Effects of routine handling in the husbandry of common eland

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DISSERTATION THESIS

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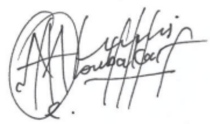
Declaration

I, Abubakar Sadiq Musa, hereby declare that I have completed this thesis entitled "***Effects of routine handling in the husbandry of common eland***" independently, except for the jointly authored publications that are included. In the case of such publications, my specific contributions have been clearly stated at the start of the relevant publication chapter. Furthermore, I confirm that proper acknowledgement has been provided within this thesis for any references made to the works of others. I also ensure that this work has not been, nor is it currently submitted for any other degree to this or any other university. All information sources have been quoted and acknowledged.

In Prague, March 1st, 2024



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Abubakar Sadiq Musa

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Abstract

In today's global wildlife enterprise, there lies a big opportunity for game farming, which is also gradually shifting to intensification. One crucial aspect that determines the success of intensification of large bovids such as common eland is the ability to tame, handle, and manipulate them with ease without compromising their welfare. It is in light of this that the present study evaluates possible ways and factors that would affect or improve the ease of handling, social interaction, and harmony of common eland in an intensive management system. These aspects are crucial as they impact overall animal welfare. Two research processes led to the entirety of the present study, the first being an evaluation of the effect of immunocastration in ameliorating agonistic behaviour, ease of handling as well as the effect thereon on the activity budget of two groups consisting of 30 mixed-sex growing common eland. Here, the hypothesis focused on the males, where half of the males were immunocastrated, and the rest and females served as control. The second study was a longitudinal study that generally investigated the effect of routine handling and social dominance on the blood biochemistry parameters of growing eland. Overall, immunocastration had no significant influence on most of the response variables, including activity budget, social behaviour, social rank, agonistic behaviour and temperament during handling. However, there were changes in activity between the pre- and post-vaccination periods, which were attributed to changes in the immunological response to immunocastration that were mild to cause a significant change. Likewise, there was a decrease in aggressive behaviour in the juvenile, which upholds the recommendation for this practice from an early age. The temperament was, however, not influenced by immunocastration, but it improved over time, highlighting the importance of routine handling in habituating such large, non-domestic bovids in an intensive management system. The blood health biochemistry parameters were not influenced by social rank. However, most of these parameters were influenced as a result of handling stress but were all within the reference range recommended by ZIMS. Thus, this study has paved the way, giving optimism to including common eland in domestic intensive game production. However, it has also opened a wide research gap for further exploring the effect of conventional domestic husbandry management techniques on the productivity and welfare of eland and other related antelopes.

***Keywords**: Domestication; Habituation; Immunocastration; Intensive management; Welfare.*

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List of abbreviations

ALB – Albumin

ALP – Alkaline Phosphatase

ALT – Alanine aminotransferase

AMLY – Amylase

AWIN – Animal Welfare Indicators

BUN – Blood Urea Nitrogen

BWAP – Bristol Welfare Assurance Programme

Ca – Calcium

CBI – Clutton-Brock index

CHOL – Cholesterol

CK – Creatine Kinase

CREA – Creatinine

DS – David's Score

fAM – faecal Androgen Metabolite

GLOB – Globulin

GLU – Glucose

GnRH – Gonadotropin-Releasing Hormone

h' – Landau's linearity index

HPG – Hypothalamic-Pituitary-Gonadal axis

I&SI – Inconsistencies and Strength of Inconsistencies

LDH – Lactate Dehydrogenase

LH – Luteinising Hormone

P – Phosphorus

PD – Proportion of Dominations

PUFA – Poly Unsaturated Fatty Acid

SFA – Saturated Fatty Acid

SR – Social Rank

TBIL – Total Bilirubin

TP – Total Proteins

tTri – Triangular Transitivity

ZIMS – Zoological Information Management System

CHAPTER 1

Introduction

1.1. General introduction

The husbandry of animals has been a longstanding traditional practice throughout the history of humanity (Diamond, 2002). Since time immemorial, humans have manipulated several animal species for their benefit, resulting in a broad spectrum of domesticated animals. Domestication is still an ongoing process, with more wild animals being added to the list of domesticated species, particularly with the increasing human population, decreasing habitat for wildlife, and the direct need for the conservation of threatened species. Before this age, humans had used several mechanisms in selecting and breeding animals through the cautious observation of better performance, better symmetry in structure of species, growth, temperament, high breeding and calving rates, and submissiveness (Diamond, 2002). As a result, we have achieved several standard husbandry practices for different species. In recent years, some wild animals that have been recognised to have good domestication potential, such as the common eland (*Taurotragus oryx*), have also undergone gradual steps from ranching (or what is known as game ranching) to game farming, and more recently, intensification of production practices (van der Merwe et al., 2021).

The gradual progress toward the domestication of wild animals has also seen the adoption of husbandry practices common to domestic animals. In South Africa, for example, the game industry has significantly transitioned from animals being initially managed in game parks to managed alongside domestic animals in mixed farming systems and now managed intensively alone (Sommerville et al., 2021). Though this gradual transition has allowed farmers to understand the basic principles of wildlife management in captivity, this has opened a wide gap in research to understand the impacts of novel management practices on the welfare of these animals in captivity. Thus, it would be crucial to understand the impact of these husbandry practices on their products, performance, and welfare, particularly in an age where animal welfare is of utmost concern. A key aspect of husbandry management of captive wild animals is to tame and adapt them to humans in such a way as to ease stress of both the animals and the handlers.

Routine handling is one crucial approach for conditioning wild animals to humans and domestic husbandry techniques. It is the gateway through which animals can be manipulated for health examination, routine management, transportation, assisted reproduction technologies, and research (Ceacero et al., 2014; Pennington et al., 2013). Meanwhile, the purpose for which the handling is done can significantly affect the animal's welfare. For example, this can be invasive, such as surgery or blood collection, or non-invasive, such as assessing body condition and morphometry. Anaesthesia and sedatives have been used to reduce stress on wild animals during handling but these agents have their downside, affecting the recovery of animals, causing a change in behaviour post-anaesthesia, have significant cost implications, and can sometimes lead to death if not correctly administered (Arnemo et al., 2006; Mentaberre et al., 2010). It is recommended that anaesthesia should only be considered if all other available options have failed (Soulsbury et al., 2020). Castration is another method of conditioning male animals to ease manipulation and management by decreasing their aggressiveness and calming them (Price et al., 2003). Additionally, castration of male animals help in controlling indiscriminate breeding to maintain population size base on available resources, and also prevent continuation of poor genetic traits (Needham et al., 2017). Meanwhile, over the last three decades, the ethical concern about physical methods of castration, such as surgical castration, has ushered in new alternatives, such as immunocastration, and this has proven effective in controlling agonistic behaviours such as musth in elephants, aggressive behaviour in camels, and conditioning horses during equestrian sports (Malmgren et al., 2001; Ghoneim et al., 2012; Bertschinger & Lueders, 2018). Thus, this approach may also show potential in controlling behaviour and conditioning antelopes, such as common eland, in captivity for easier management and better performance in an intensive management system.

The common eland has been successfully tamed in Africa and other parts of the world, and the initial domestication attempt dates back to 1892. It was first introduced outside of its range of origin to Eastern Europe (specifically at the Askanya Nova Zoo; Treus & Lobanov, 1971) and were even conditioned for milking, which was used not only for food but also as a prophylactic treatment for some ailments due to its rich nutrient content compared to milk from other species (Treus & Lobanov, 1971). Moreover, their large body size, high carcass dressing percentage, low intramuscular fat with a high proportion of polyunsaturated fatty acids, adaptability to different climatic conditions, drought tolerance and ability to use high-tannic vegetation (wider range than domesticated species) and adaptability to essential domestic husbandry practices have been a few of the motivations driving consideration for its domestication (Flack, 2013; van Vliet et al., 2016). Internationally, the breeding of common eland has largely been in a rangeland and extensive management with some farm having to cull about 100 animals yearly in Southern Africa (Hoffman & Wiklund, 2006). The common eland is currently categorised as Least Concern according to IUCN and has been enlisted among 32 wildlife species to be considered farm animals in South Africa (Sommerville et al., 2021). Though they have been deemed to be tamed, there is a need for further studies on how to condition them to routine management in an intensive management system and to assess the impact of such a management system on their welfare.

1.2. Aims of the thesis

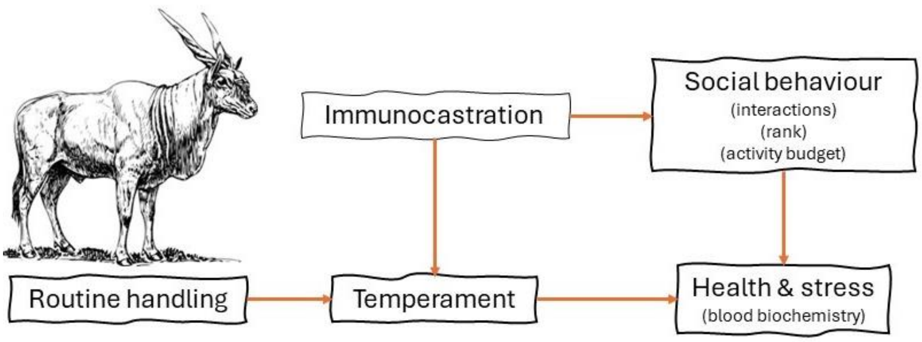

Figure 1.1. Conceptual framework showing the general aims.

The present study was motivated by the gradual increase in intensification of common eland management practices in captivity due to its docility, adaptability, and productivity. As such, it aims to examine the effect of welfare-friendly husbandry management techniques on the behaviour, health, and welfare of common eland in captivity (Figure 1.1). With this, this work seeks to achieve the following objectives:

- Examine the effect of routine handling on the temperament of common eland.

- Examine the effect of immunocastration on the activity budget, social and agonistic behaviours of common eland in captivity.

- Evaluate the effect of using immunocastration to improve the temperament of the eland during handling.

- Examine the effect of routine handling and social dominance on the blood biochemistry parameters of common eland.

1.3. Research questions

The present study seeks to address the following questions:

- Will routing handling improve the temperament and habituation of eland to restraint for routine management procedures in captivity?

- Will immunocastration positively influence common eland's agonistic and social behaviours in an intensively managed social group?

- Will immunocastration help improve the temperament and habituation of common eland during routine handling?

- To what extent will social and handling stress impact common eland's health blood biochemistry profile?

1.4. Chapters overview

The present study consists of eight chapters. Chapters 1, 2 and 3 are general introduction, literature review, and methods respectively. These three chapters are general aspects of the thesis which give a broader overview of the main aims, objectives, and research outputs. The thesis consists of three articles which are published in Web of Science-indexed journals. The research outputs are all connected and within the scope of the aims and objectives of the study.

The first research output (Chapter 4 – **Musa et al. 2024a**) of this thesis, entitled "*Activity and social behaviour of common eland and the effect of immunocastration thereon*", evaluates the effect of using immunocastration to ameliorate agonistic behaviours of common eland in an intensive management system. The research design adopts a prototype of most domestic intensive husbandry management systems, comprising two age categories or groups. Within these age groups, 50% of the males were immunocastrated while the others were left as control. Females were included in the design to simulate a mixed-herd system of management. The study tested the effect of immunocastration treatments and period (pre- and post-vaccination) on common eland's activity and social behaviour. The output from this study was published in *Applied Animal Behaviour Science* (https://doi.org/10.1016/j.applanim.2024.106189).

The second research output (Chapter 5 – **Musa et al. 2021**), entitled "*Habituation of common eland (Taurotragus oryx) to intensive routine handling and the effect of immunocastration thereon*", is connected to Chapter 6. Here, immunocastration and routine handling were seen as a potential tool in ameliorating agonistic behaviours and improving habituation toward humans during handling, a crucial criterion for such intensively managed game species. The study evaluates the effect of immunocastration on the temperament during routine handling, the order in which animals present themselves for handling, and potential changes along the study period while taking other factors such as age, sex, body mass, and faecal androgen metabolites as fixed factors. The output from this study was also published in *Applied Animal Behaviour Science* (https://doi.org/10.1016/j.applanim.2021.105294).

The third research output (Chapter 6 – **Musa et al. 2024b**), entitled "*Effects of temperament during handling and social rank on the blood biochemical parameters of common eland (Taurotragus oryx)*", aims to assess the impact of handling and social dominance on the overall health and blood biochemical profile, serving to evaluate the welfare implications of such practices and the confinement of eland in an intensive system. The research findings were published as a short communication in *Veterinary Research Communications* (https://doi.org/10.1007/s11259-024-10296-1).

Finally, Chapters 7, 8, and 9 offer a general discussion, conclusion, and implications of the study for the intensive husbandry of eland, and references respectively.

Note: The referencing formats and guidelines of all the published articles were maintained according to the respective journal formats in the corresponding chapters.

CHAPTER 2

Literature review

Wild animals are a core part of the global ecological system and a valuable resource for humans. Since time immemorial, wild animals and the products derived from them, such as meat, milk, and skin, have played an important role in the development of man and the evolution of human civilisation. This, in turn, has resulted in the domestication of pets and today's livestock animals (Harding & Teleki, 1981; Diamond, 2002). Archaeologically, gazelle in the Middle East and ancient Egypt and some antelope species, such as eland in East Africa, were found to be kept for meat production. Also, reindeer in the Arctic were used for food, clothes, and tool production. Moreover, in China, wild animals such as deer (Wang et al., 2019) and bears have been kept in captivity for the production of antlers and bear bile for more than 3000 years, for medicinal purposes (Huang & Li, 2006; Bacon, 2008).

That said, the interest in game production has increased exponentially globally. It has largely been motivated by the increase in human population and agricultural land use, as well as the demand for wildlife products for traditional medicine, human consumption, ecological management, or just based on a need for conservation. These caused a great challenge to wildlife and have threatened some animals to extinction. Meanwhile, the social and ecological value of wild animals is also increasingly being recognised by scientific institutions, the government, and the public (Clucas et al., 2008). Thus, this has facilitated more scientific and sustainable modalities of using wildlife resources such as game ranching, ecotourism, or trophy hunting (Lindsey et al., 2007, 2009). Furthermore, the International Union for the Conservation of Nature and Natural Resources (IUCN) has put forward a strategy that ensures the sustainable use of wildlife resources as part of its core theme, ensuring that wild animals are used in a more welfare-friendly approach that would also contribute to the economic development of developing countries (IUCN, 2000; Abensperg-Traun, 2009).

The husbandry of wild animals or game farming is the most intensive form of wildlife production involving intensive management and care to supply wild animal products to the market (Abensperg-Traun, 2009). In recent years, this has become a very lucrative business due to the high value (e.g. trophy) and preference placed on wildlife products; thus, this is one of the key drivers that is motivating South African farmers to convert their livestock farms to game farms, thereby contributing to wildlife production (Taylor et al., 2020). In Southern Africa, most game farmers are currently adopting sophisticated breeding technologies and a more intensive approach to game production. Though the industry has faced tremendous challenges because of the recent global life-threatening zoonotic diseases such as Ebola and COVID-19, that led to a collapse in tourism, the prospects in the game industry are still promising (van der Merwe et al., 2021).

2.1. Eland as a model species for intensive game production

The common eland has been described as the most suitable antelope species for domestication (Woodford, 2000). This recommendation was made not only by virtue of its large body mass but also because of its calm temperament and ease of habituation to humans, making it a good trait for taming (Wirtu et al., 2005). The common eland is internationally well-represented in captive populations and is used for game meat production in South Africa and Namibia, with some farms culling over 100 heads in a feedlot annually (Hoffman & Wiklund, 2006). They are also used in ecotourism in game parks and zoos and are also found across the globe in Europe and the Americas, in private farms, research farms, and zoos. People passionate about wildlife have recently also started to have common eland in their private farms, which are purchased from zoos or other private breeders in Europe. These promising assurances support that the common eland is considered as Least Concern by the IUCN. In 1892, a game researcher in Askanya Nova, Ukraine, first attempted to domesticate eland from four cows and four bulls from Africa (Treus & Lobanov, 1971). This initial attempt would later pave the way for future consideration of common eland for domestication not only in its original range but in other marginal environments differing greatly in climate, which might not be possible for breeding conventional livestock. This unique adaptability to different environments, tolerance to tannic vegetation, water independence, and flexibility in adapting to traditional cattle-like management systems create a promising atmosphere for considering investing in intensive breeding of eland in captivity (Pappas, 2002).

Taxonomically, eland are part of the tribe *Tragelaphini* (Khademi, 2017), which is described based on their spiral horn. This family consists of two genera (*Tragelaphus* and *Taurotragus*), nine species, and eighteen subspecies, among which are the Sitatunga, Nyala, Bushbuck, Kéwel, Mountain Nyala, Lesser Kudu, and Greater Kudu within which three are considered critically endangered (Estes, 1991; IUCN SSC Antelope Specialist group 2016). Until recently, eland were the only species separated in the *Taurotragus* genus (Harper, 2001). However, eland were reassigned to the genus *Tragelaphus* following molecular phylogenetic studies and proof that the genus can hybridise with the greater kudu (*Tragelaphus strepsiceros*) and the sitatunga (*Tragelaphus spekii*; Flack, 2013). Meanwhile, some still refer to the genus as *Taurotragus*; firstly, on the basis that it separated from *Tragelaphus* at the end of the Late Miocene; secondly, because the molecular evidence proposing to include them into *Tragelaphus* was done based on mitochondrial DNA; and lastly, the mentioned hybrids were infertile (Khademi, 2017). Thus, this is a subject for future investigation. The genus *Taurotragus* is further divided into two species with five sub-species (Khademi, 2017). For the interest of domestication and intensive breeding, the common eland (*Taurotragus oryx*) is the species of interest. Though three subspecies (which are distinctly distributed across Africa) are recognised in this species (Livingstone's eland, Cape eland and Patterson's eland), the difference has been attributed to phenotype rather than genetics (Kingdon & Hoffmann, 2013).

The husbandry of eland has seen the adoption of cattle husbandry practices which seems suitable. Aside from this, they also show much similarity in anatomy and physiology. Just like the *Bos indicus*, the common eland male and female both have horns, though the latter has a spiral in its horn. There is distinct sexual dimorphism a few months after birth in common eland, with males having spirals thicker from the base of the horncore designed for wrestling and that of females being more elongated designed for stabbing and protection against predators. These horns can range from 43 to 70 cm, with that of females being longer. Body length can range from 200 to 345 cm, and weight can range from 400 to 940 kg, with males being heavier (Pappas, 2002). Some farmers have also reported a weight of up to a tonne under intensive husbandry management. Eland have no specific breeding season, with females exhibiting oestrus ranging from 21 to 26 days and lasting 2-3 days. The gestation period of eland was recorded on average as 276 days with birth weights of 34.9 and 27.9 kg for male and female calves, respectively, at the Askanya Nova Institute (Treus & Lobanov, 1971). These values were similar to the findings of Posselt (1963) in southern Africa. Furthermore, Lightfoot (1977) noted that eland have a better calving percentage than cattle but would be more promising in the future with intensive management and selective breeding to help avoid calve mortality.

2.2. Significance and benefits of intensive breeding of common eland

Intensive management of common eland helps ensure humane and ethical farming practices such as adequate enclosures, proper veterinary care, and access to good natural forage, all of which are essential requirements for sustainable captive breeding. These practices generally promote the animals' well-being while enhancing the quality of their products. One of the primary benefits of intensive management for eland meat production is reduced pressure on wild populations. Overhunting and habitat loss are significant threats to these animals in their natural habitats. Moreover, the sustainable, intensive production of eland for meat, milk, or ecotourism can be economically viable by providing local communities with an alternative income source.

By breeding eland in captivity for meat, we can help alleviate the demand for wild-caught animals, thus contributing to their conservation. There are numerous benefits to the intensive breeding of game animals, either for meat, milk, aesthetic products such as skin, medicine, trophies, ecological management, conservation, or addressing climate change. The common eland, when compared to other domestic animals, performs better when placed in challenging environments. Eland is an intermediate feeder, which means they can browse and graze, which makes them better off thriving in environments with plants containing higher toxins and tannins that can be deleterious to other domestic animals (Retief, 1971). In the intensive management system, however, there would be a need for supplementation with feed and minerals to meet their requirements, which on the extensive system could be sufficed by a wide range of flowering plants, shrubs, and forbs such as *Acanthospermum, Bidens, Combretum, Commiphora, Grewia, Rhus, Tagestes, Tarchonanthus and Ziziphus* (Skinner & Smithers, 1990; Fabricius & Mentis, 1990). The feeding and adaptability to this wide range of plant species might account for the tolerance of eland to a wide range of ecto- and endoparasites such as trypanosomiasis, a disease known to be transmitted by the tsetse fly (Posselt, 1963; Hansen et al., 1985). Common eland is also a water-independent species since they obtain most of their water from their diet (Skinner & Smithers, 1990).

Intensive wildlife management of common eland (*Taurotragus oryx*) in captivity for meat production represents a unique approach to conservation and sustainable food production. The common eland has been hypothesised to serve as a potential substitute for cattle in feedlot production to produce game meat given the growing demand to reduce the effect of greenhouse gasses on global warming and also consumer demand for healthy meat (Hoffman & Wiklund, 2006; Bartoň et al., 2014; Cromsigt et al., 2018). A comparative study on chemical, physical and sensory characteristics of meat from eland and Fleckvieh cattle (*Bos taurus*) finished under similar controlled conditions of feeding and management shows that common eland meat had lower intramuscular fat and collagen but a high amount (50% more) of polyunsaturated fatty acids (PUFA). Moreover, the ratio of polyunsaturated fatty acids to saturated fatty acid (PUFA/SFA) in common eland was double that of cattle, despite being fed the same diet. It exceeded 0.4, the minimum ratio considered to reduce the risk of coronary disease in humans (Wood et al., 2008). Further studies have confirmed these findings with recommendations on improving the tenderness of eland using pelvic suspension and wet ageing in the commercial processing of eland carcasses to improve overall meat quality (Needham et al., 2019, 2020). Generally, intensive wildlife management of common eland in captivity for meat production can be seen as a multifaceted approach that addresses food security, rural development, and conservation in southern Africa. When conducted responsibly and ethically, it will offer a sustainable alternative to traditional livestock farming, promoting both environmental and social benefits while providing a nutritious and flavourful source of meat.

