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Captive bolt or free-bullet? An influence of fallow deer slaughter on behaviour, physiology and meat quality

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled "Captive bolt or free- bullet? An influence of fallow deer slaughter on behaviour, physiology and meat quality" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 15t[h] August 2024

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Aline dos Santos Lanes

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Abstract,

This study explores the impact of captive bolt and free-bullet slaughter methods on the behaviour and meat quality of farmed fallow deer (*Dama dama*). Altogether 20 fallow deer were studied divided into two groups of 10 animals in each according to treatment. The behavioural analysis indicated a significant increase of stress-related behaviour such as aggression and alertness in deer slaughtered by the captive bolt method. In contrast, the free-bullet method, which involved less human interaction, was associated with a higher percentage of movement behaviour and reduced stress behaviours. The physical attributes of meat quality were measured on muscles *Biceps femoris*, *Longissimus lumborum* and *Triceps brachii*. The analyses revealed that while there were no significant differences in all three muscles pH between the two slaughter methods, the captive bolt technique resulted in significantly tougher meat only in the *Biceps femoris* muscle measured by Warner-Bratzler shear force (WBSF). The free-bullet method resulted in less carcass and external damage measured by extent and number of body parts with bruises, highlighting its potential for producing higher-quality meat with less physical trauma to the animals. Other measured parameters like cooking loss and meat colour remained comparable in all three muscles based on slaughter method. The study highlights the importance of specific slaughter methods to minimise stressful aggressive behaviour resulting in bruises on carcass. On the other hand, there were no differences in three muscles pH and some physical attributes, but it can be caused by fact, that in both slaughter days, there was slaughtered five animals in each group. Therefore, those findings have practical implications for the farmed deer meat industry, particularly in optimizing slaughter practices. Future research should involve modification in number of animals slaughtered by each method to explore hormonal changes.

Key words: behaviour, deer meat quality, stunning,

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List of the abbreviations used in the thesis

ATP - Adenosine Triphosphate

a* - Redness of meat

b* - Yellowness of meat

L* - Lightness of meat

BCS - Body Condition Score

BF - *Biceps femoris*

DFD - dark, firm, and dry

LL - *Longissimus lumborum*

PSE - pale, soft, and exudative

TB - *Triceps brachii*

WBSF - Warner-Bratzler Shear Force

WBSF N - Warner-Bratzler Shear Force in Newtons

JLMN – Generalised Linear Mixed Model

LSMEANs – Least-squares-mean

1. Introduction and Literature Review

1.1. Introduction

The fallow deer (*Dama dama*) has become increasingly significant within the game industry due to its rich nutritional profile and high-quality meat (Ivanović et al. 2020). Despite this, concerns about how different slaughter methods influence both meat quality and animal welfare are growing (Hafiz et al. 2015; Viegas et al. 2012). While the effects of slaughter techniques have been extensively studied in traditional livestock, there is a distinct lack of comprehensive research on game species like fallow deer, which are not accustomed to human interaction and handling (Zidon et al. 2009). Fallow deers have unique behavioral and stress responses, making the impact of slaughter methods on them less predictable (Bykowska 2018).

The choice of slaughter method, including stunning, plays a critical role in determining both the physiological responses of the animal and the ultimate quality of the meat (Mulley et al. 2010). It is well-established that these procedures must be tailored to each species to ensure optimal outcomes. Pre-slaughter stress in cattle, encompassing transportation, handling, and environmental factors, can significantly impact meat and carcass quality (Choe 2018).

Research has shown that the method of stunning significantly influences the physical and biochemical properties of meat (Mulley et al. 2010). For instance, Gregory (2008) highlighted how the rate of post-mortem pH decline, a crucial determinant of meat quality, is affected by stunning methods. Rapid pH decline can lead to undesirable meat qualities such as pale, soft, and exudative (PSE) conditions, while a slower decline can cause dark, firm, and dry (DFD) meat, both of which are less favorable (Gregory 2008). Furthermore, stress induced during pre-slaughter operations can exacerbate behavioral responses, increasing the likelihood of carcass damage and impacting meat attributes like color, pH, and tenderness (Bourguet 2011).

In the context of fallow deer, some farms have integrated handling into routine management, leading to slaughter within controlled environments using captive bolt methods. On-farm slaughter can reduce pre-slaughter stress by eliminating transportation and unfamiliar environments (Hultgren et al., 2018). However, challenges remain in terms of food safety, waste management, and public health (Troeger 2004). The implementation of on-farm slaughter varies across species and production systems, with some animals still being transported to abattoirs (Needham & Hoffman 2022). While captive bolt stunning is common in conventional livestock processing due to its effectiveness in ensuring rapid unconsciousness and minimizing suffering, it may be less appropriate for fallow deer. Fallow deer have a natural flight response and are prone to heightened stress when restrained (Adzitey 2011).

Conversely, the free-bullet method, often used in game hunting, allows deer to remain in a more natural state until slaughter, potentially reducing stress. However, this method's success depended on some factors, such as, shooting accuracy include firing position, time available, aiming point, target visibility, and shooter experience (Aebischer et al. 2014).

The variation in pre-slaughter stress and killing efficacy between these methods inevitably leads to inconsistent meat quality. The game meat industry globally faces challenges related to this variability, and scientific studies that provide data to support species-specific guidelines are instrumental in addressing these issues.

To fill this research gap, this study aims to compare the effects of captive bolt and free-bullet slaughter methods on the behavior and meat quality of fallow deer (*Dama dama*). By gaining insights into the effects of each method, this research could contribute to optimizing slaughter practices and enhancing meat quality within the game meat industry.

1.2. Literature Review

1.2.1. The game industry and fallow deer

In recent years, the game industry has seen significant expansion, driven by increasing consumer demand for exotic and sustainable meat products (Branciari & Ranucci, 2022). Fallow deer, originally native to the Mediterranean region, have been introduced to various parts of the world, including Europe, North America, South Africa, and Australasia, due to their adaptability and relatively low maintenance requirements (Carpio et al. 2017). Game farms have capitalized on this, promoting fallow deer as a premium meat source, fallow deer have become an important species in game management and farming across Europe and New Zealand. Their population in Europe has increased five-fold since 1984, with a corresponding six-fold increase in harvest (Bijl & Csányi 2022).

The economic viability of fallow deer management in mixed landscapes has been demonstrated in Sweden, where the commercial value of hunting outweighs crop damage costs (Menichetti et al. 2019). However, as populations continue to grow, there is a need for consistent monitoring and responsible management to balance the economic benefits with potential negative impacts on agriculture and ecosystems (Bijl & Csányi 2022). Fallow deer have demonstrated remarkable adaptability to various environments, contributing to their widespread distribution globally. Their ability to thrive in diverse habitats, including arid regions, is evidenced by dental microwear texture analysis, which reveals their selective feeding strategies (Berlioz et al., 2017). Recent studies have explored the distribution and dynamics of fallow deer (*Dama dama*) in tropical and subtropical regions. In Tasmania, Australia, a previously stable population has shown rapid growth, with an 11.5% annual increase from 1985 to 2019, expanding to cover 27% of the island's land area (Cunningham et al. 2022). In tropical Brazil, fallow deer maintained their reproductive seasonality despite different climatic conditions (Pizzutto et al. 2019). In South Africa, fallow deer are abundant and show promise as a protein source, with males exhibiting higher carcass weights and dressing percentages than females (Fitzhenry et al. 2019). Historically, humans have played a crucial role in fallow deer distribution, repeatedly translocating populations across the Mediterranean, Europe, and globally (Baker et al. 2024). This long-term human intervention has resulted in fallow deer being simultaneously considered wild, domestic, endangered, and invasive in various regions, highlighting the complex relationship between humans and this species.

Fallow deer farming has gained importance as a sustainable and environmentally friendly meat production method. Venison from fallow deer is considered a healthy meat, low in fat and cholesterol, and high in protein (Bykowska 2018). The quality of venison is influenced by various factors, including sex, age, slaughter method, housing system, and feeding practices (Bykowska 2018). Advancements in health management and welfare practices have significantly contributed to the success of deer production systems, particularly in New Zealand (Wilson, 2002). Additionally, fallow deer farming has minimal environmental impact compared to traditional livestock farming, making it an attractive option for sustainable meat production (Kilar 2021).

Preslaughter handling of deer and other non-traditional meat species presents unique challenges. While some deer are still slaughtered on-site, others are transported to abattoirs, with journey times averaging 4.8 hours (Pearce et al. 2023). Handling systems are regularly used, especially for habituated deer, to minimize stress during transport and slaughter (Needham & Hoffman 2022). Preslaughter handling typically involves herding, loading, and transportation, similar to traditional livestock (Needham & Hoffman, 2022). However, challenges persist, including the need for deer-specific vehicles and experienced hauliers (Pearce et al. 2023). Group size during transport significantly affects bruising rates (Pearce et al. 2023). To address these issues and ensure animal welfare, the European Union has implemented regulations for deer farming, covering aspects such as housing, feeding, handling, and transport (Urošević et al. 2019). These standards aim to improve deer farming practices and product quality.

1.2.2. Slaughter methods for deer and the importance of stunning

Animal stunning is a crucial step in the meat processing industry to minimise the pain and stress experienced by animals during the slaughter process. Stunning before slaughter can be defined as a technical process subjected to each single animal to induce unconsciousness and insensibility in animals so that slaughter can be performed without avoidable fear, anxiety, pain, suffering, or distress (European Food Safety Authority (EFSA, 2006). The purpose of stunning animals is to help prevent them from any pain or affliction during and after bleeding, ensuring humane treatment and compliance with animal welfare standards. The Humane Slaughter Act requires that all inspected animals be rendered insensible to pain before exsanguination, that it induces immediate insensibility before the bleeding process, and that the animal must remain unconscious until it dies as a result of bleeding (HAS 2013). The whole slaughter process, including stunning, must respect the freedoms of animal welfare, especially these 2 freedoms described in the Animal Welfare Act. (2018): Freedom from fear and distress; freedom from pain, injury, and safety.

The effectiveness of stunning is evaluated using various physical and neurological indicators to confirm that the animal has been rendered unconscious and will not regain consciousness before death. Effective stunning should cause the immediate collapse of the animal, with the inability to stand or move indicating that brain function has been sufficiently disrupted (Comin 2023). An absent corneal reflex, assessed by gently touching the eye to check for blinking, is a reliable indicator of unconsciousness (Verhoeven et al. 2014). In terms of neurological indicators, vocalization after stunning is a sign of retained consciousness and pain perception, which indicates ineffective stunning (Contreras-Jodar et al. 2023). The onset of tonic (rigid) and clonic (jerking) seizures following stunning is typically observed and serves as a positive sign of a successful stun. The absence of these seizures may suggest that the electrical current or mechanical impact did not adequately disrupt brain function (Comin 2023).

