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Effects of immunocastration on the social interaction and activity budget of common eland (*Taurotragus oryx*)

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled "**Effects of immunocastration on the social interactions and activity budget of common eland (*Taurotragus oryx*)**" independently. All texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references according to the citation rules of the FTA.

In Prague April 2019

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Abubakar Sadiq Musa

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Abstract

Common eland are social animals with hierarchical rank being largely determined by body mass, age, muscularity, matrilineal genealogy and aggressiveness. The bull needs to be aggressive in order to increase in social hierarchy and dominate over others for a better resource-holding potential and mating opportunities. However, this can affect eland management and handling in captivity. In light of this, this study was designed to examine if immunocastration can influence the social rank, aggressive behaviours, affiliative interactions and thus the activity budget of common eland. To test this, 30 common eland were divided into two groups of sub-adults (G1, n=15, 182.9 ± 59.37 kg, ≈ 2 years old) and calves (G2, n=15, 94.18 ± 24.76 kg, ≈ 6 months old). Each group consisted of males (n=10) and females (n=5). Improvac® was used for the immunocastration of five randomly selected males per group and administered at two doses of 2ml/animal subcutaneously in the shoulder area using a Sterimatic® needle guard system fitted with a Stericap®, at four weeks between vaccination and three months before slaughter. All occurrence sampling was done fortnightly to record dyadic social interactions while activity budget behaviours were observed through scan sampling. Social interactions were processed in DomiCalc (matrix manipulation and analysis software) to examine the linearity and hierarchy of the groups, and the proportion of total, dominance, aggressive and affiliative interactions were established. Generalised linear models were designed to test the effects of immunocastration on the behavioural patterns. Both groups showed linear social hierarchies ($p<0.001$). The immunocastrates had higher dominant rankings ($p<0.001$) with reduced aggressive behaviours ($p<0.001$) and less affiliative behaviours ($p<0.01$). There was no difference between the activity budget for all behaviours except for social behaviour, which was reduced ($p<0.001$) in immunocastrates. This study suggests that active immunization against GnRH is a practical and non-invasive alternative to physical castration in the behaviour management of common eland bulls.

Keywords: Aggression, Castration, Dominance, Improvac, Social rank

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List of the abbreviations used in the thesis

AR	Affiliative interaction received
AS	Affiliation started
AgR	Aggressive received
AgS	Aggressive started
DK	Drinking
DAgS	Dominant aggressive interaction started
DAgR	Dominant aggressive interaction started
DNAgR	Dominant–not aggressive started
DNAgS	Dominant–not aggressive started
DR	Dominance received
DS	Dominance started
ET	Eating
FSH	Follicle stimulating hormone
GM	Grooming
GnRH	Gonadotropin-releasing hormone
h'	Strength of linearity
I&SI	Inconsistency and Strength of inconsistency
LY	Lying
LH	Luteinising hormone
NAgS	Not-aggressive started
NAgR	Not-aggressive received
Oi	Occurrence of interactions
PL	Playing
SR	Social rank
ST	Standing
t_{tri}	Triangle transitivity
WK	Walking
WR	Wrestling

1. Introduction and Literature Review

1.1. Introduction

The common eland [*Taurotragus oryx* (Pallas 1766)] is a social animal and the second largest African antelope, after the giant eland (*Taurotragus dabianus*). It is endemic to East and Southern Africa (Pappas 2002) but it has been successfully bred in various part of the world. The interest in farming common eland for meat is motivated by their large body size and lean meat (Barton et al. 2014). According to Scherf et al. (2000), under the auspices of FAO, common eland is considered the best antelope for domestication. Despite being previously described as a calm animal (Gentry et al. 2009), its handling and management can be quite stressful and dangerous to both the handler and the animal due to it alertness, flightiness and muscularity (Wirtu et al. 2005). However, castration may decrease the potential incidence of such agonistic and aggressive behaviour in farm animals (Godfrey et al. 1996; Price et al. 2013).

Immunocastration influences animal behaviour by interrupting the pituitary-gonadal axis, thereby preventing the production of androgenic hormones from the reproductive organs. Traditionally, various methods of castrating farm animals have been developed (Bouissou 1983). At present, with the advancement in technology and the efforts to improve animal welfare, immunocastration is an alternative to physical castration (Melches et al. 2007; Sales 2014). In contrast to physical castration, immunocastration works using vaccines which stimulate antibody production that bind Gonadotropin-releasing hormone (GnRH) and prevent its action on the pituitary gland (Needham et al. 2017).

There is a decrease of the immunocastrate’s testosterone level which consequently affects their activity (Amatayakul-Chantler et al. 2013). Such extraneous changes in a group of social captive animals, such as eland, can have an effect on their hierarchy, social interaction and activity. Dominance and hierarchy in a social group, substantially determine the animal’s resource-holding potential and mating in breeding animals (Wirtu et al. 2004; Pelletier & Festa-Bianchet 2006; Ceacero et al. 2012; Horová et al. 2015).

Despite several studies on the effects of immunocastration on growth and meat quality in livestock (Lowe et al. 2014), information is rarely available on it use in wildlife, as well as its effects on social interaction and activity budget of captive animals. Providing such information may ease the management of captive antelope, particularity for non-breeding bulls meant for meat production.

1.2. Literature Review

1.2.1. Common eland

1.2.1.1. Origin, distribution and description

The name "eland" originates from the Dutch word meaning "elk" and was given to these antelope by early Dutch settlers in southern Africa (Hillman 1979). The taxonomic nomenclature of eland has undergone a number of changes from the initial *Antilope oryx* to the present *Taurotragus oryx* (Pallas 1766). It is an antelope belonging to the order Cetartiodactyla, family Bovidae and subfamily Bovinae. The common eland and the giant eland are the only antelope in the tribe Tragelaphini (or spiral-horned antelope), to be given a generic name other than *Tragelaphus*. They were initially placed within the genus *Tragelaphus* based on molecular data (Essop et al. 1997).

Based on fossil records, the bovids first appeared around 20 million years ago (Pappas 2002). Some eland fossils have been discovered in France, but the most thorough paleontological record of the common eland was traced to sub-Saharan Africa (Essop et al. 1997). The common eland (*Taurotragus oryx*) is now considered native to southern and eastern Africa (Figure 1) and at present, three distinct subspecies are recognised, all of which are considered "least concern" by IUCN (2016). These subspecies are: *Taurotragus oryx oryx* in southern Africa, *T. o. livingstonii* (Sclater 1864) in East-Central Africa and *T. o. pattersonianus* (Lydekker 1906) in Tanzania (Groves et al. 2011). Eland are widely distributed and numerous in game reserves within these regions, with only those in the Burundi considered extinct (IUCN 2016). With the growing interest in game meat, and the efficient adaptability of the common eland to varied climate (Kotrba 2002; Yahya 2018), eland have been exported out of its home-range to countries such as Russia and the United States of America, where they are farmed commercially (Furstenburg 2016). Eland can also be found in Asia, Europe, America, in animal and wildlife research institutes and zoos, where they have also been used as a model animal for physiological research for other antelope (Wirtu et al. 2005; Pennington 2009).

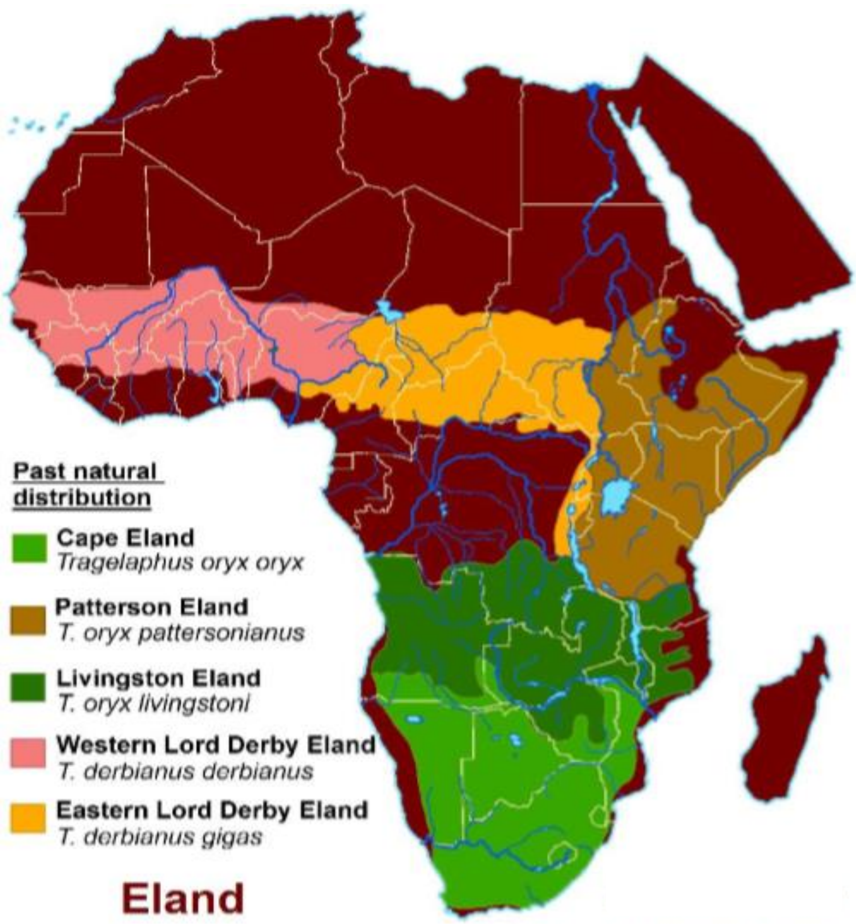

Figure 1. Map showing the original distribution of the subspecies of common eland (*Taurotragus oryx*) in Africa. Source: Furstenburg 2016

The common eland has been described as a "cow-like" animal due to its large body size and the success that has been achieved in taming it (Pennington 2009). Male eland are exceptionally larger and more muscled than females, which can be attributed to efficient androgenic functioning in the male, creating a distinct sexual dimorphism within this species (Underwood 1979). In mature bulls, height at wither averages 163 cm (151-183 cm) while in cows it averages 142 cm (125-153 cm). Bulls can weigh an average of 500-600 kg (450-942 kg) at maturity, and mature cows weigh an average of 340-445 kg (317-470 kg) (Estes 1991).

Pelage colour varies amongst eland subspecies, from dark grey-brown to reddish-brown, but males tend to turn blue-grey due to alopecia as they mature which then exposes the skin (Hillman 1979). In both sexes there is a crest of hair which runs from the nape to a less prominent hump on withers. Moreover, there is a tuft of hair on the forehead of both sexes which is particularly dense and darker in the male, likely linked to testosterone production (Hosking & Withers 1996). Both sexes have a dewlap but that of the males gradually enlarges with maturity (Kingdon 1997; Groves et al. 2011). The common eland typically has 2-15 transverse white stripes, which are more prominent and conspicuous anteriorly on the body (Haltenorth & Diller 1980). As one moves more south of Africa, the fur colour is lighter, and stripes are less pronounced on the animals as compared with animals in northern areas (Skinner & Smithers 1990). All eland subspecies have a black spot on the posterior upper region of the forelegs, and a dark dorsal stripe running down the spine (Posselt 1963).

Both sexes of common eland have spiralled or corkscrewed horns, but the horns of males are relatively shorter, thicker, tighter and with more prominent spirals (Figure 2). Horn length of males' averages 54 cm (43-67cm; Estes 1991), while the females' horns are longer, thinner with loose spirals (Figure 3) and average 60.5 cm in length (51-69.6 cm; Estes 1991).

Unlike other temperate ungulates that are mostly seasonal breeders, the common eland can breed all year round, even when exported out of its native environment. Just like cattle, the eland cows have been monitored to ovulate on average every 21-26 days and lasting 2-3days (Nowak 1999; Pennington 2009). On average, males mature at 4 years of age while female mature earlier at 2.5 years of age under extensive natural condition. The eland cow can calve at any time of the year in captivity due to adequate availability of feed, but in the "wild" they have a peak breeding and calving season to coincide with the periods of optimal food availability (McNaughton 1990). The eland cow can breed successfully at approximately 2.5 years of age and gestation lasts 271 ± 2.9 (SE) days (Dittrich 1972; Pappas 2002). Calves are usually naturally weaned at 6 months of age and their life span is up to 25 years in captivity. Analysis of the eland cow's milk was observed to be very rich in fat, protein and lactose with 11%, 8.2% and 4.5%, respectively (Pennington 2009).