Aside from meat, eland also produce rich, nutritious, and healthy milk which is an essential goal for its intensive breeding. Findings from different parts of the world and management systems have shown that eland milk is better than that from cattle. The first notable attempt to milk eland cows commenced as early as 1950 when 21 milking cows were trained and conditioned for milking (Treus & Lobanov, 1971). One of the cows was recorded to produce seven litres daily, amounting to 637 litres in her seventh lactation. The milk produced was found to have a higher amount of dry matter, fat, albumin, protein, non-digestible protein, lactose, calcium, and phosphorus than that in cattle milk (Treus & Lobanov, 1971). The higher amount of fat was an important trait for future consideration in cheese production. Supporting these findings, Hall (1975) reported that eland can produce good-tasting, rich, creamy milk of up to 15 pounds daily. Eland milk is also rich in anti-bacterial properties, which help improve its shelf life for up to 8 months compared to cattle, which can deteriorate in a similar condition in a few days (Uspenskii & Saglanskii, 1952).

2.3. Some challenges and requirements of intensive management of eland

Though interest in game production has spawned, intensive wildlife production has its share of challenges, just like conventional livestock production. Among these challenges are space limitation, animal physiology and behaviour, handling for examination, diseases, management requirements, and the impacts of these challenges on the welfare of the animals. Like cattle, eland are large bovids that require vast space which is difficult in intensive management. Lack of adequate space can increase the chances and rate of agonistic behaviours that can be detrimental to subordinate individuals, which might result in injuries, skin damage, and possible health complications. High stocking density can also result in poor production parameters such as reproductive performance, growth, and quality of products. Moreover, eland have extraordinary jumping ability despite having a large body mass like the cattle (Retief, 1971). It is reported from several pieces of literature that they can jump a height of 1.5m from a range of standing positions, which can be up to 3m for young animals (Hillman, 1979; Kingdon & Hoffmann, 2013). Meanwhile, they tend to lose this jumping ability over time with domestication and habituation to captive settings unless agitated or frightened (Posselt, 1963).

Generally, intensive management of eland requires an understanding of their ecology to help ensure the facility is appropriately designed to provide the appropriate space for the animals to exhibit their normal social behaviour for good welfare. In the wild, eland are gregarious and nomadic animals. They live in groups of 8 to 12 individuals and a herd of up to 70 animals; even up to 500 to 1000 animals during sporadic migration have been recorded (Pappas, 2000). They are also non-territorial, but their social structure is strongly influenced by a dominant hierarchy and mainly consists of females, calves, and juveniles staying together. In contrast, adults stay in a small separate group (Estes, 1991). During oestrus periods, females reorganise themselves in small groups where they come in contact with dominant males for breeding (Underwood, 1981). In captivity, these social structures should be simulated as much as possible for efficient productivity. At the Common Eland Research Facility under the management of the Faculty of Tropical Agrisciences at the Czech University of Life Sciences Prague, eland are housed in a barn of 230 m^2 with a paddock of up to 1.3 ha. This barn is divided into two large pens, each with a feeding alley. Each of these pens, which are 7.2 x 18.3 m, has been used to accommodate up to 30 individuals at a time of mixed ages and thus size (Needham et al., 2022). These animals were shown to be in better condition, though, with *ad libitum* feeding and supplementation with concentrates (Needham et al., 2022; Dearman, 2023). Bothma and Van Rooney (2005) suggest that 20-100 ha should be sufficient for a breeding population of a bull, a sub-adult bull, and 15 cows. This is, however, challenging considering the increasing human population and the need for land for crop-based agriculture.

When designing an enclosure for mega-fauna such as the common eland, it is crucial to take into consideration the potential herd size and the provision of ample space that would be sufficient and would help reduce the incidence of conflicts and possible injuries. The facility should be designed with different compartments to accommodate different age groups, sexes, and physiological states of animals, such as pregnancy, calving pens, and sick animals, if possible. This is particularly important for common eland females, which mostly seclude themselves from the herd for calving and conceal their calves (Underwood, 1979). After calving, the dam and the calves quickly bond through imprinting. Thus, this would be difficult in a mixed herd system where there is no provision for such facilities. In such circumstances, allosuckling or milk theft is most prevalent, with the implication that the maternal calves might find it challenging to suckle from its dam. According to Lightfoot (1977), this accounts for most of the calf mortality in eland production system. To avert this challenge, a facility should consider a calving pen where a calf can easily suckle (especially the needed colostrum) from its dam without difficulty for the first few days of life. When introduced into the herd, this would help build the required immunity and health. However, in a mixed-sex farm where such provision would be difficult, calves can be provided with high-quality feed, and this can be done with the help of a creep feeder, which only the calves can access (Carvalho et al., 2019). As stated earlier, the eland are agile jumpers despite their large body size. With this in mind, it is crucial to construct the outdoor fence relatively high, at least 2.4 to 3 m (De Vos, 1997). Fences can be built as straining posts, line posts, or droppers using strong wooden frames or galvanised pipes. Wooden frames are subject to potential destruction by animals or weather. In such circumstances, a wooden frame can be incorporated with an electric fence to help deter animals (Bothma & Van Rooney, 2005). Furthermore, wire fencing can be used together with an electric fence in an environment where there is the possible danger of attack by predators (De Vos, 1997).

2.3.1. Understanding eland behaviour and social structure for effective husbandry management

The behaviour of animals evolves and changes over time, like physiology and morphology, in response to complex environmental changes to aid survival and increase reproductive success in their native environment. This is different for captive mammals living in an environment entirely different from their natural habitat in which they must adjust to the management procedures in the captive setting. Thus, this can potentially lead to the modification and gradual change in genetic and phenotypic expression in the behaviour of the animals with great divergence as compared to the wild counterpart (McPhee, 2004). Given the captive setting, the long-term objective of keeping the animals in captivity will determine the behavioural modification to suit such objectives. For zoo biologists, maintaining the natural behaviour of captive-bred animals is the top priority in either in-situ or ex-situ systems. This would be paramount when such animals are reintroduced to their natural habitat. Meanwhile, for husbandry management aiming at game production, the top priority is to improve the habituation to humans and reduce the wildness of the animals because such wildness can have great implications in their handling, manipulation, and stress response, which can invariably affect the quality and quantity of its product, such meat and milk (Hoffman & Wiklund, 2006). In the context of wildness in captivity, the common eland has generally been described in broad literature as an animal that can easily be tamed and accustomed to management procedures similar to that of cattle (De Vos, 1997). However, species-specific behaviours need to be synchronised and adapted to the management procedure for successful reproduction, growth, and development.

Common eland are social animals and one of the crucial phenomena to consider while managing eland in captivity is the influence of social dominance in their social organisation (Wirtu et al., 2004; Vymyslická et al., 2015). Within the social system of eland, social dominance is displayed through several communication systems, which include visual display, olfactory cues, auditory signals, and tactile movement (Kiley-Worthington, 1977). In dyadic interactions, these communications further translate into postural, cutaneous, orientation, and protective movement, resulting in agonistic, affiliative, or submissive behaviours (Kiley-Worthington, 1977). Postural movements are characterised by an increase in postural tonus, with an animal showing head and tail elevation in response to an external stimulus of aggression or warning. At the same time, lower postures are associated with fear, sick, or submissive animals (Figure 2.1).

Common eland exhibit protective movement by coordinating all sense organs in directing their head towards a threat; these include head pointing, head lowering, clashing, and wrestling. These are executed to protect themselves from threats like predation or conspecifics. Sexual dimorphism in eland is distinct from an early stage of life which can easily be differentiated from the nature of the horn as early as six months of age, with the males having a short and thick spiral from the base of the horn modified for wrestling and fighting against predators while females have long and sharp pointing horn adapted for stabbing (Pappas, 2002). These horns also aid in browsing trees and shrubs. Lastly, orientation and cutaneous movement can be characterised as explorative or affiliative behaviours that help toward the harmony and cohesion of the social group; these include sniffing, flehmen, lip-licking, grooming, tail wagging while suckling, pawing, and head rubbing (Kiley-Worthington, 1978). Kiley-Worthington (1977) extensively detailed these behavioural mechanisms in a herd of eland at the Pretoria Zoo, South Africa.

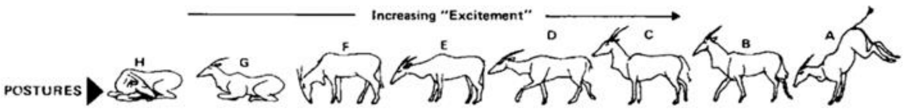

Figure 2.1. Changes in postural tonus with an increase in excitement (Kiley-Worthington, 1977).

In an intensive management system, animals are mostly provided with all needed resources and do not need to spend much energy in search of food. However, even in an intensive system, competition among conspecifics still occurs where animals battle to get the best resource-holding potential, such as access to supplementary feed, space, and mating opportunity in a mixed-sex herd (Vymyslická et al., 2015). Generally, access to resources in eland is influenced by age, sex, body mass, time spent in the herd, matrilineal genealogy, nature of weaponry such as horns, and physiological states such as sickness or pregnancy (Côté, 2000; Horová et al., 2015; Wirtu et al., 2015). To attain dominance, animals engage in dyadic or triadic agonistic interactions, resulting in winners and losers. A series of these competitions continues until a stable hierarchy is established where everyone knows their place in the social group. The consequence of such establishment is to reduce the risk of costly behaviours (Cransac & Aulagnier, 1996). Over the years, several algorithms have been developed in vast literature to test the linearity and transitivity of social groups and the order of dominance (hierarchy) of individuals (David, 1987; Clutton-Brock et al., 1979; Bayly et al., 2006; Bang et al., 2010; Shizuka & McDonald, 2012). Generally, keeping large bovids in captivity requires an understanding of these mechanisms. For example, Vymyslická et al. (2015) found that high-ranking individuals in a group of eland prevented low-ranking herd mates from gaining access to supplementary feed, which ideally was meant for the weak animals and the young, and suggested providing supplementary feed at multiple feeding points and in excess.

2.3.2. Understanding eland physiology and behaviour as a tool for stress and welfare assessment

The transition of wild animals to rangeland, captivity, or intensive management requires an understanding of their biology and ecology, and this is thus to make sure that their welfare is not compromised considering the tenet of animal welfare freedom. Animal welfare is very important in the global animal industry, in livestock production, zoos, or intensive breeding of game animals. Over the past decades, there has been a substantial increase in scientific studies on animal welfare, which has led to increased public awareness and concern about the quality of life of animals kept in intensive systems (Jones et al., 2022). This increasing awareness has shown the need to evaluate the welfare conditions of animals and to provide information on the various factors that can influence their living conditions (Main et al., 2003). Generally, animal welfare is a measure of how an animal is adapting to the condition of the environment in which it lives. A good environment will provide an atmosphere where an animal can express and execute all its biological needs without undue stress (Richmond et al., 2017). A key aspect in the animal welfare assessment is the measurement of the determinants that help define the welfare state of an animal as either good welfare or deprived. In this regard, several on-farm welfare assessment protocols have been developed for several livestock species and working animals through projects and programs such as the Bristol Welfare Assurance Programme (BWAP; Main et al., 2007), Welfare Quality® project (Canali & Keeling, 2009; Blokhuis et al., 2010), The Five Freedoms (Farm Animal Welfare Council, 1993; Mellor, 2017; Webster, 2001) and Animal Welfare Indicators (AWIN; Dalla Costa et al., 2016). Overall, the welfare indicators in these protocols have been categorised as animal-based or resource-based indicators, and the principles therein can be adapted to emerging intensive game production such as in eland and other game species. Pioneering research in animal welfare emphasises resources-based indicators; however, recent emerging research in animal welfare has shown a dramatic shift toward animal-based indicators because these give direct information about the welfare state of the animals. Animal-based indicators measure the behavioural, physiological, immunological, and physical response to the intervention of the various resources in the animals' environment (Whittaker & Marsh, 2009; Broom, 1991).

One approach to examining animal welfare involves defining benchmarks in the physiological profile of the animals, including their overall health and blood biochemistry. These benchmarks serve as references, and deviations from them can be linked to stress arising from the animals' difficulty adapting to environmental challenges. As such, the routine evaluation of the health status of captive game herds is an inevitable part of animal husbandry and welfare, which is particularly crucial when confined to an area out of their natural habitat (Amadori et al., 1997; Miretti et al., 2020). Blood biochemistry parameters are essential in understanding animal welfare, health, and physiological conditions, such as deficiencies and disorders (Xie et al., 2022; Kumar et al., 2023). The first sign of changes in the homeostatic equilibrium is increased cortisol levels due to stress. This, thus, leads to a metabolic increase in glucose production to sustain the energy supply to cells toward powering the body system to combat the influence of stressors. In a chronic stress situation where much of the stored energy is depleted, significant pressure is exerted on the extrahepatic tissues to help produce energy from noncarbohydrate substances such as cholesterol, triglycerides, and phospholipids (Kumar et al., 2023). Some studies have shown that an increase in stress, such as heat stress, can lead to a rise in cholesterol levels, which is attributed to an increase in the metabolism of fatty acids to help produce energy (Mundim et al., 2007). Protein blood biochemistry, such as albumin, total protein, and globulin, have also increased significantly due to stress (Kumar et al., 2023; Ribeiro et al., 2016, 2018). Enzymes such as aspartate aminotransferase, gamma-glutamyl transferase, and alanine aminotransferase can also reflect the rate of metabolic activities in a stressed or ill animal (Helal et al., 2010). However, it should be noted that the interpretation of biochemical profiles is complex as it is, influenced by other factors such as age, sex, body weight, and physiological management such as handling, which should all be taken into consideration during interpretation (Gomide et al., 2004; Kumar et al., 2023).

2.3.3. Challenges during handling and restraining eland/bovids for examination

Managing large bovids places considerable emphasis on handling, a crucial aspect of husbandry. Handling and restraint play a pivotal role in various management activities, including the transportation of animals, health examinations, assisted reproductive techniques, and the transfer of animals to different facilities within the farm. This task becomes especially challenging when dealing with wild captive large bovids due to their large body size, instincts, and the wilderness environment they are accustomed to (Wirtu et al., 2005). Numerous challenges come with the handling of large bovids, and the kind of facilities or equipment used for the handling or restraint can determine the severity of the stress response of the animal. Besides the nature of the facilities, factors such as handler experience, training, odour, agitation, and noise can also exacerbate the fear and stress response of animals during handling (Grandin, 1999). During handling, animals undergo stress, inducing fear in them. This stress-induced fear response can result in heightened agitation, often leading to injuries. The animals commonly endure severe pain, bruises, and injuries as they attempt to escape the handling system (Grandin, 1998). These bruises and injuries impact carcass quality and contribute to significant losses in meat and skin quality. Additionally, such physical harm or rough handling can suppress immunity, decrease conception rate, and reduce weight gain; all these can serve as indicators of compromised animal welfare (Hixon et al., 1981; Kelley et al., 1981; Brando & Norman, 2023).

In an intensive husbandry system, the challenges of handling animals for routine examination begin from herding the animals in the barn to the raceway or directly to the squeeze chute. Some animals are inherently calm, while others can be provoked and agitated by the smallest changes or presence of humans. In this case, understanding the factors that result in fear-motivated behaviours in animal species is crucial (Grandin, 1999). This is thus important because such novel sounds or changes at the onset of the handling can lead to a stampede, resulting in a big casualty and injury. Common eland are also very sensitive to cues and show a good sense of alertness to the slightest pitch of sound in their environment or the approach of humans (Kiley-Worthington, 1978; Bordes et al., 2018). Herding such animals to the raceway will also require understanding the flight zone or flight distance of the animals, which is the zone or distance within which an animal feels safe, and stepping into this zone, an animal is perceived to be in danger. This zone can be influenced by several factors, including dominance and the extent of tameness or wildness of an animal (Grandin, 1999). The animal handlers and veterinarians need to understand these factors to avoid panicking the animals, which can result in injury or destruction of the farm structure and facilities.

To avoid injury within the raceway, the raceway needs to be designed with material that would not predispose the animals to injury. Metal or wooden materials with sharp edges should be avoided (Grandin, 1998). Likewise, slippery floors along the raceway should be avoided, which can agitate the animals. Animals should also be herded with priority within similar age groups. This would help avoid aggression toward submissive animals, which might otherwise result in fatal injuries. Moreover, at the Common Eland Research

Facilities at CZU, it was realised that nursing mothers always feel stressed when they are separated from their calves during routine handling; in such a situation, the calf can be handled first followed by its dam, this would help motivate the dam to move through the raceway with ease to catch up with her calf. The raceway design should incorporate minimal escape space, especially considering that prey animals, like eland, which are typically agile and prone to flight, may easily leap over an open enclosure (i.e., without a roof). During regular handling, animals can be guided through the raceway and squeeze chute multiple times without restraint, promoting a sense of calmness until they perceive the process as non-threatening and harmless (Pennington et al., 2013).

In recent years, there have been improvements in equipment design for handling or restraining species such as bovids and cervids without or with minimal drug-induced immobilisation or sedatives to enable more frequent handling. Currently, there are manual or hydraulic-operated squeeze chutes. With the advancement in innovative technology, more improvements with a focus on specifications (e.g., age, physiological states such as for pregnant females, milking, artificial insemination, and semen collection) and welfare-friendly approaches would help greatly (Fuentes et al., 2022). In any case, fatalities can result if these facilities are not adequately designed or operated. The age, animal species, and the purpose for which the facilities are needed (e.g., blood collection, reproductive techniques, or routine health examination) should be considered while designing a squeeze chute. This is crucial because if an animal is not properly restrained, particularly during invasive procedures such as blood collection, an animal can panic, resulting in injury to both the personnel, veterinarian and the animal. In a study conducted by Huhnke et al. (1999) on identifying injuries sustained by cattle calves, 20% of the injuries sustained were observed to result from handling in the squeeze chute. Simultaneously, it is crucial to refrain from applying excessive pressure in the squeeze chute, as this can prompt animals to resist pain, potentially causing physical injuries and harm to internal organs.

2.4. Some husbandry techniques to help improve the intensive management and welfare of eland

2.4.1. Improving temperament and habituation of animals to routine handling and management

The temperament and habituation of animals, whether wild or already domesticated, form the essential criteria for animal taming, husbandry, and the selection process for domestication. This aspect is closely tied to the performance and productivity of animals, making it a crucial trait in facilitating the transition of animals into domestication (Wirtu et al., 2015). The concept of temperament or personality has been a fascinating topic even in human psychology because of its impact on defining individuals or groups regarding job satisfaction, productivity, risk aversion behaviour, social stress, and disease susceptibility and severity (Réale et al., 2007). There has been a vast array of definitions and interpretations of temperament in the broader literature, with some viewing it as copying style (Koolhaas et al., 2010), while others as personality (Gosling, 2001; Réale et al., 2007). Generally, temperament can be described as individual behavioural differences that are repeatable and consistent over time and across situations in response to stressors; thus, this idea covers numerous traits, such as stress response to novelty situations, aggressiveness, aversion to taking risks, sociality, and exploration (Box, 1999; Lowe & Bradshaw, 2001; Dall et al., 2004). According to Réale et al (2007), temperament must be "consistent over time" and does not imply that the traits cannot change with environmental conditions, age, or management. Still, the differences in individuals within or between populations are primarily maintained.

Narrowing down the idea of temperament to domestic animal breeding and game production at large, it is crucial to include the assessment of temperament in selection and breeding programs because of the significance of selecting animals with docile temperament. Docility in captive animals helps increase animal welfare by reducing agonistic behaviour and stressful handling during routine management, transportation, and the risk to animal keepers or handlers (Rushen et al., 1989). Moreover, several studies have shown that temperament is related to the productivity of economically important traits such as meat and carcass quality in beef cattle (Müller & von-Keyserlingk, 2006; Coombes et al., 2014), milk yield in dairy animals (Sutherland et al. 2012; Hemsworth et al. 2000), reproduction, as well as health (Burdick et al., 2009, 2011). Temperament has also been found to significantly influence the growth in feedlotted beef cattle, showing that animals with good temperament scores have better feed conversion efficiency, performance, and growth rates, thereby prompting the recommendation to select potential feedlot animals based on good temperament before entry into feedlot system (Burrow & Dillon, 1997). With this, it became apparent that the impact of temperament should be given significant consideration, particularly in measuring the specific indicators that help define such behavioural traits in an individual or population.

It should be noted that temperament is a qualitative trait, which makes it difficult to measure due to the complexity of accessing the behavioural response that helps define the temperament of specific animals. In the exhaustive reviews of Réale et al. (2007) and Finkemeier et al. (2018), these authors suggested that temperament should not be viewed or interpreted as a bimodal variable but rather a continuum of two extremities between aggressive/peaceful, active/inactive, dominant/submissive, explorative/unexplorative, bold/shy, or less social/highly social. In the last 45 years, various techniques have emerged for assessing temperament. As outlined by Burrow (1997), in cattle, these methods can be categorised as either restrained or non-restrained, including the dominant test, dairy cow scoring system, maternal temperament assessment, and free movement test. Thus, this principle can also be applicable in eland. There is a growing emphasis on adopting more objective measurement approaches. In farm or captive animals, the restrained and non-restrained approaches have been measured through pen score, chute score, and more objectively through exit velocity or flight speed (Burdick et al., 2011; Adamczyk et al., 2013; Finkemeier et al., 2018). In the pen score, the reactivity of an animal or a group of 3 or 5 animals is measured in response to the approach of an observer. A docile animal will remain calm when approached and scored 1, while an animal with a poor temperament will react out of fear on approaching its flight zone, which can be scored 5 (Hammond et al., 1996). For the chute score, the temperament of an animal is analysed when restrained in a squeeze chute with the head also restrained. Then, the reactivity of the animal is accessed by an observer in a continuum ranging from calm and nervous to panicked (Pennington et al., 2013). Nevertheless, the responsiveness of the animal depends on the intensity of the exercise to be administered, with greater reactivity anticipated in response to invasive procedures such as blood collection. Thus, this should be taken into consideration during the interpretation of results.