Various national and international bodies provide guidelines and regulations for stunning animals. Animal welfare organizations such as the World Organisation for Animal Health (OIE) advocate for the humane treatment of animals during slaughter. The OIE provides guidelines and recommendations to ensure the welfare of animals, including the use of stunning methods (OIE 2019). Pre-slaughter stunning is required by EU law as ethical, and in many other countries Grandin (2020). Different countries may have their own specific regulations and guidelines regarding pre-slaughter; however, the overall objective is to promote the well-being of animals in the context of food production.

Recent research on deer slaughter methods highlights, particularly for fallow deer, various approaches across different regions, reflecting ongoing developments in humane and efficient practices. For instance, helicopter-based shooting has been widely used in Australia, with studies showing varying results depending on the species and procedures followed (Hampton et al. 2021). A novel approach using aerial culling with shotguns has demonstrated improved efficiency and welfare outcomes for fallow deer in South

Australia (Bradshaw et al. 2023). In addition to these methods, electrical stunning is another common method, particularly in smaller operations. This involves passing an electric current through the animal's brain, inducing immediate unconsciousness. Electrical stunning is less commonly used in deer compared to other livestock but is recognized for its efficiency in rendering the animal insensible before slaughter.

In white-tailed deer, conducted electrical weapons showed potential for short-term immobilization with minimal long-term physiological consequences, offering an alternative to chemical immobilization (Grunwald et al. 2024) Recent research has explored alternatives to traditional stunning methods for animal slaughter. While carbon dioxide (CO2) stunning is widely used, it can cause aversion and discomfort in animals (Atkinson et al. 2020).

Meanwhile, traditional methods like on-farm or off-farm slaughtering are still common for farmed deer, while wild harvesting through hunting and culling remains prevalent for game species (Needham & Hoffman 2022). In addition, traditional slaughter of reindeer in Yakutia occurs in corals during cold weather, with specific guidelines developed to ensure meat quality and safety (Ershova et al. 2022). In Africa and Australia, free-bullet shooting is commonly used for harvesting wild game species and kangaroos, while some deer farms have intensified operations to allow for more efficient slaughtering within enclosures (Needham & Hoffman 2022). Free-bullet shooting is commonly used for harvesting wild game species, including deer, in extensive systems or natural environments (Needham & Hoffman 2022). While lead-based bullets have been traditionally used, there is a growing focus on transitioning to lead-free alternatives due to environmental and health concerns (Hampton et al. 2021; Hampton et al. 2023). captive bolt devices, powered by blank cartridges, are commonly used in abattoirs but can suffer from performance variations that may impact animal welfare (Grist et al. 2020). The slaughter process generally involves stunning, exsanguination, and carcass processing, with neck cutting being a common method for halal slaughter (Anil & Al-Teinaz 2020). Optimization of slaughter point locations in reindeer farms has been studied to reduce transportation distances and improve economic efficiency (Bazaeva 2019). These diverse approaches reflect the importance of adapting slaughter methods to regional conditions and specific animal species while maintaining high welfare standards and meat quality.

1.2.2.1. Free-Bullet

For non-traditional meat species like cervids, antelope, and kangaroos, free-bullet shooting is often used for slaughtering, while ostriches and some deer farms follow more conventional livestock handling procedures (Needham & Hoffman 2022). Free-bullet provides a quick and effective method of killing as it requires minimal or no restraint of the animal and can be used to kill from a distance without interrupting the normal behaviour of the animal (HSA 2014). The method is especially advantageous for extensively managed, wild, or agitated animals, such as adult bovines and deer, which may be difficult or dangerous to manage in confined spaces. Recent advancements in free-bullet stunning have highlighted its potential to induce unconsciousness with minimal stress, thanks to its ability to stun from a distance without direct handling or restraint (Velarde et al. 2015).

The free-bullet fired from a rifle of an appropriate calibre used by a good marksman is capable of reliably killing adult cattle through the massive transfer of energy and the resulting damage to the brain (Blokhuis 2004). The free-bullet first stuns and then kills the animal in very quick succession (Manson 2006). Both large and small calibre rifles can be effective, with small calibre potentially offering safety advantages by retaining the bullet within the skull (Schiffer et al. 2014). Low-energy pistol ammunition in rifles and .410 shotguns with steel or porcelain shots are recommended as safer alternatives to high-powered firearms for close-range use (Whiting & Will 2019). However, lead-based bullets pose significant risks of carcass contamination through fragmentation, potentially affecting wildlife scavengers and human consumers (Hampton et al. 2023). Physical hazards, such as bullet particles and bone fragments, introduced during the killing process can disperse widely in carcasses, raising food safety concerns (Nkosi et al. 2022). When lead bullets fragment upon impact, they contaminate game meat with lead particles, which can be ingested by consumers (Thomas et al. 2022). Despite these risks, lead ammunition remains widely used in many countries, including Australia, where research on its impacts is lacking (Hampton et al. 2018). These findings suggest a need for refined killing methods and a transition to lead-free ammunition in deer management and hunting practices.

The effective use of a free-bullet firearm involves precise shot placement aimed to penetrate the skull in a frontal position and proceed through the brain into the upper neck, following the line of the vertebra. This will cause catastrophic injury to the midbrain and brainstem leading to immediate death (HAS 2023). Successful head/upper neck shot results in immediate loss of consciousness and allows bleeding. (Hultgren J. et. al. 2022). However, when animals are startled by gunfire, they often experience a flight response, resulting in a chaotic scramble that can cause injuries and loss of muscle glycogen due to excessive running (Hampton et al. 2021). Acute stress immediately before slaughter can lead to pale, soft, and exudative (PSE) meat, while chronic stress may result in dark, firm, and dry (DFD) meat with high pH (Gonzalez-Rivas et al. 2019).

The skills of a marksman play a critical role in the effectiveness and humane aspects of free-bullet shooting. An experienced marksman can significantly reduce the risks of panic and injury among animals by ensuring precise shot placement, which minimizes the suffering and stress inflicted on the animals (Hampton et al. 2021). Accurate shot placement not only increases the likelihood of an immediate and humane kill but also affects carcass and meat quality. Improperly placed shots can lead to unnecessary pain and suffering, as well as contamination of the meat with blood and other tissue damage, ultimately impacting meat quality and processing efficiency (Hampton et al. 2023). Additionally, the use of a silencer can mitigate some of the stress associated with gunfire by reducing noise, which can decrease the likelihood of panic responses among the herd and improve the overall welfare of the animals. The reduction in noise also helps maintain better shot accuracy by minimizing the disruption caused by the initial gunfire, thus enhancing both the efficacy of the harvest and the quality of the resulting carcasses (Grunwald et al. 2024). Therefore, the combination of skilled marksmanship, precise shot placement, and the use of silencers can contribute significantly to more humane and effective shooting practices, positively influencing both animal welfare and meat quality.

1.2.2.2. Captive bolt

Captive bolt devices are widely used for stunning animals before slaughter, including deer (Sharman 1983). These devices employ blank cartridges to propel a piston onto the animal's head, causing rapid brain dysfunction and unconsciousness (Grist et al. 2020). Captive-bolt devices work in a similarly to firearms, although unlike free-bullet firearms, the bolt remains captive within the barrel. This bolt strikes the animal's head (percussion) which causes concussion. A penetrative captive-bolt stunner also fires a retractable bolt into the animal's head allowing the bolt to penetrate the cortex and midbrain of the animal (HAS 2023). captive bolt stunning thus involves the use of a penetrating or non-penetrating bolt, depending on the species and desired outcome. Both methods aim to disrupt brain function swiftly (Gregory et al., 2008). A penetrating captive bolt, with a sufficiently high bolt speed, in a good state of repair, and used properly will stun an animal. Death may result as a consequence of the physical damage to the brain caused by penetration of the bolt, but death is not a guaranteed outcome (Appelt 2007).

Captive bolt stunning is a widely employed method in animal slaughter, designed to induce immediate unconsciousness and minimise pain and distress. However, research has revealed significant variations in cartridge performance, which can affect stunning effectiveness and animal welfare. The effectiveness of stunning depends on factors such as device type, bolt speed, and kinetic energy (Kameník et al. 2019). Recent studies have found significant variations in blank cartridge performance, which can affect stunning efficacy and animal welfare (Grist et al. 2019; Grist et al. 2020). Lower charge cartridges showed greater variation in weight, propellant volume, and velocity compared to higher charge cartridges (Grist et al. 2019). Some cartridges even split upon firing, potentially compromising stunning effectiveness (Grist et al. 2020). Proper maintenance, regular cleaning, and safety considerations are crucial for optimal performance of captive bolt devices (Baier & Willson 2020). The design and calibre of captive bolt pistols can vary, influencing the stunning efficacy and animal welfare outcomes. Modern captive bolt devices are engineered for precision and reliability, contributing to the method's consistent use in commercial slaughter facilities (Lücking, et al., 2024). Factors such as cartridge weight, propellant fill volume, and velocity can vary within cartridge boxes (Grist et al. 2019). A study comparing Eley and Accles and Shelvoke cartridges found that the latter showed greater variation in velocity, kinetic energy, and mechanical stability (Grist et al. 2020).

To ensure humane slaughter, it is essential to monitor stunning effectiveness by checking for loss of reflexes and verifying correct shot placement (Kameník et al. 2019). International and national regulations provide guidelines for the application of captive bolt stunning to ensure humane slaughter practices. Organizations such as the World

Organisation for Animal Health (OIE) establish standards that promote the effective and ethical use of this stunning method (OIE 2019).

Guidelines for assessing stunning methods emphasize the importance of immediate onset of unconsciousness, absence of avoidable pain, and duration of unconsciousness until death (More et al. 2018; Authié et al. 2013). Proper handling and well-designed facilities are essential for minimizing stress during transport and slaughter of deer and other low-volume farm animals (Bornett-Gauci et al. 2006; Grandin 2020). The guidelines stress that all stunning methods must be regularly maintained, and operators trained to ensure effectiveness. Compliance with these practices is critical to meet animal welfare standards (EFSA 2013; HSA 2014).

Effective stunning requires proper device selection, maintenance, and positioning (Kameník et al. 2019). In deer, the brain is located high in the head. The optimal point for stunning is in the middle of the forehead, at the intersection of two imaginary lines drawn from the eyes to the top of the base of the opposite ears. In stags, this spot is found between, or in some cases just behind, the antlers. The muzzle of the stunner must be held at right angles to the skull (Figure 1) (HSA 2014).

