate pH values and L* values as well as the rate of pH decline and water-holding capacity.

5.2 Introduction

Also known as the brindled gnu, blue wildebeest (*Connochaetes taurinus*) are a hardy species that are known to be more tolerant of stressful environments, as evident in their better survival in harsher environments than most other species (La Grange, 2006; Hoffman *et al.*, 2011; Furstenburg, 2013). In South Africa, as the sixth and seventh most hunted species for the purpose of trophies and meat respectively, blue wildebeest generate an average annual income of approximately 2.2 million ZAR (Munzhedzi, 2018).

Blue wildebeest are a difficult species to capture and cull as they are skittish, not allowing potential threats within a distance of 40-150 m, and often use speed and agility to confuse potential predators (La Grange, 2006; Furstenburg, 2013). Gender identification is also more difficult in blue wildebeest when compared to some other ungulate species such as impala, as both males and female blue wildebeest have well developed horns and similar colouring. From the limited research conducted on blue wildebeest, literature has indicated that the meat yield and

meat quality from blue wildebeest can be considered similar to that of other ungulate species (Hoffman *et al.*, 2011; Van Heerden, 2018).

Blue wildebeest are known to have a heavy forequarters and lighter hindquarters therefore, when shot whilst running at full pace, their upper body goes down quickly and their hind legs, carried by momentum, force the body into a tumble, as would occur during a helicopter-cull (Furstenburg, 2013). This poses a possible problem when considering certain culling methods, such as *via* helicopter. However, little research, if any, has been conducted on comparing the influence of culling method on the ante-mortem stress and consequent effect of meat quality parameters for this species.

Historically, the influence of ante-mortem stress on meat quality has been underestimated however, recent research conducted on various mammalian species and has shown the deleterious effects of ante-mortem stress on meat (Ferguson & Warner, 2008). Culling methods should therefore be efficient and aimed at minimizing the stress experienced by the animal (Veary, 1991; Hoffman & Laubscher, 2009a). Stress is known to result in meat that is dark, firm and dry (DFD) or pale, soft and exudative (PSE), although the latter is less likely to occur in ungulates (Swanepoel *et al.*, 2016). Dark, firm and dry meat is characterised by an increased ultimate pH 24 hours (pH_U) post-mortem (normally > 6.0) which, in turn, results in an increased water-holding capacity and darker coloured meat (Shange *et al.*, 2019). Meat characterised by a DFD condition is often less desirable to consumers (Viljoen *et al.*, 2002), therefore it is beneficial to reduce the ante-mortem stress experienced by culled animals, not only to manage welfare aspects but also to ensure an optimized marketing potential for the game meat.

The purpose of this investigation is to determine the influence of various commercial culling methods on blue wildebeest meat quality. The influence of the methodologies will be determined by comparing the physical meat quality and proximate composition of the meat samples obtained from the animals culled using a helicopter-, day- and night-culling methods. Associations between meat quality and serum cortisone and cortisol hormone levels, as determined in Chapter 3, will also be discussed.

5.3 Materials and Methods

Ethical approval for the trail was obtained from the Stellenbosch University Animal Care and Use Ethical Committee (ACU-2018-6598).

5.3.1 Experimental location and animals

This trial was conducted on the ROMACO Ranch in the Limpopo province. Meat samples were collected from 25 mature blue wildebeest cows that were older than 8 years and at the end of their optimal breeding life span.

5.3.2 Experimental treatments

The blue wildebeest were culled using three different methods, i.e. helicopter-cull, day-cull and night-cull.

- *Helicopter-cull* N = 8 blue wildebeest cows.
  The helicopter method was conducted between 06:00 and 12:00 and involved the use of a small helicopter to chase the animals being targeted by a marksman seated in the helicopter. The animals were shot in the head/high neck with a shotgun loaded with SSG lead bullets. The animals were exsanguinated in the field and vehicles were used to collect the culled animals and transport them to the abattoir within an hour of death.
- *Day-cull* N = 9 blue wildebeest cows.
  The day-cull method was conducted between 11:00 and 18:00 and used vehicles to chase and herd the animals before they were shot with a .308 rifle in the head/high neck by a marksman located on the back of the vehicle. The animals were exsanguinated in the field and loaded onto the vehicle to be transported to the abattoir within three hours of death.
- *Night-cull* N = 8 blue wildebeest cows.
  The night-cull was similar to the day-cull, however, was conducted at night, from 21:00 until 03:00, using one million candlelight spotlights.

For more details on the respective culling methods, please refer to Chapter 3.

5.3.3 Blood sampling and hormone analysis

Blood samples were collected in the field, immediately post-mortem from the jugular vein in lithium heparin vacutainer tubes. The blood samples were cooled to 4 °C and centrifuged at 4000 rpm for five minutes and allowed to settle. The serum was transferred into clearly marked cryotubes and stored at -20 °C.

For the hormone assays, standards and serum samples were prepared as described in Chapter 3. Serum samples were analysed using Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS). For further details on the blood hormone analysis, please refer to Chapter 3.

5.3.4 Processing of carcasses

The culled animals were transported to an on-farm registered abattoir (Certificate number: 2/4G) within three hours of death; all animals were still in a pre-rigor state when offloaded. Upon arrival

at the abattoir, blue wildebeest were offloaded from the vehicles and suspended from one hind leg and the *dead weight* was recorded. The animals were skinned and eviscerated, organs, skin, trotters and heads were placed into a crate to be separated and individually weighed to obtain organ weights. The carcasses were suspended by both hind legs and weighed to obtain the *warm carcass* weights before being clearly tagged and placed into a cold room, at 4 °C, for 24 hours.

5.3.5 pH profiles

At death, in the field, initial pH readings (pH_I) were taken at the location of the last rib in the LTL (*Longissimus thoracis et lumborum*) muscle of each animal using a Crison pH 25 pH meter (Crison Instruments, Barcelona, Spain). Throughout the first 24 hours post-mortem, various pH and temperature readings were taken at regular intervals; hourly for the first four hours post-mortem, thereafter every two hours until 12 hours post-mortem and then every four hours until processing, at 24 hours post-mortem. The ultimate pH reading (pH_U) was taken at processing.

Calibration of the pH meter was done before every session of readings, and was conducted as in Chapter 4. Care was taken to ensure the electrode was rinsed with distilled water and dried between each carcass reading. Varying temperature conditions had an effect on pH values therefore, the measured pH readings were adjusted to a constant temperature (4 °C) as in Chapter 4.

Ultimate pH values were used to classify the meat samples as either Normal ($5.30 \leq pH \geq 5.80$), High ($5.80 < pH > 6.00$) or DFD ($pH \geq 6.00$).

5.3.6 Meat sample collection and physical analysis

At processing, the chilled carcass was weighed to obtain the *cold carcass* weight. Thereafter, only the left LTL muscle was removed from the carcass for the purpose of this study. The muscle was de-membraned in preparation for physical and proximate analysis. Half the LTL was vacuum-packed and stored at -20 °C for proximate analysis. Two steaks, approximately two centimetres thick, were cut from the other half of the muscle for the purpose of physical analysis. Physical analysis, conducted as described in Chapter 4, commenced immediately after muscle removal.