Figure 2. A mature common eland (*Taurotragus oryx*) bull Source: Wildlife Stud Services: www.ws2.co.za

Common eland occurs in wide variety of open vegetation such as: open grassland with shrubs along drainage lines, montane grassland, woodland and savannah (Pappas, 2002; Codron et al. 2007). They avoid deep and dense forest vegetation to be conscious and alert and thus avoiding predators, they are also not found in true desert regions (Groves et al. 2011). They are non-territorial, and females have a wider home-range (174-422 km^2) than males; but they do maintain a herd hierarchy (Hillman 1979; Wirtu et al. 2004). They are nomadic in nature and migrate from one region to another based on food availability and season.

In view of their feeding habit, the common eland are considered intermediate feeders. They usually browse on forbs from the family Compositae as well as young foliage from trees. They also graze during the wet season when grasses are abundant

(Buys 1990). They exhibit crepuscular feeding behaviour, which implies feeding in the early morning and late evening in a bid to avoid harsh weather of the day and predators (Lewis 1978). They have an efficient ability of water conservation and tolerance to water scarcity, as they obtain much of their water from their feed (Skinner & Smithers 1990). Unlike cattle, they are asymptomatic and have been observed to be tolerant to trypanosomiasis (Pappas 2002). However, they are susceptible to the deadly Theileriosis, myiasis and a variety of ticks (Young et al. 1980).

Figure 3. A group common eland cows (*Taurotragus oryx*) Source: Wildlife Stud Services: Source: www.ws2.co.za

1.2.1.2. Domestication and husbandry

Nowadays, common eland are recommended for domestication by FAO (Scherf 2000) but the most referenced efforts toward the domestication of common eland is that of the Askanya Nova in Ukraine, where elands have been successfully bred since 1892 (Treus & Lobanov 1971). In East and South Africa, there were several attempts to domesticate common eland in first half of 20th century (Carles et al. 1981). The initial focus of research into taming of wild bovids in national parks fell on cross-breeding and domestication, with the eland being one of the first species under investigation (Mossman & Mossman 1976). However, there has been many challenges with the domestication of wild bovid due to less substantive knowledge of their behaviour, reproduction physiology and nutritional requirement, and their game meat products. Consumers have also become increasingly interested in the ethical, disease status and environmental benefits concerning game farming (Hoffman & Wiklund 2006).

Over the recent years, there has been an increase in interest into the farming of game animals for meat production e.g. common eland. The advantages quoted are the large size of the animal, the palatability of the meat, its docility in captivity and it being an intermediate feeder (Codron et al. 2007). Eland were reported to be handled like cattle in a pen (Bothma 1996); however, due to its flighty nature and extraordinary jumping ability, further modifications to typical cattle handling are required. Such modification may be seen at the Czech University of Life Science's Research Farm, were the initial facilities were meant to house cattle, but after some adjustments it has sustained the farming of common eland since 2006. Considering the increasing interest and knowledge in the breeding of eland, the future of eland farming seems positive. The first practical experiences about farm breeding of common eland in the Czech Republic were reported by Hrouz (1995). The fact that eland need a larger area to meet their feeding habits only apply to eland that are receiving no supplementary feeding; therefore, eland can be kept in the same farm size as cattle, given they receive adequate feed (Barton et al. 2014).

Much effort and studies are ongoing on the reproductive technology of common eland, in oestrous detection, oestrous synchronization, embryo transfer, semen collection, artificial insemination. These studies are motivated due to the large body size of common eland and the ease with which their reproductive organs can be manipulated (Pennington 2009). In the studies of Dresser et al. (1985), the common eland was used as a surrogate mother to bongo, which signifies a great potential of species-rescue for the tragelaphini tribe.

1.2.1.3. Behaviour, social and agonistic interaction

Common eland are social animals that live in herds of 25 to 60 animals, but groups up to 500 individuals have been reported when the environment is favourable (Estes 1991). Their social groups have been described as having a fluid structure and apparently no stable long-term relationship (Groves et al. 2011). Relatively long-term relationship exit between cows and their calves and between calves in nursery. As calves grow beyond two years, they usually divide into natural groups based on age, sex and affiliation (Hillman 1987). Larger grouping sizes are usually triggered by the females when they are in oestrous (Pappas 2002). However, males stilll spend much of their time in multi-male groups; even in the presence of oestrous females. Males may stay in the group or move individually, while the females and juveniles stay very close. Many antelope exhibit territorial behaviour, but eland do not (Underwood 1981). Eland exhibit a social organization much similar to that of the Tragelaphini, but this is modified by its large body mass.

Females usually calve when there is abundant food and the calf is hidden for a period of two weeks, after which it is introduced into the herd. Calves exhibit a phenomenon called allo-suckling were calves of other cows suckle from another cow that is not their mother, this behaviour can affect the chance of survival of the true calf from that particular cow (Bartoš et al. 2001). Aggregation between nursing females is often motivated by their calves. Calves often stay close exhibiting much of affiliative behaviours such as reciprocal grooming, playing and play-fighting (Kiley-Worthington 1978).

The social and agonistic interactions of common eland have been detailed and extensively studied by Kiley-Worthington (1978) in a herd of common eland at the Pretoria Zoo, South Africa. The study affirms that olfactory cues, visual display, and auditory signals are the observed means of communication in common eland. These communications can be seen as postural, protective, orientation movements, and movements related to cutaneous irritation. However, for a communication to be ensured, there must be cause and effect of such behaviours. Generally, these communication displays in common eland, as in most ungulates, can be translated into agonistic, affiliative or submissive behaviour.

In postural movements, Kiley-Worthington (1978) observed that an increase in the postural tonus, that is increase in the elevation of the head and the tail in response to stimuli (Figure 4), 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. This can be seen in Figure 4 below, showing a gradual change in posture and increase in excitement from H to A.

Figure 4. Changes in postural tonus with increase in excitement (Kiley-Worthington 1977).

Protective movements are movements relating to threat and protection of the animal, either from conspecific species or predators. The animal is always alert and uses all sense-organs to coordinate the horn towards the threat. These include head lowering, horn pointing, horn clashing, and wrestling, in order of increasing intensity. Elands develop horns within a few weeks of birth in both sexes and as the horns develop, there is a distinct sexual dimorphism in view of their functions. The horns of male eland, along with the help of the strong muscular neck, are used for fighting and wrestling to establish dominance in rut, or against other species. According to Pappas (2002), the spiralled horn and the thick muscular neck both appears to help avoid fight injuries. Female eland have longer and thinner horns which are ideal for delivering quick stabs, mostly in defence against predators. Unlike other ungulates, ritualization in the fight seems to be uncommon. The horns in eland are also used in grooming, scratching and aiding in browsing of shrubs and trees (Kiley-Worthington 1978).

Orientation movement in common eland relates to visual and olfactory signals in responses to stimuli. Such movements includes sniffing, movement of the mouth, flehmen, lip-licking and movements of the head and eyes in a particular direction. Such display helps other animals to locate potential sources of danger, but oestrous females may also exhibit head turning towards the flank directed at the male approaching the rear before mating. Eland also perform flehmen, usually in response to pheromones released from the other animal urination. Both sexes perform flehmen in response to urination from either sex or even themselves, but much of the feedback is usually towards a urinating female, during which the male extends its head and nose toward the urine or genital of the female and sniffs to detect if she is in heat. Thus, this action can trigger competition between males of which the dominant finally succeeds in mating if the female is in true heat otherwise abstained (Kiley-Worthington 1978).

Cutaneous irritation movements are related to social affiliative behaviour such as grooming, pawing, head rubbing, ear flicking, tail wagging in calves while suckling, head shaking and tossing.

1.2.2. Dominance and aggressive behaviour in ungulates

The study of dominance and social relationships in animals has been a topic of interest for ethologists since 1920s (Drews 1993). Animals such as eland have evolutionarily developed visual and sensory cues and mechanisms for lateral exposure preference to take note of its surroundings in order to avert predators and threat from conspecifics (Bordes et al. 2018). Social dominance within a group of social animals is seen as a way to facilitate access to food resources, especially during food scarcity (Appleby 1980). Thus, great research interest has been focused on the importance of social rank to competition for mating, as well as larger or better food resources under natural and captive conditions. However, because the observation of aggressive behaviour is difficult under natural conditions, much research on ungulates has depended on observations of animals at baiting sites or in captivity (Clutton-Brock et al. 1979; Cassinello 1995). Factors which seem to influence or necessitate the establishment of dominance in ungulates include resources such as food (Ceacero et al. 2012), mates and breeding opportunity (Šarova et al. 2017), and territory (Richard et al. 2014). For an individual to achieve dominance they engage in aggressive behaviour and their individual success depends on age, sex, space, time spent in a herd, size and nature of “weaponry” such as horns and antlers, body mass and, matrilineal genealogy (Cote 2000, Horová et al. 2015).

Winners and losers in an aggressive context are often easily identified, and in such dyadic interactions the winner is referred to as “dominant” while the loser is referred to as “subordinate”. Based on who wins against whom, all or most individuals can be ranked in a dominance hierarchy (Bang et al. 2010). Many dominance hierarchies observed in nature have been found to be completely or nearly linear (Chase et al. 2002; Chase & Seitz 2011). In this context, linearity means that the top-ranking individual dominates all other individuals, the one with second-highest rank dominates all individuals besides the top ranker and so on, with the lowest-ranking individual (i.e. subordinate to) being dominated by all others (Schmid & deVries 2013). Another means of analysing the linearity of a group structure is the triangle of transitivity. This follows the rule stating that when individual X dominates individual Y, and Y dominates Z, then X dominates Z (Wirtu et al. 2004; Shizuka & McDonald 2012). The linearity and triangle transitivity are fundamentally equivalent when dominance relations of all dyads are known. However, they differ in that the triangle of transitivity is based in the dominance relationships among sets of three players (triads) that all interact with each other (Shizuka & McDonald 2012).

Aggression serves a great variety of social functions, and it is hard to find any part of the social life of bovids where aggression is not involved (Walther 1984; Rajagopal et al. 2010). Also, aggression has shown to be a powerful mechanism for intra-sexual and natural selection amongst males, leading to sexual dimorphism in the size and shape of the horns and body proportions (Lundrigan 1996; Perez-Barberia et al. 2002; Bro-Jorgensen 2007; Wronski et al. 2010). In the study of Lincoln et al. (1982), it was found that weaponry plays a vital role in determining the rank in a social group of red deer. Stags who were castrated prematurely lost their antlers and subsequently demoted to the lowest level of the hierarchy and they never regained their position after the rut season.

In ungulates, competitive interactions can be divided into those in which opponents aggressively engage with each other, but no physical contact is involved (non-contact dominant interactions) and fighting that usually involves contestants butting and locking horns or antlers and engaging in a serious pushing contest (Jennings & Gammell 2013; Blanka & Yanga 2014). Common types of non-contact interactions include vocalisation contests (Clutton- Brock & Albon 1979), displacement interactions, horn or antler displays (Alvarez 1993; Jennings et al. 2002) and parallel walks, were animals display their dominance through increase in girth and showing their muscularity (Clutton-Brock & Albon 1979; Jennings et al. 2003). Similar to non-contact interactions, fights also contain a variety of distinct actions such as the jump clash, charge, slam, butt and push (Clutton-Brock & Albon 1979; Estes 1991; Alvarez 1993). It is generally known that males communicate their strength, dominance or aggressiveness via horn or antler displays.