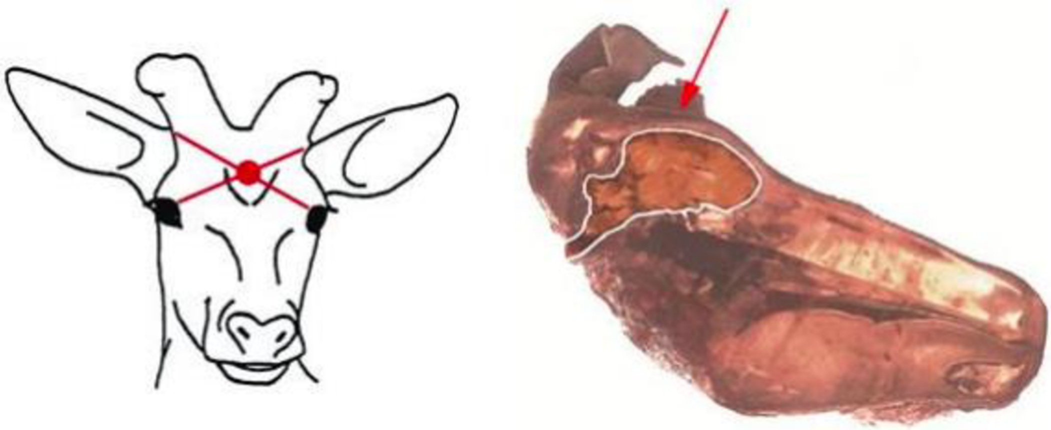

Figure 1 *Correct position for captive bolt placement in deer (left), and lateral view illustrating the trajectory of the captive bolt (right). Source: Humane Slaughter Association (HSA 2014)*

1.2.3. Physiology of the muscle and meat quality

The physiological state of muscle tissue significantly influences meat quality. Muscle glycogen is a polysaccharide that serves as a primary source of energy for muscle contractions. Glycogen, a branched glucose polymer, plays a crucial role in muscle energy metabolism. It serves as a key substrate for ATP production during high energy demand, impacting work capacity and force generation (Katz 2022). Glycogen metabolism involves phosphorylase for breakdown and glycogen synthase for synthesis, with recent findings challenging traditional views on their regulation (Katz 2022). While primarily considered a local glucose reserve, glycogen utilization in brain and muscle occurs even when glucose is available, suggesting additional functions (Manchester 1980). Glycogen metabolism influences energy yield from ATP by sequestering inorganic phosphate, maximizing energy at sites of rapid ATP consumption (Manchester 1980). In muscle, glycogen provides energy both anaerobically and aerobically, supporting maximal exertion and sustained activities (Haller 2008). Recent research has expanded our understanding of glycogen's roles in energy sensing, metabolic pathway integration, and cellular responses to hormonal stimuli (Greenberg et al. 2006), highlighting the complexity of glycogen metabolism regulation.

Muscle glycogen and ATP are fundamental to both muscle function and meat quality. Glycogen acts as a primary energy reservoir during muscular activity, while ATP is essential for muscle contraction and relaxation (Hargreaves & Spriet 2020; Haller 2008). The depletion of these crucial resources due to exhaustion can significantly impact meat quality, particularly through changes in post-mortem muscle metabolism Specifically, reduced glycogen and ATP levels can lead to undesirable alterations in meat texture, colour, and overall quality (Stempa & Bradley 2020). During exhaustion, ATP levels drop significantly, impairing muscle contraction and relaxation. This can result in tougher meat texture and reduced overall meat quality. ATP depletion also impacts post-mortem muscle pH, contributing to issues like dark, firm, and dry (DFD) meat, which is less desirable in the market (Ijaz et al., 2021). Research by Rosenvold et al. (2001) emphasizes that lower glycogen content at slaughter is associated with higher ultimate pH, darker meat color. For instance, low glycogen content can contribute to the development of Pale, Soft, and Exudative (PSE) meat in pigs, characterized by its pale appearance, softness, and increased water-holding capacity.

1.2.4. Animal behaviour during slaughter and its impact on meat quality

The intricate relationship between animal behavior and meat quality is a pivotal aspect of the livestock industry. Stressful pre-slaughter conditions can significantly alter animal behaviour, resulting in the release of stress hormones like cortisol. This stress has been shown to negatively impact meat quality, affecting attributes such as tenderness, colour, and water-holding capacity (Warriss et al. 1994). Proper handling techniques, characterised by gentle and low-stress approaches, contribute to positive animal welfare outcomes and, subsequently, better meat quality. Recent studies emphasise that proper handling techniques, which include gentle and low-stress methods, are crucial for enhancing animal welfare and improving meat quality. Calm handling practices have been demonstrated to reduce stress hormone levels and mitigate adverse effects on meat properties (Grandin, 2020; Bittante 2023).

Stress and pain lead to physiological stress, such as changes in heart and respiratory rate, body temperature and blood pressure, which occur when the animal is exposed to adverse conditions (Fernandez-Novo et. al. 2020). Stress can affect drip loss, meat colour, ultimate pH, and cause meat anomalies (Ijaz et al., 2021). Pos-mortem pH and temperature play crucial roles in determining meat quality. Higher initial pH is associated with various quality attributes like water holding capacity and shear force (Kim et al. 2016). Early post-mortem muscle temperature, pH, and structural damage affect beef quality, particularly tenderness (Bruce & Ball 1990). These findings underscore the importance of proper animal handling, stress reduction, and careful management of post-mortem conditions to ensure high-quality meat production.

Lairage time influences blood lactate concentration, skin blemish scores, and meat colour, with longer lairage resulting in darker, less red and yellow meat (Dokmanović et al. 2017). On-farm slaughter of deer can effectively reduce lairage time and associated stress compared to conventional transport to abattoirs. Studies have shown that deer transported to slaughterhouses may spend up to 68.9 hours in lairage (Pearce et al., 2023). On-farm slaughter minimizes exposure to unfamiliar environments, reduces handling stress, and eliminates or shortens transport times (Hultgren et al. 2018). This approach is particularly beneficial for unhabituated deer, as only those accustomed to handling are typically transported to abattoirs (Needham & Hoffman 2022). Research on bison, another less domesticated species, demonstrated that on-farm slaughter could mitigate injuries, muscle damage, and trim losses associated with handling and transport (McCorkell et al. 2013). However, implementing on-farm slaughter for deer requires addressing challenges such as food safety, waste management, and veterinary inspections to ensure compliance with regulations (Hultgren et al. 2018).

The social environment can also influence animal behavior and, subsequently, meat quality. Social stressors, such as aggression within groups, may impact the physiological responses of animals and influence the composition of muscle tissues (Teixeira et al. 2014). Aggression and social stress among deer before slaughter can lead to significant carcass damage, impacting overall meat quality. Aggressive interactions often result in physical injuries such as bruising and lacerations, which can directly affect the visual and textural quality of the meat, such injuries not only degrade the meat but also contribute to increased levels of stress hormones, further compromising the physiological state of the carcass (Carrasco-García et al., 2020). The quality of meat is significantly influenced by post-mortem changes in muscle tissue composition, affecting tenderness, color, and water-holding capacity (Hughes et al. 2014). This social aggression is common in most if slaughter methods except in free-bullet which requires no pre-slaughter handling (Needham & Hoffman 2022). Effective management strategies that minimise aggressive behavior and social stress are crucial for maintaining high meat quality and reducing carcass damage, ensuring better outcomes for both animal welfare and meat production (Velarde 2015).

Pre-slaughter conditions, including handling and social mixing, can significantly impact meat quality across various animal species, potentially resulting in defects such as PSE and DFD meats (Karabasil et al. 2019). The occurrence of PSE and DFD meats is particularly sensitive to these conditions. PSE meat results from acute stress before slaughter, leading to rapid glycogen depletion and a subsequent decrease in meat pH, which causes pale coloration and a soft texture (Bittante 2023). Conversely, DFD meat arises from chronic stress or inadequate pre-slaughter handling, resulting in higher pH levels and dark, dry meat due to glycogen retention and reduced water-holding capacity (Mia et al. 2023). In wild game, hunting practices and animal characteristics influence meat quality, with pH measurements used to categorize meat as DFD ($pH \geq 6.2$), intermediate DFD ($5.8 \leq pH < 6.0$), or high-quality ($pH < 5.8$) (Viganò et al.

2019). Research indicates that deer are susceptible to developing DFD meat, characterized by high pH values (>6.2) and altered color, tenderness, and water-holding properties due to the high stress levels (Wiklund et al. 2007; Wiklund et al. 1995). According Wiklund et al. (2007) the factors influencing DFD occurrence in deer include pre-slaughter stress, poor nutritional status, and transport conditions.

Recent studies emphasise that optimising pre-slaughter conditions - such as minimising stress through proper handling, transport, and environmental control - can mitigate these issues. Optimal stocking density during transport and appropriate lairage times are essential to reduce stress and improve meat quality (Hoffmana & Fishera 2011). Meat colour significantly influences consumer perception of quality, although it may not always accurately reflect freshness or flavour (Winstanley 1979). The pigment myoglobin primarily determines meat colour, which can be affected by various factors such as animal age, diet, and processing methods, specially stress immediately before slaughter (Winstanley 1979). Consumers associate bright cherry-red colour with fresh meat wholesomeness, while cooked colour indicates doneness (Suman & Joseph 2013). Deviations from expected colours, as seen in DFD and PSE meat, can lead to product rejection and economic losses.

Stressful conditions during the pre-slaughter phase trigger physiological responses in animals, including the release of stress hormones such as cortisol. Elevated cortisol levels are associated with changes in muscle metabolism that can impact meat quality attributes (Arsenoaia & Malancus, 2023; Stronskyi et al., 2021). Research indicates that animals subjected to stress before slaughter exhibit variations in meat quality, including colour changes, decreased tenderness, and altered water-holding capacity, these changes are attributed to the metabolic effects of stress on muscle glycogen levels and pH (Mia 2023). Elevated cortisol levels can lead to rapid glycogen depletion in muscle tissues post-slaughter, pH decline, and changes in muscle structure. The alterations impact the texture, juiciness, and overall palatability of the final meat product (Gao, 2008).