One steak was weighed and suspended in an inflated polythene bag and hung in a refrigerator unit at 4 °C, caution was taken to avoid the meat from touching the sides of the bag. After 24 hours the sample was patted dry using absorbent paper and weighed, and *drip loss* was calculated as a percentage of the initial sample weight.

The second steak was left to bloom for 30 minutes before a Color-guide 45°/0° (BYK-Gardner GmbH, Gerestried, Germany) colorimeter was used to obtain the relevant colour parameter values. A total of five readings of each parameter were taken per meat sample and averages were calculated for the L*, a* and b* values. These values were used to calculate the Chroma and Hue angle values as in Chapter 4.

This same sample that was used to obtain the colour readings, was then weighed, and placed into a cooking bag that was sealed and placed into the water bath (80 °C) for one hour. The bag with the cooked sample was removed from the water bath, drained of liquid, and cooled at 4 °C. The cooled meat sample was patted dry and weighed to determine the *cooking loss*. *Cooking loss* was also calculated as a percentage of the initial sample weight. Cylindrical corers (1.27 cm Ø) were used to cut samples from the chilled meat samples, parallel to the fibre direction. The cut sample cores were then used to test the shear force in a Warner-Bratzler (kg/1.27 cm Ø). A minimum of six shear force readings were taken per animal and converted to Newton before an average shear force (N) value was calculated per animal culled. For more information regarding the meat quality analysis and equations used, refer to Chapter 4.

5.3.7 Proximate analysis

The frozen LTL samples were thawed thoroughly at 4 °C over-night and trimmed of excess fat, where applicable, before being cut into smaller pieces. The sample was then homogenised and thereafter placed into individual bags, vacuum-sealed and immediately frozen again (-20 °C) similar to Chapter 4. The following proximate analysis was performed:

- The moisture content (g/100g) per sample was determined according to the AOAC Official method 934.01 (AOAC International, 2002c).
- A Leco Nitrogen/Protein Determinator (FP528 – Leco Corporation) was used to determine protein content (g/100 g) according to the Dumas combustion method stipulated by the AOAC Official Method 992.15 (AOAC International, 2002b).
- Total fat content (g/100 g) was determined using a rapid solvent extraction method as described by Lee *et al.* (1966).
- The ash content (g/100 g) was determined as per the AOAC Official Method 942.05 (AOAC International, 2002a).

5.3.8 Data recorded

5.3.8.1 Exposure to culling activity

The camps that were targeted for this trial had been recently used for culling procedures, targeting impala culled for the purpose of previous chapters, therefore the blue wildebeest had been recently exposed to culling activities. Exposure scores were categorized as in previous chapters, regardless of which species was targeted in the previous culling activity.

- ‘No prior’: No exposure in at least a year.
- ‘Some’: Exposure to culling activity.
  Once or twice with 24 hours break between the culls.
- ‘Recurrent’: Herds that had been culled from consecutively and/or several times.

5.3.8.2 Chase Time

Time was recorded from the time the herd became aware of the culling team in the camp, until the animal was shot and killed. If multiple animals were culled from a herd in succession, the timer was left to run on. The chase time was divided into three categories: 'short', 'medium' and 'long'. Caution was taken to avoid chasing a single herd for long periods of time multiple times in a day. There was also an attempt to limit the length of chase times to less than 45 minutes if the animals were relaxed. However, if the animals fled and were chased intensely, there was an attempt to limit the chase time to 30 minutes.

- 'Short': > 5 minutes.
- 'Medium': 5 – 20 minutes.
- 'Long': > 20 minutes.

5.3.8.3 Number of shots required

The number of shots was categorized as either 'single' or 'multiple'. A shot that was fired but completely missed the animals was not considered as an extra stressor as multiple animals were culled within a herd and this was accounted for in the exposure scores.

- 'Single': Included all animals that were shot and killed using one bullet.
- 'Multiple': Allocated to all animals that were shot and injured by a bullet. It was required that these animals were to be shot again to kill them.

5.3.9 Statistical Analysis

All animals that were injured during the culling procedures were removed from the statistical analysis as they showed to be influential outliers although they will be discussed. The data was analysed using XLStat version 19.2 where descriptive statistics were utilised to explore the data and multi-factorial ANOVA analysis was performed to compare the helicopter-, day- and night-culling methods. Due to the small sample size and unbalanced nature of the data, prior exposure to culling activity, chase time and number of shots, could not be analysed although, these were considered when analysing and discussing outliers. Non-linear regression was used to determine the pH decay model. Fisher's least significant difference post-hoc tests were used to compare culling method differences and calculate LSMeans, and a p-value of less than 0.05 was considered significant.

5.4 Results

Many of the observations could not be statistically analysed due to a too small sample size and the unbalanced nature of the data, therefore only the influence of culling method will be considered in detail within this investigation.

5.4.1 The influence of culling method on carcass weights and organ yields of blue wildebeest

All organ weights were presented as a percentage of the dead weight and are presented in Table 5.1. Dead carcass weight differed between culling methods so that the day-culled animals had a lighter body weight than the helicopter- and night-culled animals (Day: 153.78 kg vs. Helicopter: 188.55 kg and Night: 180.68 kg; $p \leq 0.05$). The warm and cold carcass weights were also lighter for the day-culled animals than the helicopter-culled animals ($p = 0.01$). There were also tendencies observed that the day-culled animals had lighter warm and cold carcass weights than the night-culled animals ($p = 0.08$). Conversely the contribution of the head and tongue weights to the overall carcass weight were higher for the day-culled animals when compared to that of the helicopter-culled animals (Day: 7.51 % vs. Helicopter: 6.71 %; $p = 0.01$). Once the tongue was removed from the head, it was also weighed - results showed that the day-culled animals had a higher proportion of tongue weight to carcass weight than that of the night-culled animals (Day: 0.30 % vs. Night: 0.27 %; $p = 0.03$). Tendencies were also reported for the contribution weight of the trotters to the overall carcass weight whereby the helicopter-culled animals exhibited lower values when compared to the day- and night-culled animals (Helicopter: 1.98 % vs. Day: 2.16 % and Night: 2.17 %; $0.05 < p \leq 0.10$).

The contribution of the gastrointestinal tract (GIT) to the overall carcass weight was lower in the helicopter-culled animals than for the day- and night-culled animals (Helicopter: 19.24 % vs. Day: 22.81 % and Night: 23.18 %; $p \leq 0.05$). A tendency was noted for the heart contribution to the overall weight of the carcass to be higher for the day-culled animals than the night-culled animals (Day: 0.66 % vs. Night: 0.60 %; $p = 0.07$). It was also seen that the day-culled animals had heavier livers relative to their dead weight than the night-culled animals (Day: 1.14 % vs. Night: 1.02 %; $p = 0.05$). Overall, the organs made up a smaller percentage of dead weight in the helicopter animals than the day- and night-culled animals (Helicopter: 22.91 % vs. Day: 27.24 % and Night: 26.26 %; $p \leq 0.05$).

Table 5.1 A comparison on the carcass weight (mean ± SE) and percentage organ contribution to dead carcass weight (mean % ± SE) of blue wildebeest, as influenced by helicopter-culling, day-culling or night-culling.