1.2.3. Castration and behavioural management in ungulates

Castration has long been practised traditionally in domesticated animals, principally aimed at reducing or ameliorating aggressive behaviour in livestock and companion animals (Thüer et al. 2007). This technique is also particularly helpful to prevent unwanted or indescriminate breeding in farms which do not have subunits to separate different sexes. Mostly, castration on-farm has been practiced in goats (Rajkumar et al. 2017), sheep (Cloete et al. 2012), cattle (Fisher et al. 2001) and pigs (Needham & Hoffman 2015). With the increasing interest in game meat, taming and domestication of wild ungulates, castration may aid in ensuring the accomplishment of various objectives relating to animal performance and management. The end result of all castration process is to prevent testosterone production and reproductive functioning, and various methods have been developed to achieve it (Oliveira et al. 2016).

1.2.3.1. Basic methods of castration

Castration in animals can basically be achieved by physical, chemical or immunological method (Stafford & Mellor 2005; Oliveira et al. 2016). Physical castration is done by either the use of Burdizzo clamps (closed-crushing), surgery (orchiectomy) or application of elastic rings to obstruct the supply of blood to the testicle (Bretschneider 2005; Dnekeshev & Kereyev 2013; Cloete et al. 2012). Chemical castration can be achieved through intratesticular injection of chemical compounds that causes the destruction of testicular cells. Thus, caustic or osmotic substances, such as lactic acid (Fordyce et al. 1989), $CaCl_2$ (Matins et al. 2011) and NaCl (Andrade et al. 2014) are used in chemical castration. Immunocastration is a minimally invasive approach of castrating animal which uses a vaccine to stimulate the immune system to produce anti-GnRH antibodies, thus suppressing androgenic hormone production (Amatayakul-Chantler et al. 2013). According to Thompson (2000), while immunocastration in some instances can be reversed, physical castration is permanent if performed correctly.

Looking at physical castration, the gonads can be removed surgically from the scrotum or by forcing a rubber ring around the neck of the scrotum (ring/band castration). The use of rubber ring results in ischaemia and sloughing of the testicles and scrotum due to necrosis (Winter 1996). In another way, a segment of scrotal tissues, blood vessel and nerves can be destroyed using the Burdizzo clamp. However, just as in immunocastration, the testicles in budizzor castration remains intact. Consumers, particularly in developed countries, have raised concern on the invasive approaches of physical castration as they are often performed without pain mitigation. Thus, this prompted the use of sedatives and pain-relieving drugs (Melches et al. 2007). However, this result to another cost implication and not all farmers are willing to comply with regulations. Furthermore, such drugs are somewhat ineffective in a large-scale commercial enterprise and not food-safe.

In the study of (Melches et al. 2007), there was body weight lost and decreased feed intake of male sheep surgically castrated under sedation with local anaesthesia due to immediate and frequent pain responses when compared to the burdizzo technique under the same anaesthetic treatment. Burdizzo castration resulted in a quick response to pain (acute) when compared to band castration, but recovery occurred faster and with less challenges in Burdizzo castration than band castration. Generally, band and surgical castration result in a compromised recovery and the animal suffers acute or chronic pain from ischemia, infection, and abscesses (Needham et al. 2017).

Over the years, farmers have traditionally become acquainted with these different castration techniques and regulations in certain countries mandate a specific time period in the animal’s life in which some castration techniques should be used.

However, some regulations do not stipulate categorically when pain mitigation should be applied. The welfare codes under Mutilations Regulations (Permitted procedures, England 2007) dictates that, ram-lambs, buck-kids and calves in the United Kingdom must be castrated before seven days of age using rubber rings. This regulation also dictates the use of anaesthetic when these animals attain 12 weeks of age and above, or when other techniques are to be used. Australia, New Zealand and Canada, as well as most developed countries, also have similar regulations, but with virtually no formal enforcement in Africa currently (Needham et al. 2017). There are no strictly defined regulations in the castration of wild ungulates; however, the AMVA (2012) promotes the reduction/elimination of pain during routine management practices such as dehorning, tail docking and castration.

Immunocastration has been most effective in ensuring a win-win situation as reported by most studies (Needham et al. 2017). Thus, this implies ensuring safety and welfare and at the same time reaping high productivity of animals (Price et al. 2003). The basic mechanism of immunocastration lies on blocking the action of gonadotropin-releasing hormone (GnRH). Gonadotropin-releasing hormone is conveyed to the anterior pituitary by the hypophysial portal systems when produced. In these vessels the GnRH are most liable and prone to attack by antibodies. Thus, in the presence of much antibodies specific to GnRH, GnRH would bind to the antibodies which eventually neutralizes their action (Figure 5). This is achieved by preventing the diffusion through capillary or masking the binding site on GnRH which ultimately prevent it from binding to the anterior pituitary (Thompson 2000).

The immunocastration vaccine called Vaxstrate (Peptide Technology, Ltd, NSW, Australia) was the first available vaccine against GnRH approved in Australia, for the suppression of oestrus in heifers (Hoskinson et al. 1990). However, Vaxstrate was abandoned in 1996 due to severe adverse reactions and poor efficacy in the field. The second vaccine developed was called Improvac® (Zoetis™). It was specifically introduced for the immunocastration of male pigs, to prevent boar taint. It then served as an avenue for the development of Equity® (Zoetis™) which was used in horses against GnRH. For cattle, Bopriva® (Pfizer, Animal Health) was design in 2007 and has proven to be effective and safe in numerous studies in bulls (Theubet et al. 2010).

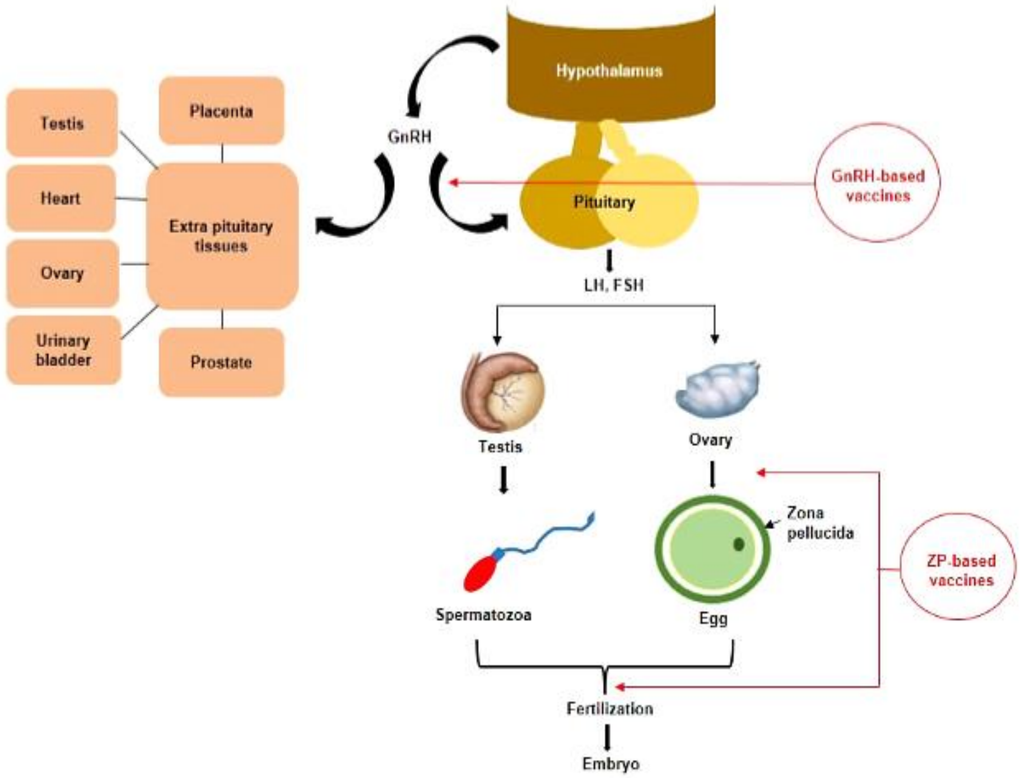

Figure 5. Brief schematic representation of the processes targeted by the anti-GnRH vaccine (immunocastration). Antibodies against GnRH will inhibit production of LH and FSH from anterior pituitary thereby leading to inhibition of spermatogenesis and androgen production in males (Gupta & Minhas 2017).

These vaccines have similar immunological properties but differ in the adjuvant used, which implies that either of these products can be used in other animal species as well (Curtis et al. 2002; Killian et al. 2009; Needham et al. 2018).

Unlike in surgical castration, where equipment which can also injure the operator in the process of struggling with the animal or requires additional technique training, immunocastration employs the simple use of injection needle and syringe. Needham et al. (2018) validated the use of Sterimatic® and Stericap® system which shown to be friendly, feasible and safe system for the commercial administration of Improvac® and with no adverse reactions to vaccinations at the injection sites. Thus, immunocastration is simple to perform and prevents the pain associated with physical castration techniques.

1.2.3.2. Immunocastration and its application

Much success has been achieved with the use of immunization against GnRH from a welfare point of view, as immunocastration has been used in reducing pain and stress compared to physical castration (Needham et al. 2017). Aside from the stress experienced by physically castration, the animals are also prone to infection and myiasis (Muniz et al. 1995). This leads to morbidity and even mortality of physically castrated animals, thereby posing more challenges to the farmer by increasing the unit cost of his production. Unlike physical castration, the immunocastration procedure can be carried out without the presence of a veterinarian, who is required to administer sedation or anaesthesia for surgical procedures (Needham et al. 2017). Therefore, much of the problems related to physical castration have been circumvented with immunocastration. Producers can also wait until later in the growth of the animal to handle them, potentially integrating the vaccination schedule with other routine activities such as vaccination and deworming.

The control of aggressive behaviour is a big challenge to farmers because aggression not only exacerbates dyadic agnostic interaction between animals, but it often results in destruction of farm infrastructure. Immunocastration has been effective in the control of aggressive behaviour, thereby decreases fighting-related injuries, carcass bruising and quality issues of the hides in male animals (Godfrey et al. 1996; Dunshea et al. 2001). In the studies of Bell et al. (1997), immunocastration was found to be effective in heifers and cows in the control of unwanted pregnancies and suppression of oestrus cycle. This study also reported a decreased in agonistic behaviour of immunocastrated females, thus preventing injuries and improving their welfare. In bulls, immunocastration has been successful in decreasing physical activity, aggressive and sexual behaviour (Huxsoll et al. 1998; Janett et al. 2012). According to Amatayakul-Chantler et al. (2013), the administration of Bopriva® was more effective in decreasing testosterone levels within 14 days after the booster, thereby controlling aggression and sexual behaviour. Immunocastration has also been effective in population control in red tail deer (Rutberg et al. 2013) and could possibly be used in game reserves and zoos to aid in stabilising and maintaining population control, particular where euthanasia is not permitted.

In some mammals, androgenic hormones trigger the production or release of obnoxious compounds (e.g. androstenone in boars); these compounds often serve as pheromone in attracting mates or in territorial marking, as in some wild ungulates. Unfortunately, these pheromones often affect the sensory quality of the meat. Boar taint and buck odour are the two main challenges in the meat industry that have been successfully tackled through immunocastration in research studies (Claus et al. 2007; Godfrey et al. 1996). Ülker et al. (2009) Investigated the effects of a recombinant ovalbumin GnRH vaccine in goat bucks and found it to significantly decrease odour score associated with buck meat. In a similar study, Bonneau et al. (1994) also found that fat androstenone (major cause of boar taint) concentrations were reduced to approximately 210 ng/g which was much below 500 ng/g for human sensory detection (Bonneau et al. 1992). Immunocastration and castration in general, results in increased fat deposition due to decrease in testosterone production. According to Needham et al. (2017), It is important to consider vaccination timing with regards to slaughter in order to achieve the desired fat deposition on the carcass, which has a large influence on profitability of a carcass.

1.2.3.3. Influence of physical castration and immunocastration on behaviour

One of the basic objectives of castration is to control and reduce the level of aggressive and sexual behaviours in animals. Physical castration often leads to intermittent and aggravated level of cortisol which is related to stress from incessant pain and these animals display pain-behaviours (Earley & Crowe 2002; Sutherland et al. 2013). With physical castration, Lincoln et al. (1972) found a decrease in aggressive behaviour in European red deer, and stags that were castrated at the peak of rut suddenly shed their antlers, which affected their dominance and the rank position of the treated animal. These treated animals could not regain their position in the hierarchy even after the rut season where other individuals had return to velvet.