1.2.5. Stressful behaviours of deer before and during the slaughter process

The humane treatment of deer before and during the slaughter process is essential for ensuring animal welfare. Stressful behaviours in deer can indicate fear, discomfort, and suffering, making it crucial to identify and minimise such behaviours. Behavioural indicators serve as valuable tools for assessing stress in deer. Observational studies have identified specific behaviours associated with stress, including restlessness, vocalization, and attempts to escape or avoid certain situations. These behaviours are crucial for gauging the emotional well-being of deer in different contexts (Gregory et al. 2008). Different handling practices, such as the use of corrals, chutes, and transportation methods, and darkness can elicit specific stress-related behaviours in deer. Understanding these behavioural indicators provides insights into the effectiveness of handling protocols and opportunities for improvement in animal welfare (Grandin 2020).

During the pre-slaughter process, it is possible to observe some behaviours that can influence directly in quality meat, and levels of cortisol in the blood. Deer often display signs of restlessness and agitation in response to pre-slaughter handling or transportation. Research has indicated that these behaviours are indicative of stress and may influence the physiological responses that impact meat quality (Gregory et al. 2008). Slaughter can increase vigilance behaviour and short-term stress responses in fallow deer, though physiological effects appear to be temporary (Pecorella et al. 2016). Deer may exhibit restlessness and agitation in response to unfamiliar surroundings, handling, and transportation. This behavior can be exacerbated if animals are not acclimated to human presence or handling procedures (Grandin 2000). Vocalization and attempts to escape confinement are common stress responses observed in deer during pre-slaughter procedures. These behaviours can be assessed as indicators of the animal's emotional state and the effectiveness of handling practices (Grandin 2020).

The stunning phase is a critical aspect of the slaughter process, and the choice of stunning method can influence stress levels in deer. Research has explored the effectiveness and welfare implications of different stunning techniques, aiming to minimise stress and ensure humane slaughter (Velarde et al. 2015). Observations of deer behavior during the slaughter process have identified behaviours such as reluctance to enter the stunning area, increased alertness, and changes in posture as indicators of stress. Understanding these responses contributes to refining slaughter practices (Gregory et al., 2000). Research has shown that restlessness, agitation, vocalization, and other indicators of fear and distress can be observed in deer during pre-slaughter handling. Proper stunning techniques and equipment are essential to minimise distress during the stunning and exsanguination phase.

The observed behaviours during pre-slaughter operations provide critical insights into the stress responses of fallow deer. Ensuring the humane treatment of deer before and during the slaughter process is essential for maintaining animal welfare (Grandin 2020). Deer are known to exhibit heightened vigilance and flight responses when sensing human presence or perceiving a threat. Studies, such as those by Lashley et al. (2014), indicate that deer demonstrate increased alertness, frequent head raising, and scanning behaviours when humans approach with firearms. Just before the shot, deer often display freeze or startle responses. This moment of intense alertness can be associated with physiological stress indicators, such as elevated heart rates. The immediate aftermath of a free-bullet shot can vary. If the shot is precise and hits vital organs, the deer typically collapses swiftly with minimal movement. However, suboptimal shots can lead to escape attempts, prolonged suffering, and erratic movements.

Recent studies indicate that deer subjected to the free-bullet method exhibit minimal stress-related behaviours prior to the shot. According to Grandin et al. (2020), deer in their natural environment display normal behaviours such as grazing and walking up until the moment of impact, suggesting low pre-slaughter stress levels. However, post-shot behaviours can vary based on shot placement and immediate physiological responses. Hampton et al. (2021) analysed the behaviours of deer immediately after being shot with a free-bullet. They reported that a well-placed shot results in rapid collapse and minimal post-shot distress, characterized by a quick loss of consciousness. Conversely, poorly placed shots can lead to prolonged distress behaviours, including vocalization, struggling, and attempts to flee.

Deer subjected to the captive bolt method exhibit a range of behaviours associated with handling and restraint, post-stun behaviours are generally minimal if the captive bolt is applied correctly. Correctly applied captive bolt results in immediate loss of consciousness and a rapid cessation of voluntary movement. However, misapplication can lead to incomplete stunning, resulting in signs of distress, as described by Meyer (2015) stress assessment during transitional states of consciousness can involve behavioral and physiological measures, including movement, vocalization, heart rate changes, and sympathetic nervous system activity.

1.2.6. Concern about the stunning process on deer

The stunning and slaughter of deer involve a critical intersection of animal welfare, regulatory compliance, and ethical practices. Deer slaughter methods encompass a range of techniques, including captive bolt, and free-bullet. Captive bolt as prevailing, but often pre stunning handling is stressful and May have negative impacts on animal physiology and meat quality (Warriss, P. D. 1990). Stunning process have prompted the development of monitoring practices to assess the effectiveness of stunning in real-time on deer. Observational studies and audits play a vital role in identifying areas for improvement and ensuring that stunning methods align with ethical and welfare standards (Grandin 2020).

Concerns about the stunning process in deer extend to regulatory compliance and ethical frameworks. Welfare considerations during stunning extend beyond the method itself. Pre-stunning handling practices and the design of stunning facilities play crucial roles in minimizing stress. Research has explored stress mitigation techniques and optimal handling protocols for deer, emphasizing the importance of a low-stress environment (Gregory et al. 2008), such as the used of specific handling pens, drop-floor cradles, and dim lighting during yarding operations (Mattiello 2009). Extensively farmed animals, especially game like a deer can have high level of stress and even injure themselves during handling (Grandin, 1997).

Research may investigate the impact of distant slaughter methods on animal welfare, considering factors such as immediate unconsciousness, stress levels, and the potential for missed shots leading to prolonged suffering. Understanding the welfare implications is crucial for ethical slaughter practices (Grandin 2020). They use of distance slaughter methods can be particularly advantageous in reducing handling-induced stress, as it allows deer to remain in their natural environment up until the moment of the shot. Research into the impact of distant slaughter methods on animal welfare emphasizes the importance of achieving immediate unconsciousness and minimizing stress levels. Studies by Hampton et al. (2021) have investigated the effectiveness of free-bullet stunning, highlighting its potential to reduce prolonged suffering when shots are accurately placed. However, the risk of missed shots remains a concern, necessitating skilled operators to ensure humane outcomes.

Further advancements in stunning practices continue to evolve, driven by ongoing research and technological innovations. Recent studies have focused on the refinement of both captive bolt and free-bullet methods to enhance welfare standards. For example, advancements in captive bolt technology aim to improve the precision and consistency of stunning, thereby reducing the incidence of incomplete stunning and associated distress (Rodriguez et al. 2021). Similarly, research into free-bullet methods emphasizes the need for advanced training and marksmanship to ensure the humane treatment of deer during distant slaughter. These developments underscore the dynamic nature of animal welfare research and the continual effort to align stunning practices with the highest ethical standards.

2. Aims of the Thesis

The study aimed assess the behavioural responses and the overall impact of different slaughter methods on the meat quality of fallow deer. Specifically, to:

- Evaluate the effects of two slaughter methods, captive bolt, and free-bullet, on fallow deer (*Dama dama*) behaviour prior and during slaughter.
- Quantify the impact of the slaughter method on physical meat quality.
- Describe the carcass damage associated with each slaughter method.

2.1 Hypotheses

H1: Fallow deer subjected to the captive bolt method will not exhibit higher levels of pre-slaughter stress, as assessed by behaviour, compared to those subjected to the free-bullet method.

H2: The meat quality of fallow deer slaughtered using the captive bolt method will not differ from that of deer slaughtered using the free-bullet method. Specifically, meat from deer slaughtered with the captive bolt method due to the handling and stunning process, potentially affecting tenderness, temperature, and pH levels.

H3: Carcass damage will not be more extensive in fallow deer slaughtered with the captive bolt method compared to those slaughtered with the free-bullet method.

3. Methods

3.1. Experimental location and animals

The research was conducted in mid-January 2024, over a 4-day period at a commercial fallow deer farm located in Mnich near Kardašova Řečice, situated in the South Bohemian Region of the Czech Republic. The farm, approximately 110 kilometres south of Prague, specializes in the farming and management of fallow deer. All experimental activities and its extent were done within competence of the Institutional Animal Care and Use Committee at the Czech University of Life Sciences, Prague within accreditation no. MZE-13122/2022-13114 following all legal acts.

The study involved a total of 20 farmed fallow deer spikers (19 months old) as part of annual herd reduction, which were randomly divided into two treatment groups of 10 animal per group. Each group underwent a different slaughter method, either free-bullet or captive bolt. Ten deer were slaughtered per day to facilitate efficient data collection and minimise stress on the animals. The meat processing was conducted on the subsequent day.

3.2. Slaughter protocol

The slaughtering was conducted on two separate days, January 16th, and January 26th, 2024. On both days, the slaughter commenced with the free-bullet method (5 animals), followed by the captive bolt method (5 animals), and follow the slaughter protocol described in Figure 2. The animals were separated into their respective groups several days prior to slaughter. For the free-bullet method, the animals were in an open field with the freedom to move around. The shooter was positioned 35-80 metres away to ensure an effective and humane kill. The total time required to shoot all ten fallow deer both days of slaughter was 3 minutes and 24 seconds, with the shot placements being targeted at the head. During the slaughter days, animals were head-shot with a CZ 700 rifle (calibre 0.308 Winchester, Brno Rifles, Czech Republic) with optics from the middle of the paddock (the shooter was propped up on a table with their rifle on a stand) consecutively (one bullet in the barrel and 4 in the cartridge magazine) to ensure the minimum shooting time necessary for all animals. All five animals were exsanguinated directly after the last shot.

In contrast, for the captive bolt method, the animals were first herded from their paddock into the handling corridor (6 m wide and 200 m long), grouped into batches of five, and before slaughter driven further thorough corridor into the holding pen within the barn with the handling facilities. They were then led one by one into a stunning box where the captive bolt stunning was carried out. This method ensured that each animal was stunned individually and ensured accuracy of stunning. The total time used to slaughter all 10 fallow deer was 39 minutes and 18 seconds for the two days, following the correct position of stunning on deer at the head.

Immediately following stunning or shooting, the animals were exsanguinated by cutting a major blood vessel, typically the carotid artery or jugular vein, with a knife to allow bleeding. This process is critical for both animal welfare and meat quality. The slaughter process for both methods was recorded to analyse animal behaviour and assess stress levels. This analysis is crucial for determining which method induces less stress, according to the guidelines by Daly et al. (2006).