	Helicopter	Day	Night	Range	p-value	CV
Dead Weight (kg)	188.55^{a}±4.44	153.78^{b}±9.13	180.68^{a}±8.49	111.20-214.60	0.01	0.15
Warm Carcass (kg)	101.75^{a}±3.47	81.43^{b}±6.41	95.05^{ab}±5.25	55.80-115.80	0.03	0.18
Cold Carcass (kg)	100.15^{a}±3.54	79.83^{b}±6.18	93.20^{ab}±5.18	55.20-114.60	0.03	0.18
Head (+ tongue, %)	6.71^{b}±0.13	7.51^{a}±0.27	7.11^{ab}±0.17	6.08-8.69	0.03	0.09
Trotters (%)	1.98±0.04	2.16±0.07	2.17±0.09	1.82-2.68	0.14	0.10
Skin (%)	8.99±0.28	9.11±0.36	8.65±0.21	7.21-10.82	0.52	0.09
GIT (%)	19.24^{b}±0.63	22.81^{a}±1.46	23.18^{a}±0.95	16.49-28.13	0.03	0.16
Tongue (%)	0.29^{ab}±0.01	0.30^{a}±0.01	0.27^{b}±0.01	0.24-0.34	0.09	0.10
Heart (%)	0.63±0.03	0.66±0.01	0.60±0.03	0.48-0.77	0.19	0.10
Lungs (%)	1.03±0.07	1.12±0.09	1.04±0.06	0.79-1.63	0.62	0.19
Liver (%)	1.10±0.03	1.14±0.05	1.02±0.05	0.85-1.37	0.14	0.12
Kidneys (%)	0.20±0.01	0.21±0.01	0.20±0.01	0.17-0.25	0.56	0.10
Spleen (%)	0.27±0.03	0.24±0.02	0.22±0.04	0.16-0.39	0.29	0.22
Organ Total (%)	22.91^{b}±0.74	27.24^{a}±1.66	26.26^{ab}±0.94	19.75-35.30	0.04	0.15

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation
%: a percentage of dead weight values

5.4.2 The influence of culling method on the pH parameters and profiles of blue wildebeest meat

In this study, the measured pH values were adjusted for temperature (4 °C) as in Chapter 4 and an exponential decay model ($y = a + be^{-ct}$) was fitted to the adjusted values. From this, the a-, b-

and c-constants were determined (Table 5.2). The R^2 values of the exponential decay models for individual animals were not ideal and varied between 0.03 and 0.92.

Tendencies suggested that the adjusted pH_I was higher in the LTL muscles of the night-culled animals than the helicopter-culled animals (Night: 7.42 vs Helicopter: 6.99; p = 0.06). Culling method did not have an effect on the adjusted pH_U of the LTL muscles. The a-constant was lower in the exponential decay models of pH data collected from the helicopter-culled animals than the day- and night-culled animals (Helicopter: -7.58 vs Day: 5.66 and Night: 5.52; $p \leq 0.05$). The b-constant was also affected by the culling method where the muscle pH profiles of the helicopter-culled animals had higher values and much larger within-group variance than that of the day- and night-culled animals (Helicopter: 14.56 vs. Day: 1.68 and Night: 1.69; $p \leq 0.05$).

Table 5.2 A comparison on the pH parameters (mean ± SE) of the blue wildebeest carcass, as influenced by helicopter-culling, day-culling or night-culling, and measured at the last rib in the LTL muscle.

	Helicopter	Day	Night	Range	p-value	CV
pH_I (Adjusted)	6.99±0.15	7.26±0.15	7.42±0.13	6.49-7.99	0.15	0.06
pH_U (Adjusted)	6.04±0.06	5.86±0.11	5.87±0.07	5.56-6.38	0.27	0.04
a	-7.58^{b}±0.52	5.66^{a}±0.17	5.52^{a}±0.41	-32.82-6.46	0.04	20.93
b	14.56^{a}±5.91	1.68^{b}±0.23	1.69^{ab}±0.07	0.64-39.72	0.07	1.73
c	1.19±0.59	2.02±0.54	2.50±0.59	0.01-4.51	0.30	0.83

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation
$y = a + be^{-ct}$ where $a = pH_U$ and b and c indicative the rate of decay

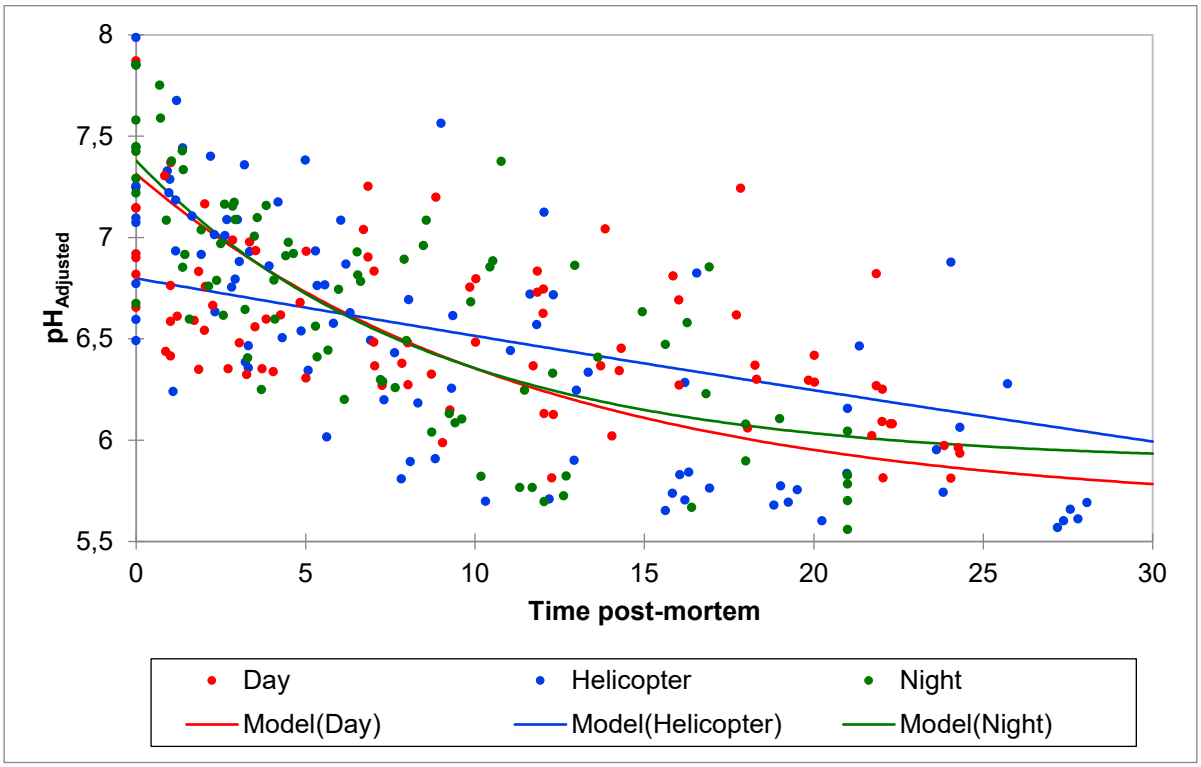

Figure 5.1 Non-linear regression showing the influence helicopter- (R^2 = 0.28), day- (R^2 = 0.63) and night-culling (R^2 = 0.59) on the pH profiles of the LTL muscles of day-culled blue wildebeest.