Pen and chute scores are considered subjective approaches to assessing temperament and, therefore, subject to human error. If such methods are adopted, it is encouraged to have multiple trained observers from which the inter-observer reliability or a single observer from which intra-observer reliability of the observation will be tested (Bokkers et al., 2012). A more reliable and objective approach to assessing temperament is the escape velocity or flight speed, which is the speed (m/s) at which an animal covers a given distance in a specific time, and it is measured when an animal is released from a squeeze chute (Curley Jr et al., 2006; King et al., 2006; Burdick et al., 2011). There are no standards for the required distance, but in most studies, the escape distance has been set to be between 1 and 2 metres (Burrow et al., 1988; Parham et al., 2019). In the study of Burrow (1991), cattle having an escape velocity below 1.9 m/s were considered calm, while those having a record of more than 2.4 m/s were considered to have a poor temperament. Meanwhile, other researchers have used different approaches to assessing the temperament of animals using a categorical three or five-point scale, with animals walking out gently labelled as calm while animals jumping out and trotting off labelled as having a poor temperament (Curley Jr et al., 2006; King et al., 2006). Aside from pen score, chute score, and exit velocity, there are other approaches or markers, such as eye white percentage (Core et al., 2009) and the nature of hair whorl (Lanier et al., 2001; Grandin et al., 1995) that have proven reliable with high repeatability and correlation with tested response variables. It is recommended to integrate various behavioural, physiological, and endocrine responses to gain a more comprehensive understanding of animal temperament.

The holistic understanding of these temperament determinants is crucial for the husbandry of both domestic and captive wild species. In captive wild species, such as common eland, the measurement of temperament will play a significant role in the selection process for potential parent stock for breeding programs. Meanwhile, since poor temperament is subject to modification through behavioural training, even for aversive conditions, it is necessary to train and condition animals so that they will familiarise themselves with such techniques. For example, even the wildest eland were conditioned and habituated to aversive reproductive techniques such as gamete collection and embryo transfer (Wirtu et al., 2009), oestrus synchronisation (Pennington et al., 2013), and milking (Treus & Lobanov, 1971). Generally, an animal's temperament is closely linked to its fear response. An animal with a negative experience during a past aversive procedure will likely approach such procedures with apprehension. At the same time, some animals may exhibit poor temperaments but possess valuable genetic traits for desired productivity. As such, regular handling and behavioural training can help condition such animals. Research indicates that routinely handled animals tend to habituate to even aversive procedures, displaying lower cortisol levels, reduced exit velocity, and improved growth and carcass quality (Cooke et al., 2009; Basczcak et al., 2006; King, 2006).

2.4.2. Immunocastration as a husbandry technique in breeding and behaviour management towards an improved welfare

Castration is a husbandry management practice that dates back several centuries. This practice entails rendering an animal impotent or sexually infertile. Castration is mostly common in male animals, while female animals are said to be spayed. It is the process of removing the gonads of an animal to prevent it from reproducing. The castration of domestic male animals has been traditionally carried out to control population, aggressiveness and other unwanted behaviours, reproductive diseases, and breeding of unwanted traits, and to improve meat quality. This practice has also been used to condition farm animals for a good temperament to make them calm as a source of farm power. Other than in traditional livestock species where castration is common in male animals, in Arabian sports, immunocastration has been used in bull camels to prevent unwanted sexual and aggressive behaviour (Ghoneim et al., 2012), which is also applicable in equestrian sports (Malmgren et al., 2001). In wild animals, such as cervids and most bovids, castration has also been carried out to make them calm for ease of handling and to improve meat quality. This practice has also been used in elephants to control unwanted behaviour during musth, which can be dangerous to animals, handlers and properties (Bertschinger & Lueders, 2018). More recently, castration has also been used to control the pest populations of synanthropic animals such as Capybara (*Hydrochoerus hydrochaeris;* Rosenfield et al., 2019). Castration is a beneficial and crucial technique in managing domestic, companion, and wild animals. Still, the physical method through which it is done has called for concern due to the negative impact on the welfare of animals.

Castration is generally categorised into physical, chemical, or immunological methods (AVMA, 2014). For physical castration, several methods have been identified, mostly connected to the scale of the farming, the farming system, and the size and age of the animal species. One common approach to physical castration is the surgical removal of the testicles, which is mainly carried out by a veterinarian or well-trained personnel. Analgesics or tranquillisers occasionally accompany this practice to ease the pain of the animals, which most farmers overlook to save cost and the belief that animals do not have pain sensations at a younger age. Meanwhile, even if adopted, there is also the additional concerns that these drugs are not food safe, that recovery of the animal in a herd environment is poor and complicated, and that estimating doses per animal is inaccurate unless weighed, and thus often not very effective. Physical castration can also be carried out using a Burdizzor castrator, which is applied slightly above the scrotum to clamp the spermatic cord and blood vessels (Melches et al., 2007). Thus, this requires competence to carry it out successfully and with minimal stress to the animal (Robertson et al., 1994). Physical castration can also be done using a rubber band, which is released at the neck of the scrotum. The application process is rapid and does not require as much competence as the previous. However, rubber rings are not recommended for heavier or suckling animals as this would cause discomfort, preventing them from suckling and resulting in weaker animals (Molony & Kent, 1997). Generally, physical castration (particularly surgical castration) has caused concern globally due to welfare issues. This is mainly because animals subjected to this system of castrations are left with complications such as inflammation or open wounds, which can result in myiasis and infection (Neto et al., 2014). In Europe, there has been a road map to end surgical castration, particularly in the pig industry, since 2018. However, about 63% of male pig castration is still done surgically due to a lack of alternatives that are cheap and which produce products that appeal to consumers' tastes and preferences.

Chemical castration came into existence to ameliorate the challenges of animals due to physical castration. This method is also a bloodless method of castration that has the potency of destroying parenchymatous tissues. It involves injecting the testicles using certain chemicals such as lactic acid, zinc gluconate, calcium chloride, or formaldehyde, which destroys testicular tissues, thereby rendering the animal sterile (Capucille et al., 2002). This system of castration is less expensive and requires less technical expertise. Although this system of castration is also effective in inducing sterility, it also has some side effects associated with it, which include anaemia, loss of libido, osteoporosis, causes depression, and also painful (Vinke et al., 2008). The limitation of the systems mentioned above of castration prompts scientists to usher in a more welfare-friendly alternative. One such recent alternative is immunocastration, a more welfare-friendly option that uses an immunological approach to target the hormones produced along the hypothalamic-pituitary-gonadal axis (HPG axis). The basic principle is to create antibodies against, usually, gonadotropin-releasing hormone (GnRH), leading to a blockage in communication between the hypothalamus and anterior pituitary, thereby leading to a decrease in luteinising hormone (LH) and follicle-stimulating hormone (FSH) production. Failure in the production of FSH and LH gradually leads to atrophy and dysfunction of the gonads, which renders an animal (both male and female) infertile (Wicks et al., 2013).

The advancement in adjuvants played a major role in commercialising anti-GnRH vaccines (Reyes & Patarroy, 2023). Vaxstrate® was the first commercial anti-GnRH vaccine launched in the late 1980s. This vaccine consisted of a conjugate of anti-GnRH and ovalbumin using an oil emulsion adjuvant (Hoskinson et al., 1990). However, this vaccine was withdrawn from the market in 1996 due to its high incidences of side effects on animals, causing about 40% of the treated animals to develop abscesses. Secondly, the multiple doses required for its efficacy contributed to its less patronage. After Vaxstrate, the growing concern about animal welfare and meat quality, majorly motivated by consumer preference, led to the development of a new anti-GnRH vaccine called Improvac®. Improvac® was mainly developed for the male pig industry to control objectionable odours in meat boar taint, aggressive behaviour, and improve their welfare. Improvac® comprises of a modified form of a GnRH peptide conjugated to a carrier protein with a water-soluble adjuvant diethylaminoethyl (DEAE-Dextran). Zoetis (formerly Pfizer Ltd., CSL Limited, Parkville, Victoria, Australia) manufactures this vaccine. Since its launch in 1998, it has been selling commercially in Australia, New Zealand, Mexico, South Africa, Brazil, and the Philippines. In May 2009, Improvac got approval for use in the European Union (Kress et al., 2019). For now, it is the only approved anti-GnRH vaccine in the EU. Improvac is also approved in the US (approved by the U.S. Food and Drug Administration) and Canada under the trade name Improvest®. Improvac has been the most successful reproductive control vaccine. However, it still faces a lot of hurdles to approval from many other countries, and consumers in countries where it is approved are apprehensive about buying meat products from immunocastrated animals due to fear of residual effects (Aluwé et al., 2020; Di-Pasquale et al., 2020). Improvac has also been successfully used in other domestic animals, such as sheep, goats, bulls, chickens, and horses, to ensure efficacy, better performance, and improved welfare than other forms of castration (Needham et al., 2017). Meanwhile, other vaccination products such as Bopriva® (for cattle) and Equity ® (for horses) have been launched specially for some categories of animal species, differing in their adjuvant rather than active ingredient.

Behaviourally, immunocastration has been shown to significantly reduce sexual and detrimental agonistic behaviours in a group-housed intensive management system (Ahmed et al., 2021; Brewster & Nevel, 2013; Noya et al., 2019). As stated earlier, one of the primary objectives of castration is to render an animal impotent and decrease testosterone production, and depending on the approach or method of castration adopted, efficacy can be seen through the sexual behaviour exhibited by the animal afterwards. In this regard, several studies have shown immunocastration effectively reduces sexual behaviour, such as interest in females, mounting, and flehmen behaviours (Jago et al., 1999; Janett et al., 2009; Needham et al., 2017). In terms of animal well-being or welfare, immunocastration has demonstrated the ability to decrease the occurrence of aggressive and agonistic behaviours, while preserving the advantage of not imposing excessive stress on treated animals, as seen in traditional physical castration (von Borell et al., 2020; Ahmed et al., 2021). This is crucial, particularly in a group house mixed-sex intensive system where there is a space limitation. Incidences of agonistic behaviour arising from dominant individuals can significantly affect the welfare of other herd mates. Agonistic behaviour will affect welfare and product quality, such as the quality of meat and skin (von Borell et al., 2020), and largely economic return on investment. With immunocastration, these challenges have been overcome, as reported in several extensive reviews (Needham et al., 2017; Ahmed et al., 2021). For behavioural activity, it is expected that immunocastrated animals would experience a decrease in activity, which can be attributed to a reduction in sexual and aggressive behaviour due to suppressing androgenic hormones (Dunshea et al., 2001). Meanwhile, the proportionate decrease in aggressive and sexual behaviour can proportionately lead to the allotment of more time to feeding behaviour, leading to high-fat deposition, supported by the lack of the appetite-suppressive effect of testosterone. However, these can be controlled through proper vaccination schedule timing and the age at which immunocastration is conducted before slaughter.

Unlike in cervids, where there is more literature related to immunocastration, mainly motivated by large-scale venison production, the predominant focus of employing immunocastration in the management of antelopes has primarily revolved around the aspects of game meat and breeding control (Needham et al., 2022). Previously, hormonal implants and anti-GnRH treatment using Deslorelin were tried in gerenuk (*Litocranius walleri walleri*), scimitar horned oryx (*Oryx dammah*), and dorcas gazelle (*Gazella dorcas*) with no positive effect in decreasing aggressive behaviour which was attributed to the downregulation resulting from a tonic surge in LH production that was sufficient for testosterone production (Penfold et al., 2002). However, having seen the positive impact of immunocastration in the domestic livestock industry, particularly in ruminants that may be used as model species for endangered wild ruminants, the use of immunocastration in captive game animals such as eland may potentially contribute to managing their behaviour and help decrease aggressive behaviour and make them calm for routine handling in an intensive management system.

CHAPTER 3

General materials and methods

3.1. Description of the study area

The entire study was conducted at the Common Eland Research Facility. This farm is located in Lány (Central Bohemia region, Czech Republic, 13°57' E, 50°07' N) and is managed by the Faculty of Tropical Agrisciences at the Czech University of Life Sciences Prague (CZU). Lány has an altitude of 421 m above sea level, and according to the Köppen–Geiger classification, the area has a cold temperate climate, which is characterised as fully humid with relatively cool summers. The eland farm consists of a paddock area (2.5 ha; divided into two paddocks) and a barn, which is 230 m^2 with deep litter made using straw bedding. The internal part of the barn is divided into two large pens, each measuring 18 m × 7.5 m, and in between the pens is a main door that leads to the barn. Upon entering the barn, a passage separates both pens; along the pens are feeding areas. This passage leads to the paddocks, though each pen also has a door through which animals can exit to either the left or right side of the paddock (Figure 3.1). The paddocks are fenced with wooden poles at a height of 3 m and an electric fence has been placed along the bottom of the wooden fence to deter the animals from destroying the fence.

3.2 Management of the experimental animals

The experimental animals (common eland, *Taurotragus oryx*) used in this study are all European-born. Their origin stems from the wild population of eland which was captured from the tropical transboundary areas of Kenya, Uganda, Tanzania, Cameroon, and some parts of West Africa and transported to Europe between 1967 and 1972, and settled in Dvur Kralove (Vágner, 1974). Later, in 2002, CZU obtained five eland from this nuclei stock, which would later be the parent stock of the CZU eland farm. The records indicate that almost 330 animals have been born from this group of parent stock. Exchange of breeding animals with local zoos is done to increase genetic diversity. Generally, animals are housed indoors from December to April, during which the environmental temperature is mostly below zero, and can be detrimental to the animals being below their thermoneutral zone.

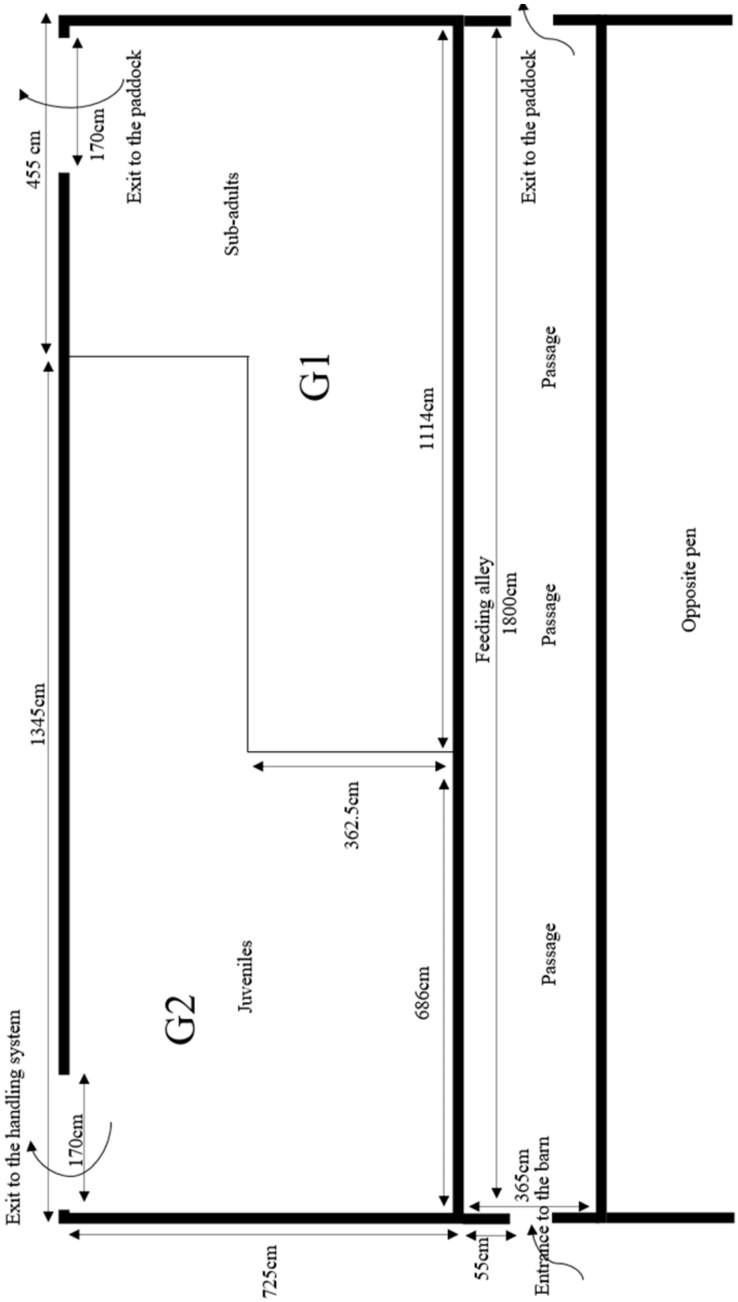

Figure 3.1. The design and dimension of the barn which housed the two groups of common eland during the immunocastration study.

The barn helps maintain the temperature between 2 to 10°C with the help of the straw bedding and the animal-generated heat. However, animals are allowed access to the paddock freely when the temperature is suitable. The animals are intensively managed, and breeding is regulated with a breeding bull placed in between females during the spring, which leads to uniformity in calving in the subsequent spring. Each animal gets an ear tag immediately after birth and an electronic chip to help digitalise growth and productive data such as weight and average daily gains, which are measured monthly since birth. Animals are fed ad libitum with corn silage and lucerne haylage mix. Nutritional details are shown in Table 3.1. They are also fed meadow hay and supplemented with oat grains and commercial pelleted cattle feed, which is high in protein (19.05%) and is provided at an estimated live body weight of 1%. Animals are also given free access to mineral block as a supplement, and water is also provided *ad libitum*.

Table 3.1. The nutrient composition of the various fed ingredients analysed by NIRS and XRF.

	Compound feed	**Pasture**	**Hay**	**Silage**
Crude protein (%)	19.05	21.54	10.28	4.57
Moisture (%)	10.20	8.55	15.31	58.42
Fat (%)	2.37	4.60	2.75	
Fibre (%)	7.04	18.44	20.86	
Starch (%)	28.64	14.34	15.64	
ADF (%)				14.04
NDF (%)				26.00
Ash (%)	7.22	7.47	6.82	2.91
Ca (%)	1.24	1.11	0.92	0.93
P (%)	0.64	0.64	0.13	0.07
K (%)	0.51	1.35	1.07	0.65
S (*ppm*)	694	3176	289	219
Si (*ppm*)	3402	30632	8870	9833
Mn (*ppm*)	45	90	99	98
Cu (*ppm*)	15	19	4	15
Fe (*ppm*)	366	3265	278	178
Zn (*ppm*)	120	158	42	26
Mo (*ppm*)	6	11	14	15

3.3 Experimental design and data collection

All experimental procedures conducted during this study involving husbandry and management of animals were accredited by the Czech Republic Ministry of Agriculture. Ethical clearance on the use of experimental animals was approved by the Czech University of Life Sciences' Animal Welfare and Clearance Committee (details of all clearance can be found in each publication). Moreover, the study also adhered to all guidelines for the ethical treatment of animals in applied animal behaviour and welfare research (Sherwin et al., 2003). Generally, two experiments led to the entirety of this thesis. The first fieldwork commenced from November 2018 to March 2019. This study was part of a postdoctoral project on the use of immunocastration in common eland (Development of International Mobility of Research Staff at CZU Prague, Ministry of Education, Youth and Sports: CZ.02.2.69/0.0/0.0/16_027/0008366). The second longitudinal study commenced immediately after the first study (from April 2019) until December 2022.

Figure 3.2. Subcutaneous administration of immunocastration vaccine (Improvac®). Photo credit: Animal Physiology and Behaviour Research Team – APB Team.

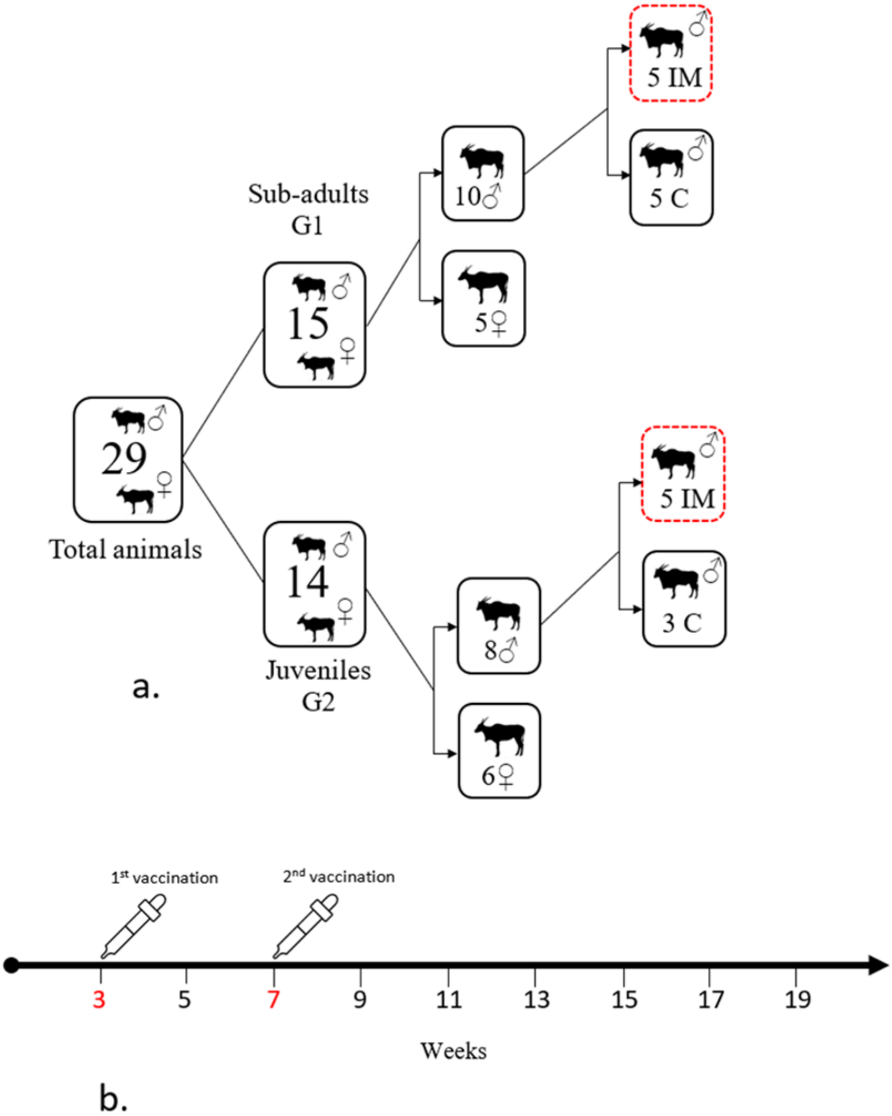

Figure 3.3. Showing the layout (a) and timeline (b) of the first study on immunocastration.