In the study of Fisher et al. (2001) on 14-month and 9-month bulls investigating the effects of surgical or banding castration on stress responses and behaviour of bulls, no differences were observed in the activity pattern (lying, ruminating, grazing and walking) between the treatment group and the control non-castrated group. However, bulls that were surgical castrated exhibited more leg-stamping and tail-swishing than band-castrated or intact animals in the hours immediately after castration. However, the band castrated group had a high amount of cortisol, signifying that they were also more stressed than the control group. Comparing surgical castration to immunocastration, Price et al. (2003) observed that active immunization against GnRH reduces the incidence of aggressive behaviour in male beef cattle in relation to intact bulls, but surgical castrates were least aggressive.

Agonistic behaviour related to seasonal breeding in immunised goat bucks was found to be intermediate between surgically castrates and entire bucks (Godfrey et al. 1996), while sexual behaviour was completely reduced. In a study of Jago et al. (1997), immunization against GnRH of Friesian bulls at 2.5, 4, or 7.5 months of age only resulted in a delay of sexual and social behaviours, and thus it was concluded that there is no practical reason to immunize before 7.5 months. Huxsoll et al. (1998), immunocastrated beef bulls at 1, 4, or 6 months of age, and finally gave a single booster at 12 months of age, the bulls were slaughtered at 16.4 months of age. Feedlot gain in GnRH-immunized bulls from this study were similar to control bulls, whereas aggressive behaviour was reduced. This is a positive for the Brazilian beef industry, because they tends to practise late castration at 18 to 24 months of age to take advantages of male steroid hormones, the associated growth and feed efficiency as much as possible (Needham et al. 2017).

In summary, agonistic and aggressive behaviour in common eland and related antelope needs to be studied for successful and effective intensive management, handling and breeding. In light of the negative effects and perception of physical castration, as well as the cost of pain-mitigation, immunocastration may pose a better management strategy for male common eland raised in captivity for meat production. Immunocastration of common eland may thus ease management by preventing the destruction of farm structure and implements, easing handling, ensuring the safety of young animals and heifers, and controlling indiscriminate breeding by preventing unwanted traits to breed through in the herd.

2. Aims of the Thesis

There is a continuous increase in concern towards the welfare of domestic animals and the use of castration in reducing the aggressive behaviour for good management and improving meat quality. However, immunocastration has proven to be an alternative to physical castration but with little study of its effect on the behaviour of game animals, despite an increase in preference for game meat. On this background, this study aimed at evaluating:

1. The effect of immunocastration on the social interaction of common eland.
2. The effect of immunocastration on the activity budget of common eland.

2.1. Research questions:

- Will immunocastration influence the social rank of immunocastrated male common eland?
- Will immunocastration influence the aggressive behaviour of immunocastrated male common eland?
- Will immunocastration influence the affiliative behaviours of immunocastrated male common eland?
- Will immunocastration influence the activity Budget of immunocastrated male common eland?

2.2. Hypotheses:

H_0 Immunocastration will not influence the social rank of male common eland.

H_0 Immunocastration will not influence the aggressive behaviour of male common eland.

H_0 Immunocastration will not influence the affiliative behaviour of male common eland.

H_0 Immunocastration wil not influence the activity budget of male common eland.

3. Methods

3.1. Description of experimental site

The study was conducted at the Czech University of Life Sciences Eland farm located at Lány (50°7'41.704"N, 13°57'31.370"E) in the Central Bohemia region, Czech Republic. According to the Köppen–Geiger classification, which was updated by Rubel and Kottek (2010), the study area has a temperate cold climate characterised as fully humid with a cool summer. This area is located at an altitude of 421m above sea level. The eland farm consists of a barn (230m^2) with deep litter straw bedding. It is divided into two by a central corridor with a feeding alley (Figure 6). Animals are separated into either the left or right part of the barn using galvanised fencing. The experimental animals were kept on the left side of the barn, which was divided into two groups (Figure 6) of either sub-adults (group 1) and calves (group 2). Trained personnel on eland management was routinely present to feed and control the animals.

3.2. Animals, husbandry and experimental design

The study was approved by the Institutional Animal Care and Use Committee (Czech University of Life Sciences Prague). The study was conducted from November 2018 to March 2019, during winter. In the course of this study, animals were completely housed in the barn and the internal temperature of the barn ranged from 2 to 10 °C. Thirty common eland of two age groups were used for the study. The mean (± SD) weight of the two groups at the start of the trial were 94.18 ± 24.76 kg (n=15) and 182.9 ± 59.37 kg (n=15) and for the calves (≈6 months old) and sub-adults (≈1-2 years old) respectively. Each group consisted of males (n=10) and females (n=5). A detail description of the groups is presented in Appendix l. The animals were fed a regular basal diet consisting of 60% corn silage, 30% lucerne haylage, 7% meadow hay and 3% barley straw this was provided *ad libitum*. They also received supplementation of concentrate feed (19 % crude protein) twice daily. The concentrate diet consisted of wheat, barley, soybean meal, minerals and vitamins. Each pen had an automated drinker and animals had access to water *ad libitum*. One male calf died during the first week of the study due to reasons unrelated to the trial and has thus been omitted from data from this point.

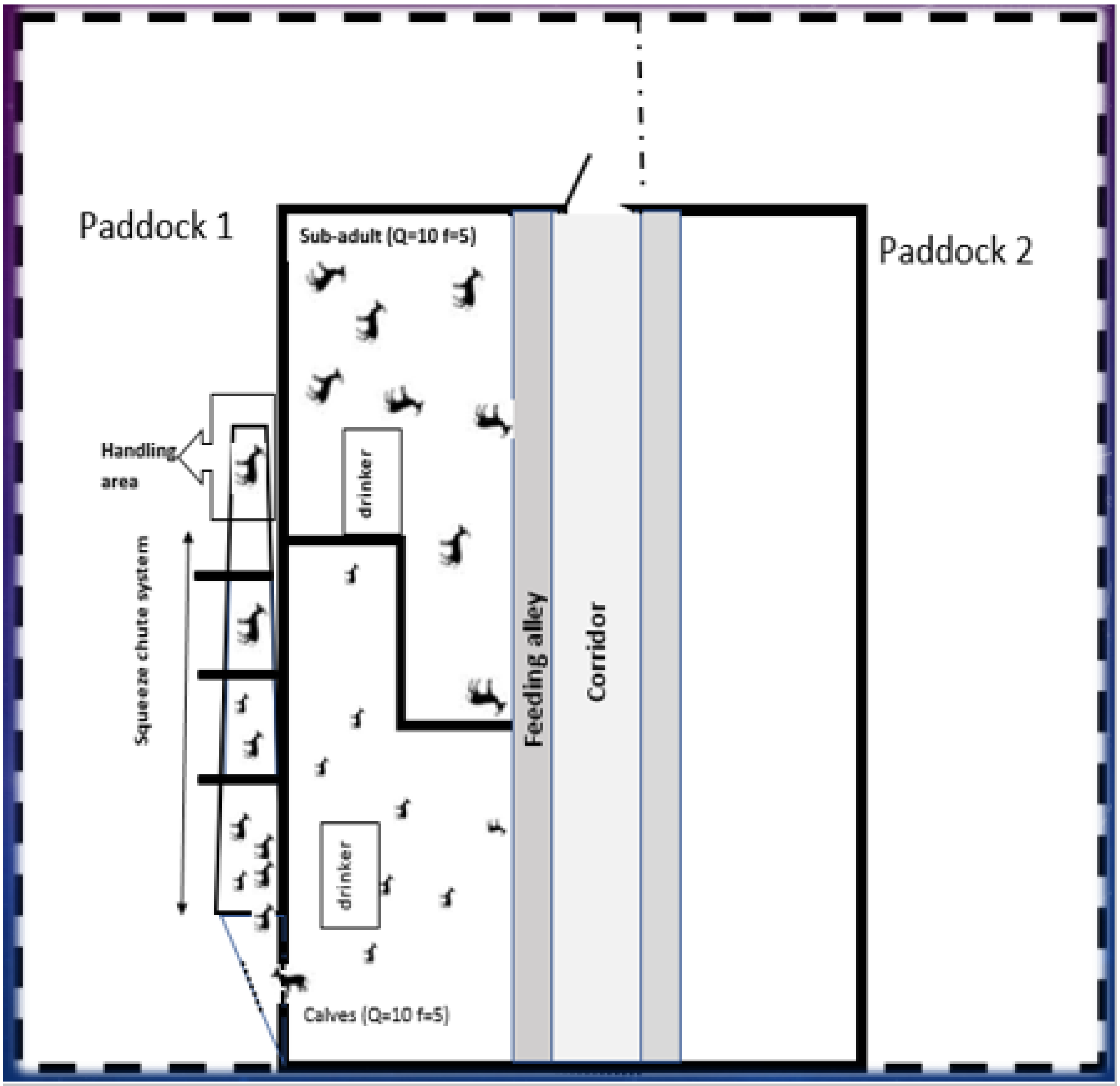

Figure 6. A brief schema showing the design of the experimental farm with two pens in which the experimental animals were kept in two group (sub-adult and calves) in the left part of the barn.

3.3. Handling, identification and weighing of experimental animals

For ease of handling, examination and sample collection from the animals, a squeeze chute system was used. A picture of this this chute system is presented in Appendix IV. One outlet of the barn is directly linked to the chute system. At the onset of every handling, the calf group were passed individually through the alley of the chute, which is further divided by three movable sliding doors/dividers into four corridors (**I, II, III, IV**). Animals are individually separated in each corridor before entering the weighing corridor/box and finally the squeeze of the chute, where they were restrained. A digital Gallagher weighing system (TW-1 Weight Scale G02601, Gallagher, Hamilton 3240, New

Zealand) was used to weigh the animals in corridor III. All animals were initially ear tagged at birth, but at the onset of the study, the animals were further ear tagged with a digital chip (Appendix **II**) which is identified by the Gallagher weighing system. To weigh an animal, it was gradually lured out of the barn with concentrate feed into corridor **I**, **II** and finally restrained in corridor **III** in which the Gallagher load bars are fixed at the base of a platform. Initial weight was used to stratify the animals and randomly allocate them to treatments.

Figure 7. Complete view of the squeeze chute system used to handle the eland at the Czech University of Life Science's Research Farm, Lány.

3.4. Administration of immunocastration vaccine

Immunocastration of the experimental animals against GnRH was done using the anti-GnRH vaccine called Improvac® (Reg. no. G3643, Act 36/1947; Zoetis Animal Health). 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. Male calves (n=5) and sub-adult (n=5) were randomly selected from the two age groups and administered with the vaccine at a dose of 2ml/animal/dose subcutaneously on the shoulder area, using a Sterimatic® needle guard system fitted with a Stericap® (Figure 8 and 9). This needle guard system was also validated as effective for Dohne Merino ram lambs by Needham et al. (2019). At the squeeze, the eyes of the immunocastrated animal were covered using a non-transparent garment, the head was also held firmly by an assistant (Appendix ll, figure 9). Experimental animals receiving the vaccine were vaccinated at day 14 and day 42, after the commencement of the study.