5.4.3 The influence of culling method on the water-holding capacity of blue wildebeest meat

The amount of weight lost due to drip and cooking loss was used to determine the water-holding capacity of the meat samples. These values were calculated as a percentage weight loss per parameter.

Culling method had no influence on drip loss ($p > 0.05$) however, cooking loss was higher in the meat samples from the helicopter-culled animals when compared to that of the day- and night-culled animals (Helicopter: 48.53 % vs. Day: 38.20 % and Night: 35.85 %; $p < 0.01$). The total moisture loss was also higher for the meat samples collected from the helicopter-culled animals than the other treatment groups ($p < 0.01$). The relevant results are displayed in Table 5.3.

Table 5.3 A comparison on the water-holding capacity parameters (mean ± SE) of the blue wildebeest carcass, as influenced by helicopter-culling, day-culling or night-culling, and measured at the last rib in the LTL muscle.

	Helicopter	Day	Night	Range	p-value	CV
Drip Loss (%)	1.12±0.10	1.18±0.11	1.42±0.28	0.67-3.19	0.48	0.41
Cooking Loss (%)	48.53^{a}±1.99	38.20^{b}±0.90	35.85^{b}±0.94	31.42-55.81	<0.01	0.16
Total Moisture Loss (%)	49.58^{a}±2.00	39.38^{b}±0.96	37.26^{b}±1.03	33.00-56.74	<0.01	0.15

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

5.4.4 The influence of culling method on the tenderness of blue wildebeest meat

Shear force values obtained were converted to Newton and considered an indication of meat tenderness. Culling method had an influence on shear force values where the meat samples obtained from the night-culled animals had higher shear force values than that of the helicopter-culled animals ($p = 0.034$). Tendencies also indicated that the meat samples obtained from the day-culled animals had lower shear force values than that of the night-culled animals ($p = 0.09$). All culling methods also exhibited high variances (Table 5.4).

Table 5.4 A comparison on the shear force and colour parameters (mean ± SE) of the blue wildebeest carcass, as influenced by helicopter-culling, day-culling or night-culling, and measured at the last rib in the LTL muscle.

	Helicopter	Day	Night	Range	p-value	CV
Shear Force (N)	32.90^{b}±2.92	34.73^{ab}±2.06	40.24^{a}±1.59	17.52-45.76	0.08	0.19
L*	30.57±0.80	31.58±1.15	31.18±0.36	27.50-36.48	0.71	0.08
a*	10.54±0.58	10.60±0.49	10.45±0.22	8.04-13.00	0.97	0.12
b*	7.88±0.48	8.07±0.51	8.54±0.20	6.07-10.60	0.56	0.15
Chroma	13.18±0.72	13.34±0.68	13.50±0.29	10.08-16.77	0.93	0.13
Hue	36.74^{b}±0.99	37.04^{b}±0.89	39.51^{a}±0.18	33.03-42.71	0.06	0.07

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

5.4.5 The influence of culling method on the colour of blue wildebeest meat

As mentioned previously, the colour parameters analysed were L*, a*, b*, Chroma and Hue angle values. All colour parameters were similar between treatment groups except for the Hue angle values (Table 5.4). The meat samples from the night-culled animals had higher hue angle values than those observed for helicopter- and day-culled animals (Night: 39.51 vs. Helicopter: 36.71 and Day: 37.04; $p \leq 0.05$).

5.4.6 The influence of culling method on the proximate composition of blue wildebeest meat

The only influences of culling method observed for proximate analysis was for fat (%) and ash (%) (Table 5.5). Fat content was higher in the meat samples from the helicopter-culled animals than night-culled animals (Helicopter: 1.70 % vs. Night: 1.31 %; $p < 0.01$). Fat content also showed a tendency to be higher in the meat samples from the day-culled animals when compared to that of the night-culled animals (Day: 1.53 % vs. Night: 1.31 %; $p = 0.06$). The average ash content in the meat samples obtained from the night-culled animals was 1.23 % which was higher than that of day-culled animals (1.18 %; $p = 0.04$). The meat samples from the day-culled animals also showed higher ash content than helicopter-culled animals (Day: 1.18 % vs. Helicopter: 1.13 %; $p = 0.04$). The meat samples collected from the helicopter- and night-culled animals also differed in ash content ($p < 0.01$).

Table 5.5 A comparison on the proximate composition (mean ± SE) of the blue wildebeest carcass, as influenced by helicopter-culling, day-culling or night-culling, and measured at the last rib in the LTL muscle.

	Helicopter	Day	Night	Range	p-value	CV
Moisture %	76.33±0.30	76.02±0.26	76.16±0.21	74.91-77.45	0.69	0.01
Protein % 'g/100g'	22.25±0.54	22.10±0.26	22.28±0.13	20.57-25.46	0.93	0.04
Fat %	1.70^{a}±0.05	1.53^{ab}±0.11	1.31^{b}±0.07	1.01-2.07	0.01	0.18
Ash %	1.13^{c}±0.01	1.18^{b}±0.02	1.23^{a}±0.02	1.09-1.29	< 0.01	0.05
Total	100.97±0.13	100.96±0.20	101.09±0.17	100.21-102.14	0.83	< 0.01

^{ab} Means with different superscripts within the same row differ significantly ($p \leq 0.05$)
CV - coefficient of variation

5.5 Discussion

Research on various ungulate species has provided evidence that culling method potentially affects meat quality (Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009a; Hoffman & Laubscher, 2009b; Hoffman & Laubscher, 2010). It has been assumed that the differences in meat quality found between culling methods were primarily due to stress however, this has always been an assumption.

The hormone concentrations that were determined for the blood samples obtained from the blue wildebeest in Chapter 3 differed significantly between culling methods. The blood samples obtained from the helicopter-culled animals had the highest serum concentrations of cortisone and cortisol (stress hormones). The serum glucocorticoid hormone levels followed the expected diurnal pattern, but based on the previous studies, the exact serum glucocorticoid concentrations were unknown. Therefore, it was unclear whether the measured concentrations of the hormones were in fact elevated from their normal, baseline concentrations as present under stress-free circumstances. It was assumed that the helicopter-culled animals were exposed to ante-mortem stress during the culling operation. It was, thus, expected that the meat samples from the helicopter-culled animals would exhibit the relevant stress-associated traits, the results of this trial supported this expectation.

5.5.1 The influence of culling method on the carcass and organ yields of blue wildebeest

As mentioned for the impala, the culling-related stress was considered as acute stress and therefore was not expected to have any detrimental effects on carcass yield in this trial. Since all animals were culled using head/neck shots, no losses of meat were anticipated due to bullet damage.