In the first study, 29 animals were selected, all from the same herd. This study examined the effect of immunocastration on the behavioural management of common eland. The 29 experimental animals were divided into two groups: Juvenile (G1, ~6 months old, 94.18 ± 24.76 kg) and subadult (G2, ~1.5 years old, 182.9 ± 59.37 kg). Each group comprised of males and females. The juvenile group comprised eight males and six females, while the subadult group comprises ten males and five females. Five males were immunocastrated (IM) subcutaneously (Figure 3.2) within each group, while the rest served as a control (C). It should be noted that only males were castrated based on the requirement of the study, while females were included in the design to maintain a natural herd-like structure. Figures 3.3a and 3.3b show the schematic presentation of the study design and the study's timeline, respectively. At the onset of the study, the animals were weighed, randomised, and allocated to their respective pens. Data collection and immunocastration treatment commenced two weeks after animals were conditioned to their various pens and the routine handling system. Further details of the research design and experimental treatment are elaborated in Chapters 4 and 5.

As mentioned earlier, the second longitudinal study considered the effect of routine handling, social structure, and management practice on the ontogeny, behaviour, and the possible impact of such effects on the welfare of eland in captivity. These were all geared towards understanding the impact of such management on the domestication of common eland. It started with the routine handling of eleven calves that were born in 2019. Thereafter, every animal born was included in the research design. Until 2022, there were more than 80 animals from which data was collected. A few animals died during the early days of life due to poor immunity and access to milk from the dam as a result of the prevalence of allosuckling/allonursing, which is common in eland (Hejcmanová et al., 2010). Generally, data were collected from the experimental animals once a month, and on subsequent days, social interactions were recorded while the animals were closed in the barn.

During the monthly routine handling, the experimental animals are herded into one pen, individually passed through a raceway, and finally restrained in a squeeze chute where their biological samples and morphometric measurements are taken and recorded. Their temperament or stress response as they move through the restraining system was recorded individually. The details of the temperament score and procedure for recording this data are elaborated in Chapter 5.

3.3.1 Biological sample collection

The biological samples collected during this study were blood and faeces. Blood was collected when an animal was confirmed to be appropriately restrained in the squeeze chute. The head was restrained and blindfolded using a headcover by a handler to prevent agitation while collecting the blood. The blood was mostly collected from the right side of the neck (Figure 3.4). Blood collection was mostly done by a veterinarian or a well-trained person within the team. The neck is adequately disinfected before the blood is collected via the vena jugularis. Blood samples were collected using Vacutainer (Vacuette® Single Use Needle 20G x 1" 0.9 x 25 mm) needles and an adapter to prevent stress to the animal and haemolysis and allow for multiple tubes to be filled within a short time. Blood tubes (Vacuette® tube 8 ml LH Lithium Heparin Separator 16x100) were then labelled and placed in an icebox until further processing and analysis in the lab.

Figure 3.4. An animal properly restrained for blood sampling. Photo credit: Animal Physiology and Behaviour Research Team – APB Team.

After blood collection and morphometric measurement, animals remained restrained for faecal sample collection. Faeces were collected directly from the animal's rectum, and new gloves were used per animal to prevent cross-contamination of samples. The collected samples were placed in a plastic bag, labelled, and placed in an ice box, and later processed in the Laboratory of Animal Sciences at the Faculty of Tropical Agrisciences for coprology and hormonal analysis.

3.4. Sample processing and analysis

Blood samples were immediately processed when returned to the lab. They were centrifuged at 2800 rpm for 15 minutes; afterwards, the supernatants were decanted into Eppendorf tubes and stored in a freezer at -20°C until further analysis. Further details of the various biochemistry parameters analysed during the study can be found in Chapter 6.

The faecal samples collected were also processed, where 4 g was used for coprological analysis, and the rest of the samples were weighed (for moisture content) and subjected to drying using a lyophiliser (Freeze dryer L10-55, Gregor Instruments s.r.o. CZ-25101 Říčany, Czech Republic). The freeze-drying of the faecal samples were done for 72 hours (<-40°C; >100 Pa). Afterwards, the faeces were reweighed and moisture content was calculated. They were then pulverised and stored. They were later analysed for reproductive hormonal metabolites (more detail in Chapter 5).

3.5. Data processing and statistical analysis

All collected data were entered in Excel, processed, and then analysed using the appropriate software that suited the study's objective. Social interaction data were first analysed using DomiCalc software (Schmid & de Vries, 2013). Afterwards, all other statistical analyses were conducted in IBM® SPSS® Statistics 28 (IBM, Armonk, New York).

CHAPTER 4

Activity and social behaviour of farmed common eland (*Taurotragus oryx*), and the effect of immunocastration thereon

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Activity and social behaviour of farmed common eland (*Taurotragus oryx*), and the effect of immunocastration thereon

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4.1. Abstract

Maintaining game animals under intensified captive conditions for production or ecotourism purposes has increased over recent years. This is especially true for the common eland, which was identified as an ideal species for captive production conditions due to its temperament and adaptability. However, little has been done to understand their social behaviour under captive conditions, especially when implementing management tools such as immunocastration, which is often used in wildlife in zoological gardens for contraception and may also aid in easing the management of eland males within mixed-sex herds in captivity, but which could potentially have adverse effects on the hierarchical rank of the immunocastrated animal, and thus welfare from a social perspective. Therefore, this study monitored the social behaviour and hierarchical structure of captive common eland and the effects of immunocastrating male eland on their social rank, aggressive *vs* affiliative behaviours, and activity budget. Thirty common eland were divided into two groups consisting of sub-adults (n = 15; ≈ 2 years old) and juveniles (n = 15; ≈ 6 months old). Each group consisted of five intact males, five immunocastrated males, and five females. Vaccinations (Improvac®, 2mL per dose) were administered to the males assigned to the immunocastration treatment during week 3 and again in week 7 of the study. Behavioural observations were conducted every two weeks from week 1 of the study, for five months, using all occurrence sampling for dyadic social interactions and scan sampling for activity budget behaviours. The hierarchical structure of the two groups was examined, and the rates and proportion of dominant, aggressive and affiliative interactions were established using DomiCalc. The pre-vaccination period and the control animals (not vaccinated) were used as categories of reference. Overall, immunocastration reduced aggressive behaviour in juveniles, with no effect on activity and other agonistic behaviours. However, juveniles and subadults decreased their feeding behaviour post-vaccination and were more active socially. Immunocastration can potentially be used for controlling agonistic behaviour in farmed common eland.

Keywords*: aggression; antelope; anti-GnRH vaccine; dominance hierarchy; immunocastration; social interactions.*

4.2. Introduction

The use of non-domesticated megafauna for meat production instead of traditional livestock (*i.e.*, as an alternative farming species) may help meet the protein requirements of the growing human population, especially in areas not suitable for traditional livestock production due to vegetation and/or water availability and may thus even play a role in addressing global warming (Cromsigt et al., 2018). The game (wildlife) ranching industry has become one of the fastest-growing agricultural sectors in southern Africa (Taylor et al., 2020), shifting focus to the intensification of their production systems. The common eland (*Taurotragus oryx*) has been identified as an ideal antelope species for domestication (Woodford, 2000) and game meat production as an alternative farming species due to their drought and disease resistance (Skinner, 1966), as well as their lean meat (Bartoň et al., 2014). This is not only in the countries of origin like South Africa but also internationally; for example, in Eastern Europe, eland were introduced as early as 1892 and have been used for meat and milk production (Treus, 1968; Treus and Lobanov, 1971). However, little is known about the management of such game species within more intensive farming systems and, consequently, their welfare.

Eland are social animals (Estes, 1991) living in fission-fusion dynamics (Groves et al., 2011), but these groups also show hierarchical social ranks (Wirtu et al., 2004) determined by body mass, age, muscularity, matrilineal genealogy, and level of aggressiveness (Kiley-Worthington, 1978; Cransac and Aulagnier, 1996). Social rank is important as it determines access to space, food and opportunities for mating, etc., especially in captive conditions (Ceacero et al., 2012). For a group of animals with a social hierarchy, the linearity of the hierarchy generally contributes to stability (Shizuka and McDonald, 2012), which is favourable for the domestication of social species in comparison to territorial ones, since species with stable dominance ranks typically show less aggressivity (Price, 2003). Furthermore, the social rank of an animal may give an indication of their ease of handling, influencing the captive management strategy of a particular species (Barroso et al., 2000). Olfactory cues, visual display, and auditory signals are the observed means of communication in common eland (Kiley-Worthington, 1977; Bordes et al., 2018) and can be translated into agonistic, affiliative, or submissive behaviour. Aggressive behaviours may be unfavourable in captivity, but changes in the behaviour and social rank of an animal may also be detrimental to their welfare, as subordinate animals may be stressed and suffer injuries from more dominant animals (Grandin, 1999).

Public awareness and demands regarding improving animal welfare have also increased, especially regarding practices like physical castration of male animals, which is typically done in several livestock species to ease management and improve meat quality in slaughtered animals. Immunocastration of male livestock has been identified as an alternative to physical castration for suppressing aggressive behaviour, controlling reproduction and improving meat quality by preventing the production of androgens (Needham et al., 2017). This decrease in androgen production has a positive effect on decreasing agonistic behaviours in some livestock (Needham et al., 2017), but the effect on rank and social behaviour after immunocastration is poorly documented. Immunocastration could provide a useful management tool in captive male common eland to control breeding and decrease aggressive behaviours amongst male animals, with minimal effects on meat quality (Needham et al., 2020). In captive wildlife, mixed-sex herds are common, where breeding females and males are maintained together year-round with excess stock for culling. Thus, suppression of testosterone production in excess males for culling may influence their social behaviour and rank, thereby impacting their welfare from a behavioural/social perspective. Common eland are not considered to be seasonal breeders under adequate environmental and nutritional circumstances, and indeed, the captive herd at the Common Eland Research Facilities of the Czech University of Life Sciences in Prague experiences year-round-breeding with females maintaining regular oestrus cycles under intensive production conditions, and thus breeding males would be expected to maintain elevated serum testosterone levels all-year-round.

Although the effects of immunocastration may improve the management of captive eland, its effects on their behaviour and social rank should be thoroughly studied before considering it as a potential future management tool within mixed-sex herds. The objectives of this study were, therefore, to describe the social behaviour of males from two age classes in mixed-sex herds of common eland and to evaluate the effects of immunocastration on their activity budget, the hierarchical structure of the herd, and their social interactions.

4.3. Methods

4.3.1. Animal husbandry and experimental design

The experimental animals were maintained at the CZU Common Eland Research Facilities (Lány; 50°7'42"N, 13°57'31"E) under the management of the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague (Permit: 63479_2016-MZE-17214). The experimental protocol was approved by the Institutional Animal Care and Use Committee (permit no. CZU 20/19). Further details regarding the history and structure of the herd and their husbandry can be found in Bartoň et al. (2014) and Needham et al. (2020). Throughout the study, which was conducted for 17 weeks between November 2018 to March 2019, the eland were housed within a cattle fattening barn (230 m^2) on straw-deep bedding and had *ad libitum* access to the same mixed feed ration routinely fed at the facilities (corn silage, lucerne haylage, meadow hay, straw and mineral supplement) and water. During the whole study period, each pen also received a commercial high-protein pelleted cattle feed (19 % crude protein) at a level of 1 % of the total body weight per pen, recalculated and adjusted fortnightly. This supplement was provided twice per day across the feeding alley, which all animals had access to. Thirty common elands (*Taurotragus oryx*) were divided into two equal-sized adjacent pens according to their age: sub-adults (approximately 2 years old, according to birth records; n = 15) or juveniles (approximately six months old, according to birth records; n = 15). These groups represent the two stages at which producers should typically select animals for culling. Each age group pen housed five females, five control males who remained intact, and five males who were immunocastrated (immunised against GnRH) during the course of the study. The females were included in the groups in order to keep social structures similar to those commonly observed in the wild (Hillman, 1987) and to simulate normal management procedures of a mixed-sex herd system. After creating the experimental groups, these were allowed to acclimatise for two weeks before data collection commenced.

Custom handling facilities were used for the safe handling and weighing of the eland, which were already habituated to it prior to the start of the study (Musa et al., 2021). Each animal was weighed every two weeks using a digital Gallagher weighing system (TW-1 Weight Scale G02601, Gallagher, Hamilton 3240, New Zealand). During the first weighing event before vaccination (pre-vaccination), male animals from both groups were allocated to either the immunocastration treatment or control group, balanced for initial weight. The mean (± SD) weights of the two groups at the start of the study were 94 ± 25 kg and 183 ± 59 kg for the juveniles and sub-adults, respectively.

Immunocastration against GnRH was performed using Improvac® (Zoetis Animal Health; New Jersey, USA) following the protocol developed by Needham et al. (2019a) and under the advisement of a wildlife veterinarian. Two doses of the vaccine were administered per animal using a four-week inter-vaccination period (vaccination 1 in week 3 of the study, and vaccination 2 in week 7). Each dose (2 mL) was injected subcutaneously into the shoulder area using a Sterimatic® needle guard system fitted with a Stericap®. No injection site reactions were found throughout the study. According to the manufacturer, the effects of immunocastration using Improvac® appear after a lag period post-second vaccination, which is typically between one and two weeks in the case of pigs. In the case of Bopriva®, an immunocastration vaccine from Zoetis™ designed for cattle utilising the same technologies with only the adjuvant differing, this lag period is typically up to nine days.

4.3.2. Behavioural observations

Behavioural observations were conducted on nine occasions: during week 1 and then every second week during the 17 weeks of the study. Each observation event (a total of seven hours) was distributed over two days (*i.e.*, 3.5 hours per pen each day) from 9:00 to 12:30 and 12:30 to 16:00, rotating morning and afternoon observation times between the two pens over the two days. The observer was previously trained regarding the correct identification of common social interactions between eland (Table 4.1) and performed all the behavioural observations during the study. Animals were identified using large, numbered ear tags and their unique body markings. The animals were observed from a distance in such a way that the presence of the observer did not influence their normal behaviour. Regular feeding of the animals was done one hour before the commencement of the observation, and the animals were allowed to settle for one hour after the entry of the observer into the barn.

Behavioural monitoring was done by direct observation since recordings would not guarantee an adequate identification of the animals involved in every interaction. Following Altman (1974), "all occurrences sampling" was used for recording social interactions, while "scan sampling" was used for the activity budget. For the "all occurrence sampling", the social behaviours recorded were grouped into affiliative, dominant-not aggressive and dominant-aggressive (Tabe 4.1). The dominant interactions were coded in a dyadic dominance/submission way, following the methodology by Ceacero et al. (2007). For each agonistic encounter, the interaction was observed until the end, and the winner and loser were recorded. Scan sampling was additionally used to record the activity budget of each animal every 15 min. Therefore, 28 activity budget observations per animal were recorded every week. The daily budget activities recorded included eating+drinking, lying, standing, walking, and social activities (affiliative, dominant, and playing).

Table 4.1. Ethogram indicating the social interactions between common eland recorded in this study, grouped into three categories: affiliative, dominant-not aggressive, and dominant-aggressive.

Categories	Behaviours	Description
Affiliative	Grooming	Grooming of one individual by another or each other (in that case, the interaction is considered reciprocal)
	Flehmen	Lip curl in response to urination by another individual
	Mounting	Mount another individual in a non-sexual context
Dominant (not aggressive)	Passing	Sudden alertness/moving away when another animal is passing
	Threatening	With head or horns on approaching, without contact with the threatened individual
Dominant (aggressive)	Pushing	Pushing another animal to displace it with any other part of the body different than the horns
	Horning	Pushing another animal to displace it with the horns
	Wrestling	Locking/clashing of horns with certain force and alacrity

4.3.3. Behavioural data processing

Two male juveniles within the control group of the juveniles died at the beginning of the study due to reasons unrelated to the study and were thus omitted from the analyses.

The behavioural datasets were pooled into two study periods: pre-vaccination for the data collected between weeks 1 and 7, and post-vaccination for the data collected between weeks 8 and 17. The same approach was used for the body growth data: mean weight and average daily gains (ADG) were calculated for each study period.

The dominant interaction data were processed separately for each fortnightly observation event, and separately for each group. Dominance matrices were created and analysed using DomiCalc matrix manipulation and analysis software (Schmid and de Vries, 2013). To test the assumption that the interactions were significantly linear, linearity tests (h'; de Vries, 1995) with 10000 randomisations were carried out on each matrix. As this h' index does not fully account for the unknown dyadic interactions, triangle transitivity tests (*tTri*; Shizuka and McDonald, 2012) with 1000 randomisations were also performed. Subsequently, the social rank of the individuals in each group was calculated for each matrix using the Inconsistency and Strength of Inconsistency (*I&SI*) method, which provides a hierarchical ordering that minimises the number of inconsistencies and simultaneously minimises the strength of these inconsistencies (Schmid and de Vries, 2013). The formula *(1–rank)/(n+1)* was used for transforming the I&SI linear rank obtained for each group and period into a social rank value (*SR*) within the interval]0,1[with high-ranked animals having larger *SR* values. *n* represents the number of individuals and *rank* the position of a given animal in the linear hierarchy obtained. This allows for comparing groups of different sizes. The *SR* values were further transformed through the arcsine of the square root to fit a normal distribution. For each individual, a final *SR* value was finally calculated as the average of the values obtained along each study period (pre- and post-vaccination).

The activity budget data was expressed as the percentage of occurrence of each of the defined activities for each individual during each study period. The total number of interactions where an animal was involved (*T*), the number of interactions started (*S*) and received (*R*), dominant interactions started (*Dom_S*) and received (*Dom_R*), dominant non-aggressive interactions started (*DNAgg_S*) and received (*DNAgg_R*), dominant aggressive interactions started (*Dagg_S*) and received (*Dagg_R*), affiliative interactions started (*Aff_S*) and received (*Aff_R*) were processed as rates (occurrence per hour; *i.e.*, *T/h, Dom_S/h, Dom_R/h, DNAgg_S/h, DNAgg_R/h, Dagg_S/h, Dagg_R/h, Aff_S/h, and Aff_R/h*) and also calculated for each study period (pre- and post-vaccination). Finally, the relative occurrence of certain types of interactions [*Dom_S/T, Dagg_S/S, Dagg_S/Dom_S, Aff_S/S, Aff_S/(Aff_S+Aff_R), Dom_R/R, Dagg_R/R*; computed as percentages] were also calculated for each observation event.

4.3.4. Statistical analyses

All analyses were performed in IBM© SPSS© Statistics (version 29.0 for Windows; IBM, USA), with a focus placed on determining the effects of the treatment on the behaviour of the immunocastrated males compared to that of the control ones. Females were excluded from the analyses. Histograms were built for initial inspection of the variables involved in the models, and normality tests were conducted (Shapiro-Wilks). Generalised Linear Mixed Models

(GLMM) were designed to determine the effects of the immunocastration treatment and period (*i.e.*, time) on the behavioural patterns of the animals in the study. A data structure based on age *Group* and *ID* as subjects was used. The behavioural traits previously described [Growth (*Weight*, *ADG*); Activity budget (*Eating*, *Walking*, *Lying*, *Standing*, *Social* in %); Social rank; Rates of social activities either started or received (*T/h, DI/h, DR/h, DNAgI/h, DNAgR/h, DAgI/h, DAgR/h, AfI/h, AfR/h*; and Ratios of social activities either started or received (*DI/T, DR/T, DNAgI/T, DAgI/T, DAgI/DI, AfI/(AfI+AfR)*)] were the target variables in the models. Normal distribution with an identity link function was used for some of the models, while gamma distribution with a log link function was used for others (see the respective results tables). For each model, the independent variables *Period* and *Treatment* were tested for multicollinearity, which was always quite low (always lower than 0.5).

4.4. Results

Due to the relatively short duration of the study, body growth was not significantly different when comparing the pre-and post-vaccination periods, neither in juveniles (Table 4.2) nor in subadults (Table 4.3). More importantly, body growth was not negatively influenced by the immunocastration treatment in any of the studied age classes.

On the other hand, changes in the activity budget of the common eland between the pre-and post-vaccination periods were highly significant. Post-vaccination, both juveniles and subadults dedicated less time to feeding (32 *vs*. 25% and 38 *vs*. 27%, respectively) and more time to social activities (5.1 *vs*. 6.4% and 3.3 *vs*. 7.7%, respectively) than in the pre-vaccination period.

Juveniles also performed more lying (28 *vs*. 41%) and less standing (30 *vs*. 18%), while subadults performed more standing (19 *vs*. 22%) and less lying (29 *vs*. 25%) in the post- compared to the pre-vaccination period. However, again, no treatment effect was observed to influence the activity budget.

Pre-vaccination and Control animals (not vaccinated) were taken as categories of reference. Thus, the t-values indicate an increase or decrease in the variable after the vaccination and in the immunocastrated animals, with respect to the category of reference.

Pre-vaccination and Control animals (not vaccinated) were taken as categories of reference. Thus, the t-values indicate an increase or decrease in the variable after the vaccination and in the immunocastrated animals, with respect to the category of reference.

Table 4.2. Generalised Linear Mixed Models showing the effects of immunocastration treatment in the growth, activity budget, social rank and social behaviour in a group of juvenile common eland. Period (pre- and post-vaccination) is also included in the models.