All animals were tagged for identification, their body condition was measured by palpation according to the body condition score chart for deer (Audigé et al, 1998) and the fallow deer were weighed post-bleeding. Evisceration was performed on-site at the farm. After evisceration, pH and temperature measurements were taken using a pH and integrated temperature probe for automatic adjustment XS pH PC70 Waterproof Meter (XS Instruments, Italy) in three muscles of the carcass: TB, LL, and BF. Measurements were repeated over a 48-hour period, beginning 1 hour post-mortem, described further below. As measurements commenced prior to skin removal, the areas of interest were cleaned, hair was removed, and incisions using knife were made in the muscle to facilitate the insertion of the pH meter probe. These measurements are essential for monitoring the biochemical changes that occur post-mortem, as per the guidelines of Ferguson and Warner (2008).

After evisceration, all carcasses were transported to the slaughterhouse. The vehicle used for transportation was either dedicated to meat transport. At the slaughterhouse the carcasses underwent several procedures:

- Skinning and removal of the head and feet were performed.
- A veterinary inspection was conducted to ensure the health and safety standards of the carcasses.
- Upon arrival at the slaughterhouse and after processing, the fallow deer carcasses were placed in cooling chambers maintained at a temperature between 0-4°C. This temperature range helps inhibit bacterial growth and preserve meat quality. The carcasses remained in the cooling chambers for approximately 24 to 30 hours.
- The whole cold carcasses were weighed before meat processing.
- Meat processing and meat sample collection were carried out one day after the cooling process, 24 hours after the carcasses were placed in the cooling chambers. This waiting period allowed for the conditions to stabilize, providing a consistent environment for the subsequent meat quality analyses. This approach aligns with recommendations by Tadic (2022) to ensure reliable and repeatable results.

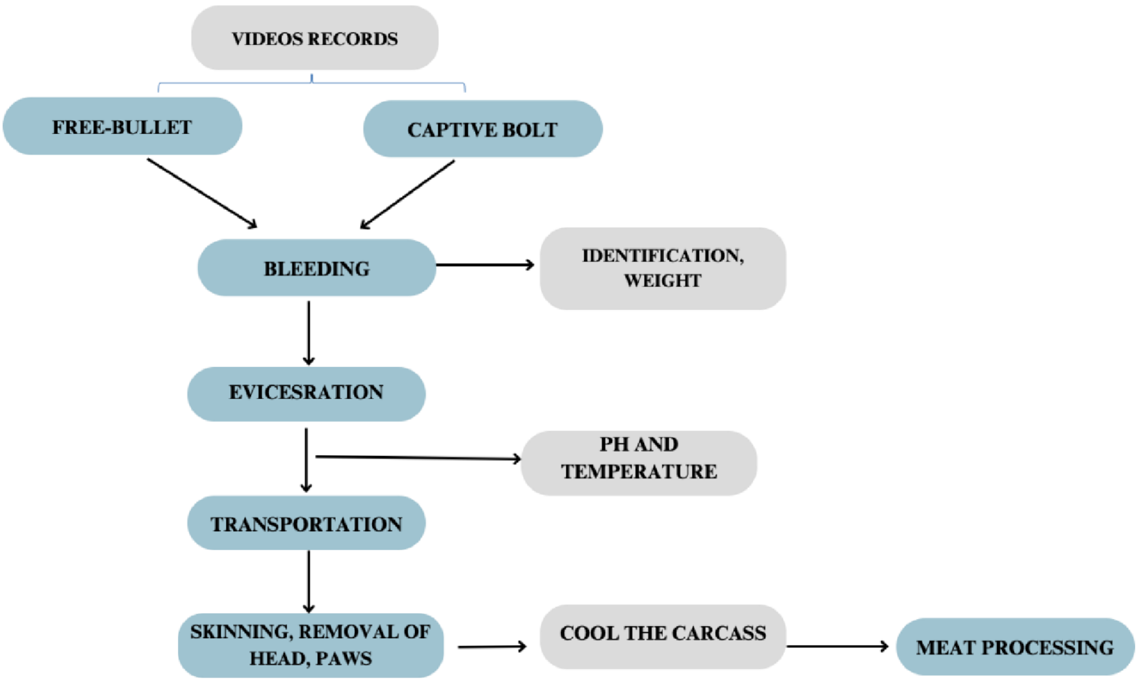

Figure 2 *Slaughter protocol used during the data collection; free-bullet slaughter was always done first and then captive bolt on the respective data collection days.*

3.3. Behavioural analyses at slaughter

Behavioural observations were recorded during all the shooting, handling or stunning process using video cameras strategically placed to capture all entire procedure. After recording videos during the slaughtering process, the behaviour analysis aimed to understand which method affects more the behaviour. Video analysis was conducted using The Observer XT 17 software (Noldus Information Technology, Wageningen, The Netherlands). The focus of the analysis was on identifying stressful behaviours, utilizing the following behaviour variables:

- **Movement Behaviours**: Includes activities such as walking, jumping, running, and stopping.
- **Aggression behaviours**: Encompasses interactions with other exhibiting aggression and flights.
- **Alertness behaviour**: Identifies signs of stress and fear, including vocalizations, alertness, restlessness, fleeing, staring, and attempts to escape.

The recorded videos were meticulously analysed using Noldus The Observer XT 17 to identify and quantify the frequency and duration of the specified behaviours. This software allows for detailed observation, coding, and analysis of behavioural data, providing comprehensive insights into animal behaviour. The analysis aimed to determine which stunning method induced more stress, thereby affecting the overall meat quality. There was not technically possible to recognise individuality of recorded animals, therefore analysis was treated and evaluated as overall response per group.

3.4. pH and temperature measurements

The pH and temperature measurements were initiated one hour post-mortem using an XS pH PC70 Waterproof Meter (XS Instruments, Italy), equipped with an integrated temperature probe for automatic adjustment. This ensured precise and accurate readings of both pH and temperature, focusing on three specific muscles: TB, LL, and BF. The three muscles chosen for this study represent different anatomical locations and functions within the deer, thereby providing a comprehensive overview of the slaughter method's impact. Measurements were taken thorough a 48-hour post-mortem period, starting 1 hour after death. Data were collected every hour for the first four hours, then every two hours until 12 hours post-mortem, and subsequently every four hours until the end of the study according to Calitz et al. (2020). The final measurement was taken at 48 hours post-mortem. The reasons for this schedule are as follows:

• 1 hour after death: Initial post-mortem period, assesses immediate changes in muscle biochemistry, tracking the initial rate of pH decline and temperature.

• 4 hours after death: With the onset of rigor mortis, measurements every two hours provide insights into muscle stiffening and biochemical changes.

• 12 hours after death: As rigor mortis progresses, measurements every four hours capture late-stage biochemical changes and the onset of aging processes.

• 24 hours after death: Measurements at this stage are crucial for evaluating pH and temperature stabilization, reflecting further biochemical changes and the progression of meat quality indicators.

• 48 hours after death: The final pH measurement determines the ultimate pH, which is critical for assessing meat quality, including colour, tenderness, and water-holding capacity.

3.5. Carcass damage

During the slaughter process, various types of physical damage can occur, including bruises, fractures, and blood splash in the muscle. To evaluate the extent of physical damage to carcasses and to compare the effects of different slaughter methods, each carcass was photographed to visually document its condition. High-resolution images were captured from multiple angles to ensure thorough coverage of all potentially damaged areas from the outside of carcass, and if bruises or blood splash occurred, also inside the carcass. These photographs served as the primary tool for analysing and comparing the extent of damage resulting from different slaughter methods.

A damage scale of 0 to 5 was developed to ensure consistent and objective evaluation of carcass damage, this protocol was adapted from Smith (2009):

- **0**: No visible damage.
- **1**: Minor superficial damage (small bruises, blood splash, slight abrasions)**;**

- **2**: Moderate damage (larger bruises, minor cuts, blood splash, abrasions);
- **3**: Significant damage (extensive bruising, deeper cuts, blood slash).**;**
- **4**: Severe damage (multiple deep cuts, large areas of bruising, broken bones);
- **5**: Extreme damage (major structural damage, severe haemorrhaging, multiple fractures).

External damage on carcasses was assessed by evaluating the number of body parts (side, legs, neck, and back) where the severity of lesions or injuries occurred A common scale from 0 to 5 was used.

Internal damage was evaluated by the presence or absence of blood splash (petechial haemorrhages) on the diaphragm, which can be indicative of stress or poor handling before slaughter:

- **0**: No blood splash present.
- **1**: Blood splash present.

3.6. Collection and analysis of meat samples

After 24 hours of cooling in the chamber, the carcass was weighed, and meat processing was initiated. For meat quality analysis, samples were collected from the carcass and weighed. Samples were collected from all slaughtered fallow deer following three areas: TB, LL, and BF. The samples were subsequently placed in labelled plastic bags and stored in a refrigerator until complete 48 hours post-mortem, at which point the final pH and temperature measurements were taken. Following these measurements, the samples were subjected to physical analysis, which included colour assessment, shear force testing, and cooking loss evaluation.

3.7. Colour

Colour is a crucial quality attribute that significantly influences consumer perception and acceptance of meat. The colour of muscle samples from each slaughter method was analysed for all 20-fallow deer, specifically focusing on TB, LL, and BF. Following the protocol outlined by Needham et al. (2019) each meat sample was cut into three 2 cm thick steaks, the steaks were then allowed to rest for 45 minutes on a table to permit the meat to "bloom"; this process allows oxygen to interact with the myoglobin in the meat. The colour of each steak was then measured using a CM-700d Spectrophotometer, (Konica Minolta, Osaka Japan, aperture diameter, 8mm illuminant: D65, observer angle: 100 and specular component: 0% UV) from Institute of Animal Science. For each sample, six colour measurements were randomly taken at different positions and then averaged for each of the L*, a* and b* values.

This instrument provides precise colour readings by assessing three key parameters:

- L* (Lightness): Indicates the brightness of the meat, where higher values denote lighter colours.

- a* (Redness): Measures the intensity of the red colour, with positive values indicating red and negative values indicating green.

- b* (Yellowness): Assesses the degree of yellow in the meat, with positive values representing yellow and negative values representing blue.

These colour measurements offer an objective evaluation of meat appearance, which is essential for understanding how different slaughter methods impact the visual quality of meat.

3.8. Cooking loss and Warner-Bratzler shear force

After the initial measurement of colour, cooking loss followed by shear force was conducted to evaluate the effect of slaughter method on moisture retention during cooking and level of tenderness. The meat samples were weighed from the TB, LL and BF muscles to establish baseline weights before cooking. Each meat sample was then placed into a labelled cooking bag, and a thermometer was inserted into the muscle to monitor internal temperature (Figure 3).