The differences seen between the dead-, warm- and cold carcass weights showed the helicopter-culled animals were heavier than the day-culled animals. There were no significant differences between the helicopter- and night-culled animals for the carcass weights however, a difference was noted between the day- and night-culled animals for dead weight. Although not significant, tendencies were observed suggesting differences between the warm- and cold carcass weights of the day- and night-culled animals. It is worth noting that although differences are observed between treatments for the warm- and cold carcass weights, when calculated as a percentage of the dead weight, no differences are observed between the helicopter-, day- and night-culled animals. This suggests that the weight differences were primarily due to the size of the animals culled and not due to organ weight differences. Although statistically significant, these differences carcass weights were not considered meaningful and could be attributed to variation within the population or that the larger animals were easier to target from a helicopter as well as at night. The dead weights of the day-culled animals were lighter than Furstenburg's (2013) expected 170-200 kg and Hoffman *et al.*'s (2011) 170±43.4 kg however, the carcass weights of

all treatment groups exceeded the expected 88 kg (Hoffman *et al.*, 2011) most probably because these cows were all old, mature animals whilst that of Hoffman were various ages.

5.5.2 The influence of culling method on the pH parameters of blue wildebeest meat

As discussed in Chapter 4, there is a natural decline of muscle pH at death due to a lack of oxygen and circulation after exsanguination. Acute stress from culling activities, has been shown to result in lower ultimate pH values of the muscles 24 hours post-mortem than less stressed animals. This leads to meat with a reduced water-holding capacity and being paler in colour (Swatland, 2004) although this is primarily applicable to pigs. Chronic ante-mortem stress, on the other hand, results in an increased ultimate muscle pH (i.e. at 24 hours post-mortem), ensuing dark, firm and dry (DFD) meat in ruminants (Wiklund *et al.*, 2001; Guàrdia *et al.*, 2005; Adzitey & Nurul, 2011; Shange *et al.*, 2019).

As the helicopter-culled animals were chased intensively prior to being shot, there was an expectation that they would have a decreased initial LTL muscle pH value due to a higher lactate concentration (Cockram *et al.*, 2011). This was the case; and a tendency was also seen for the LTL muscles of the night-culled animals to have a higher initial pH value than that of the helicopter-culled animals. Lactate concentrations were however, not measured in the current investigation; therefore, this remains an assumption. The initial muscle pH values in this study were higher than the mean 6.60±0.55 found by Hoffman *et al.* (2011) in a study that included blue wildebeest bulls and cows of various ages which could have accounted for the large variation in their study. Similar studies were conducted on other ungulate species; Kritzinger *et al.* (2003) found that muscles of the night-culled impala had significantly higher pH values 45 minutes post-mortem than the day-culled animals although, this was attributed to increased day-time activity.

Serum concentrations of glucocorticoids, cortisol and cortisone, were measured for these animals and discussed in Chapter 3. Results provided evidence that the helicopter-culled animals were more stressed than the day- and night-culled animals. This led to the expectation that the muscles in the helicopter-culled animals would show higher pH_U values. However, there was a weak positive correlation between serum glucocorticoid levels and muscle pH parameters in this investigation (Table 5.6). No statistical differences were detected between the treatment groups for ultimate muscle pH values, although this could be attributed to the high variation between animals between and within the main treatment groups (Table 5.2).

The LTL of the helicopter-culled animals, however, was characterised by a pH_U value indicative of DFD meat, which can potentially be the reason for the unexpected correlations seen in Table 5.6. From Figure 5.2 it is evident that none of the helicopter-culled animals had a normal LTL pH_U and half the animals from this treatment had DFD classed meat. Helicopter-culling is typically characterised by a low wounding percentage and high efficiency rate of culling (number of animals per time unit) which can be attributed to increased visibility, the ability to get close to the animal to take a shot as well as increased agility of the helicopter and marksman during the

chase (Hampton *et al.*, 2014). While helicopter-culling is known to create some stress as referenced in Hampton *et al.* (2017), chronic stress, as determined in the current investigation was not anticipated and could potentially be attributed to influential factors other than the culling method. The helicopter-culled animals were chased at high intensity for short periods of time which could have resulted in reduced glycogen levels, also a known cause of DFD meat (Guàrdia *et al.*, 2005; Adzitey & Nurul, 2011), however, as glycogen was not measured, this theory remains speculation.

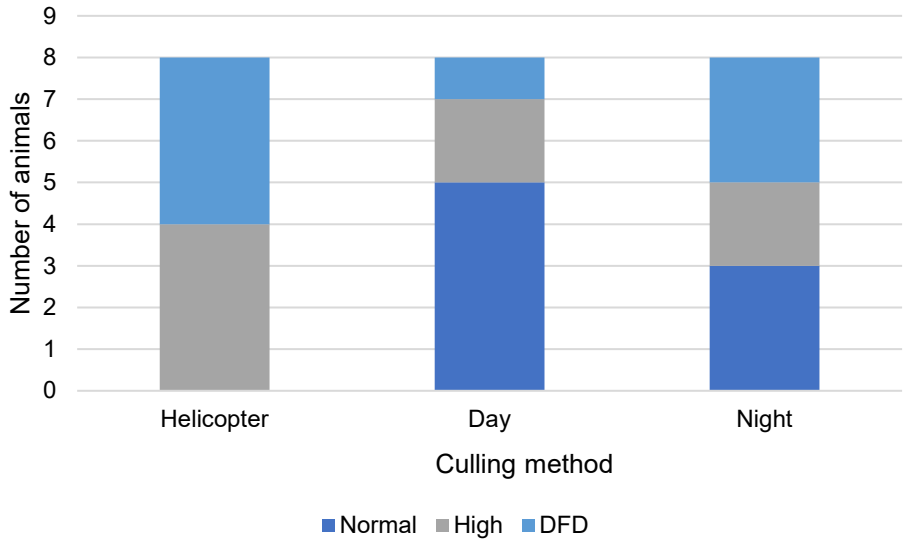

Figure 5.2 Histogram showing the number of occurrences of normal, high and DFD classed pH_U values of the LTL muscles of helicopter-, day- and night-culled blue wildebeest.

In the current investigation a large amount of culling was done in a short period of time and the helicopter-culling involves an intense chase. It is possible that the blue wildebeest were unintentionally chased whilst the impala were being targeted thus resulting in a prolonged state of stress. To better understand the outcomes of this investigation, further research is required on exposure to prior culling activity. Similar studies would benefit by ensuring the animals are not exposed to other culling activity for a period of time before being targeted, Veary (1991) suggested a minimum of two days. Similar recommendations could be made for the other culling methods although these methods did not seem to have been as affected by previous exposure within this study.

Table 5.6 The correlation coefficient values indicating the relationship of serum cortisone and cortisol levels of culled blue wildebeest with pH_I and pH_U values as well as drip and cooking loss measured from samples from the LTL muscle.

	Cortisone	**Cortisol**
pH_I (Adjusted)	-0.12 (p = 0.69)	-0.15 (p = 0.61)
pH_U (Adjusted)	-0.05 (p = 0.87)	-0.07 (p = 0.82)
Drip Loss (%)	029 (p = 0.32)	0.41 (p = 0.15)
Cooking Loss (%)	0.18 (p = 0.54)	0.04 (p = 0.89)

Significant differences were seen for the a- and b-constants of the pH exponential decay models. The a-constant was lower for the muscle pH profiles of the helicopter-culled animals than that of the day- and night-culled animals whereas the b-constant was higher in the exponential decay models of the muscles of the helicopter-culled animals than the other two treatment groups. The pH decline (b-constant) was observed to be extremely high for muscles of some animals but low for others within a treatment group, there is no clear trend as to the reason for this variation.