	Distribution	**Period**	**Treatment**
Weight	Linear	t=1.708; p=0.111	t=-0.313; p=0.759
ADG	Linear	t=0.754; p=0.465	t=0.338; p=0.741
Eating%	Linear	t=-3.594; p=0.003**	t=0.975; p=0.347
Walking%	Gamma	t=0.421; p=0.681	t=-0.544; p=0.596
Lying%	Linear	t=5.356; p<0.001 ***	t=-1.723; p=0.109
Standing%	Linear	t=-4.030; p=0.001**	t=1.498; p=0.158
Social%	Gamma	t=6.780; p<0.001***	t=-0.752; p=0.467
Social Rank	Linear	t=0.323; p=0.752	t=0.319; p=0.755
T/h	Gamma	t=-0.511; p=0.618	t=0.455; p=0.656
Aff_S/h	Gamma	t=2.112; p=0.055	t=-0.381; p=0.709
Dom_S/h	Linear	t=-0.582; p=0.571	t=0.433; p=0.672
DAgg_S/h	Linear	t=-0.443; p=0.665	t=0.024; p=0.981
DNagg_S/h	Gamma	t=-0.779; p=0.453	t=0.172; p=0.867
Aff_R/h	Gamma	t=1.350; p=0.200	t=0.369; p=0.718
Dom_R/h	Linear	t=-1.210; p=0.248	t=0.107; p=0.916
DAgg_R/h	Linear	t=-0.947; p=0.361	t=0.244; p=0.811
DNagg_R/h	Linear	t=-1,223; p=0.243	t=-0.077; p=0.940
Dom_S/T	Linear	t=-0.392; p=0.701	t=0.243; p=0.812
DAgg_S/T	Linear	t=-0.208; p=0.838	t=-0.307; p=0.764
DAgg_S/Dom	Gamma	t=0.326; p=0.750	t=-3.843; p=0.002**
Aff_S/Aff_T	Gamma	t=0.924; p=0.372	t=-1.049; p=0.313
Aff_S/T	Linear	t=-0.208; p=0.838	t=-0.307; p=0.764
Dom_R/T	Linear	t=-0.697; p=0.498	t=-0.145; p=0.887
DAgg_R/T	Linear	t=-0.613; p=0.550	t=0.010; p=0.992

T – Total number of social interactions. *S* – Started interactions. *R* – Received interactions. *Aff* – Affiliative interactions. *Dom* – Dominant interactions. *DAgg* – Dominant, aggressive interactions. *Nagg* – Dominant, not aggressive. *Aff* – Affiliative interactions.

The hierarchy within both age groups was significantly linear and transitive throughout the study period (Table 4.4), except for the first week during the pre-vaccination period when the juveniles' group did not show significant linearity in their hierarchy. Thereafter, the hierarchy was always significantly linear, as also observed in the subadults' group all throughout the study. The period did not affect the social rank of the animals in any of the studied groups, and, again, it was also not affected by the immunocastration treatment.

Table 4.3. Generalised Linear Mixed Models showing the effects of immunocastration treatment in the growth, activity budget, social rank and social behaviour in a group of sub-adult common eland. Period (pre- and post-vaccination) is also included in the models.

	Distribution	**Period**	**Treatment**
Weight	Linear	t=1.458; p=0.163	t=1.040; p=0.313
ADG	Linear	t=-1.200; p=0.247	t=1.276; p=0.219
Eating%	Linear	t=-5.891; p<0.001***	t=0.492; p=0.629
Walking%	Linear	t=6.177; p<0.001***	t=-0.389; p=0.702
Lying%	Linear	t=-2.479; p=0.024*	t=1.463; p=0.162
Standing%	Linear	t=1.498; p=0.153	t=-1.293; p=0.213
Social%	Linear	t=3.930; p=0.001**	t=-0.289; p=0.776
Social Rank	Linear	t=0.063; p=0.951	t=0.771; p=0.451
T/h	Gamma	t=0.115; p=0.909	t=0.331; p=0.744
Aff_S/h	Gamma	t=2.727; p=0.014*	t=0.463; p=0.649
Dom_S/h	Gamma	t=-0.508; p=0.618	t=0.831; p=0.418
Agg_S/h	Linear	t=0.250; p=0.806	t=0.214; p=0.833
Nagg_S/h	Gamma	t=-0.759; p=0.458	t=1.008; p=0.328
Aff_R/h	Linear	t=1.664; p=0.114	t=0.956; p=0.352
Dom_R/h	Linear	t=-0.458; p=0.653	t=-1.616; p=0.125
Agg_R/h	Linear	t=0.462; p=0.650	t=-0.708; p=0.488
Nagg_R/h	Linear	t=-0.884; p=0.389	t=-1.750; p=0.098
Dom_S/T	Linear	t=-0.959; p=0.351	t=0.535; p=0.600
Agg_S/T	Linear	t=0.165; p=0.871	t=-0.172; p=0.866
Agg_S/Dom	Linear	t=1.504; p=0.151	t=-0.294; p=0.773
Aff_S/Aff_T	Linear	t=1.369; p=0.189	t=-0.932; p=0.365
Aff_S/T	Linear	t=0.165; p=0.871	t=-0.172; p=0.866
Dom_R/T	Linear	t=-0.366; p=0.719	t=-1.069; p=0.300
Agg_R/T	Linear	t=0.760; p=0.458	t=-0.369; p=0.716

T – Total number of social interactions. *S* – Started interactions. *R* – Received interactions. *Aff* – Affiliative interactions. *Dom* – Dominant interactions. *DAgg* – Dominant, aggressive interactions. *Nagg* – Dominant, not aggressive. *Aff* – Affiliative interactions.

Table 4.4. Strength of the linearity and triangle transitivity of the social hierarchies calculated for the two groups of captive eland: juveniles and sub-adults. Significant results confirm the existence of a linear hierarchy.

Week	*h'*				*tTri*			
	Sub-adults	**p**	**Juveniles**	**p**	**Sub-adults**	**p**	**Juveniles**	**p**
1	0.649	<0.001	0.314	0.125	0.826	<0.001	0.556	<0.001
3*	0.631	<0.001	0.507	0.003	0.922	<0.001	0.691	<0.001
5	0.548	<0.001	0.416	0.028	0.898	<0.001	0.648	<0.001
7*	0.459	<0.001	0.499	0.002	0.912	<0.001	0.679	<0.001
9	0.516	<0.001	0.532	0.002	0.883	<0.001	0.792	<0.001
11	0.637	<0.001	0.423	0.015	0.958	<0.001	0.522	<0.001
13	0.629	<0.001	0.379	0.046	0.916	<0.001	0.643	<0.001
15	0.669	<0.001	0.418	0.023	0.753	<0.001	0.654	<0.001
17	0.509	<0.001	0.462	0.006	0.778	<0.001	0.577	<0.001

h' – Strength of linearity in the order of social hierarchy (Schmid and de Vries, 2013).

tTri – Triangle transitivity (Shizuka and McDonald, 2012).

* – Indicates the weeks when the vaccination was performed. Data collected until the second vaccination was pooled as pre-vaccination. Data collected after the second vaccination was pooled as post-vaccination.

Finally, immunocastrated and control males showed very similar behavioural displays in terms of interaction rates or the relative frequency of different social interactions. Only one effect was found between the two periods, with the rate of affiliative interactions started per hour within the subadults' group being higher in the post-vaccination period (0.46 *vs*. 0.86). The only effect of the immunocastration treatment was observed in the juveniles' group for the proportion of dominant interactions started which were aggressive, which decreased during the post-vaccination period (median of 69% in the control animals and 53% in the immunocastrated animals; Figure 4.1). All the other studied social interactions were not affected by the immunocastration treatment.

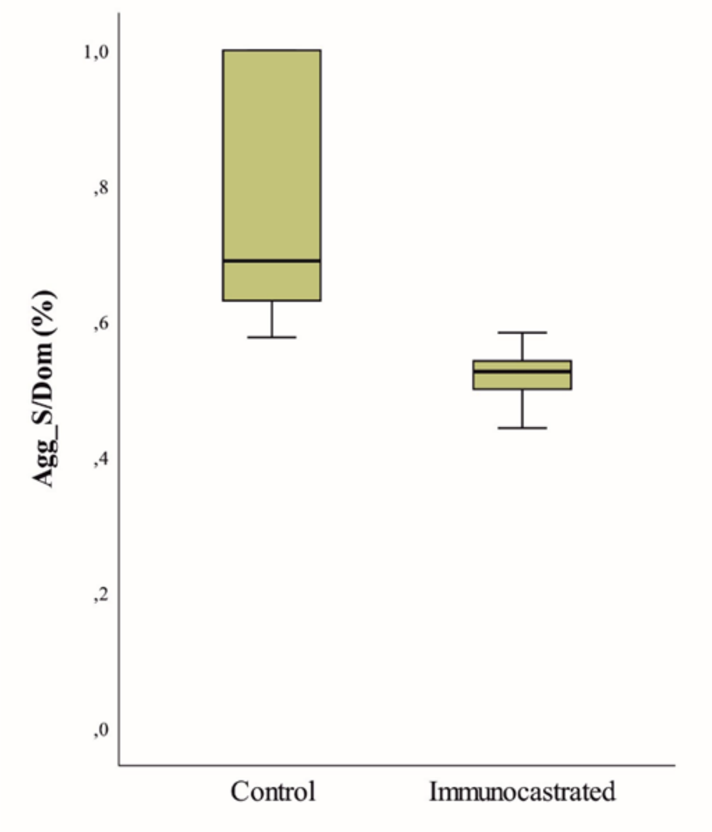

Figure 4.1. Effect of the immunocastration treatment on the kind of dominant interaction performed by juvenile common elands. Y-axis refers to the proportion of the dominant interactions started by an animal which are aggressive (see Table 4.2).

4.5. Discussion

Physical and surgical castration not only affects the physiology and development of animals due to the prevention of androgen production, but it also results in pain and distress in livestock (for which farmers are reluctant to bear extra cost for analgesics and which require withdrawal periods before slaughter) often exacerbating the decrease in their growth and development (Prunier et al., 2006) and influences their normal behaviour linked with androgen production and pain (Melches et al., 2007). During immunocastration, there is a significant increase in the GnRH-antibody titre as a result of the second vaccination, which decreases androgen production, also changing the animal's physiology and can thus also change their growth, physical and social activity (Janett et al., 2012). However, depending on the vaccination schedule, the study duration, and the species, significant effects on, for example, body growth can be minimal (Needham et al., 2017). Immunocastration in the present study shows no adverse effects on physical activity and social behaviour, as well as the body weight and ADG of male common eland. This is a positive output since immunocastration as a panacea to physical and surgical castration is principally carried out to control breeding and welfare-compromising agonistic behaviours and to improve meat quality while aiming to achieve minimal effects on growth and normal behaviour (Needham et al., 2017). Thus, the application of immunocastration looks promising, particularly in captive mixed-sex herds of antelope, where budget and space limitations are common, as with other farmed animals (Thompson, 2000; Ahmed et al., 2022). Meanwhile, immunocastration was found to slightly reduce aggressive behaviour in juveniles but not in sub-adults, suggesting a stronger effect during early development. Moreover, the decrease in aggressive behaviours of the treated animals did not imply changes in the group stability since the linearity tests confirmed that both groups remained stable, with significant linearity and transitivity, signifying harmony in the social groups. The social rank was also not affected by immunocastration in both age groups and remained stable from the pre-vaccination and throughout the post-vaccination period. Thus, the immunocastration effect at different ages is an issue that needs to be further studied.

Irrespective of the immunocastration treatment, there were significant changes during the study period, with both age groups experiencing a decrease in feeding behaviour and an increase in social behaviour over time. Subadults were generally more active, with a higher proportion of standing and decreased lying activity compared with the juveniles during the post-vaccination period. Subadults were also more affiliative during the post-vaccination period than the pre-vaccination period, which means a progressive increase in the proportion of affiliative interactions such as grooming (either started, received, or reciprocal) in order to foster more cohesion and stability in the social group given the limited space and the cost for engaging in conflict (Šárová et al., 2016). Such a phenomenon can be described by the "grooming for stability hypothesis", where the long-term fitness of individuals in a social group is promoted by a network of stable positive relationships (van Schaik and Aureli, 2000).

One of the welfare concerns that motivated the use of immunocastration in farm animals is the uneasiness, pain and distress induced by physical or surgical castration, which can affect their growth and differential body development (Álvarez-Rodríguez et al., 2017). It is generally expected that there should be a decrease in growth and development in castrated animals (either via immunocastration, surgical, or physical castration) as compared to intact animals due to a decrease in the anabolic effect of testosterone which contributes to muscle development (Laron, 2001). However, the effects of immunocastration on growth and development are dependent on a number of factors, including the timing of the vaccinations relative to the physiological development of the animal, the growth rate of that species, and the duration of the study (or fattening) period. Due to variations in these factors, differing results are reported in the literature. For example, cattle studies conducted on calves of similar ages have reported no significant difference in the weight gain of calves after immunocastration (Cook et al., 2000; D'Occhio et al., 2001; Janett et al., 2012). On the contrary, Noya et al. (2019) reported a decrease in weight gain and stunted sexual development in immunocastrated calves compared to intact males; however, a third booster dose before slaughter, which may have a greater effect on growth suppression (Giriboni et al., 2020).

Within the present study, the activity budget of the male common eland were also not affected by immunocastration. Studies have shown that immunocastrated pigs increase their feed intake after the second vaccination due to the lack of androgenic suppression of their appetite by increasing their meal durations (Weiler et al., 2013). Descriptive data collected on sheep also showed a tendency for immunocastrated lambs to increase ingestion activities (Needham et al., 2019b). In bull castrated surgically, some studies have found a significant decrease in dry matter intake and overall daily intake, which were attributed to acute tissue trauma associated with the castration (Ting et al., 2003; Carragher et al., 1997). However, neither feed intake nor feed duration was assessed in the current study, which should be considered for future studies. Moreover, common eland under extensive conditions (*i.e.*, "the wild") are considered crepuscular animals, which means that they exhibit more feeding or activity behaviour during the early hours of the day and late evening, and rest during the day when the conditions are not favourable (Lewis, 1978). Thus, future studies focused on feeding behaviour *per se* should account for this. While the present study did not show any effect of immunocastration on activities such as walking, laying, and standing, Ting et al. (2003) and Carragher et al. (1997) observed a high proportion of standing, leg stamping, reduced laying posture, and tail swishing in bulls which were all attributed to the pain resulting from surgical castration. Thus, further strengthening the need for welfare-friendly alternatives such as immunocastration.

Immunocastration had no effect on the agonistic interactions of the eland in the present study but was effective in reducing aggression in the juveniles (Figure 1). Whilst it is difficult to compare behavioural studies using different species and ethograms, studies involving immunocastration agree that it decreases aggressive behaviour (summarised in Needham et al., 2017). The effects of immunocastration on animal behaviour have largely been studied in pigs up until now due to consumer pressure to ban surgical castration in commercial piglets. Common eland are social animals and non-territorial that live in different social groups in the wild (Kiley-Worthington, 1978). In captivity, particularly in a mixed herd intensive system, it is expected that animals would engage in agonistic interactions until linearity and harmony are established in the social group in order to reduce the cost of engaging in conflict. Research has shown that behavioural effects are experienced after the second vaccination in pigs (Rydhmer et al., 2010), with immunocastrates being less active in social and aggressive behaviours in a group-housing situation (Price et al., 2003). Zamaratskaia et al. (2008) also reported less social, aggressive and manipulating behaviour (nibbling/pushing) in immunocastrated pigs, while Brunius et al. (2011) observed decreased mounting by immunocastrated compared to intact males. Additionally, immunocastration was also found to decrease the aggressive and sexual behaviour of bulls (Price et al., 2003). However, in the present study, the subadults (irrespective of immunocastration treatment) were more affiliative post-vaccination, which likely helped in reducing the incidence of costly agonistic behaviour.

In the present study, the social rank of the animals in both age groups and periods was not affected by immunocastration. This can be attributed to the social structure of both eland age groups, which was significantly linear and relatively stable during the study, with the exception of the initial data collection period for the juveniles (Table 4.4). This stability is not surprising in species with hierarchical social rank and fission-fusion dynamics, which need continuous re-organisation of the hierarchy as the group changes (Wirtu et al., 2004; Aureli et al., 2008). This process may be slower in groups of juveniles with lower social experience (Cransac and Aulagnier, 1996; Vymyslická et al., 2015). Immunocastrated eland in the present study did not increase their aggressive, dominant interactions to maintain their social rank, which implies that they displayed more non-contact agonistic interactions (*e.g.*, threatening and passing). Moreover, the animals in the present study were all drawn from a mixed-sex large population of common eland where all animals already co-exist together. Hence, this could suggest that all the animals had already established their roles within the recently formed group and did not attempt to challenge the dominant individual. This lack of challenge might also be attributed to the fact that the effects of immunocastration were not visibly apparent, unlike surgical castration, which can cause injuries leading to illness and decreased strength in the animals, potentially making them a weak target

(Prunier et al., 2006). Additionally, as in the case with cervids, immunocastration did not have a visibly significant effect on horn size, whilst red deer (*Cervus elaphus*) stags who were immunocastrated, prematurely lost their antlers and subsequently fell to the lowest level of the hierarchy, never regaining their position after the rut season even when other individuals had loss their antlers and return to velvet (Lincoln et al., 1982). In contrast to cervids, common eland (especially captive populations) are not seasonal breeders, with mating happening throughout the year under good conditions, which may contribute to the relative stability of their social ranks. However, further investigation into the relationships between age, social rank, and androgen secretion patterns is needed for common eland males. The application of immunocastration in adult males and their social networks should be considered as well. Whilst castration of adult males is not common for commercial production purposes, this may have important implications in zoological gardens or other animal parks where common eland are maintained for tourism.

4.6. Conclusion

Immunocastration in the present study has shown the potential of decreasing the incidence of aggression in juveniles of common eland without negatively affecting their activity and social behaviour, thereby supporting literature considering such treatment from a young age. Therefore, the use of immunocastration on male common eland may be considered a valuable tool for managing mixed herds of common eland with different purposes (slaughter *vs*. breeding) while simultaneously improving their welfare in captivity. Future studies should focus on the effect of immunocastration on the behaviour and productivity exclusively within a cohort of treated animals (*i.e.*, not mixed-sex groups) while simultaneously considering age and androgen production monitoring following immunisation against GnRH to further understand the relationships between social behaviour and changes in endocrine physiology. Additionally, the effects of immunocastration in groups of adult common eland deserves continued attention.

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Conflicts of interests

None

Ethics statement

The experimental protocol was approved by the Institutional Animal Care and Use Committee (permit no. CZU 20/19) at the research facilities of the Czech University of Life Sciences Prague (Ministry of Agriculture of the Czech Republic permit no. 63479_2016-MZE-17214). The number of animals selected was done to meet the minimum requirements for a study of this nature, and all procedures were performed in such a way as to minimise their impact on the welfare of these animals (ASAB, 2020).

Software and data repository resources

None.

4.7. References

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CHAPTER 5

Habituation of common eland (*Taurotragus oryx*) to intensive routine handling, and the effect of immunocastration thereon

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Habituation of common eland (*Taurotragus oryx*) to intensive routine handling, and the effect of immunocastration thereon

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5.1. Abstract

The temperament of captive common eland (*Taurotragus oryx*) during handling is crucial for their management, as they have been identified as an ideal antelope for game meat farming, and are well-represented in captive antelope populations world-wide. Generally speaking, common eland are considered undomesticated, and thus maintain their natural prey instincts, making it necessary to habituate them to routine handling. Immunocastration can be implemented as part of the herd management strategy in mixed or single-sex eland groups, and might reduce aggressive behaviour of males by blocking the production of testosterone, thereby improving docility for ease of handling. Within this study, data was collected to determine the influence of routine handling and immunocastration on the temperament of common eland during handling. Twenty-nine common eland were divided into two groups: sub-adults (n = 15; ca. 2 years old; 182.9 ± 59.37 kg) and juveniles (n = 14; ca. 6 months old; 94.18 ± 24.76 kg), and they were routinely handled every two weeks over a four-month period (10 handling events). Within each age group, females, males, and immunocastrated males were present. Immuno- castration treatment was administered during the second and fourth handling events (2 mL Improvac®/animal). During handling, animals were individually driven through the raceway and finally restrained in a squeeze chute, where routine biological samples and measurements were collected. Faecal androgen metabolite (*fAM*) concentrations were measured for each event, for males only. Each animal was focally sampled for specific behaviours during the handling. A temperament score was obtained by observing the general state of the animal from the point of entering the raceway, until its exit from the squeeze chute. Generalized linear mixed models tested the influence of immunocastration, week, group, *fAM* concentration, and body weight on the temperament score. Immunocastration did not affect the temperament score, the general state in the squeeze chute, the order of entering the handling system, and the chute exit. However, the temperament score and the state in the squeeze chute improved over time (with animals being less nervous). Moreover, animals with low temperament scores (calmer) entered the system earlier, and exited the system calmly over the repeated handling period. The results show that routine handling through a raceway and squeeze chute system leads to habituation and improves the ease of handling in common eland. However, immunocastration did not affect the temperament of the male common eland during handling.

Keywords: *Antelope; Anti-GnRH; Castration; Handling; Taurotragus oryx; Welfare.*

5.2. Introduction

The increased intensification of wildlife farming motivates the need to handle large undomesticated ungulates, such as the common eland (*Taurotragus oryx*). Such large ungulates need to be trained to ease routine handling, transportation, reproductive examinations, administration of medical treatments, biological sample collection, and slaughter, with minimum stress (Hemsworth, 2003). Common eland are inherently calm animals, except in circumstances when poorly handled or exposed to novel situations, then they tend to be flighty to counteract the influence of such stressors (Pennington et al., 2013). It is thus necessary to habituate these animals to handling, as forceful handling can result in serious injury (Bergvall et al., 2017), thereby impairing the welfare of the animals and resulting in the culling of animals with good breeding potential, as well as predisposing the handlers to injury.

The ease with which an animal can be handled is associated with their temperament (Pennington et al., 2013; Schütz et al., 2016; Parham et al., 2019). As such, priority should be given to achieving docile temperaments in farm and captive animals, because of its effect on important production parameters such as milk production (Sutherland and Dowling, 2014), meat quality (Fordyce et al., 1988), average daily gain (Voisinet et al., 1997), and their overall welfare. Temperament is an essential qualitative management parameter, particularly for large ungulates such as common eland, which cannot be handled as easily as in comparatively small ungulates (Rice et al., 2016). Fordyce et al. (1982) defines temperament as the behavioural, stress and physiological responses of an animal to being handled by humans, while Koolhaas et al. (2010) explains it as the response patterns in reaction to a stressor. Generally, temperament entails the idea that individual behavioural patterns are repeatable over time and across situations, and thus covers numerous traits, such as sociality, aggressiveness, avoidance of novelty, and aversion to taking risks (Reale et al., 2007). The appropriateness of measuring temperament has been quite challenging, due to the qualitative nature of its determinants. Escape velocity, flight distance, and chute scores have typically been used to measure temperament during

handling, and have given a reliable association with some productive parameters, as seen in different species of farm animals (Fordyce et al., 1982; Della Rosa et al., 2018; Parham et al., 2019). More so, different methods have been adopted to ensure docile temperaments and ease of handling of farm animals, which include the selection and breeding for specific temperament traits, castration, use of sedatives, behavioural training and routine handling (Burrow and Corbet, 2000; Lansade et al., 2008; Core et al., 2009; Ceacero et al., 2014).