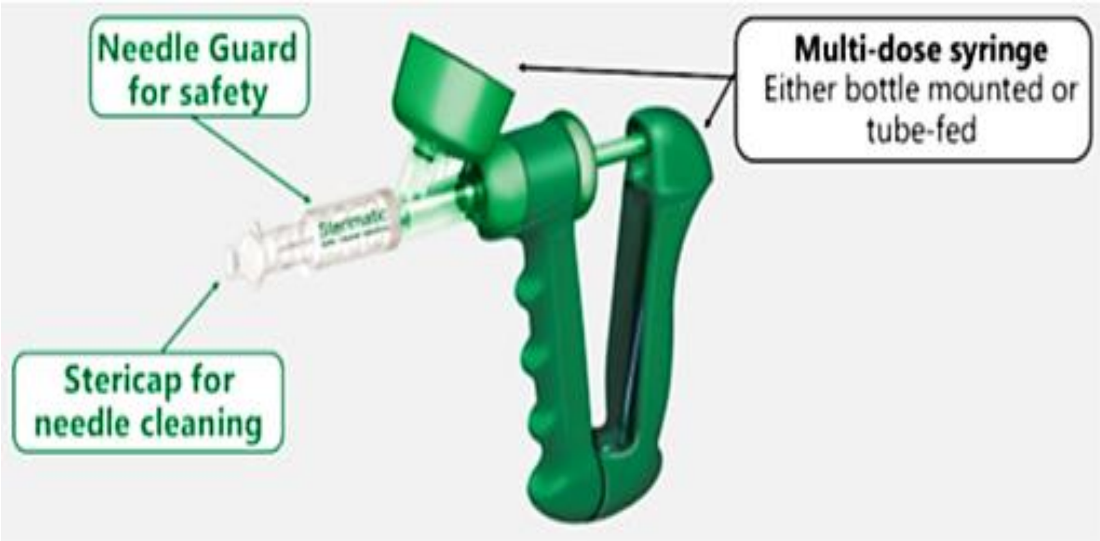

Figure 8. Illustration of the Sterimatic® safety needle guard system fitted with a Stericap® and mounted to a multi-dose vaccinator (http://www.fwi.co.uk/advertisement/sterimatic-how-to-guide-for-injecting-cattle-and-sheep.htm)

3.5. Behavioural evaluation and data collection within the barn

Behavioural observations were grouped into social interactions and activity budget behaviours. In accordance with Altman (1974), "all occurrences sampling" was adopted for recording the social interaction behaviours, while "scan sampling" was used for the activity budget. Animals were identified using ear tags and body markings, with binoculars (Canon 10X30 IS) used when needed. The observer was properly trained prior to the commencement of the study on the correct identification of interactions. Animals were observed from a distance in such a way that the presence of the observer did not confound or influence the behaviour of the experimental animals. In each day of the observation, feeding and management of the animals were done before the commencement of the observation and the animals were allowed time to settle after the entry of the observer. Behavioural observation was done for two days starting the day after the animals were handled for samples collection. A total of seven hours (9:00-16:00 hours) of instantaneous behavioural observations were performed each day. The activity budget observation periods were alternated between the groups (sub-adult and calves) and five observation sessions were covered each day. A total of 10 observations per group were covered each week. Thus, 90 sessions (45 sessions for calves and 45 sessions for sub-adults) which is equivalent to 126 hours for the whole study.

Figure 9. Administration of Improvac® at the shoulder area using a Sterimatic® needle guard system fitted with a Stericap®

Recordings of the social interactions were based on dyadic dominance/submission displays of behaviour. In this section, the methodologies by Ceacero et al. (2012), Vymyslická et al. (2015) and Horová et al. (2015) were modified and used.

Table 1. Ethogram of the social interactions and behaviours recorded according to three categories, affiliative (grooming, flehmen, mounting), dominance (threatning, passing, wrestling, yielding, pushing, yielding) and aggressive (wresling, pushing, yielding).

Categories	Behaviours	Description
Affiliative	Grooming	Grooming of individual by another or of each other
	Flehmen	Lip curl in response to urination or pheromone by another
	Mounting	Being mounted by another individual of either sex
Dominance	Threat	Threatening with head or horn on approaching
	Passing	Sudden alertness/moving away when another animal is passing
Dominance + Aggressive	Wrestling	Locking/clashing of horn with much force and alacrity
	Pushing	Pushing from behind/beside
	Yielding	Heating/butting of another

The behaviours recorded were grouped into two categories (I) Dominant and (II) Affiliative. Dominance behaviour was further disclosed into aggressive and not aggressive dominant interactions: not aggressive dominant interactions included those displays without contact, like threatening and passing. Aggressive dominant interactions included those when there was a direct contact between individuals in a dominance display, like pushing, yielding and wrestling.

Table 2. Ethogram describing the activity budget behaviour recorded while examing the effects of immunocatration on the activities of the two different age groups of common eland, compared to non-immunocastrates.

Behaviour	Description
Eating	When an animal is eating from the feeding alley
Walking	Walking from one point to another within the pen
Standing	Standing with or without rumination, head up/down
Lying	Lying with or without rumination, on sternum/lateral
Drinking	Drinking of water from the automated drinker
Playing	Playing with another or stereotypic playing with material
Wrestling	Locking horn and fighting
Grooming	Grooming of self or each other

Grooming, mounting and flehmen were included as affiliative behaviour. Table 1 and 2 gives a detailed description of the behavioural ethograms of the social interactions evaluated.

3.6. Data processing and statistical analyses

3.6.1. Data processing

A dominance matrix was created to determine whether a hierarchy existed in either of the groups. These matrices originated from loss and win tables, which were filled in during observations (Schmid & de Vries 2013). For each agonistic encounter, the interaction was viewed till the end; the winner and loser were recorded, the dyadic interactions in the win and loss tables were analysed using DomiCalc (matrix manipulation and analysis software; Schmid & de Vries 2013). The linearity of the studied groups was analysed by two different methods. The first method was the Landau's (1951) linearity index modified by de Vries (1995) (h') which allows to test whether the assumption of the linearity (h') is statistically significant. The value of h' varies from 0, indicating the absence of linearity, to 1, indicating complete linearity (Cafazzo et al. 2010). However, this index does not fully account for the unknown dyadic interactions as does the triangular transitivity (t_{tri}). The triangle transitivity is based in the dominance relationships among sets of three players (triads) that all interact with each other and is more effective in taking the null or unknown dyadic interactions into consideration (Shizuka & McDonald 2012). The triangle transitivity (t_{tri}) ranges from 0 to 1, although the interpretation is more complex. Still, the method also provide an associated p-value. Both analyses were performed using the software DomiCal (Schmid & de Vries, 2013). Linearity tests (h') were carried out with 10000 randomizations and the triangle transitivity (t_{tri}) tests with 1000 randomizations. The social rank order obtained from I&SI (inconsistency and strength of inconsistency) was used. This method provides a hierarchical order that minimises the number of inconsistencies and simultaneously minimise the strength of these inconsistencies. The formula 1–rank/n+1 where "n" represents the number of individuals was used for transforming the I&SI linear rank obtained for each groups into a social rank value ranging between 0 to 1 (0 < SR <1); closer to 0 and closer to 1 represents the most submissive and dominant, respectively. The social rank values were finally transformed through arcsine of the square root to fit a normal distribution.

The occurrence of interactions (Oi), dominant interactions started (DS) and received (DR), dominant–not aggressive started (DNAgS) and received (DNAgR), dominant aggressive started (DAgS) and received (DAgR), aggression started (AgS) and received (AgR), not-aggressive started (NAgS) and received (NAgR), affiliative started (AS) and received (AR) were also computed. Thereafter, rates (occurrence per hour) of these events were calculated (e.g. Oi/h, DS/h, DR/h, DNAgS/h). Finally, the proportions between the different types of interactions to the occurrence of interactions (DS/Oi, DR/Oi, DNAgS/Oi, NAgS/Oi), the proportion of aggression started to dominance started (AgS/DS) and affiliation started to total affiliation [AS/(AS+AR)] were also calculated. These calculations were used to examine variations in the social behaviour at individual's level.

The activity budget included eating (ET), drinking (DK), lying (LY), standing (ST), walking (WK), wrestling (WR), playing (PL) and grooming (GM), and was calculated for each individual and each sampling period, and expressed as percent of the total observations for that period. Due to the low frequency of occurrence of PL, WR and GM, they were collectively grouped as social behaviours.

3.6.2. Statistical analysis

All analyses were performed in IBM© SPSS© Statistics (version 25.0 for Windows; IBM, USA). Generalized Linear Mixed Models (GLMM) were designed to determine the effects of the immunocastration treatment on the various behaviours of the animals in the study. Group (age) and animal ID were allocated as subjects and Trial (week of study) as a repeated measure within the model. The behavioural traits previously described for activity budget and rates/ratios of social activities were the target variables in the models. *Body mass* at every given trial/week, *Age* at the start of the experiment, *ADG* for the two-week period between trials, and *Trial* were planned as fixed factors and *Group* as random. The interactions "Treatment*Trial" and "Treatment*Weight" were also included in the models since they were considered to potentially to have an influence in the behaviour of the animals. Histograms were built for initial inspection of the variables involved in the models, and normality tests were conducted (Kolmogorov-Smirnov). While *Body mass*, *Age*, *Social rank* and *ADG* were normally distributed, most of the behavioural variables recorded were not, and thus they were transformed using either an identity link function or into a gamma distribution with a log link function. Finally, the independent variables selected to enter in the models (*Body mass*, *Age*, and *ADG*) were tested for multicollinearity. The Variance Inflation Factor was high for *ADG* (VIF=4.951) and thus this variable was excluded from the models. The final model for each behavioural variable studied was selected after a traditional stepwise backward selection procedure. The threshold for significance was always considered as $P<0.05$.

4. Results

A total of 3351 dyadic interactions (sub-adults=1884, calves=1467) were observed. The studied groups showed a linear and transitive hierarchy throughout the study period (Table 3). Only the hierarchy for the group of calves was not linear during the first trial/week (the week prior to immunocastration).

Table 3. Strength of the linearity and triangle transitivity of the social hierarchies calculated for the two groups of captive elands studied. Significant results confirm the existence of a linear hierarchy.

Trial	***h'***				***t_{tri}***			
	SA group	**p-value**	**C group**	**p**	**SA group**	**p-value**	**C group**	**p-value**
Pre	0.649	<0.001	0.314	0.125	0.826	<0.001	0.556	<0.001
1	0.631	<0.001	0.507	0.003	0.922	<0.001	0.691	<0.001
2	0.548	<0.001	0.416	0.028	0.898	<0.001	0.648	<0.001
3	0.459	<0.001	0.499	0.002	0.912	<0.001	0.679	<0.001
4	0.516	<0.001	0.532	0.002	0.883	<0.001	0.792	<0.001
5	0.637	<0.001	0.423	0.015	0.958	<0.001	0.522	<0.001
6	0.629	<0.001	0.379	0.046	0.916	<0.001	0.643	<0.001
7	0.669	<0.001	0.418	0.023	0.753	<0.001	0.654	<0.001
8	0.509	<0.001	0.462	0.006	0.778	<0.001	0.577	<0.001

h' – Strength of linearity (after Schmid and de Vries 2013).

t_{tri} – Triangle transitivity (after Shizuka and McDonald 2012).

P <0.05

The social rank (Table 4) was positively affected by the body mass (t=5.254) and age (t=3.242). Immunocastrated animals had higher social rankings than the controls (t=2.066, Figure 10), while the interaction Treatment*Body mass was negative for the immunocastrated animals (t=-2.318, Figure 11).

Table 4. Effects of immunocastration, as well as other experimental and individual factors, on the social rank and the rate of display of social interactions (started and received) by calves and sub-adult male elands (n=18).