Studies have indicated that ante-mortem stress is often accompanied by increased serum cortisol concentrations and higher ultimate muscle pH values due to a decreased acidification potential resulting from glycogen mobilisation and a build-up of lactic acid in the muscles (Hoffman & Laubscher, 2009b). The ultimate muscle pH values were within a normal range for the day- and night-culled animals while the LTL muscles of the helicopter-culled animals exhibited values suggesting a DFD condition. The camps where the helicopter-culling procedures were conducted had been recently used for culling impala using similar culling procedures, it is possible that this stressed the blue wildebeest prior to them being targeted subjecting them to stress of a more chronic nature. Meyer *et al.* (2008) indicated that ungulates are able to retain memories and can be habituated to human behaviour, whether positively or negatively - this indicates that it was likely that the blue wildebeest had an existing memory of the helicopter-culling activities. This memory could have resulted in increased chronic ante-mortem stress of the animals compared to animals who had no prior exposure to helicopter-culling methods.

In Chapter 3, three influential outliers were identified, in the current chapter the outlier that was previously notated as (a) was not an outlier however, it was observed that the muscles samples from this animal had a pH_U of 6.27 which is a possible indication of ante-mortem stress caused by the injury and extended chase time (45 minutes) of this animal. However, the meat from the remaining two outliers from Chapter 3, was similar to that of the other animals.

In this trial there was a total of five blue wildebeest that were injured during the culling procedures, one of which has already been discussed. Of the remaining injured animals, only two showed pH_U values above 6.0. One of which was a cow that had been severely injured and was therefore highly stressed and removed from the analysis of this investigation. The other animal was one that was culled during a night-cull but had been previously exposed to recurrent culling activity which possibly resulted in long-term stress. It is worth noting that these animals, except the one that was removed from the study, were not classified as influential outliers in this trial.

5.5.3 The influence of culling method on the water-holding capacity of blue wildebeest meat

As discussed, there is a natural acidification of muscles post-mortem and consequently, moisture loss from the meat due to proteolysis as well as a decrease in the electronic charge of the proteins (Kritzinger *et al.*, 2003; Huff-Lonergan & Lonergan, 2005). When animals are exposed to acute ante-mortem stress, the ultimate pH of the muscle is lower than in animals that are not exposed to stress. This means that the meat is more acidic in stressed animals, which results in increased proteolytic activity and thus, a decreased water-holding capacity of the meat (Hoffman & Laubscher, 2009b). The opposite is expected when animals are exposed to chronic stress where there is consequent glycogen depletion in the muscles resulting in higher ultimate muscle pH. Higher ultimate muscle pH results in the extracellular spaces in the muscle being smaller due to a decreased rate of proteolysis in the muscle and thus, there is a higher water-holding capacity, whilst at the same time the muscle protein have stronger electronic charges which strengthen the Van der Waal's forces (Viljoen *et al.*, 2002; Warris, 2010). In the current investigation water-holding capacity was analysed as drip loss-, cooking loss- and combined moisture loss percentage (Drip Loss + Cooking Loss).

Since the pH_U values of the muscles from the helicopter-culled animals were higher than 6.0, it was expected that the meat from the animals of this treatment group would have an increased water-holding capacity. Although the correlations shown in Table 5.7 indicate an extremely weak positive correlation between ultimate pH and water-holding capacity, of this investigation however a correlation of 0.75 was observed between the rate of decay (b-constant) and water-holding capacity. Therefore, as the muscles from helicopter-culled animals had the fastest decay rate, it was anticipated that the meat samples from the helicopter-culled animals would have the lowest water-holding capacity. This expectation was supported by the data. The meat samples obtained from the helicopter-culled animals had the most cooking loss and total moisture loss which did not support the expected meat quality associated with DFD meat. It is worth noting that of the helicopter-culled animals, no meat samples from this treatment group had a normal pH_U (Figure 5.2).

Table 5.7 The correlation coefficient values indicating the relationship of pH_U and b-constant values with water-holding capacity, tenderness and colour parameters of culled blue wildebeest, regardless of culling method.

	pH_U	**b**
Total moisture loss %	0.04 (p = 0.90)	0.75 (p < 0.01)
Shear Force (N)	0.27 (p = 0.36)	0.01 (p = 0.97)
L*	-0.71 (p = 0.01)	-0.19 (p = 0.53)
a*	-0.31 (p = 0.28)	0.33 (p = 0.25)
b*	-0.46 (p = 0.10)	0.14 (p = 0.63)
Chroma	-0.39 (p = 0.16)	0.27 (p = 0.36)
Hue	-0.30 (p = 0.29)	-0.19 (p = 0.53)

No significant differences were noted for muscle pH parameters between the day- and night-culled animals resulting in no differences for the water-holding capacity parameters in the meat samples from the animals of these treatments. These results were similar to those found by Hoffman and Laubscher (2010) where they observed no differences in water-holding capacity of day- and night-culled gemsbok.

The drip loss values in the current investigation of the meat samples were similar to those found by Van Heerden (2018) (average of 1.6 %) however, were lower than those found by Hoffman *et al.* (2011) who found a mean value of 4.05 %. Similar to the current investigation, Van Heerden (2018) analysed animals from the Limpopo province however Hoffman *et al.* (2011) analysed animals from the Free State province - this suggests a possible influence of ante-mortem stress as well as the environment in which they were processed.

5.5.4 The influence of culling method on the tenderness of blue wildebeest meat

Both pH and water-holding capacity of meat have been shown to influence the perceived tenderness of the meat (Guignot *et al.*, 1994; Hoffman & Laubscher, 2010; Hughes *et al.*, 2014a). There is no clear expectation as to the effects of stress on tenderness of meat although, Silva *et al.* (1999) indicated that chronic stress, leading to DFD meat, causes high variation of tenderness. A more accurate indication of tenderness is possibly pH_U values of the muscles. A muscle pH_U of 6.0 has been shown to result in a high shear force of the meat, suggesting that the meat would be extremely tough. As the pH of the muscle is increased further, the meat becomes more tender

again (Purchas, 1990). However, the correlation values presented in Table 5.7 show weak positive correlations of ultimate pH and rate of decay to tenderness.

The average pH_U value of the meat samples from the helicopter-culled animals was 6.04 which led to an expectation that the meat from these animals would be extremely tough. The results from the current investigation were not in agreement with this expectation (Table 5.4). Hughes *et al.* (2014a) found evidence suggesting that with an increased water-holding capacity, meat samples become more tender. Therefore, there was a conflicting expectation that since the meat samples obtained from the helicopter-culled animals had the highest water-holding capacity, those meat samples would also be the most tender, as found; the meat samples obtained from the helicopter-culled animals had the lowest shear force. These values were similar to those found by Van Heerden (2018).