Figure 3 *Meat samples were placed in plastic bags, attached to wooden rods, and submerged in a water bath set to 80°C. On the left side of the image, a thermometer is inserted into the meat to monitor and control the cooking temperature.*

The water bath was maintained at a controlled temperature of 80°C. This consistent water temperature is critical to ensure uniform cooking across all samples, as variations can affect the meat's texture and quality (Lawrie 2006). The cooking process continued until the internal temperature of the meat reached 75°C, at which point the water bath was stopped. This target internal temperature was selected based on established guidelines for achieving thorough cooking while preserving meat quality (Ferguson 2008).

Post-cooking, the meat samples were re-weighed to determine the amount of water lost during the cooking process. This step is essential for assessing the water-holding capacity of meat, which significantly influences its juiciness and overall quality. Following the weighing, the samples underwent shear force testing.

For the WBSF measurement, the cooked samples were cooled to room temperature, six cubes, measuring 1x2cm, were then cut from each sample for each muscle group (TB, LL, BF) to determine shear force. It was crucial to ensure that the samples were free from visible connective tissue to obtain accurate shear force readings (Daly et al. 2006). The prepared samples were then placed in the sample holder of a Texturometer or Universal Testing Machine 3365 (Instron Canton, MA, USA), fitted with a standard Warner-Bratzler blade, at a crosshead speed of 100mm/ minute, which measured the force required to shear through the meat.

The WBSF blade cut each cube perpendicular to the direction of the muscle fibre. The average of the shear force measurements for the six sub-samples per muscle was calculated. This analysis is vital for understanding the relationship between cooking-induced water loss and meat tenderness, two critical attributes of meat quality (Gregory, 2007).

3.9. Statistical analyses

For statistical evaluation SAS System V 9.4 (SAS Inst. Inc., Cary, NC) and SPSS (version 29.0 for Windows, IBM, USA) were used. The normality of data distribution was tested by 'UNIVARIATE' statement. The data on Body condition score (BSC), Animal and Carcass weights, muscle Physical attributes and Carcass damage were tested using a Generalized Linear Mixed Model (GLMM) with MIXED procedure. The BSC, Animal and Carcass weights were included as a dependent variable to control, that both groups were equally selected and there were no differences in condition and sizes. The Physical attributes (cooking loss; Warner-Bratzler Shear Force and colour - L*, a*, b*) of three muscles (TB, LL, BF), and Carcass damage (Extent of bruises; number of parts of body with external damage - side, legs, neck, back; and presence of blood splash on diaphragm - yes or no) were included as a dependent variable. The explanatory fixed effects of class variables were 'method of slaughter' (captive bolt vs. free-bullet) and 'date' (two terms of slaughter with seven days difference). The significance of each fixed factor in the GLMM was assessed using an F-test. The least-squares-means (LSMEANs) were used to find differences between the tested fixed effects. For multiple comparisons, the Tukey-Kramer adjustment was used. Linear regression was used to evaluate the effect of the treatment (captive bolt vs. free-bullet) on the pH of the three studied muscles during the 48-hour monitoring period. Treatment, date of slaughter, temperature and time were included in the model as predictors. Collinearity between the predictors was assessed by the Variance Inflation Factor; time and temperature showed certain collinearity ranging between 3 and 4 in the three models, which is statistically acceptable. Thus, both variables were included in the regression models. The behavior data was analyzed using descriptive statistics, the mean durations of different behaviors were assessed for each method using Microsoft excel.

4. Results

4.1. Influence of slaughter method on fallow deer (*Dama dama*) behaviour

The descriptive analysis of behaviours revealed differences between the two slaughter methods. The behaviours were categorised into three types: movement behaviours (including walking, jumping, and running), alertness behaviours (including staring, alertness, attempting to escape, and restlessness), and aggressive behaviour. The data indicated a difference in the frequency of alertness behaviours between the slaughter methods. Specifically, alertness behaviours were observed to be more frequent in the captive bolt method compared to the free-bullet method. In contrast, when examining movement behaviours, the percentage of time spent exhibiting these behaviours was higher in the free-bullet method than in the captive bolt method. Aggressive behaviours were not observed in the free-bullet method. However, aggression was noted in the captive bolt method, as detailed in the Table 1.

Table 1 Overall observation of behaviours between slaughter methods

Method	Slaughter duration (sec)	Slaughter duration (sec)	Aggression (%)	Alert (%)	Moving (%)
Captive bolt 1	0:21:54	1314,00	1,97	74,79	23,24
Captive bolt 2	0:15:41	941,00	2,12	88,10	9,79
Free-bullet 1	0:01:03	63,00	0,00	81,25	18,75
Free-bullet 2	0:02:24	144,00	0,00	53,87	46,13

Percentage of the time observed behaviour dedicated to each by slaughter method, 1 is first day of slaughter and 2 is second day of slaughter.

4.2. Influence of slaughter method on pH and temperature

Regression analyses was conducted to assess the relationship between pH in three muscles (TB, LL, BF) and various factors, including slaughter method (free-bullet and captive bolt), date of slaughter, time of monitoring, and temperature. There was no significant effect of muscle pH (TB, LL, BF) and the slaughter methods, with p-values of 0.094, 0.781, and 0.402, respectively, indicating that the method of slaughter does not significantly influence the muscle pH. However, a significant relationship was found between pH in the muscles (TB, LL, BF) and the date of slaughter, with p-values of 0.005, 0.001, and 0.008, respectively. This suggests that the date of slaughter affected pH in both slaughter methods. Additionally, the time of monitoring showed a significant correlation with muscle pH (TB, LL, BF), with p-values of 0.008, 0.018, and 0.080, respectively, indicating that pH changes over time during monitoring and there was no interaction between the slaughter methods for pH and monitoring time. Furthermore, there was an effect of temperature on muscle pH in the TB and LL. However, there was no effect of temperature on pH in the BF muscle. This indicates that temperature affected pH in the TB and LL muscles but not in the BF muscle, as described on Table 2.

Table 2 Analyse of pH and temperature between muscle and slaugher method

	Muscle											
Parameters of the model	TB				LL				BF			
	B	Std. Error	t	sig	B	Std. Error	t	sig	B	Std. Error	t	sig
(Constant)	5,987	0,117	51,361	0,000	5,933	0,114	51,920	0,000	5,880	0,122	48,199	0,000
Treatment	-0,017	0,045	-0,383	0,702	-0,012	0,044	-0,279	0,781	0,037	0,045	0,841	0,402
Date of Slaughter	-0,017	0,045	2,851	0,005	0,124	0,044	2,808	0,005	0,123	0,045	2,749	0,006
Time of monitoring (hour)	-0,015	0,006	-2,670	0,008	-0,018	0,006	-3,323	0,001	-0,016	0,006	-2,680	0,008
Temperature	0,006	0,004	1,683	0,094	0,007	0,003	2,309	0,022	0,005	0,003	1,490	0,138

Model correlation coefficient R=0.485 for TB; R=0.580 for LL; R=0.497 for BF. Predictors: (Constant), Temperature, Date of slaughter, Treatment (free-bullet and captive bolt), Time of monitoring (hour after the slaughter) and b is dependent Variable: pH

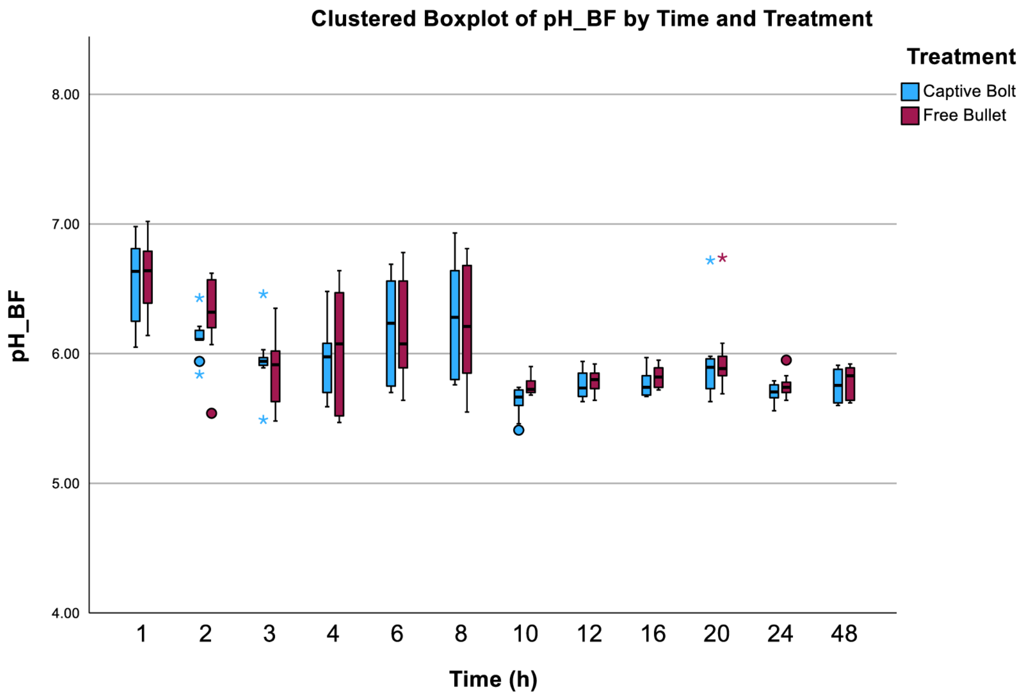

Figure 4 *pH of BF muscles monitored during 48 hours for both slaughter methods.*