Target variable	Function	Significant fixed effects	
Social rank	Linear	Body mass	$F_{1,148}$=48.017; P<0.001
		Age	$F_{1,148}$=10.508; P=0.001
		Trial	$F_{8,148}$=3.078; P=0.003
		Group	$F_{1,148}$=8.567; P=0.004
		Treatment*Body mass	$F_{1,148}$=5.375; P=0.022
		Treatment	$F_{1,148}$=4.270; P=0.041
Social interactions (/h)	Linear	Body mass	$F_{1,152}$=23.755; P<0.001
Affiliative interactions started (/h)	Gamma	Body mass	$F_{1,144}$=26.382; P<0.001
		Trial	$F_{1,144}$=2.898; P=0.005
Dominant interactions started (/h)	Gamma	Body mass	$F_{1,142}$=25.574; P<0.001
		Treatment*Body mass	$F_{1,142}$=12.932; P<0.001
		Trial	$F_{1,142}$=5.404; P<0.001
		Age	$F_{1,142}$=12.137; P=0.001
		Treatment	$F_{1,142}$=9.402; P=0.003
		Group	$F_{1,142}$=5.468; P=0.021
Dominant–aggressive interactions started (/h)	Gamma	Age	$F_{1,136}$=7.859; P=0.006
		Trial	$F_{8,136}$=2.142; P=0.036
		Body mass	$F_{1,136}$=4.329; P=0.039
Dominant–not aggressive interactions started (/h)	Gamma	Age	$F_{1,34}$=17.121; P<0.001
		Trial	$F_{8,34}$=5.875; P<0.001
		Body mass	$F_{1,34}$=5.510; P=0.020
		Group	$F_{1,34}$=3.971; P=0.048
Dominant interactions received (/h)	Gamma	Age	$F_{1,151}$=38.922; P<0.001
Dominant–aggressive interactions received (/h)	Gamma	Age	$F_{1,137}$=12.486; P=0.001
		Trial	$F_{8,137}$=2.705; P=0.009
Dominant–not aggressive received (/h)	Gamma	Trial	$F_{8,124}$=3.959; P<0.001
		Age	$F_{1,124}$=10.681; P=0.001
		Group	$F_{1,124}$=6.191; P=0.014
		Body mass	$F_{1,124}$=4.594; P=0.034

Body mass and age also affected the rate of display of social interactions (Table 4). Heavier animals performed higher amounts of social interactions per hour (t=4.874), started more affiliative (t=5.136), dominant (t=3.096), dominant–aggressive (t=2.081) and dominant–not aggressive interactions (t=2.347) and received less non-aggressive interactions (t=-2.143).

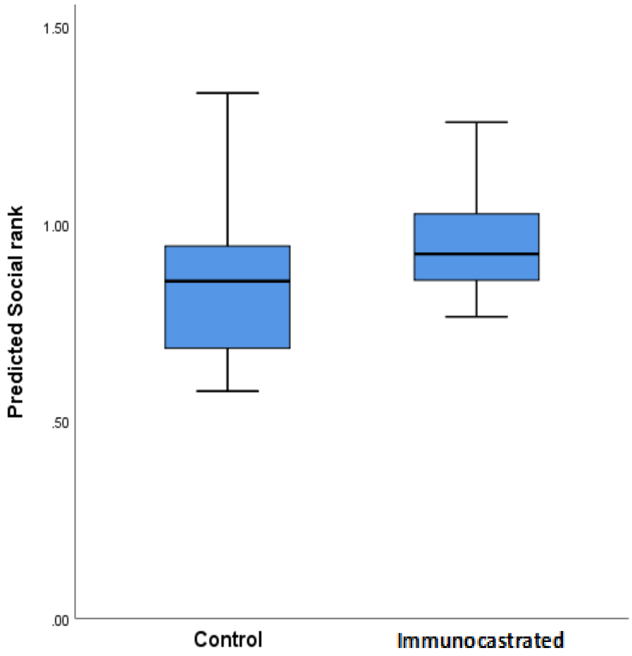

Figure 10. Effects of immunocastration on the predicted social rank of male eland along the trial period. Immunocastrates have a relatively higher social rank as compared to the control.

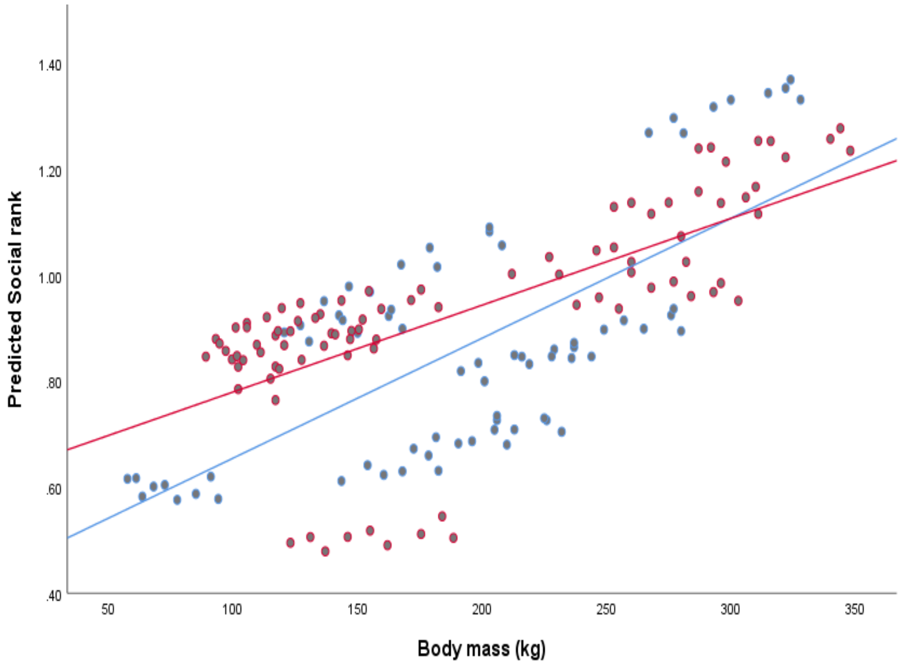

Figure 11. Effects of body mass on the predicted social rank of male common eland along the trial periods. Treatment always in red.

Table 5. Effects of immunocastration, and other experimental and individual factors, on the ratios between social interactions started and received s in calves and sub-adult male elands (n=18).

Target variable	Function	Significant fixed effects	
Dominant interactions started / Total interactions started	Gamma	Body mass	$F_{1,143}$=17.654; P<0.001
		Trial	$F_{1,143}$=5.099; P<0.001
		Age	$F_{1,143}$=11.915; P=0.001
		Treatment*Body mass	$F_{1,143}$=5.393; P=0.022
		Group	$F_{1,143}$=4.689; P=0.032
Dominant–aggressive interactions started / Total interactions started	Gamma	Body mass	$F_{1,137}$=11.992; P=0.001
		Trial	$F_{8,137}$=2.217; P=0.030
Dominant–aggressive interactions started / Dominant interactions started	Linear	Treatment	$F_{1,143}$=17.847; P<0.001
		Treatment*Body mass	$F_{1,143}$=6.998; P=0.001
		Trial	$F_{1,143}$=3.207; P=0.002
		Age	$F_{1,143}$=4.735; P=0.031
Affiliative interactions started / Total interactions started	Gamma	Trial	$F_{8,144}$=5.701; P<0.001
		Body mass	$F_{1,144}$=7.427; P=0.007
Affiliative interactions started / Total affiliative interactions involved	Linear	Age	$F_{1,157}$=24,508; P<0.001
		Treatment	$F_{1,157}$=6,437; P<0.012
Dominant interactions received / Total interactions received	Gamma	Age	$F_{1,141}$=17.405; P<0.001
		Body mass	$F_{1,141}$=15.233; P<0.001
		Trial	$F_{1,141}$=2,246; P=0.027
		Group	$F_{1,141}$=4,008; P=0.047
Dominant–aggressive interactions received / Total interactions received	Gamma	Age	$F_{1,137}$=28,521; P<0.001
		Trial	$F_{1,137}$=3,022; P=0.004

No significant model was found for the rate of affiliative interactions received per hour.

Older animals started more dominant (t=3.484), dominant–aggressive (t=2.803), and dominant–not aggressive interactions (t=4.138) and received less dominant (t=-6.239), dominant–aggressive (t=-3.534) and dominant–not aggressive interactions (t=-3.268). Immunocastration also had an effect on this behavioural display: immunocastrated animals displayed more dominant interactions per hour (t=3.066,

Figure 13), and the interaction term for “Treatment*Body mass” was negative for the immunocastrated animals (t=-3.596, Figure 14).

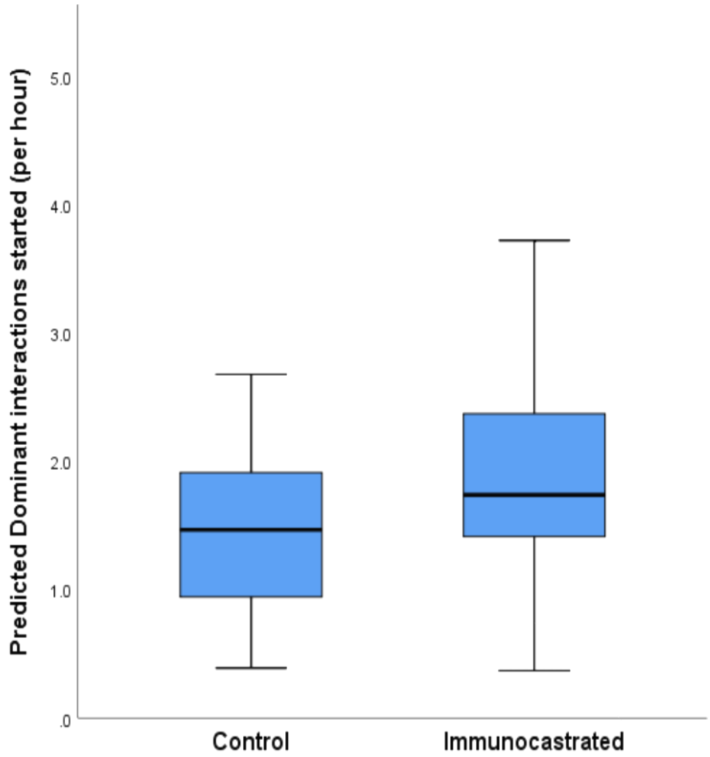

Figure 12. Effects of immunocastration on the predicted dominant interaction started (per hour) in male common eland along the trial period. Immunocastrates were higher in dominant interaction started which is related to non-contact dominance interaction.

Body mass also increased the proportion of dominant interactions started by an animal (related to the total amount of social interactions started, t=3.729), the proportion of dominant–aggressive interactions started (t=3.463), and the proportion of affiliative interactions started (t=2.725). From the total amount of social interactions received, heavier animals also received less dominating ones (t=-3.903). Regarding age, older animals started a higher proportion of dominant interactions (related to the total amount of social interactions started, t=3.452), a lower proportion of dominant–aggressive interactions (related to the total amount of dominant interactions started, t=-2-176), a higher proportion of affiliative interactions (related to the total amount of affiliative interactions where the individual was involved, t=4.951), and received a lower proportion of dominant (t=-4.172) and dominant–aggressive behaviours (t=-5.340) relative to the total amount of social interactions received. Immunocastrated animals displayed a lower proportion of dominant interactions than control ones (related to the total amount of social interactions started, t=-2.322; Figure 18). They also displayed a lower proportion of dominant–aggressive interactions (related to the total amount of dominant interactions started, t=-4.225; Figure 19); which was also positively affected by the “Treatment*Body mass” interaction (t=2.262, table 5). The proportion of affiliative interactions (related to the total amount of affiliative interactions where the individual was involved) were also lower in Immunocastrated animals (t=-2.537; Figure 11).