5.5.5 The influence of culling method on the colour of blue wildebeest meat

Game meat is naturally darker in colour than that of domestic species, and various studies have attributed this to the stress associated with culling procedures (Von La Chevallerie & Van Zyl, 1971; Von La Chevallerie, 1972; Kritzinger *et al.*, 2003; Hoffman & Laubscher, 2009b) as well as due to increased myoglobin content (Hoffman, 2000a; Kritzinger *et al.*, 2003). Hughes *et al.* (2014a) also found that the perceived colour of meat, as influenced by light scattering properties, was influenced by the water-holding capacity.

The LTL muscles of the helicopter-culled animals of the current investigation showed pH_U values that were typical of DFD meat. There was therefore an expectation that the L* values would be lower in the meat samples obtained from the helicopter-culled animals than that of the day- and night-culled animals. Contrary to expectations, no statistical differences were seen between the meat samples from animals from different treatment groups for the L*, a*, b* or Chroma colour parameters. However, the correlations in Table 5.7 showed a correlation coefficient of -0.71 between ultimate pH and L* values thus supporting that higher ultimate pH results in lower L* values in meat. The lack of difference between culling was potentially due to the high variation between the animals within each treatment group. All the aforementioned parameters' values were similar to those found by Van Heerden (2018).

Studies have shown that DFD meat has lower hue angle values than normal and PSE meat (Adzitey & Nurul, 2011) therefore, the meat samples collected from the helicopter-culled animals were expected to conform to having the lowest hue angle values. The results of the current investigation supported these findings (Table 5.4).

5.5.6 The influence of culling method on the proximate composition of blue wildebeest meat

The moisture and protein content of the meat samples from the animals in the current investigation were similar to that of Hoffman *et al.* (2011) although, the fat content found in that

study was lower (1.06 %) than the current investigation (Table 5.5). The animals observed in Hoffman *et al.* (2011) were males and females of different ages, thus providing a possible explanation for the lower average fat content of the meat. All the blue wildebeest in the current study were mature cows and Hoffman *et al.* (2009) found that female animals have higher fat content in their meat than that of males.

5.6 Conclusions

The meat obtained from various blue wildebeest culled using different methods exhibited characteristics commonly associated with DFD meat. DFD meat is usually as a result of chronic ante-mortem stress which was not expected to be a factor in this investigation where the focus was on acute culling-associated stress. The meat samples obtained from the helicopter-culled animals showed high ultimate pH values as well as decreased water-holding capacity and the most tender meat. However, conclusions could not be made regarding the helicopter-culling method in this study due to the results indicating that the animals possibly experienced chronic stress. Throughout the investigation, outliers were found however, all were from the helicopter-cull group thus emphasising the excessive amount of stress those animals had experienced. No differences were observed between the meat samples obtained from the day- and night-culled animals of this study suggesting that both culling methods were acceptable methods for commercial use.

Due to the large within-group variation in the meat samples of the treatments, more accurate conclusions could be deduced from the correlation of the meat quality attributes to ultimate pH and rate of pH decay. To conclude, meat samples with a higher ultimate had lower L* values while meat samples with a faster rate of pH decay had a reduced water-holding capacity.

It would be beneficial to analyse the influence of culling-associated activities on the stress levels of the remaining herd members post-cull. This will determine if these animals experience residual stress and what an optimal rest period would be before the herd is culled from again to reduce the occurrence of chronic stress.

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Chapter 6

General conclusions and recommendations

This study investigated the effects of culling method (i.e. helicopter-, day- and night-culling) on ante-mortem stress experienced by impala (*Aepyceros melampus*) and blue wildebeest (*Connochaetes taurinus*), and the potential resulting influence on meat quality parameters. Serum concentrations of the glucocorticoids, cortisol and cortisone, were determined in blood samples collected immediately post-mortem, and related to meat quality characteristics. A comparison of shot placement was also analysed to determine if the use of a head/neck or body shot had an influence on the meat quality of day-culled sub-adult impala rams. Ultimately the research conducted in the current investigation elucidated on the effects of culling methods on impala and blue wildebeest meat quality, highlighting the associated stress and the influences thereof. This study will benefit the industry by ensuring animal welfare is maintained during culling procedures and meat quality is of an optimal standard for the commercial market. This study may also assist in improving the profitability of the game meat market by ensuring sceptical consumers that the animals were culled in the most humane manner without negatively affecting the meat quality.

6.1 The influence of culling method and shot placement on the ante-mortem stress and consequent meat quality of sub-adult impala rams

Hormone analysis indicated that serum cortisone levels were elevated in animals culled at night, when compared to that of the day- and helicopter-culled animals. Glucocorticoid hormone concentrations are naturally regulated according to a diurnal rhythm, and in impala, the night-time serum cortisone and cortisol levels deviated from the expected levels reported for these animals during night periods.

There was a tendency for pH_U values to be lowest in the meat samples from the night-culled animals, therefore indicating a typical acute ante-mortem stress response. Contrary to the expected influence of acute ante-mortem stress, drip loss and total moisture loss were lowest in the meat samples obtained from the night-culled impala thus indicating that the meat samples from the night-culled animals had the highest water-holding capacity. A higher water-holding capacity is usually indicative of more tender meat, which was the case in this study. Tendencies showed that the meat samples from the night-culled animals had the lowest shear force values and were thus considered to be the most tender meat.

The *Longissimus thoracis et lumborum* (LTL) muscles of the helicopter- and day-culled animals did not differ in terms of meat quality except for the pH_I values and the b-constant values of the pH exponential decay model. The LTL muscles of the day- and night-culled animals had lower initial pH values when compared to that of the helicopter-culled animals although, it was postulated that the higher lactate concentrations recorded may potentially be ascribed to an

increase in the duration of physical activity. Lactate concentrations were not measured therefore, this remains speculation.

Studies of a similar nature would also benefit in quantifying lactate and glycogen concentrations in the muscle and blood samples post-mortem. Studies have indicated that lactic acid could be used as an indication of meat quality and ante-mortem stress. More research regarding the influence of lactic acid and glycogen levels at death, on meat quality could provide further understanding of reported results on pH and water-holding capacity in this study.

It was concluded that the night-culled impala had experienced increased levels of ante-mortem stress than the day- and helicopter-culled animals. The day- and helicopter-culling methods showed to have minimal influences on the meat quality obtained from the impala and could therefore, be considered appropriate methodologies for commercially culling impala.

There may also be residual stress on the remaining herd members although it is unknown whether the animals can smell the blood or if they are able to comprehend what has happened when their herd member has been shot. Further neurological research would be beneficial in determining the impact of culling procedures on the remaining herd members.

The shot placement during day-culls appeared to have an influence on pH_l and b-constants (rate of change) of the pH decay models fitted. The LTL muscles of the head/neck shot animals had higher initial pH values however, the reasons for which were unknown and require further research. The meat samples obtained from the body-shot animals had a higher percentage of cooking loss but there was no difference between meat samples from the treatment groups when analysing for total moisture loss. Shot placement also showed to have no effect on shear force or colour parameters of the meat samples, suggesting that shot placement does not have an influence on meat quality; it should be recognised though that most of the animals shot in the shoulder region dropped quickly and that if this was not the case, the results might differ.