Chemical immobilisation is quite successful in eland, for collecting biological samples or routine management procedures (Wirtu et al., 2005; Allan, 2015); however, it requires specific expertise, it is not cost-effective, can interfere with normal reproductive physiology (Loskutoff and Betteridge, 1992) and in extreme cases, it can lead to mortalities. In some studies, operant and classical behavioural training have been used to train animals for handling (Valenchon et al., 2017), but changes in routine handling can excite these animals, as they cannot be completely tamed. The combination of behavioural training together with handling in a hydraulic chute system after a mild sedative was successfully used by Wirtu et al. (2005) to handle female eland for transvaginal ultrasound-guided oocyte retrieval, but the animals showed increased glucose levels which indicates that significant stress was still experienced (Phillips et al., 1998).

While various methods of castration have been documented to reduce aggressive behaviour, immunocastration is mentioned as more welfare-friendly overall (Price et al., 2003; Moreira et al., 2016). Principally, immunocastration reduces aggressive behaviour by blocking the production of testosterone by the testes (Noya et al., 2019). Decreasing the incidences of aggressive behaviour in captive male eland is beneficial for the welfare of the herd and handlers, particularly due to their large body size and horns. Besides reducing aggressive behaviour, immunocastration also improves meat quality in a number of species (Needham et al., 2017), and may also have numerous benefits for the meat production potential of captive male eland. Immunocastration can thus be used to achieve multiple objectives in the management of farm animals, including ease of handling and improving docility, considering its beneficial effects on aggressive behaviour. Meanwhile, to apply the immunocastration treatment, routine restraint or darting of the animal would be required for its application. Furthermore, androgen monitoring is typically conducted to evaluate the efficacy of immunocastration in male animals. In this regard, the quantification of faecal androgen metabolites (*fAM*) has been used as a biological marker for androgenic activity in animals (Pereira et al., 2005; Weerasekera et al., 2020), as it provides a more robust signal, by representing the cumulative secretion and elimination of androgens over several hours and thus, is less affected by episodic fluctuations, as usually seen when using blood as a hormone matrix. Thus, after a reliable validation, the evaluation of *fAM* concentrations can provide a suitable non-invasive approach to quantify changes in androgens.

In light of the increasing interest in the intensification of wildlife farming, the wide representation of common eland in captive populations, as well as the preference of immunocastration for behavioural management of male animals, the present study aimed to examining the influence of routine handling on the temperament of common eland. Furthermore, the study aimed to determine if immunocastration affects the temperament of male common eland during handling.

5.3. Materials and methods

5.3.1. Animal husbandry and experimental design

The study was conducted at the Czech University of Life Sciences Eland Research Farm located in the Central Bohemia Region, Czech Republic, from November 2018 to March 2019. The common eland were housed in a barn with deep litter straw bedding for the duration of the study. The Czech Republic Ministry of Agriculture accredited (clearance no. 63479_2016-MZE-17214) all experimental procedures involving husbandry, handling and treatment of the focal animals, while ethical clearance for the research was obtained from the Czech University of Life Science Animal Welfare and Clearance Committee (clearance no. CZU 20/19). The experimental animals were fed ad libitum with a mixed feed ration, which consisted of corn silage, lucerne haylage, meadow hay, straw, mineral supplement, and a concentrate pelleted cattle feed (19 % crude protein). Further details regarding the chemical composition of the diet are discussed in Needham et al. (2020).

Twenty-nine common eland were selected for this study, and two age groups of common eland were formed, using all of the available males and females in each of these age groups in the herd. The female animals were present in both age group pens in order to maintain their normal social structure, and thus they moved through the handling system together with the entire group. The juveniles (G1) were not more than six months old (94.18 ± 24.76 kg), and the sub-adults (G2) were not more than two years old (182.9 ± 59.37 kg), at the start of the experiment. The males from each age group were randomly assigned to either immunocastration (IM) or as a non-castrated control (C). There were eight males in the juvenile group (five immunocastrated and three non-castrated) and six females, while the sub-adult group consisted of 10 males (five immunocastrated and five non-castrated) and five females. Immunocastration against GnRH was performed using Improvac® (Zoetis Animal Health, New Jersey, USA). Improvac® contains a synthetic peptide analogue of GnRH conjugated to diphtheria toxoid, and the adjuvant diethylaminoethyl (DEAE)-dextran (aqueous, non-mineral, oil-based). The protocol developed by Needham et al. (2019) was followed for the immunisation of the experimental animals. The vaccine was administered subcutaneously at a dose of 2 mL per animal, using a Sterimatic® needle guard system fitted with a Stericap®. Two doses of the vaccine were given at an interval of four weeks between the first and second dose.

5.3.2. The squeeze chute system and temperament scoring

The squeeze chute system used was designed for routine handling and biological sample collection from eland, based on systems widely used for the handling of large ungulates. The raceway is 11.60 m long 1.05 m wide 1.85 m high, and it is divided into four sections (A–D) by three galvanised sliding doors (Fig. 5.1; Supplementary Fig. SM5.1). The raceway is covered by a roof, ensuring low-light conditions in the corridor and thus preventing the eland from jumping. Along the wall of the corridor, a narrow section has been cut out to allow the handlers to observe the animals, and insert a "touch/tapping stick" made from a bamboo stem (10 mm in diameter). This stick was only used to gently touch the animal on the hindquarter after ~ 10 s of opening a new section, to stimulate forward movement in cases when the animal was apprehensive to move voluntarily. The first section of the corridor (A) is directly attached to the barn, and accessed through a rotating door. From the barn, animals are herded in batches of three to five animals into the first part of the raceway (A), from which a single animal is allowed to enter the second section (B). This second section is designed to briefly contain a single animal while waiting for the weight of the animal ahead of it to be recorded, in the third section (C). The third section (C) is the weighing area, and consists of a platform on load bars, and an ear-tag panel reader (TW-1 Weigh Scale G02601, Gallagher, Hamilton 3240, New Zealand). After weighing, the animals enter the fourth section (D), and into the adjustable squeeze restraint chute. The squeeze restraint chute consists of two rotating doors (1.50 m long), which are padded and can be adjusted vertically, according to the height of the animal, such that the top of the door is above the shoulder of the of the animal but the upper part of its neck and head are accessible. To ensure that the animal does not jump out of the squeeze restraint chute, thick plastic curtains have been fitted to the upper section of the restraint box. Once the animal enters the chute, the two doors are wheeled closed, towards the animal. With the aid of serrated clips, the doors can be finely adjusted to squeeze the animal, thereby restraining it. After the animal has been safely restrained, the plastic curtains were opened for sample collection.

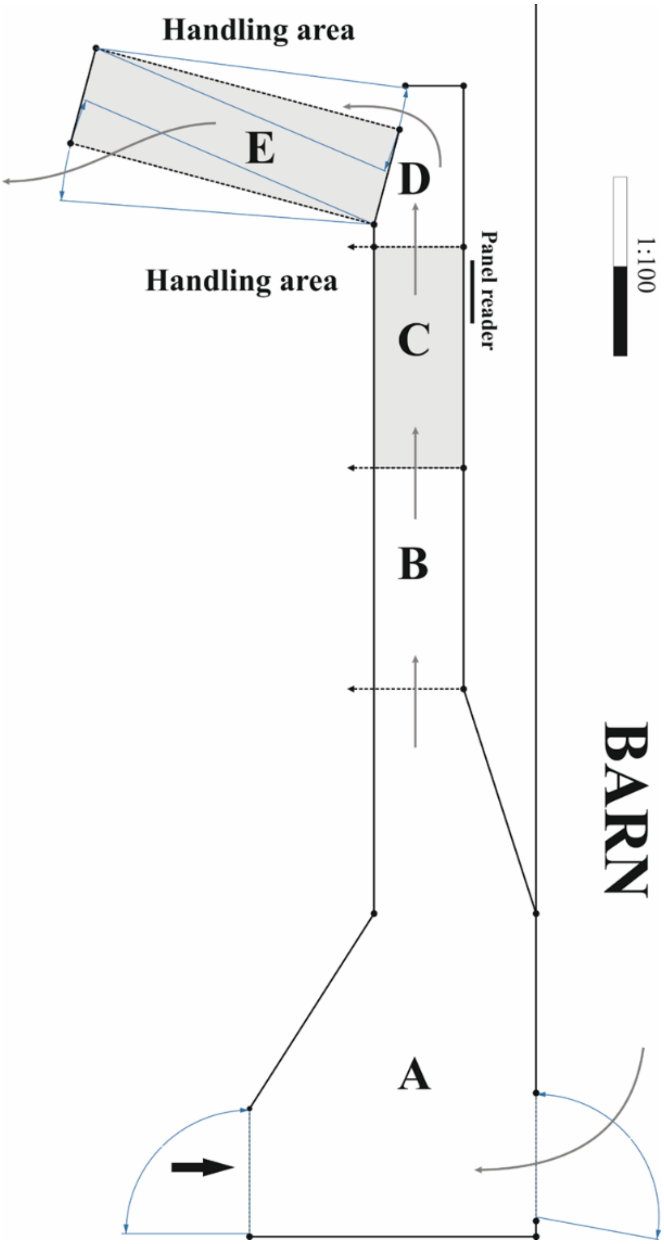

Figure 5.1. Design of the raceway divided into four sections (A, B, C and D) and the squeeze chute system (E). Animals enter the first part of the raceway (A) from the barn, after which they are individually separated from the group into section B, using sliding galvanised doors. The animal then enters the weighing system (C), and exits into the squeeze restraint (E), through section D. After closing of the rotating doors of the squeeze restraint chute, morphometric measurement and biological samples were taken. The animal exits the restraint directly into a paddock, by opening the left door. Broken lines indicate movable doors. Thin arrows indicate direction of movement of animals, while the thick arrow indicates possible entrance to the system for handlers, from the paddock.

Table 5.1. Temperament indicators used to assess the temperament of farmed eland during routine handling procedures.

Parameters	**Categories**	**Description**	**Score**
Entering the system	Voluntary	The animal walks toward the raceway without the handlers' interference.	0
	Lured	The animal is lured with a treat or sound cue.	1
	Forced	The animal is forced by handler using tapping stick.	2
State before and along the raceway	Calm	The animal does not make frequent eye contact and is not agitated.	0
	Nervous	The animal is agitated and uneasy.	1
	Panicked	Jumping, backwards and forwards movement, or aggressive toward the handler.	2
Use of tapping sticks to trigger forward movement	Yes/No	This entails if a stick was used at each of the four areas of the corridor to touch/tap the animals to move into the next section.	1/0
Movement through the system	Walking	The animal walks calmly through the raceway.	0
	Running	The animal seems to be tensed and trots from one section to another.	1
	Jumping	The animal is jumping and moving forwards and backwards.	2
Vocalisation	None		0
	Low		1
	Medium		2
	High		3
State in the squeeze chute	Calm	The animal remains calm while being measured and biological samples are being collected.	0
	Nervous	The animal is tense, uneasy and lying down.	1
	Panicked	The animal is trying to jump, pushing its head/horns towards the handler.	2
Chute exit	Walking	The animal calmly walks out of the chute	0
	Running	The animal trots off out of the chute.	1
	Jumping	The animal jumps out and trots off with high speed.	2

The recordings of individual animal behaviour within the system commenced after three step-up handling sessions conducted over three weeks, after which the handlers were confident that intensive handling could be performed regularly. During the step-up procedure, animals were handled progressively within the system once per week. Daily unrestricted free movement through the open system was allowed, with the animals voluntarily entering and exiting the barn exclusively through the handling raceway. The first handling event that involved intervention required the animals to be stopped individually within the weighing box only, and then restrained within the squeeze chute without any physical human contact.

Figure SM5.1. A side view picture of the raceway and squeeze chute handling system, showing the galvanized sliding doors, the green plastic curtain, and the blue coloured adjustable padded boards on the rotating doors.

In a second handling event, the curtains were opened once the animal was in the squeeze restraint, and a towel was placed over the animal's eyes before rectal faecal sampling was performed. The third handling event included blood sampling. The animals were offered small pieces of carrots while in the restraint during training, to provide a positive reinforcement strategy. At the end of this habituation process, it was decided that data may be collected every two weeks (each handling event being referred to as a "trial") to minimise stress and allow for an acceptable recovery time between handling for the eland.

The behaviour of each animal was recorded as they moved through the system, according to the parameters specified in Table 5.1. Each animal was focally sampled, and all of the indicative behaviours were recorded for a total of 10 handling events/trials. Scoring was done subjectively by a single trained individual throughout the study (Parham et al., 2019). The temperament scoring methodology was adapted from Schütz et al. (2016), as used for red deer, and Parham et al. (2019), as used for beef cattle. The scoring of the temperament and ease of handling takes into consideration how the animal enters the system from the barn (lured with pelleted feed, voluntary, or forced), the general state of the animal within the raceway (calm, nervous, panicked), speed of movement through the raceway (walking, running, jumping), vocalization (low, medium or high level), if the use of a tapping stick was necessary to trigger its forward movement in each section, state in the squeeze chute (calm, nervous, panicked) and finally, its exit from the squeeze chute after handling (Table 5.1). Exit velocity, which has been a primary parameter for measuring temperament by Curley et al. (2006) and Parham et al. (2019) was judged as walking, running, or jumping out of the squeeze chute.

The final dataset included 290 observations (twenty-nine eland during 10 handling trials), and 7 observations were excluded from the analyses because some of the temperament indicators could not be recorded. The temperament score (TS) for each handling trial was calculated as the sum of the sum of the indicators recorded (Table 5.1). The pooled score was obtained for each animal per trial, by summing these indicators and was used for the statistical analysis. Thus, the calculated TS may range from 0 to 17, although the actual values collected ranged from 1 to 10. This implies that an animal which measure a TS score of 0 moves through the handling system calmly, without any human intervention to move it to the squeeze chute.

Moreover, the animal is expected to be handled in the squeeze chute calmly during biological sample collection (blood and faeces) and morphometric measurement, without vigorous agitation, and finally exit the squeeze chute via walking. While at the opposite end of the TS scale, as it tends toward 17, it is implied the animal requires human intervention at each phase from the barn through the raceway to the squeeze chute, is highly agitated during handing at the squeeze chute, and finally it exits the chute by jumping out of it. The order in which the animals presented themselves for handling during the whole study period was also taken into consideration. Meanwhile, there was no human intervention/preference in the selection of animal for handling; this implies that the order of handling was not influenced by the herder.

5.3.3. Faecal steroid extraction

All faecal samples were frozen at –20 ◦C, until they were freeze-dried using lyophilization, after which they were pulverized, and sieved (1mm sieve) to remove undigested material. Steroid extraction followed the methodology of Sarmah et al. (2017). For each sample, 0.100 – 0.110 g of faecal powder was extracted by adding 3 mL of 80 % ethanol, vortexing the suspension for 15 min, and then centrifuging it at 1500 RCF for 10 min. Thereafter, 1 mL of the supernatant was transferred into Eppendorf tubes, and evaporated, using a GeneVac (Genevac Ltd, England) at 50 ◦C for 2.5 h. The samples were then stored at room temperature until analysis. At the point of analysis, the samples were reconstituted in 1 mL of 80 % ethanol, and glass beads were added to the Eppendorf tubes before vortexing for 15 s, following by sonification in a waterbath for 30 min.

5.3.4. Faecal androgen metabolite enzyme immunoassay analysis

Biological validation was performed, as described by Kamgang et al. (2020), to determine faecal androgen metabolite (*fAM*) enzyme immunoassay (EIA) suitability prior to analyses of the study samples. For the biological validation, faecal samples were collected from the breeding male in the eland herd, during an active mating period, as well as from new-born male calves (~ 2 months old). Both testosterone (T) and an epiandrosterone (EA) EIA was performed, according to Ganswindt et al. (2002). A full description of the EIA components used, including antibody cross-reactivities, is given in Palme and Möstl (1993). The overall individual median *fAM* concentration of the breeding male eland (4.32 µg/g dry weight (DW)) indicated a 138 % increase compared to male calves (1.82 µg/g DW) when using the EA EIA, while the T EIA only revealed a 77 % increase. Thus, the EA EIA was selected as more suitable for the further analysis of the study samples. Serial dilutions of faecal extracts gave displacement curves that were parallel to the established standard curve of the assay (the relative variation of the slope of the trend lines was < 3%). The sensitivity of the EA EIA was 12 ng /g DW. The inter-assay coefficients of variance (CV), of high- and low-concentration controls, were 14.51 % and 15.55 %, respectively, and the Intra-assay CV were 5.88 % and 6.98 %, respectively.

5.3.5. Statistical analyses

All analyses were performed in IBM© SPSS© Statistics (version 25.0 for Windows; IBM, USA). A Generalized Linear Mixed Model (GzLMM) was designed to determine the effects of the immunocastration treatment on the temperament of the studied animals during handling (*TS*), and to detect potential changes during the study period. A data structure based on group and animal as subjects, and trial (2-week period) as a repeated measure, was used. Body mass at every trial, treatment and trial were used as fixed factors, and group as a random factor. The interactions treatment*trial and treatment*group were also included in the model. The final model was selected after a traditional stepwise backward selection procedure. The same model was built using state in the squeeze chute and chute exit as target variables, since these indicators are especially important determinants of temperament and ease of handling, and also showed the highest variability among the recorded indicators. Finally, a similar model was built using order of handling as a target variable; however, this model also included *TS* as a fixed factor. According to the lack of normality of some of the target variables (Kolmogorov-Smirnov tests), a Gamma distribution with log function was used for the temperament model, a Poisson distribution with log function for the state in the squeeze chute and chute exit models, and a normal distribution with identity link for the order of handling model. Lack of multicollinearity between body mass, trial, *fAM* concentrations, and TS was assessed through the variance inflation factor (VIF), which reached a maximum value of 1.136.

All the suggested models were also tested when female animals were excluded from the analyses, and thus, only the immunocastrated and non-castrated males were evaluated. This procedure also allowed to include *fAM* concentrations as a fixed factor in the models, since *fAM* was not analysed in the females. However, the significant relationships found were the same when using both methods, with *fAM* concentrations never being significant in the final solved models. Thus, only the models previously described involving all the animals in the study are shown. The threshold for significance is considered as $P < 0.05$ throughout.

5.4. Results

Immunocastration treatment did not affect the calculated TS of juvenile and sub-adult males during routine handling, compared to both the control males and females. The TS was also not affected by age group or body mass. However, TS was affected by the handling trial ($\beta = -0.031$, $t = -4.959$, $P < 0.001$; Figure 5.2), decreasing with time (i.e., the animals were calmer after repeated handling). Similarly, immunocastration did not affect the state of the animals during handling in the squeeze chute or their exit from the handling system. State in the squeeze chute was affected by trial ($\beta = -0.188$, $t = -6.151$, $P < 0.001$; i.e., they were less nervous after repeated handling) and body mass

($\beta = 0.006$, $t = 4.199$, $P < 0.001$; i.e., heavier animals were more nervous during handling in the squeeze chute). Chute exit was affected by the trial ($\beta = -0.075$, $t = -3.686$, $P < 0.001$; i.e., they were less excited when exiting the chute after repeated handling). Finally, immunocastration did not affect the order of the animals entering the handling system. However, animals with low TS (i.e., more calm animals) entered the system earlier ($\beta = -0.075$, $t = -3.686$, $P < 0.001$), as well as animals with lower body mass ($\beta = -0.001$, $t = -3.071$, $P = 0.002$).

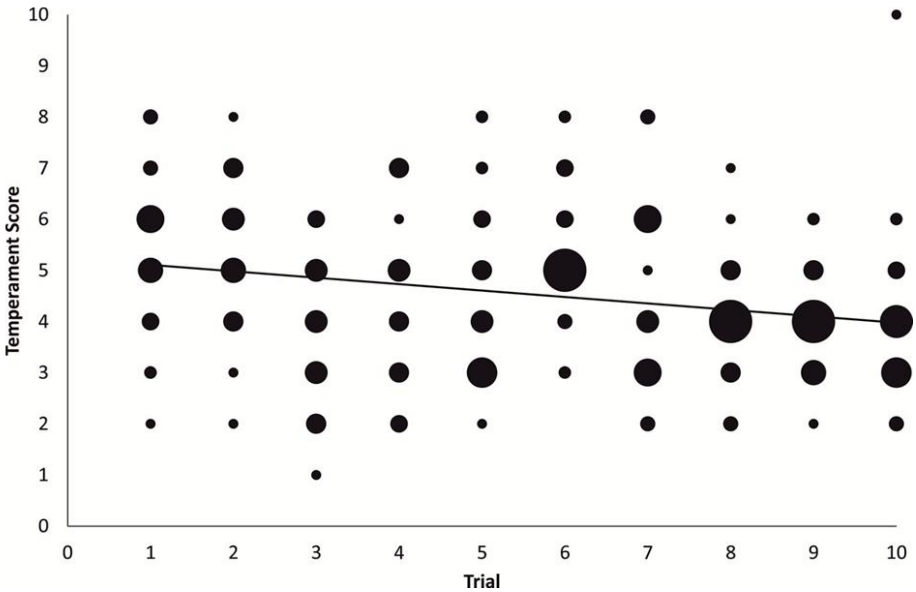

Figure 5.2. Influence of routine handling on the temperament score of common eland (n = 29) over time. Each trial was performed in two-week intervals, for a total of 10 trials. The size of the dots is proportionate to the number of observations for a particular score within each trial period.

5.5. Discussion

The present study showed that immunocastration did not influence the temperament of common eland during handling within the raceway and squeeze chute, nor the order of entering the system, and the chute exit velocity, irrespective of age. The results are in contrast with what was initially hypothesized, that immunocastrated animals would be calmer and easier to handle, and thus would have more docile temperament scores. The initial hypothesis being based on the impediment of androgen production, which is related to aggressive behaviour in other species (Brunius et al., 2011; Janett et al., 2012). However, the temperament score, state in the squeeze chute and chute exit velocity were all influenced by repeated handling, showing improved docility. This result implies that the animals were successfully habituated to the handling facility and sampling routine, as similarly reported in cattle after repeated handling (King et al., 2006). Despite habituation to the system, heavier animals had a higher aversion to entering the system, and were also more nervous in the squeeze chute.