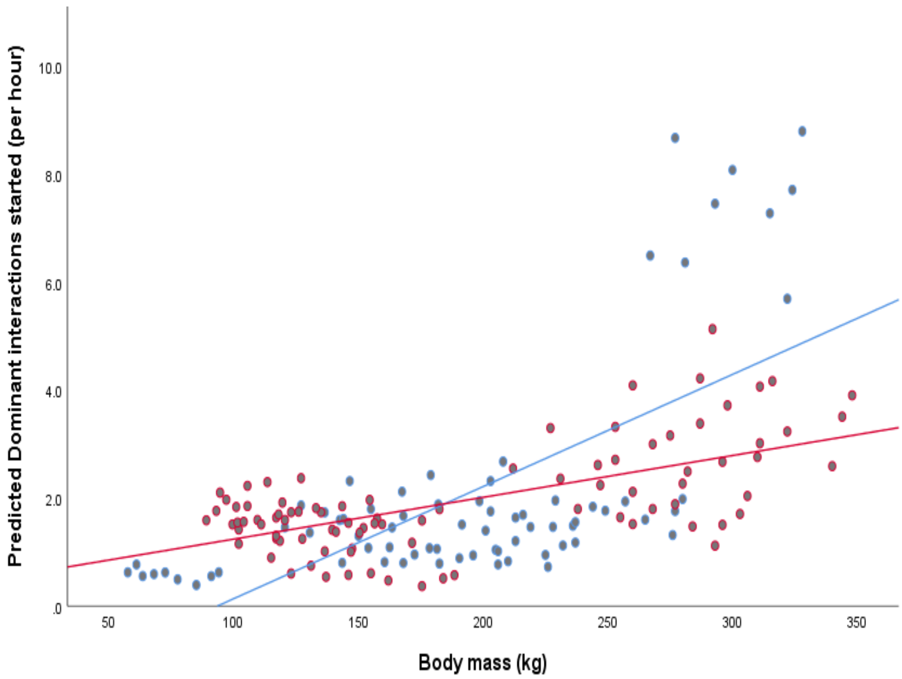

Figure 13. Effects of body mass on the predicted dominant interaction started (per hour) by male common eland along the trial period. Immunocastrates in red with less dominance interaction started signifying

Immunocastration also affected the daily activity budget of the studied animals (Table 6). Heavier animals were more frequently involved in social activities (t=76.445) and less frequently standing (t=-3.812). Older animals were also more frequently involved in social activities (t=139.673) and walking (t=2.666). Immunocastrated animals were involved less frequently in social activities (t=-2.071, Figure 5), and the interaction term "Treatment*Body mass" was positive for the immunocastrated animals (t=54.909, Figure 6). The interaction term "Treatment*Trial" was also significant: immunocastrated animals were less frequently involved in social activities than control ones during the weeks 2 ($F_{1,16}$=14.262, P=0.002) and 3 of the study ($F_{1,16}$=12.135, P=0.003), that is, the two weeks/trials after the immunocastration treatment was applied (Figure 17).

Table 6. Effects of immunocastration and other experimental and individual factors in the activity budget of calves and sub-adult male elands (n=18).

Target variable	**Function**	**Significant fixed effects**	
Eating (%)	Linear	Trial	$F_{8,154}$=23.069; P<0.001
Walking (%)	Gamma	Trial	$F_{8,148}$=13.428; P<0.001
		Age	$F_{1,148}$=7.109; P=0.009
Standing (%)	Linear	Body mass	$F_{8,152}$=14.543; P<0.001
		Trial	$F_{1,152}$=3.878; P<0.001
Lying (%)	Linear	Trial	$F_{1,154}$=7.226; P<0.001
Social (%)	Gamma	Age	$F_{1,95}$=19508.472; P<0.001
		Body mass	$F_{1,95}$=6133.733; P<0.001
		Treatment*Body mass	$F_{1,95}$=3015.008; P<0.001
		Treatment	$F_{1,95}$=67.139; P<0.001
		Trial	$F_{8,95}$=16.445; P<0.001
		Treatment*Week	$F_{8,95}$=9.219; P<0.001

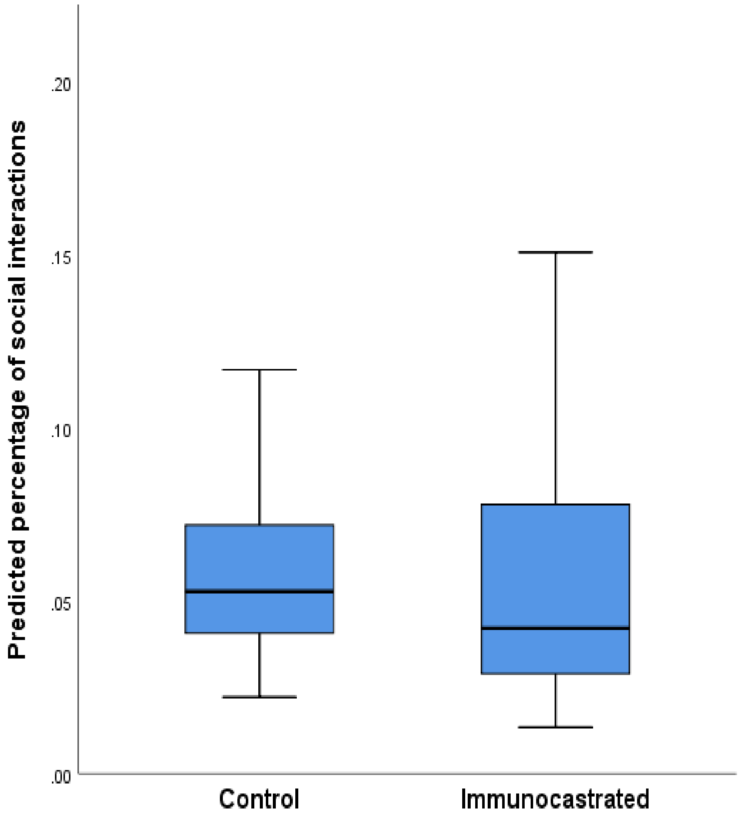

Figure 14. Effects of immunocastration on the predicted percentage of social interaction of male common eland along the trial period. Immunocastrates were less in social interaction as compared to the control.

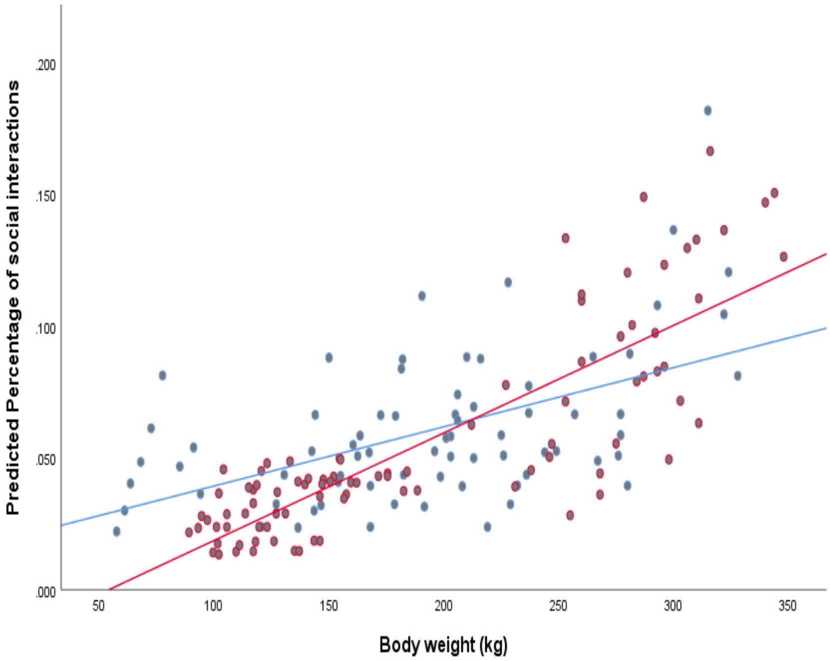

Figure 15. Effects of immunocastration on the predicted percentage of social interaction in male common eland along the trial period. Immunocastrates in red were more social with increasing weight.

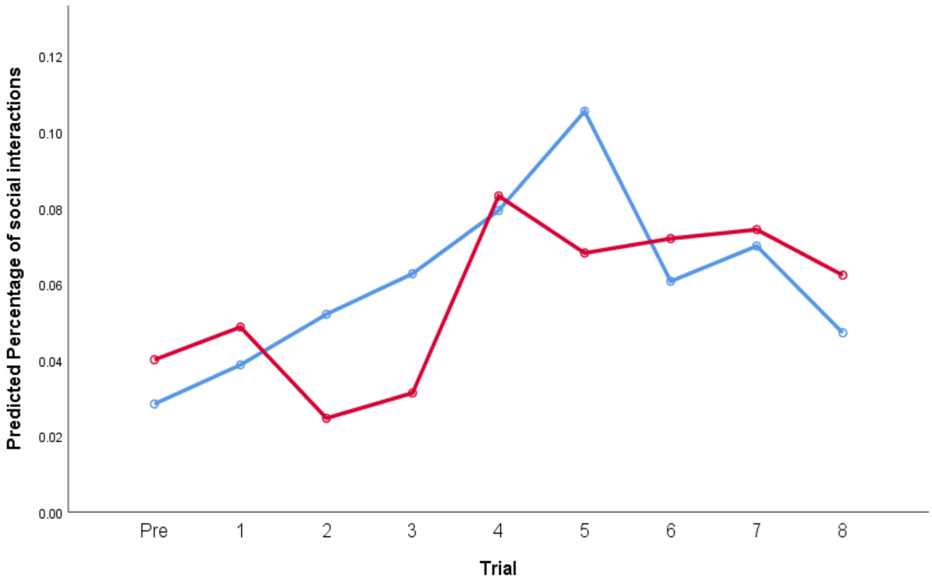

Figure 16. Predicted percentage of social interaction for male common eland along the trial period. Immunocastrates in red gradually decreases and then increases in social interaction, this was attributed to the gradual increase and decrease in the suppressive effects of the treatment.

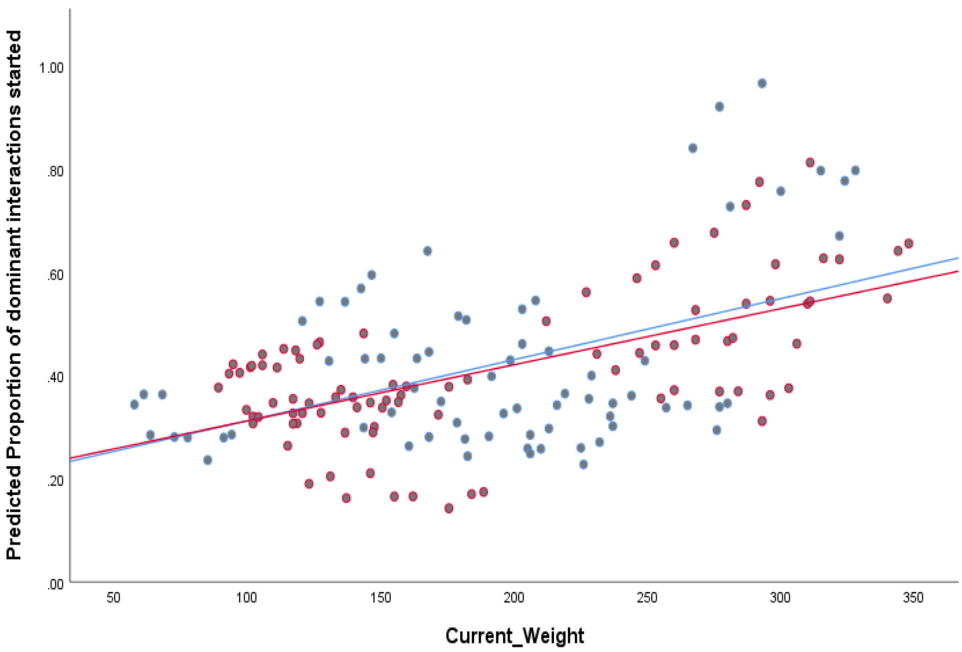

Figure 17. Effects of current weight on the predicted proportion of dominant interaction started to total interactions started for male common eland along the trial period. Immunocastrates in red

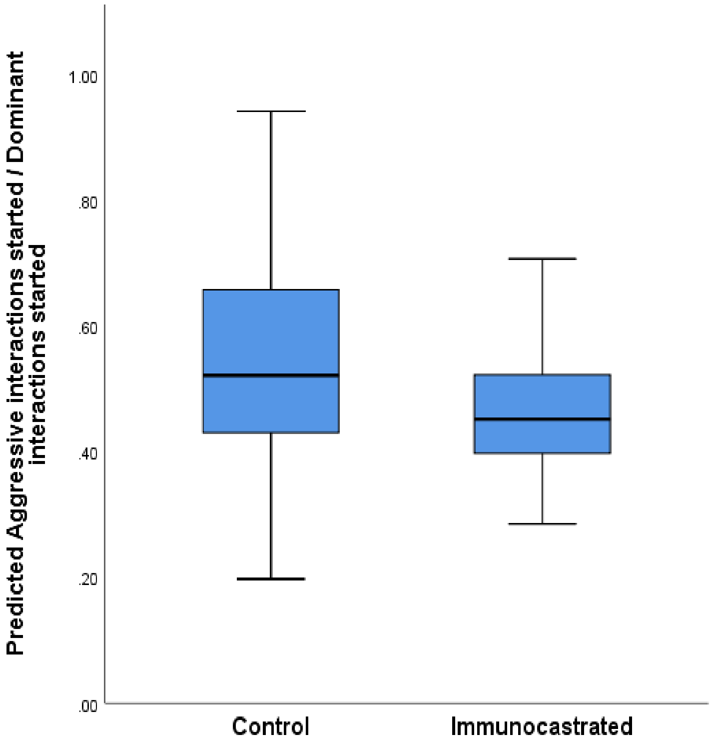

Figure 18. Effects of immunocastration on the predicted aggressive interactions started to dominant interaction started of male common eland along the trial period. Immunocastrates have low rate of aggression as compared to