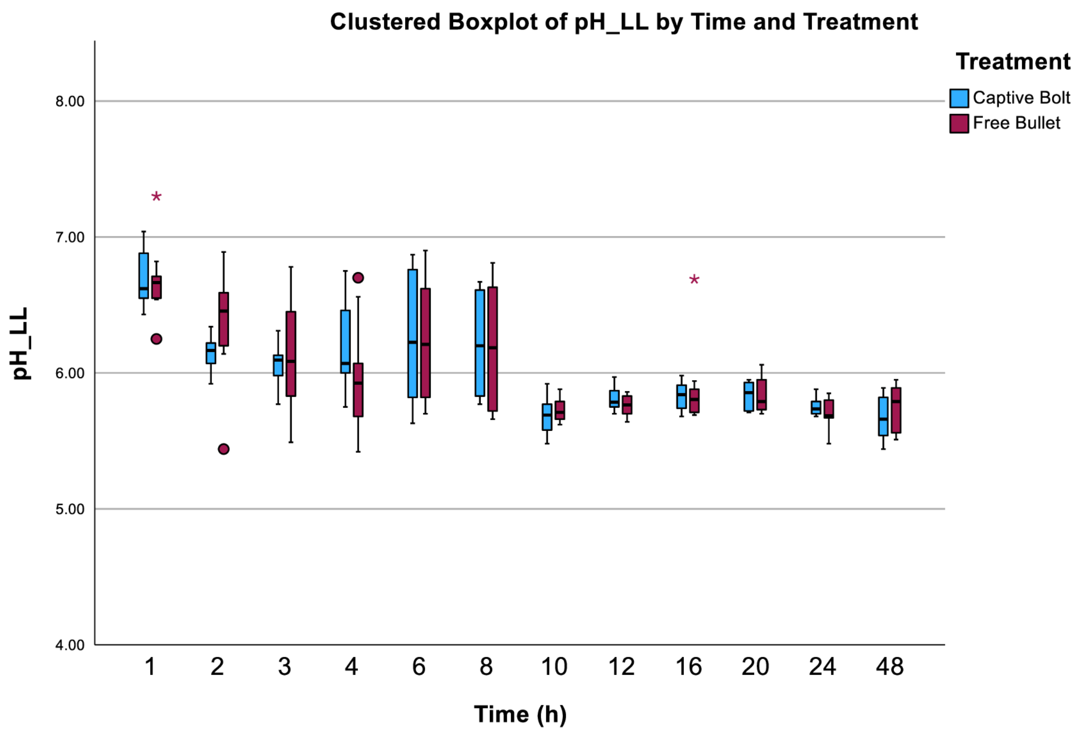

Figure 5 *pH of LL muscles monitored during 48 hours for both slaughter methods.*

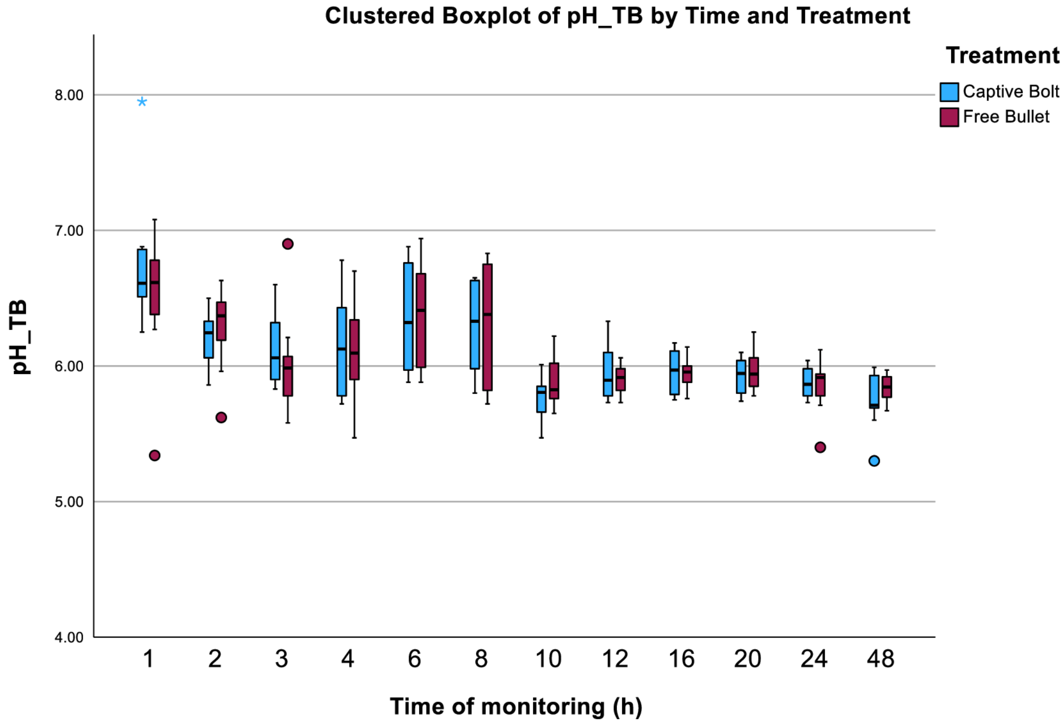

Figure 6 *pH of TB muscles monitored during 48 hours for both slaughter methods.*

4.3. Overall effect of the slaughter method on meat quality

The analysis of carcass and meat quality parameters was conducted to assess the effects of two slaughter methods: free-bullet and captive bolt. The parameters evaluated included BCS, whole carcass weight, hot carcass weight, and the characteristics of different muscles TB, LL, and BF.

In terms of colour evaluation, there are no significant differences in lightness (L*) across any muscle between the two methods. For redness (a*) a marginally non-significant difference is noted in the LL muscle ($p = 0.07$), suggesting that meat from the free-bullet method might be slightly redder, although this finding is not conclusive. No significant differences were found in yellowness (b*) across any muscle.

Based on the results of cooking loss, which indicates the percentage of weight loss during cooking, there was difference between the slaughter methods across the muscles assessed, with p-values of 0.54, 0.65, 0.98, for the TB, LL, and BF respectively. The

WBSF was used to assess meat tenderness, with higher values indicating tougher meat. There is a significant difference observed in the BF muscle ($p = 0.0013$), where slaughtering involving the captive bolt resulted in a higher WBSF, indicating tougher meat compared to the free-bullet method. No significant differences were observed in the TB and LL muscles.

The analysis revealed that the BCS was not different in animals slaughtered using the captive bolt and the free-bullet methods. Similarly, there were no significant differences in whole carcass weight and hot carcass weight between the two slaughter methods, with p-values of 0.43 and 0.32, respectively. Overall, most of the parameters measured did not show statistically significant differences between the free-bullet and captive bolt methods, except for the WBSF in the BF muscle, where meat from the captive bolt method is significantly tougher (Table 3).

Table 3 Comparison of carcass and meat quality parameters with estimates and standard errors for free bullet and captive bolt slaughter methods across different muscles.

Parameter	Muscle	Slaughter method		
		Free bullet (n = 10)	Captive bolt (n = 10)	p-value
BCS		3,15 ± 0,16	3,4 ± 0,16	0,35
Whole carcass weight		41,8 ± 1,52	40,05 ± 1,52	0,43
Hot carcass weight		21,53± 1,05	23,05 ± 1,05	0,32
WBSF (N)				
	TB	38,37 ± 2,14	41,35 ± 2,14	0,34
	LL	36,11 ± 2,89	41,27± 2,89	0,22
	BF	33,83 ± 2,76	48,85 ± 2,76	**0,001**
Cooking loss (%)				
	TB	23,65 ± 1,22	22,557 ± 1,22	0,54
	LL	26,4 ± 0,84	25,86 ± 0,84	0,65
	BF	28,14 ± 0,83	28,11 ± 0,83	0,98
L*				
	TB	37,70 ± 0,57	36,98 ± 0,57	0,38
	LL	35,04 ± 0,44	35,08 ± 0,44	0,94
	BF	35,83 ± 0,60	34,24 ± 0,60	0,08
a*				
	TB	12,43 ± 0,28	12,19 ± 0,28	0,57
	LL	13,08 ± 0,32	12,20 ± 0,32	0,07
	BF	12,89 ± 0,26	12,63±0,26	0,51
b*				
	TB	10,62 ± 0,2	10,11 ± 0,2	0,10
	LL	10,06 ± 0,82	10,93 ± 0,82	0,46
	BF	9,74 ± 0,31	9,46 ± 0,31	0,54

Mean estimates ± standard error, with the p-values indicating the statistical significance of the differences between the methods.

4.4. Influence of slaughter method on carcass damage

The overall damage of the carcasses from the free-bullet method had significantly less overall damage (1.20 ± 0.24) compared to the captive bolt method (2.40 ± 0.24), with a p-value of 0.002. Regarding the external damage, the free-bullet method also resulted in significantly less external damage (0.80 ± 0.33) compared to the captive bolt method (2.20 ± 0.33), with a p-value of 0.009. External damage often includes visible bruising or injury on the skin or outer layers of the carcass, which was more pronounced with the captive bolt method. For internal damage, the difference between the free-bullet (0.40 ±

0.13) and captive bolt (0.70 ± 0.13) methods was not statistically significant, with a p-value of 0.14 (Table 3).

Table 4 Analyse of carcass damage in both slaughter methods.

Parameter	Slaughter method		
	Free- bullet (n = 10)	Captive bolt (n = 10)	p-value
Damage	1,20 ± 0,24	2,40 ± 0,24	0,002
External Damage	0,80 ± 0,33	2,20 ± 033	0,009
Internal Damage	0,40 ± 0,13	0,70 ± 1383	0,14

Mean estimates ± standard error, with the p-values indicating the statistical significance of the differences between the methods, damage related to general lesions observed in whole carcass, external damage indicates how many parts of the carcass was affected, and internal damage indicate absence of blood splash on diaphragm.

5. Discussion

This study aims to examine the effect of captive bolt and free-bullet slaughter methods on animal behaviour and meat quality of fallow deer. Stress behaviour were prominently recorded in higher extent during the captive bolt methods in comparison to the free-bullet slaughter method. Aggression, alertness, and movement were the commonly recorded behaviours exhibited by fallow deer for the two slaughter methods. This agrees with studies that have recorded a high level of pre-slaughter stress behaviour such as alertness, and high movement in cattle in the USA (Shearer 2018). Similar behaviour was also recorded in sheep in the United Kingdom with alertness being the most recorded (Anil 2020). Aggression, alertness, and movement are characteristic to pre-slaughter behaviour, when using techniques such as captive bolt and free-bullet. These behaviours are important signs to monitor the animal's well-being and reaction to the slaughter process.

Aggression behaviour was only recorded in the captive bolt method in more than 2 % of the fallow deer during the two slaughter sessions. Other studies find similar behaviour, study by Meng et al. (20012) that revealed that handling tactics such as putting deer into small enclosures or making loud noises resulted in up to 30% of animals exhibiting aggressive behaviour. Similarly, in elk it was recorded that up to 25% of elk exhibited signs of aggression due to stress induced by poor handling practices before slaughter (Fisher et. 2006). The observed aggression behaviour among fallow deer in the captive bolt method treatment can be connected to the management before slaughter, when animals were driven into the pen for handling and contact with people, experienced overcrowding which can all contribute stress and increased aggression among the deer. In contrast, the use of a free bullet, which may involve fewer interactions and less stressful handling, is associated with no aggression in these animals.