Unlike in cattle, where heifers were found to have more excitable temperament than steers (Voisinet et al., 1997), sex (female, male or immunocastrated male) did not influence the temperament of the eland in the present study. It was postulated that the difference in temperament would be particularly evident in the sub-adult group of eland, where there is defined sexual dimorphism at this age (Kiley-Worthington, 1977). The influence of sex on temperament can be confounded by several factors, such as species-specific differences, breed, age, as well as the physiological state of the animal (Blanco et al., 2009). Voisinet et al. (1997) suggest that differences in sex would be more evident in different species, or breeds of the same species, where temperament is inherently prominent. This can be seen in the marked differences in temperament between the different sexes of so-called tropical breeds of cattle (*Bos indicus*; Elder et al., 1980). *Bos indicus* breeds have more excitable temperaments compared to *Bos taurus* breeds, and differences in the temperament between the sexes of *Bos indicus* cattle are thus more evident than in Bos taurus cattle (Hearnshaw and Morris, 1984; Fordyce et al., 1988). Additionally, the *fAM* concentrations were unable to explain the differences in temperament scores in the male common eland in the present study, suggesting that genetic and environmental factors have a larger influence on their response to handling than their androgen levels.

Noya et al. (2019) studied the influence of immunocastration on the temperament of feral bulls with different live weights, but immunocastration had no influence, which is in line with the outcome of the present study. Androgens positively influence muscle fibre hypertrophy, and thus the growth and development of animals, which makes male animals more muscular, but also potentially more aggressive (Price et al., 2003). Such attributes give male animals the opportunity to defend themselves, and to strive to surpass conspecifics in a social group by increasing their social rank (Pelletier and Festa-Bianchet, 2006), but this also makes them easily reactive to novel objects, due to their tendency for excitable temperaments. Noya et al. (2019) also suggested that the inability of immunocastration to influence temperament might be related to other factors other than decreased androgen production, such as genetics and environment. Like other social animals, common eland are sensitive to their environment, and learn to improve their chance of survival by adjusting their behaviour through postural lateralization and ritualization (Kiley-Worthington, 1978; Wirtu et al., 2005; Bordes et al., 2018). For example, Kiley-Worthington (1978) observed that an increase in the postural tonus in common eland signifies excitement and demanding attention, and are often associated with a warning or aggressive approach. Lowered postural tonus is often associated with sick, sleepy, or fearful animals, and therefore seen in subordinates and non-confident animals (Kiley-Worthington, 1978). Protective movements are related to threats, either from conspecific species or predators, and includes head lowering, horn pointing, horn clashing, and wrestling, in order of increasing intensity (Kiley-Worthington, 1978). Such postures and other social behaviours (affiliative as well as dominant) are important behavioural communicators, influencing the dominance hierarchy within social groups, and enabling the formation of larger herds which provide greater protection against predators, and access to important resources, such as food, water, and mates (Appleby, 1983; Bordes et al., 2018; Ceacero et al., 2012; Šárová et al., 2017).

Body mass also determines the chance of survival of an individual (Reale et al., 2007), which implies that an increase in body mass signifies good condition and a greater fitness for an animal to defend itself against threats (Šárová et al., 2016). In the present study, heavier animals were more nervous and had less docile temperaments during the handling process. These animals were also more muscular, and excitable behaviour can easily be elicited in such animals (Wirtu et al., 2005). In such circumstances, where heavier animals are being handled, the handling process should be done as fast as possible, to avert stress and injury (Pennington et al., 2013). Keeping these animals for a prolonged period within the handling system may also lead to the disruption of the handling process, through an increase in agitation of the group as a whole, thereby causing further stress to the entire process (Wirtu et al., 2005). Stressed animals show an increased production of glucocorticoids (King et al., 2006), and may cause bruising or injury to others and themselves (Fordyce et al., 1985), which compromises their welfare, growth, and the quality of their products, such as meat (Mondal et al., 2006).

However, overall, the eland were considered calm and easy to handle throughout the study period as seen by the range of temperament scores depicted in Fig. 5.2, where they already had relatively low cores at the onset of the study, and which gradually decreased further below the average throughout the study period with the exception of one outlier animal. This is in contrast with the initial hypothesis, that non-castrated males would be more aggressive and thus difficult to handle, but the eland herd breeding management has also prioritized the exclusion of aggressive animals. While temperament can indeed be influenced by other factors such as genetics, with routine and continuous handling, eland can gradually become habituated to the management procedures and thereby ease handling, regardless of age. Ceacero et al. (2014) found that routine and continuous handling of red deer juveniles, while ensuring minimum stress, led to the fast habituation of the juveniles to routine handling, which is also in line with the findings of Fordyce et al. (1985) and Petherick et al. (2009), who suggested that young calves of cattle should be properly managed and exposed to handling at an early age to improve their temperament, thus facilitating future handling at higher body masses.

Understanding the temperament of farmed and captive undomesticated animals is paramount, particularly when they are to be transferred or transported to a new environment (Grandin, 1997; Burdick et al., 2010). In the present study, immunocastration had no influence on the order in which the animals entered the squeeze chute system for handling, but the order of entering the system was influenced by the temperament score itself. Within the present study, animals with low temperament scores (more docile temperaments) were more willing to enter the system, unlike other animals with moderate temperament scores (less docile temperaments) which were often gently triggered to move through the raceway, using a tapping stick. Despite the aversion of the animals with higher temperament scores toward entering the system, the exit from the chute was also not affected by the treatment but was rather affected by time (the trial period). The eland were less nervous as the handling commenced, and exhibited more walking exits than running or jumping exits towards the end of the study. Considering the change in their state in the raceway, state in the squeeze chute, and nature of exit over time, the eland were able to maintain some memory of the handling procedures, and were less nervous.

5.6. Conclusion

Immunocastration has received increased attention, and is playing an important role in the management of farm animals, particularly from a welfare perspective. The present study sees no influence of immunocastration on the temperament of common eland during handling, which might be attributed to other factors such as genetics and environment. However, their temperament and habituation to handling gradually improved over time. This procedure may be implemented in zoological gardens, intensive production systems, and other captive eland populations which are handled routinely for examination or biological sample collection.

Declaration of Competing Interest

None

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CHAPTER 6

Effects of temperament during handling and social rank on the blood biochemical parameters of common eland (*Taurotragus oryx*)

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Effects of temperament during handling and social rank on the blood biochemical parameters of common eland (*Taurotragus oryx*)

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6.1. Abstract

Large herbivores are subject to handling and social stress in captivity. These may affect blood biochemical values, which motivated this research. Twelve healthy common eland (*Taurotragus oryx*) were monitored for 12 months. The animals were handled monthly, and blood samples were collected. Samples from every second month were analysed for 14 blood biochemical parameters. Temperament throughout the handling, as the summation of various behavioural responses, was calculated as a proxy of the stress generated during handling. Social behaviour was recorded each month, and the agonistic interactions were used to calculate the social rank, which was considered a proxy of social stress. Generalised Linear Mixed Models were designed to test the effects of temperament and social rank on the blood biochemical parameters while keeping sex, age, body condition, and body weight as covariates. The results show that the temperament during handling influences blood levels of albumin, alkaline phosphate, blood urea nitrogen, glucose, total bilirubin, and total protein; however, social rank has little influence, affecting just albumin. The ranges observed in the values of these biochemical parameters were still within their reference intervals, implying the absence of pathology or physiological problems during the study. The results suggest that blood biochemical values of physically restrained common eland should be carefully interpreted, even in animals already habituated to routine handling. On the contrary, social rank has low effects on the blood biochemical parameters.

Keywords: Domestication; Physical restraining; Routine handling; Social rank; Welfare.

6.2. Introduction

The number of non-domesticated ungulate species kept in captivity for conservation or production purposes is steadily increasing. These species are more susceptible than domesticated ones to handling and social stress (Hemsworth 2003), which may lead to severe effects on their physiology and health. Several parameters are influenced by handling in semi-domesticated species, like blood sodium concentrations in reindeer (Hyvarinen et al. 1976), glucose (GLU) and phosphorus (P) in woodland caribou (Karns and Chrichton 1978), or several haematological parameters in red deer (Ceacero et al. 2018). On the contrary, animals often get habituated to routine handling (Ceacero et al. 2014; Musa et al. 2021); however, these stressors can go beyond their coping level, resulting in impaired reproductive function, immunity, growth, and increased frequencies of stereotyped behaviours (Koolhaas et al. 1999). Thus, this is the basis of the first hypothesis in this study: that the stress during handling influences the blood biochemical parameters of semi-domestic common eland.

Stress can also be induced by social dominance interactions resulting from physical confrontation during conspecific competition, particularly in captivity (Brakes 2019). In such conditions, animals compete to increase their resource-holding potential, which results in agonistic interactions potentially leading to injuries (Wirtu et al. 2004; Bica et al. 2020). The effects of these stressful agonistic interactions on physiological responses have been well-studied in domestic goats, where high- and low-ranked does produce less milk (Barroso et al. 2000). In red deer, social status influences the haematological parameters through immunosuppression (Hjarvard et al. 2009; Ceacero et al. 2018). On this background, we also hypothesised that the stress derived from the social rank of each animal in the herd would influence the blood biochemical parameters of common eland.

The common eland (*Taurotragus oryx*) has been described as an ideal antelope for domestication due to its large body mass, ease of habituation to handling, and meat production potential (Woodford 2000). Depending on their exposure to handling, they might still get stressed while handling for health inspection, management, and research purposes. Moreover, few published studies have focused on the blood biochemical of common eland in the wild and captivity thus far, with few reference values available (Pospisil et al. 1984; Vahala et al. 1989). In addition, these studies utilised different immobilisation methods, leading to discrepancies. Hence, this study also aims to establish reference intervals of the blood biochemical parameters for common eland in captivity, where it is possible to control for confounding factors such as age, sex, body weight, and body condition. Overall, the present study aims to examine the effects of the stress during handling (measured through the temperament during handling) and the social stress (measured through the social rank) on the blood biochemistry parameters of common eland and contribute toward establishing reference intervals for the species.

6.3. Materials and methods

6.3.1. Experimental site and animal husbandry

The research was conducted at the Czech University of Life Sciences Prague (CZU) Common Eland Research Facilities. Animals are managed in an intensive system in a 450 m^2 barn with free access to 2.25 ha paddocks (low stocking density) and a complete feed mixture diet provided *ad libitum* inside the barn throughout the year (Musa et al. 2021). The animals in this study were managed in the same herd throughout the study. The animals are routinely handled every month; for this study, samples collected every second month for one year were selected.

The Czech Republic Ministry of Agriculture accredited all experimental procedures (clearance no. 63479_2016-MZE-17214), and ethical clearance was obtained from the Czech University of Life Sciences Animal Welfare and Clearance Committee (no. CZU 20/19). Routine handling in a custom-designed squeeze chute system with a raceway involved body weight and condition measurement, blood collection, recording of the temperament (as described in Musa et al. 2021), and recording social interactions two days after the handling. Twelve healthy animals with 135.1±2.25 kg initial body weight, 3.8±0.1 body condition score (BCS, following the 1 to 5 scale for red deer; Audigé et al. 1998), and age 9.2±1.5 months, consisting of six females six and males, were chosen for this study.

6.3.2. Assessment of the stress during handling

The stress during handling was measured as the behavioural response during handling by a trained researcher (temperament score, a proxy for handling stress; Mahre et al. 2015; Musa et al. 2021; Parham et al. 2021). The final score is the sum recorded at each step of the handling, being 0 for the less stressed animals and 17 for the most stressed ones. Then, the score is divided by 17 to get computed to a 0-1 range for easier interpretation. Since the behavioural response during the immobilisation phase was especially important for this study, this variable (scored as calm/0, nervous/1, panicked/2 as described in Musa et al. 2021) was also separately considered in the statistical analyses.

6.3.3. Social interactions and social rank

Social interactions were monitored by a trained researcher in order to obtain a social rank, which was later used as a proxy for social stress (Mahre et al. 2015; Musa et al. 2021; Parham et al. 2021). *Ad libitum* sampling (Altmann 1974) was used to continuously observe agonistic interactions for five hours (0800 – 1300) each month. Initiators and recipients of agonistic behaviours were recorded, including *Threatening* (with head or horns), *Passing* (moving away when another animal is passing), *Wrestling* (locking or clashing of horns), *Pushing* (with head or horns) and *Yielding* (butting another herdmate). Four commonly used dominance indices were computed using DomiCalc, a specialised software for the study of social interactions matrices (Schmid and de Vries 2013): David's score, Clutton-Brock index, Proportion of dominations, and the Inconsistencies and strength of inconsistencies method (I&SI).

6.3.4. Blood Samples Processing

Immediately after an animal is restrained in the squeeze chute, blood is collected from the *vena jugularis*. The area is disinfected, and blood samples were gently drawn using 18-gauge Vacuette® sterile needles with an adapter into a heparinised tube (Vacuette® Li-Heparin 8 ml). After collection, the tube was gently vortexed to avoid coagulation. The samples were then transferred to the laboratory in an ice-cold box and left to settle for at least 45 minutes before centrifugation at 2800 rpm for 15 minutes. The supernatant plasma sample was pipetted into Eppendorf tubes and stored below -18°C until analysis.

Plasma samples from every second month were analysed at the Laboratory of Animal Science (Faculty of Tropical AgriSciences, CZU), making 68 samples (four samples could not be collected). The frozen samples were thawed, vortexed, centrifuged at 12000 RCF for 2 minutes, and analysed for blood biochemical parameters: albumin (ALB), globulin (GLOB), total proteins (TP), creatinine (CREA), blood urea nitrogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase (AMYL), glucose (GLU), calcium (Ca), phosphorus (P), cholesterol (CHOL), and total bilirubin (TBIL). Analyses were done using a VetTest® Chemistry Analyser (IDEXX Laboratories, Westbrook, Maine, USA) with a standard commercial kit commonly designed for a general determination of the health status of mammals (GPH – General Health Profile; IDEXX Laboratories, Westbrook, Maine, USA).

6.3.5. Statistical analyses

Kolmogorov-Smirnov, visual examination of the histograms, and Q-Q plots were used for examining the normality of the variables analysed. Spearman´s ranked correlation showed a strong correlation for the two temperament variables ($\rho=0.412$, $p<0.001$) and very strong for the four social rank indices (all $\rho>0.802$ and $p<0.001$). Thus, Principal Component Analysis using Varimax rotation was used for both groups of variables to obtain a single factor for each one. For temperament, the selected factor explained 70.4% of the variance in the original variables. For social rank, the selected factor explained 74.8%. These factors were normally distributed and were used in further analyses. Generalised Linear Mixed Models tested the effect of temperament and social ranks on the 14 blood biochemical markers studied. A repeated measures structure was used, with individuals as subjects and months as a repeated measure. Sex, body weight, body condition, and age in days were also included in the models as covariates. All models were designed with normal distribution and identity link function, given the normality of all the variables used. Lack of collinearity was confirmed in the five continuous variables (temperament, social rank, body weight, BCS, and age) through the Variance Inflation Factor, which was always less than 5, as suggested (Kock and Lynn 2012). SPSS ver. 28.0 (IBM, Armonk, NY, USA) was used for all the analyses.

6.4. Results

The blood biochemical results were within the expected range (ZIMS 2022) for all the animals (Table 6.1). Four samples were excluded as they had extreme values, which might have resulted from inadequate sample handling. As expected, most of the blood biochemical markers studied were, to a certain degree, affected by general characteristics like sex, age, body weight, and BCS (Table 6.2). When controlling for these factors, the first hypothesis was confirmed: temperament during handling, as a proxy of the stress during handling, affected several blood biochemical markers.

Table 6.1. Descriptive statistics of the blood biochemical parameters for common eland obtained in this study, compared with reference intervals (ZIMS 2022).

Biochemical parameters	N	Mean	Median	Maximum – Minimum	Reference Interval (95%)	Reference Interval (ZIMS, 2022)
ALB (g/L)	64	34.2	35.0	30.0 – 39.0	30.0 – 39.0	13.0 – 42.9
ALP (U/L)	64	96.0	87.0	43.0 – 206.0	43.6 – 202.3	36 – 367
ALT (U/L)	64	23.5	23.0	10.0 – 38.0	10.6 – 36.1	1 – 25
AMYL (U/L)	63	455	451	301 – 600	326 – 597	30 – 750
BUN (mmol/L)	64	5.95	5.90	4.20 – 8.00	4.33 – 7.75	2.9 – 12.1
Ca (mmol/L)	63	2.36	2.41	1.59 – 2.80	1.62 – 2.67	1.7 – 2.8
CHOL (mmol/L)	64	2.12	2.05	1.52 – 3.08	1.62 – 3.02	0.65 – 2.58
CREA (μmol/L)	64	127	125	91 – 172	92 – 163	35 – 265
GLOB (g/L)	64	41.4	41.0	35.0 – 48.0	35.6– 47.38	3 – 59
GLU (mmol/L)	64	4.45	4.45	2.64 – 6.66	2.75 – 6.24	3.61– 15.65
P (mmol/L)	64	2.52	2.27	1.25 – 5.96	1.31 – 5.48	0.94 – 3.88
TBIL (μmol/L)	64	4.08	4.00	1.00 – 8-00	1.00 – 8.00	1.7 – 23
TP (g/L)	64	75.6	76.0	67.0 – 85.0	67.0 – 83.75	47 – 93
LDH (u/L)	63	399.4	367.3	213.7 – 872.0	216.4 – 825.9	352 – 1794

ALP and BUN were higher in animals with a calm temperament, while ALB, GLU, TBIL, and TP were lower in these animals. On the contrary, the second hypothesis was not confirmed since social rank, as a proxy of social stress, only affected ALB. Males had a higher, but not significant, average social rank than females (t=-5.185, p=0.197). Thus, the effect of social rank on increasing ALB values can be considered independent of sex, which was also a significant variable explaining ALB values (Figure 6.1.). Temperament and social rank did not influence other blood biochemical parameters, including AMYL, Ca, CREA, GLOB, and P. Nevertheless, certain variability was explained by the controlling factors of sex, age, body condition, and body weight. On the other hand, ALT, CHOL, and LDH were not affected by any of the studied factors.

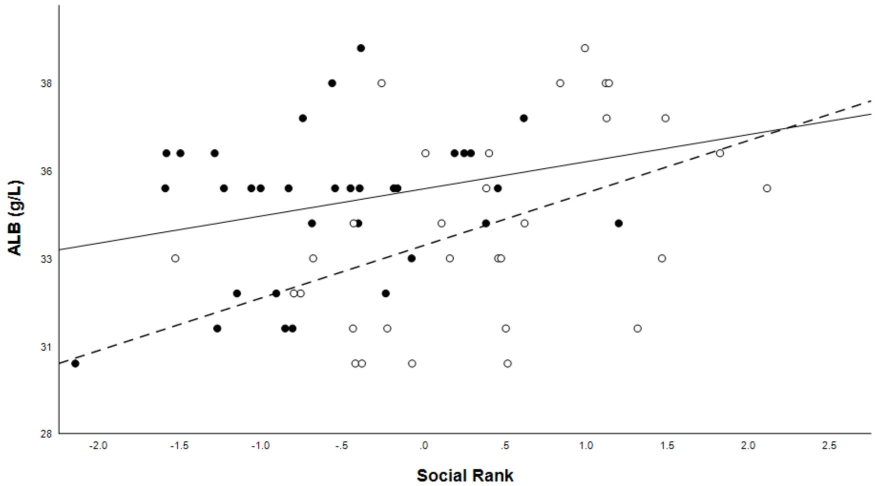

Figure 6.1 Changes in ALB concentrations (g/L) of captive common eland along the social rank gradient, separated by sex (black dots and full line for females; white dots and truncated line for males).

6.5. Discussion

Blood biochemical parameters, like electrolytes, enzymes, fat, protein, and energy-related metabolites, provide information about an animal's metabolic, nutritional, and health conditions, thereby providing insight into the welfare status of an animal. This study shows that all the values were within the reference intervals as compared to available data (ZIMS 2022), but these markers became altered in response to the studied factors when controlling for others like age, sex, weight, and body condition, which are already known to affect blood biochemistry (Kumar et al. 2022). Temperament, as a proxy of the stress during handling, influenced some enzymes, proteins, and energy metabolites, but not electrolytes, while social rank, as a proxy of social stress, only affected ALB. Other factors such as age, sex, body weight, and body condition were found to play a significant role in the blood biochemical profile of animals and their response to stress. These variables were included in the present study to increase the robustness of the models and confirm the effects independently of these confounding factors. Since these factors have already been reported in many studies, they are not further discussed.

Under acute stress, animals mobilise glucose to power the body to overcome the impact of such stressors. Handling commonly induces stress, resulting in behavioural displays that can be measured. This temperament

Table 6.2. General Linear Mixed Models showing the factors affecting the blood biochemical parameters of common eland. The table shows the β coefficients and the p-values (in brackets) obtained for the full models. P-values under 0.1 are shown, while the rest are just indicated as not significant.