The effects of firearm, behaviour prior to being shot, physiological score immediately post-mortem and chase time could not be analysed in this investigation due to a too small sample size and unbalanced nature of the data. This would provide evidence as to the effects of these, commonly overlooked, parameters on meat quality as well as to encourage the improvement of welfare protocols where necessary.

The influence of sex on the effects of stress on meat quality are unclear. It would be beneficial to compare the stress-influence on the meat of ewes vs. that of the rams that are culled using the same culling method. This could be done within a larger experiment as a sub-experiment although the sample size of the current investigation was too small.

Normally in a commercial scenario, only head and high neck shots are acceptable. In the present investigation, shoulder shot (on the impala) were also included as this is typical of shots taken by hunters and would be of value to know what the effect thereof is on the meat quality as some hunters, particularly trophy hunters, favour this shot and regularly sell the meat to the primary processing facility. In future studies, the effect of this shot placement on carcass losses

as well as meat hygiene (caused by bacteria contamination of gut shot) and safety (physical from bullet and bone fragments) could be evaluated.

6.2 The influence of culling method on the ante-mortem stress and consequent meat quality of mature blue wildebeest cows

The serum cortisol and cortisone levels immediately post-mortem were highest in the blood samples obtained from the helicopter-culled animals. At the time of the helicopter-cull, serum glucocorticoid levels are expected to be at their highest concentration, according to the typical diurnal pattern. However, the baseline concentrations of these hormones, as present in stress-free circumstances, are unknown. Therefore, for the purpose of this investigation, it was assumed that the elevated serum glucocorticoid hormones found in the blood samples obtained from the helicopter-culled animals was attributed to an activated stress response, which was then confirmed by the meat quality parameters.

Research of a similar nature would benefit in reducing the effects of the diurnal rhythm by culling only within a limited time period; although this would automatically eliminate some of the treatments such as night-culling which is commonly used during commercial culling procedures. This will assist in reducing unnecessary variation within treatment groups. Further research regarding base-line serum hormone concentrations as well as their ratios, for cortisol and cortisone would also be beneficial. Cortisone is converted to cortisol during a stress response therefore there is a shift in the balance of these hormones favouring cortisol, this would provide further insight into the stress response. The expected diurnal rhythms of this study were compiled using previous research however, it is possible that the animals from those studies had been exposed to some form of stress, therefore the figures in this study were purely used for context. To conclude on the degree of elevation of hormone concentrations more accurately, precise base-line values are required so that deviation from the norm could be calculated and analysed rather than the analysis of measured values.

Meat samples obtained from all the treatment groups exhibited ultimate pH values that are typically associated with DFD (dry, firm and dark) meat. The meat samples from the helicopter-culled animals had a pH_U above 6.0. Although this was not statistically different from the values measured in the meat samples of the day- and night-culled animals, it was considered biologically significant and was supported by other meat quality parameters. Cooking loss and total moisture loss was higher in meat samples obtained from the helicopter-culled animals which indicated a decreased water-holding capacity. The meat samples obtained from the helicopter-culled animals also exhibited a lower shear force value than that from the night-culled animals which suggested that the meat from the former was more tender. No differences were noted between the day- and night-culled animals. Overall, it was apparent that some of the animals of this trial had been exposed to chronic ante-mortem stress that resulted in increased ultimate pH values which correlated to decreased L* values as well as an increased rate of pH decay which correlated to a

lower water-holding capacity of the meat samples. To the best of our knowledge, this was the first study of its kind, focusing on blue wildebeest therefore, more research is required to verify the results of this study.

There are varying opinions about the limitations of acute and chronic stress. It would be beneficial for research to be conducted on determining acute and chronic stress boundaries, this could also allow farmers and researchers to determine over-stressed animals and eliminate the cause of the stress before the detrimental effects of chronic stress on the meat quality materialise. It has been suggested that hair samples provide reliable information regarding chronic stress, whereby the analysis of the DHEA/cortisol ratio provides insight to the long-term functioning of the HPA axis and relative stress responses. The collection and analysis of hair samples would be less invasive than other suggested samples such as blood and saliva.

It was speculated in this investigation that the chronic ante-mortem stress was caused by exposure to prior culling procedures however this could not be appropriately analysed in this study due to a small sample size. In order to improve upon this investigation and achieve reliable results, it is recommended that culling procedures are performed in such a way that animals are allowed enough resting time between hunts. Studies have found that prior experiences of animals to culling activity influences the welfare of animals that retain memory of past experiences; therefore, the wildlife industry would benefit from further research of the influence of such parameters on ante-mortem stress and meat quality.

The amount of stress that is potentially experienced by the remaining members of the herd is unknown. Since the remaining herd members are also chased, it is assumed that they are experiencing the same stress as the animals who are physically shot and killed, if a herd is culled from multiple times, it is possible that the stress experienced could become that of a chronic nature, posing welfare violations. Further research on the stress experienced by remaining herd members, post-cull, would be beneficial in understanding the implications of culling procedures on the animals that are not culled.

As pertaining to the three culling methods evaluated, there are also other factors that need to be included in the decision of which method is more appropriate. These factors were not taken into account and warrant further research. These include, costs, efficiency – both in offtake numbers per time unit as well as retrieval of animals and transport to the abattoir, terrain, etc.

ADDENDUM I

Table 1 The serum steroid hormone concentrations (mean ± SE) of impala, as influenced by helicopter-culling, day-culling or night-culling, and measured in blood samples collected immediately after death.

Hormone (nmol/L)	Helicopter	Day	Night	p-value
Androstenedione	0.139±0.004	0.236±0.019	0.165±0.002	0.306
11OHA4	0.188±0.003	0.228±0.002	0.256±0.009	0.399
DHEA	0.932±0.358	2.252±0.252	0.761±0.247	<0.01
DHT	0.135±0.006	0.122±0.009	0.157±0.008	0.855
11KA4	0.068±0.000	0.078±0.002	0.052±0.000	0.478
11KT	0.118±0.004	0.099±0.002	0.145±0.007	0.592
Progesterone	0.084±0.001	0.082±0.001	0.048±0.000	0.224
17OHP4	0.044±0.000	0.050±0.001	1.894±1.750	0.007

Table 2 The serum steroid hormone concentrations (mean ± SE) of blue wildebeest, as influenced by helicopter-culling, day-culling or night-culling, and measured in blood samples collected immediately after death.

Hormones (nmol/L)	Helicopter	Day	Night	p-value
Androstenedione	0.633±0.178	0.249±0.166	0.601±0.166	0.228
11OHA4	1.633±0.256	0.913±0.256	0.868±0.256	0.083
DHEA	2.104±0.313	1.889±0.334	1.805±0.313	0.788
DHT	0.136±0.041	0.189±0.044	0.131±0.047	0.589
11KA4	0.063±0.016	0.088±0.014	0.116±0.014	0.061
11KT	0.139±0.023	0.107±0.023	0.105±0.024	0.508
Progesterone	2.544±0.905	2.722±1.045	4.156±0.968	0.445
17OHP4	0.832±0.208	0.524±0.240	1.150±0.263	0.243