The alertness behaviour was highly observed in captive bolt method as compared to free-bullet, with more than 81% of the deer recorded to be alert. This agrees with studies done by Pecorella (2016) and Grigor et. al. (1999) on females fallow deer and red deer, respectively, where increased alertness and vigilance behaviors were recorded when animals were exposed to unfamiliar environments or pre-slaughter activities. Furthermore, male deer were noted to have higher alertness than females in pre-slaughter holding pens (Grigor et al., 1999) during the captive bolt method. Prolonged restraint before stunning is attributed with the increased stress responses, as indicated by higher cortisol levels in both sexes of deer (Grigor et al., 1999). In the captive bolt method, pre-slaughter handling (such as driving the deer into pens and proximity to people) can increase stress and alertness before the bolt is applied. In contrast, with the free-bullet method, the deer may be less stressed, without handling, because they are not in closed confinement or directly interacting with humans before the shot. During the free-bullet method, human presence was limited to only two people (shooter and person video recording), this would explain the reduced alertness of among deer before the slaughter. Even though after each shot animals moved 30-100 m away, the free-bullet method, if executed correctly, may cause less pre-shooting stress.

The movement behavior between the two slaughter methods, is highly recorded in free bullet slaughter in comparison to captive bolt. The research done by Terlouw et al. (2015) in cattle observed movement behaviours, including jumping and running in both free-bullet and conventional stunning methods. In another study by Grist (2020) the captive bolt stunning involves physically restraining the animal before the application of the stunning device, and thus the movement behaviour is reduced simply due to the confined space. The free-bullet method involves shooting the animal with a firearm, which can lead to heightened stress and alarm after the first shot. The remaining animals may react strongly to the sounds of firearm, and the deceased animal on the ground, resulting in an increase of movement behaviors such as jumping or running to escape the threat. Additionally, they simply had the space to exhibit this type of behaviour compared to those deer who were slaughtered within the handling system.

Results suggests that the methods of slaughter investigated do not have a substantial impact on the pH levels in the TB, LL, and BF muscles of fallow deer. Studies have shown that different slaughter techniques affect pH levels, with captive bolt stunning before slaughter producing meat with high ultimate pH in cattle, compared with less handling methods (Maghfiroh et al., 2014). Similarly, religious slaughter without stunning resulted in higher pH values at 24 hours post-mortem compared to traditional stunning methods (Barrasso et al., 2021). In lambs, electrical head-to-leg stunning led to lower initial pH values than captive bolt or no stunning (Petersen & Blackmore, 1982). The slaughter methods in this study do not showed significant difference of the pH in the muscles, which may be attributed to the low number of animals slaughtered at once for both methods (n = 5 per treatment per slaughter date), which could have resulted in insufficient exposure to stress-related behaviours even in free-bullet method due to response of animals after the first shot, and also the pH meter broke in the second day of slaughter that could affected the measurement. Additionally, chronic stress has been recorded to alter muscle physiology (Fushimi et al., 2023) this could have contributed to the changes the in the pH during the captive handling process.

The analyses of pH in muscles (TB, LL, BF) showed collinearity with the date of slaughter. The slaughter of fallow deer was done during the winter period to ovoid seasonal effect. Seasonality was associated with a higher risk of elevated pH in both pigs and cattle (Perre et al., 2010; Amtmann et al., 2006). Seasonal temperature differences influence the phosphorylation of sarcoplasmic proteins in pork, affecting pH decline (Zeng et al., 2021). In beef, the season duration impact meat quality, with poorer pH, electrical conductivity, and color values observed in summer for heifers and in summer and winter for bulls (Marencic et al., 2012). Temporal factors within the year can influence stress levels, handling practices, and the physiological state of animals, which in turn can affect muscle pH. Although both slaughter events occurred within the same month, they took place in different weeks with varying weather conditions. The first slaughter was conducted on a colder day with snow, whereas the second slaughter occurred on a milder day without snow. These differing environmental conditions could have contributed to variations in muscle pH, thereby explaining the observed correlations between pH and muscle samples.

The time of monitoring was collinearity with pH levels in the TB, LL, and BF muscles, indicating that pH values change over the monitoring period. In hunted red deer, it has been observed that pH values in meat decrease over time, with young and female individuals achieving ultimate pH values below 5.8, reflecting proper meat maturation (Viganò et al., 2017). Similarly, pH levels typically decrease post-slaughter, with young and female deer showing lower ultimate pH values (Viganò et al., 2017). Research on beef carcasses has shown that pH values 1hour post-slaughter can range from 6.2 or lower to 6.7 or higher, with a continuing decline to an ultimate pH level around 24 hours post-mortem (Khan & Ballantyne, 1973; Hamoen et al., 2013). This decline is attributed to the natural metabolism of glycogen in the muscle tissue. Given that the monitoring period in this study was 48 hours, the observed changes in pH over time are consistent with the expected post-mortem biochemical processes.

Temperature control during post-mortem processing is collinearity with pH in the TB and LL muscles, but not in the BF muscle. High temperatures combined with rapid glycolysis can lead to an accelerated decline in pH, resulting in protein denaturation and the development of pale, soft, exudative (PSE) characteristics in various meats (Zhu et al., 2011; Kim et al., 2014). Strobel et al. (2020) described the effective temperature control during post-mortem processing is crucial, as it significantly impacts muscle pH. The animals from this study did not experienced protein changes, due to the pH, and pH decreased under 6 for the carcass in both methods after slaughter. Proper cooling and temperature management in the processing chamber are essential for ensuring efficient glycolytic flow and subsequent pH decline.

In this study the impact of the slaughter method on physical meat quality on the muscles TB, LL, and BF from fallow deer was evaluated, including cooking loss, WBSF, and colour. The cooking loss did not show significant differences between muscle and methods. The agrees with research by Friedrich et al., (2014) compared on-farm gunshot slaughter and conventional captive bolt stunning in cattle and found no significant differences in cooking loss. Some studies have recorded a difference in cooking loss between slaughter methods. A study from Domink (2012) in roe deer found that the cooking loss was higher in muscles of female roe deer. Additionally, Agdbeniga (2014) using Kosher slaughter on beef showed a lower cooking loss and more tender beef compared to conventional slaughter methods. The evidence for significant differences in cooking loss between free-bullet and captive bolt methods remains inconclusive, as multiple studies have reported no substantial variations. The lack of difference is probably connected with lack of differences in pH. The variation in cooking loss may not be only influenced by the slaughter method; other factors, including age, pH, sex, and species, in conjunction with the slaughter method, could also affect cooking loss.

In the WBSF analysis, a significant difference was observed in the BF muscle, where the captive bolt method resulted in a higher WBSF compared to the free-bullet method. Research by Therkildsen et al. (2020) suggests that factors influencing WBSF can vary with animal age and muscle type. According to Obuz et al. (2004), the BF muscle exhibits a unique tenderization pattern, with WBSF decreasing between 40-60°C and increasing between 60-70°C, and generally showing higher WBSF values compared to the LL muscle. This can be attributed to the BF muscle's location in the hind limb, where it is highly active and contains a greater proportion of fast-twitch fibres. As a result, the BF muscle is expected to undergo rapid biochemical changes post-mortem. The prolong practice in captive bold could have influence the prolong use of BF muscle by animals refraining from restrain and causing subsequent physiological changes in the muscle, offering insights into how slaughter methods may differentially impact more active muscles.

The analysis of color parameters L* and b* showed no significant differences between muscles and stunning methods. However, the parameter a* exhibited a marginally nondifference in the LL muscle ($p = 0.07$), suggesting that meat from the free-bullet method might be slightly redder compared to that from the captive bolt, though this result is not conclusive. Yu et al. (2017, 2019) reported that the LL muscle generally displays higher redness (a*) values and better color stability compared to other muscles. Studies on various animals, including deer and poultry, have demonstrated that different slaughter techniques can impact meat color (Hafiz et al., 2015). In red deer, pre-slaughter factors such as transportation and handling can elevate plasma cortisol levels and muscle pH, potentially influencing meat color (Smith & Dobson, 1990). However, these studies do not directly address the specific impact of slaughter methods on venison color. The lack of significant differences in pH between the slaughter methods in this study may explain the absence of significant differences in meat color. Increasing the sample size could potentially reveal differences between slaughter methods and their effects on meat color.

The carcass damage results showed a significant difference in damage on captive bolt compared to the free bullet method. Regarding the internal damage in the carcass on the diaphragm region, more internal damage was observed with the captive bolt; however, the difference is not large enough to be considered significant between methods. Studies have shown that low-stress handling can reduce cortisol and lactate levels, increase initial pH, and decrease carcass damage (Peres et al., 2014). Research by Gobena & Kumsa (2020) indicated that the free-bullet method tends to cause less damage to the carcass compared to other methods, suggesting it may be a less handling promote better carcass quality. The increased damage associated with stunning using a captive bolt, as compared to a free-bullet method, may be attributed to several factors, stress behavior, contact with humans and unfamiliar environments. These stressors can lead to increased fighting among animals and higher incidences of bruising, fractures etc. The physical and psychological stress associated with these conditions may contribute to the greater carcass damage observed with captive bolt stunning.

6. Conclusions

In conclusion, this study provides an evaluation of the effects of captive bolt and free-bullet slaughter methods on the behavior and meat quality of fallow deer. The findings indicate that stress behaviours, such as aggression, alertness, and movement, were more prevalent in deer subjected to the captive bolt method, highlighting the increased stress associated with this technique, due to confinement, handling, and human interference. In contrast, the free-bullet method, which involves minimal handling and human interaction, was associated with reduced stressful behaviours including aggression toward conspecifics, suggesting it may be a more humane approach for fallow deer slaughter. These behavioral observations are consistent with existing literature on pre-slaughter stress in various animal species and underscore the importance of minimizing stress to improve animal welfare.

The analysis of meat quality parameters, including pH levels, cooking loss, WBSF, and color, revealed that the slaughter method had minimal impact on these factors, with few significant differences observed. The lack of substantial differences in pH between the methods suggests that both slaughter methods can produce comparable meat quality under controlled conditions. However, the study found a significant difference in WBSF for the BF muscle, with the captive bolt method resulting in tougher meat. While the color parameters showed no significant variation, the marginal difference in redness (a*) in the LL muscle suggests potential subtle effects of the slaughter method on meat color that could be explored further with a larger sample size.

Overall, the results of this study contribute valuable insights into the impact of different slaughter methods on both animal welfare and meat quality in fallow deer. The reduced external and internal carcass damage associated with the free-bullet method further supports its use as a potentially less stressful and more humane option with also some economic benefits (less wasted meat). These findings have important implications for the meat industry, particularly in terms of optimizing slaughter practices for game species like fallow deer to enhance both animal welfare and meat quality. Future research should focus on expanding the sample size and hormonal effects, such as cortisol.

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Appendices

List of Appendices

Appendix 1: Dataset used for analyses