Blood parameters	Sex[1]	Age	Body condition	Body weight	Social rank	Temperament
ALB (g/L)	2.140 (<0.001)	-6.056 (<0.001)	0.909 (ns)	0.038 (0.031)	0.817 (0.024)	0.892 (<0.001)
ALP (U/L)	44.495 (ns)	-1.391 (ns)	-22.767 (0.020)	0.764 (0.003)	-0.510 (ns)	-8.401 (0.053)
ALT (U/L)	0.446 (ns)	0.019 (ns)	0.235 (ns)	0.044 (ns)	0.011 (ns)	1.474 (ns)
AMYL (U/L)	-2.455 (0.090)	131.227 (<0.001)	-44.846 (0.006)	-1.351 (<0.001)	-2.707 (ns)	-0.940 (ns)
BUN (mmol/L)	0.110 (ns)	-0.438 (ns)	0.675 (0.013)	-0.006 (ns)	0.042 (ns)	-0.413 (<0.001)
Ca (mmol/L)	0.193 (0.005)	-0.384 (0.004)	-0.249 (0.002)	0.008 (<0.001)	-0.007 (ns)	0.001 (ns)
CHOL (mmol/L)	0.077 (ns)	0.221 (ns)	-0.055 (ns)	-0.001 (ns)	-0.018 (ns)	-0.004 (ns)
CREA (μmol/L)	7.247 (0.065)	20.764 (0.010)	20.480 (<0.001)	-0.147 (ns)	-0.055 (ns)	-2.163 (ns)
GLOB (g/L)	-0.862 (ns)	4.104 (0.017)	1.122 (ns)	-0.081 (<0.001)	0.058 (ns)	0.093 (ns)
GLU (mmol/L)	-0.019 (ns)	-1.048 (0.001)	0.185 (ns)	-0.007 (ns)	0.027 (ns)	0.308 (<0.001)
P (mmol/L)	-0.650 (0.012)	0.660 (ns)	0.600 (0.043)	-0.023 (0.001)	0.171 (ns)	0.025 (ns)
TBIL (μmol/L)	-0.533 (ns)	-0.921 (ns)	0.337 (ns)	-0.010 (ns)	0.231 (ns)	0.619 (0.006)
TP (g/L)	0.294 (ns)	-2.432 (ns)	2.564 (0.059)	-0.035 (ns)	-0.190 (ns)	1.385 (0.006)
LDH (u/L)	-215.985 (ns)	-491.339 (ns)	349.656 (ns)	5.598 (ns)	-112.700 (ns)	179.961 (ns)

[1] Male as a category of reference

during handling influenced many of the studied blood metabolites of common eland, with the effect on glucose being one of the most interesting. Glucose concentrations were higher in those animals that were more nervous during handling, confirming our hypothesis about handling stress and previous findings (Kumar et al. 2022). Acute phase proteins such as ALB also increased with stronger temperament reactions, as did TP, while no effect was observed for GLOB. These show directional changes in the body's immunity under stress and align with the findings of Marco and Lavin (1999), which show higher values in serum ALB and TP concentrations when animals were physically immobilised compared to chemical immobilisation. On the other hand, Murata et al. (2004) have reported not an increase but a decrease in ALB under different challenges, including stress; therefore, this effect needs further attention.

Elevated BUN concentrations are due to the high production of ammonia in the rumen, largely affected by the protein content in the diet. Calm animals showed higher BUN concentrations. However, the reason for the increase cannot be directly linked to the temperament during handling but suggests that calmer animals may have better access to feed resources. TBIL is another marker that increased with handling stress. It is a marker for cholestasis, which accompanies liver problems. A potential reason for the observed pattern can be the instantaneous breakdown of red blood cells, which are released during the acute response to stress stimuli to promote the efficient transportation of oxygen required for cellular catabolism (Marco and Lavin 1999). The cellular metabolism can increase exponentially in stressed animals, requiring enzymes to facilitate the success of this mechanism, although the temperament during handling did not influence the enzymes analysed in this study. A possible explanation for the normal enzyme concentrations is the absence of clinical pathologies and the not-so-acute stress that might appear after physical injury. Other studies have recorded higher ALT, AST, CK, and LDH concentrations in stressed animals during physical immobilisation, transportation, or handling for examination (Ali et al. 2006). Similarly, significant changes in CREA concentrations can be attributed to muscle damage (Kumar et al. 2022), which was not evident in the present study. At the same time, minerals such as Ca and P are maintained by hormonal regulation, which is usually in equilibrium in healthy individuals.

Social stress is a common phenomenon in social species. Occasionally, the agonistic interactions for access to resources may be even deadly due to the dominant-submissive relationships (Creel et al. 1996). Indeed, the effects of social stress on blood haematology have already been reported (Ceacero et al. 2018). This was the basis of our second hypothesis, which was not confirmed since social rank only influenced ALB among all of the 14 blood biochemical parameters analysed. ALB concentrations typically increase under dehydration; however, water is offered *ad libitum*, and no fluid loss, such as diarrhoea, was observed during the study. The lack of differences in other parameters can be attributed to the general harmony in the social group, as no serious agonistic interaction was recorded during the study. In a similar work, Tuchscherer et al. (1998) also found no differences in GLU, TP, triglyceride and ALP concentrations between dominant and subordinate pigs. However, significant differences appeared in TP, GLU, ALP and cortisol concentrations after adding new individuals to the group. Thus, the results suggest that the impact of social stress would be less important in well-established social groups with stable linearity and hierarchy. Another explanation may be linked to the fission-fusion social system of the species, which may make social rank not so important as in other social ungulates.

In summary, this study confirms that temperament during handling affects the blood biochemical profile of captive common eland, even if the animals involved are well used to routine handling and several individual variables were controlled for. On the contrary, effects caused by social rank were mainly rejected, except for ALB. Considering the handling technique and how habituated the animals are to it should help veterinarians interpret their results during clinical diagnosis. This study also verifies the reference values under a large sample size, establishing the reference values of 14 different blood biochemical parameters for the species. Future studies involving larger herds and species with different social systems are recommended.

The results obtained confirm the suitability of the species for domestication. Their social system allows keeping different groups with different structures, sizes and compositions and modifying them without increasing the social stress of the animals and raising welfare problems. The species can also be easily habituated to routine handling practices (Musa et al., 2021) necessary in intensive captive breeding programs, with handling having just certain stress effects on the animals, as expected, but not so strong as to challenge their welfare.

Author contributions

Conceptualisation, methodology and funding acquisition (FC), ethical clearance formalisation (RK), data collection (ASM, JK, TN, VN, JC, RK, FC), laboratory analyses (ASM, JK, VN, JC), data curation (ASM, JK, RK, FC), data analysis (FC), writing original draft (ASM, JK), writing and approving the final manuscript (ASM, JK, TN, VN, JC, RK, FC), language editing (TN).

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Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CHAPTER 7

General discussion

In today's global wildlife enterprise, there lies a big opportunity for game farming or wildlife ranching, which is also gradually shifting to intensification due to habitat loss and space limitations. In South Africa, for example, the government is pushing to expand and formalise this sector due to its growing interest in ecotourism, game meat production, and its sustainability compared to conventional livestock production (Sommerville et al., 2021). One crucial aspect determining the success of intensifying the husbandry of large bovids such as common eland is the ability to tame, handle, and manipulate them easily without compromising their welfare. In light of this, the present study evaluated possible ways and factors that would affect or improve the ease of handling, social interactions, and harmony of common eland in an intensive management system. These aspects are crucial as they impact productivity, immune function, conception rates, and overall animal welfare. The results from phase one of this study yielded two research outputs and show that immunocastration is a potentially promising tool in the behavioural management of common eland males in ameliorating agonistic behaviour, which can be detrimental in an intensive management system, particularly considering the size and wildness of such animals. Changes were, however, observed over time.

Immunocastration, in general, was observed to have no significant impact on temperament or ease of handling. However, there was habituation of the animals to handling over time due to frequent exposure to the handling system, which conditioned them to adapt to such procedures. The second study, leading to the third research output, examined how temperament during routine handling and social interaction affects health-related blood biochemistry parameters. This assessment aimed to evaluate the impact of the handling system and intensive management on the welfare of such mega faunas in captivity. All blood biochemistry parameters were within the reference range compared to information from the Zoological Information Management System (ZIMS, 2021). Temperament during handling influenced most of the blood biochemistry parameters but were all within the reference ranges of normal health profiles. Social rank, used as a proxy for social stress, was found to influence only one of all the blood biochemistry parameters, suggesting good harmony within the social group.

Immunocastration has generally been shown to be a better alternative to other welfare-comprising methods of castration, such as physical castration, in improving the performance and management of sexual and agonistic behaviours in different species of both domestic, sport, and wildlife animals (Malmgren et al., 2001; Ghoneim et al., 2012; Bertschinger & Lueders, 2018; Ahmed et al., 2022). However, no such studies have so far been conducted in common eland. Immunocastration works by interrupting androgen hormone production like testosterone, responsible for growth, muscle, and sexual development (Needham et al., 2017). An interruption of the anabolic effect of this hormone can decrease body mass due to a decrease in differential body development and an increase in fat deposition (Noya et al., 2019). Fat deposition might be exacerbated by a higher proportion of activity dedicated to feeding rather than other social activities, likely indirectly influenced by decreased testosterone levels, as seen in swine (Needham et al., 2017). In the present study, immunocastration had no significant effects on body mass irrespective of age categories and likely no effect on the level of fat deposition (more details in Needham et al., 2020). Still, it might be necessary for future studies to evaluate this.

Immunocastration largely had no effects on most of the response behavioural variables in the present study, including activity budget (Eating, Walking, Lying, Standing, Social), social rank, and rate of social (agonistic) interactions. However, there was a significant reduction in aggressive behaviour in the juveniles. Changes in activity in castrated animals can either result from the immunological impact/response of the immunocastration or the pain response from physical or surgical castration. For immunocastration, this response is mild and results in a change in activity for a few days after the second immunisation, with an increase in feeding activity due to a lack of androgenic suppression of appetite (Needham et al., 2017; Rydhmer et al., 2010). Whilst physically castrated individuals can be seen exhibiting distress and uneasiness standing for a long time, decrease in appetite, or lying for a long time in a recumbent posture due to pain response. Thus, this can be prolonged for a long period if farmers are unwilling to incorporate analgesics for such stress or physical injuries (Ting et al., 2003). Overall, in the present study, there were only changes in activity between the pre-and post-vaccination period in both treatment groups, which might signify a mild but not significant effect of the treatment. The use of immunocastration against aggressive or sexual behaviour is preferable in domestic, captive, and zoo animals but not in the wild, where such traits/qualities are necessary for maintaining herd hierarchy and fighting against predators (Francis, 1988; Creel, 2001; Bagnato et al., 2023). Here, immunocastration was only significant in reducing aggressive behaviour in juveniles, which upholds the recommendation to use this treatment from a younger age. Meanwhile, animals exhibit aggressive behaviour to maintain or increase their dominance/social rank for a better-resource-holding potential, of which such a trait would not be necessary for an intensive management system since all necessary resources are provided and under the control of the management (Bagnato et al., 2023). In this regard, controlling aggressive behaviour will help increase the ease of handling and management.

In an intensive system where eland are kept for either meat and/or milk production or in a park for ecotourism, their temperament and habituation to routine management are essential for efficient productivity. Stress resulting from struggling during human handling often has a detrimental consequence on the products derived from such animals. In the present study, the temperament or habituation of animals to humans was not significantly influenced by immunocastration. Likewise, a previous investigation by Noya et al. (2019) on feral bulls (*Bos taurus*) similarly concluded that immunocastration does not yield any notable improvement in the temperament of the bulls. To address the challenges of the temperament of feral and wild captive animals, several farmers and herd keepers have resorted to training such animals routinely to get acquainted with specific management procedures (Goddard et al., 1996; Zidon et al., 2009). This approach has been effective in common eland previously conditioned by Wirtu et al. (2005) and Pennington et al. (2013) for transvaginal oocyte retrieval without general anaesthesia. In the present study, the temperament during handling was also found to improve over time for routine handling designed for biological sample collection and manipulation for morphometric evaluation and health examination. Meanwhile, within an intensive management context, it is crucial to consider factors such as age, sex, weight, and dominance hierarchy before undertaking such procedures, particularly if animals are to be held in a holding pen before being restrained individually. In the present study, for example, females, animals with higher body mass, and young animals were more nervous when handled. In these circumstances, such animals should be handled as soon as possible to avoid causing serious agitation that can distress the whole herd/group and the handlers.

Additionally, in the second study, an evaluation into the welfare implication of the handling procedure was conducted using blood biochemistry health markers, showing that all parameters were within the reference range

(ZIMS, 2021), meaning that such a low-stress procedure can be considered as not detrimental for the welfare of common eland in an intensive management system, without the need to use anaesthesia or sedatives. In the present study, energy metabolites such as glucose, one of the most notable markers for stress, were found to be affected by handling stress and were higher in animals having higher temperament scores. However, the lack of significant change in the enzyme health-related biomarkers like ALP, AST, and LDH further justified the assertion that using such a custom-built handling system for eland as used in the present study did not compromise their welfare. Moreover, there was no notable impact of handling stress on CREA levels, which are crucial in indicating the degree of muscle damage in individuals experiencing high stress levels. Meanwhile, there were changes in the levels of ALB, BUN, TBIL, and TP. These markers are important in defining renal functions, liver function, and high ammonia production in the rumen. These notable variations could potentially be associated with nutrition, a factor not specifically controlled for in the current study, although all the animals feed on the same mixed ration. Future investigations will be essential to address these limitations. Overall, it should be noted that the temperament of an animal can also be influenced by its fear response, which may be connected to a genetic component. A crucial factor in taming or transitioning wild animals to domestication is to manage their fear response towards humans through consistent routine handling and behavioural training, as they perceive humans as potential threats or predators (Li et al., 2007; Bartošová-Víchová et al., 2007).

Another consideration in the second study of this thesis was the effect of social stress arising from social dominance on the health blood biochemistry profile to ascertain the welfare implication of managing common eland in an intensive husbandry system. Social stress arises from various phenomena, encompassing social isolation, regrouping, and overcrowding, with regrouping and overcrowding, particularly due to space constraints, being the most prominent in intensive management systems (Proudfoot & Having, 2015). In the present study, social stress did not significantly influence all the studied blood biochemistry parameters except ALB. The lack of significant influence was largely attributed to the harmony in the social group as the animals had already coexisted before the commencement of the study, which implies a linearity in the social organisation where every animal had a well-established hierarchy, which helped reduce the incidence of serious agonistic behaviours that are likely to compromise welfare (Preuschoft & Van Schaik, 2000). Similarly, other studies have reported similar findings with no significant change in physiological stress indicators in cattle cows at different stocking densities (Huzzey et al., 2012; Krawczel et al., 2012). However, significant changes in immunity and susceptibility to disease due to social stress resulting from regrouping and overcrowding have been reported in other studies, extensively reviewed by Proudfoot & Having (2015).

Other factors such as age, sex, body weight, and body condition were observed to play a significant role in the blood biochemistry profile of animals and their response to stress. These variables were included in the present study to increase the robustness of the models. In this study, the concentration of ALB, Ca, and GLU decreased while AMYL, CREA, and GLOB increased with age, with all these effects being previously reported in other studies (Roubies et al., 2006; Patel et al., 2016; 79, Kristanto & Widiyono, 2021; Husakova et al., 2014). Sex influenced most blood biochemistry parameters, with ALB, ALP, Ca, and CREA significantly being higher in females, and AMYL and P significantly higher in males. These effects have been repeatedly shown in the literature. Thus, sex was an important factor to control in the present study, especially since it was done with animals during puberty. Finally, body weight and condition also influenced the blood biochemistry parameters. Although common eland does not deposit much fat compared to other domestic animals, their musculature can clearly show variation in the levels of body condition. Thus, an increase in body weight leads to a rise in ALB, ALP, and Ca, which might signify an increase in the protease system, osteoblastic activity, and mineralisation, leading to an increase in body mass (Allen, 2003; Mohebbi et al., 2010). Surprisingly, AMYL, GLOB, and P were negatively influenced by body weight, while the rest of the parameters did not experience any significant change due to weight. Body condition positively influenced BUN, CREA, P, and TP but negatively influenced ALP, AMYL, and Ca. which can be attributed to increasing muscle mass development (Caldeira et al., 2007).

CHAPTER 8

General conclusions

The current dissertation explores the effect of husbandry management practices that are employed in a traditional livestock-intensive system on the behavioural management of common eland, driven by the growing interest in its intensification. The following overall conclusions can be drawn from the three research outputs of this study:

a. Immunocastration can be considered a potential welfare-friendly tool for the management of eland in captivity compared to physical castration since it does not affect the social behaviour and activity budgets of treated males, their hierarchical structure, or their temperament during handling, while it helps reduce aggressive behaviour in juveniles and increases affiliative behaviour in subadults.

b. Immunocastration can be used in mixed herds of eland with different breeding goals, such as in zoos, feedlots, parks, and game production.

c. Common eland can successfully habituate to routine sampling and sample collection.

d. Repeated monthly handling leads to less excitable animals, showing a progressive decrease in fear response and stress. As such, animals can be trained to voluntarily participate in the handling procedures, leading to calm animals (low temperament score) entering the handling system earlier.

e. Heavier animals are more nervous and difficult to handle. It is recommended that such animals should be handled timely to avoid agitation in the holding areas which can potentially lead to injury.

f. Even if well-habituated to handling, the inevitable physical stress affects some of the health-related blood biochemistry parameters, which should be considered by veterinarians when interpreting analytical results. However, this stress does not (and should not) result in values exceeding the reference range for the species should the correct handling procedures be followed.

g. Social stress arising from social dominance does not affect the blood biochemistry parameters.

CHAPTER 9

General references

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CHAPTER 10

Annexes

Annex 1. *Curriculum vitae* Abubakar Sadiq Musa

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EDUCATION

PhD studies **2019 – Present**

Czech University of Life Sciences Prague. Tropical Agrobiology and Bioresource Management. PhD title: Effects of routine handling in the husbandry of common eland.

Master studies **2017 – 2019**

Czech University of Life Science Prague. Sustainable Animal Production. MSc title: Effects of immunocastration on the social interaction and activity budget of common eland (*Taurotragus oryx*).

Bachelor studies **2007 – 2014**

University of Maiduguri (UNIMAID). Animal Science. BSc title: Haemato-biochemical indices of yankasa rams fed varying levels of doum palm (*Hyphaene thebaica L.*) meal.

INTERNSHIPS

Mammal Research Institute, University of Pretoria, South Africa. Endocrine research laboratory. 03/2021 – 05/2021.

University of 8 Mai Guelma, Algeria. Faculty of Natural Sciences, Life, and Sciences. 01/2023 – 03/2023.

WORKING EXPERIENCE

Laboratory Assistant. Department of Animal Science and Food Processing Laboratory, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Czech Republic. 2018 – till date.

Assistant farm manager in research farm. Department of Animal Science, Bayero University Kano, Nigeria. 2014 – 2015.

SCIENTIFIC PUBLICATIONS

1. **Musa, A. S.,** Needham, T., Kotrba, R., & Ceacero, F. (2024). Activity and social behaviour of farmed common eland (*Taurotragus oryx*), and the effect of immunocastration thereon. *Applied Animal Behaviour Science*, 106189. http://dx.doi.org/10.1016/j.applanim.2024.106189

2. **Musa, A. S.,** Kundankumar, J., Needham, T., Kotrba, R., Ny, V., Consolacion, J., & Ceacero, F. (2024). Effects of temperament during handling and social rank on the blood biochemical parameters of common eland (*Taurotragus oryx*). *Veterinary Research Communications*, 1-7. https://doi.org/10.1007/s11259-024-10296-1

3. Consolacion, J., Ceacero, F., **Musa, A. S.,** Ny, V., Kotrba, R., Illek, J., ... & Needham, T. (2024). Reproductive tract morphology and symmetry of farmed common eland (*Tragelaphus oryx*) bulls, and their relationship with secondary sexual traits and social rank. *Animal Reproduction Science*, 107438. https://doi.org/10.1016/j.anireprosci.2024.107438

4. Ny, V., Needham, T., Bartoň, L., Bureš, D., Kotrba, R., **Musa, A. S.,** & Ceacero, F. (2023). Effects of immunocastration and supplementary feeding level on the performance and blood biochemical markers of farmed yearling fallow deer (*Dama dama*). *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.13807

5. Needham, T., **Musa, A. S.,** Kotrba, R., Ceacero, F., Hoffman, L. C., Lebedová, N., & Bureš, D. (2022). Carcass and Offal Yields of Farmed Common Eland (*Taurotragus oryx*) Males, as Affected by Age and Immunocastration. *Animals*, *12*(21), 2893. https://doi.org/10.3390/ani12212893

6. Hrnková, J., Golovchenko, M., **Musa, A. S.,** Needham, T., Italiya, J., Ceacero, F., ... & Cerný, J. (2022). Borrelia spirochetes in European exotic farm animals. *Frontiers in Veterinary Science*, *9*, 996015. https://doi.org/10.3389/fvets.2022.996015

7. **Musa, A. S.,** Needham, T., Kotrba, R., Neradilova, S., Ganswindt, A., & Ceacero, F. (2021). Habituation of common eland (*Taurotragus oryx*) to intensive routine handling, and the effect of immunocastration thereon. *Applied Animal Behaviour Science*, *237*, 105294. https://doi.org/10.1016/j.applanim.2021.105294

8. Girgiri, A. Y., **Musa A. S.**, Medugu, C. I., Saleh, B., Gure, M. M. (2013). Haematobiochemical indices of Yankasa rams fed varying Levels of Doum palm (*Hyphaene thebaica L.*) meal. In: Proceedings of the 18th Annual Conference Animal Science Association of Nigeria (ASAN). Abuja, 8th-12th September 2013. pp. 379-382.

CONFERENCES, WORKSHOPS AND SUMMER SCHOOL

- International Symposium on Animal Sciences. September 2023. *Novi Sad, Serbia*. Poster presentation.

- Animal Science Days. September 2022. *Zadar, Croatia*. Poster presentation.

- Workshop: Breeding Programme Modelling with AlphaSimR and estimation of effective population size. September 2022. *Zadar, Croatia*.

- International open day student conference, Faculty of Tropical AgriSciences. November 2021. *Prague, Czech Republic*. Oral presentation.

- Animal Science Days. September 2021. *Gödöllő, Hungary*. Poster presentation.

- Workshop: Population Genetics and Genomics. September, 2021. *Gödöllő, Hungary*.

- Workshop: Introduction to tidyverse in R. Course of data visualization and filtration. September 2021. *Gödöllő, Hungary*.
- Global Biodiversity Conservation Conference. November 2021. Faculty of Tropical AgriSciences. *Prague, Czech Republic*.
- 71st Annual Meeting of European Federation of Animal Science, EAAP. September 2020. Online.
- Summer School: Euro league for Life Sciences (ELLS). Contribution of animal breeding to global food security. August 2020. University of Natural Resources and Life Sciences. *Vienna, Austria*.
- 46th Conference of Czech and Slovak Ethological Society. November 2019. *Bratislava, Slovakia*. Poster presentation.
- Global Biodiversity Conservation Conference. September 2018. *Prague, Czech Republic*.

TEACHING ACTIVITIES

IAI008Z - Practical Animal Handling, Sampling and Processing

IAI001E - Animal Production in Tropics

IAI011E - Tropical Bovid with Husbandry Guidelines

AWARDS

- 2019 - Rector's Award for graduating with Honor (Masters). Czech University of Life Sciences Prague, Czech Republic.
- 2014 - Vice Chancellor's Award (Bachelors). University of Maiduguri, Borno State, Nigeria.
- 2014 - Head of Department Award for Best Graduating Student (Bachelors). Department of Animal Science, University of Maiduguri .