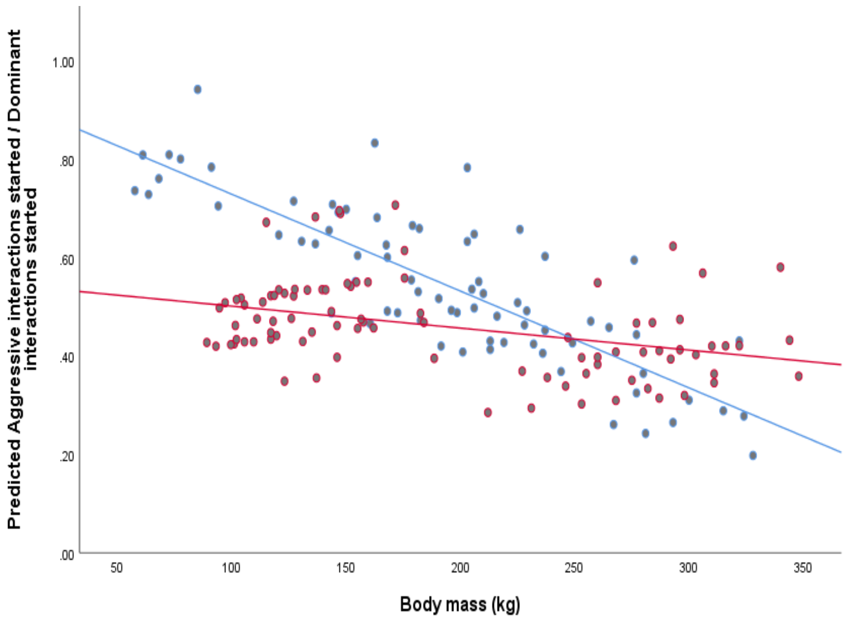

Figure 19. Effects of body mass (kg) on the predicted aggressive interactions started to dominant interaction started of male common eland along the trial period. Immunocastrates in red are lower in aggression started but increases with increase in body mass which can be attributed to increase in body mass and gradual decrease in the suppressive effects of the immunocastration

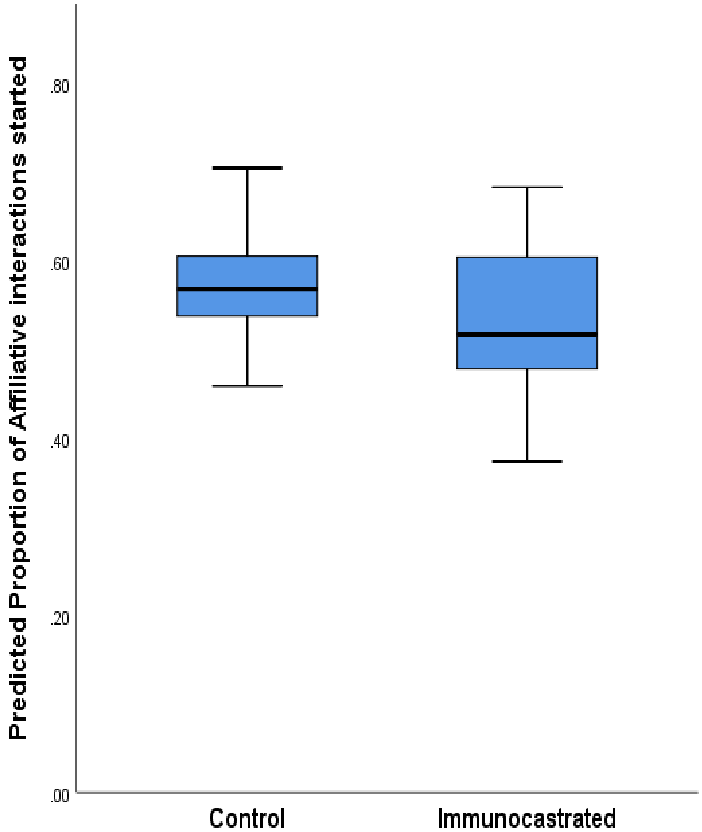

Figure 20. Effects of immunocastration on the predicted proportion of affiliative interactions started to total affiliative interactions involved of male common eland along the trial period. Immunocastrates were less affiliative as compared to the control.

5. Discussion

As in other ungulate species, the common eland are also socials animal with a relatively stable social structure in term of social hierarchy and dominance (Martin & Bateson 2007). This social organisation or structure can be affected by extraneous changes, an example of which is castration. As previously stated in Chapter 2, this study aimed at finding answers to the following question: will immunocastration influence the social rank, aggressive behaviour, affiliative interaction and the overall activity budget of common eland? The results from this study revealed a significant influence of immunocastration as on some of the earlier established hypotheses. Social rank, dominance interactions, aggressive interactions were significantly affected by the immunocastration treatment. For the activity budget, only the frecuency of social behaviours displayed were affected by the treatment. One of the most important outcomes from this study is the effectiveness of immunocastration in reducing the aggressive behaviour of the immunocastrates.

5.1. Linearity of the social groups

For a social group to be stable there is need for linearity in its structure (Schizuka & McDonald 2012). Social organisation in ungulates has been shown to be predominantly linear and transitive (Barroso et al. 2000; Cote 2000). This study also revealed similar results (Table 3), with a higher triangle Transitivity (t_{tri}) and Landau's index *h'* for the sub-adult group than the calves. According to Martin and Bateson (2007), the social organisation in groups of mammals can be relatively simple, that is either linear, nearly linear or highly complex. Both indices were low for the calves' group, which can be attributed to their age. Mainly affiliative behaviours were observed in the calf-group, as the animal are still young, and are likely still in the early establishment of their hierarchy, especially considering the changes in the group composition after the creation of the experimental groups.

5.2. Effects of immunocastration on social rank of common eland

Castration has been most effective in several studies in the reduction of androgenic hormone production, which is positively related to displaying sexual and aggressive behaviour (Price et al. 2003). Meanwhile, animals in a social group need to be aggressive for the benefit of increasing their social rank or for self-defence. In this study there was significant influence of the immunocastration treatment on social rank of both calves and sub-adult eland. Surprisingly, immunocastrated male common eland had a higher social rank than the controls (Figure 10). This is in contrary to the study of Lincoln et al. (1982) which observed that immunocastrated red deer stags (*Cervus elaphus*) were having lower social rankings which was likely attributed to the premature lost of antlers as the immunocastrated stags never retain their position even after the rut, were all other males most have lost their antlers. This study attributed the higher social rank of the immunocastrated male eland to the possession of the intact structures such as horns which are the prime structures required for wrestling and self-defence for the establishment of dominance. However, the common eland in the present studies did not increase their aggressive to obtain this, but rather they exhibited high frequency of non-contact dominance behaviour. Kiley-Worthington (1977) also observed that common eland uses visual cues in displaying their dominance through parallel walking.

The body mass and age are also a positive function of the social rank (Barroso et al. 2000), as also observed within the current study. Cransac and Aulagnier (1996) described the social hierarchy of the common eland as strongly age-based. Similarly, in this study the older individuals were having the highest rank. In the study of Vymyslická et al. (2015), on derby eland at the Bandia reserve in Senegal, the positive effect of social rank on age was clearly proven but only in the young animals that were still growing. The influence of age on social rank was significantly weaker after attainment of full body size, which suggest that other factors such as horn, condition or body mass might possibly play a role in the rank achievement of fully-grown animals (Pelletier & Festa-Bianchet 2006).

5.3. Effects of immunocastration on the aggressive behaviour of common eland

One of the primary objectives of castration is to reduce aggressive and sexual behaviours in farm animals. There is a decrease in aggressive behaviours in cattle bulls after surgical castration (Bretschneider 2005). Meanwhile, animals subjected to physical castration, such as surgical and band castration, may not only reduce their aggressive behaviour as a result of the reduction in their androgenic production but also as a result of the physical stress and pain experienced (Fell et al. 1986; Fisher et al. 2001; Melches et al. 2007; Jongman et al. 2016). In this study, immunocastrated male common eland were more involved in dominant interactions than the controls. The immunocastrated males displayed a higher proportion of dominant interaction started (/h) but less aggressive interactions started. This implies that the immunocastrated animals were more engaged in the non-contact agonistic interactions (threatening and passing) as compared to the intact animals. Eland can exhibit these behaviours for self-defence and not necessarily for aggression (Kiley-Worthington 1977). The findings from this study are thus consistent with the previous studies of Huxsoll et al. (1998) and Price et al. (2003) in bulls, and Dunshea et al. (2001) in swine, where immunocastration was effective in reducing aggressive and sexual behaviours.

5.4. Effects of immunocastration on the affiliative behaviour of common eland

Affiliative interaction substantially helps in fostering cohesion and affection of social organisations, particularly when resources are abundant (Boissy et al. 2007; Miranda de la Lama & Mattiello 2010). From Figure 11, it can be seen that the affiliative interactions started by the immunocastrates were lower than the controls, compared to the total affiliative interactions that the animals were involved in. This is not surprising as affiliative social rank is an inverse of dominant rank (Kiley-worthington 1977; King et al. 2008; Améndola et al. 2016), and it can be seen that immunocastrates had the higher social rank compared to the controls (Figure 12). It therefore implies that the least aggressive eland with the lowest dominant rank should be expected to be the most affiliative. This was observed in the course of observations of this study, were the lower ranking individuals in the sub-adult group usually groom other individuals above them in the hierarchy, either to avert aggression or to be allowed to feed conveniently. Although, grooming has also been shown to occur between individual of closely equal dominant rank in eland (Kiley-Worthington 1977).

The present findings is also in line with the ''Grooming-for-Commodity'' hypothesis which state that subordinate members in a social group groom higher-ranking animal in favour of tolerance at feeding and resting places, or tolerance and even protection of progeny (Schino 2007; Tiddi et al. 2012). This clearly explained the negative relationship between dominance and affiliation started and positive relationship with affiliation received (Šárová et al. 2016).

5.5. Effects of immunocastration on the activity budget of common eland

Non-castrated animals often engage in more activity, particularly during rut season where males roam about to mate with the female, but there is often a decrease in feeding behaviour due to the high proportion of time attributed to sexual behaviour. In this study, there was no difference between the control group and the immunocastrates in terms of activity budgets for eating, walking, standing and lying. In the studies of Janett et al. (2012) on bulls and Dunshea et al. (2001) on swine, immunocastration was effective in decreasing the overall physical activity of the immunocastrates compared to intact males. The lack of effect of immunocastration within the present study may be influenced by the to the available space in the barn, particularly for the sub-adult group, as space plays a vital role as a function of activity budget (Bouissou et al. 2001; Haley et al. 2000; Grant & Albright 2001). Fisher et al. (2001) also observed no statistical difference in lying, ruminating and walking in 14 month old bulls subjected to physical castration compared to the control intact males, but surgical castrated bulls were found to engage more in tail swishes and leg stamping which was attributed to the stress and pain from the injury. Thus, immunocastration appears to have a lesser effect on time activity budget related to resting and ingestion compared to physical castration, possibly due to the absence of pain-related behaviours after castration.

However, as evident in the differences in the social interactions from this present study, immunocastrated male common eland were less engaged in the social activity than non-castrated males between vaccinations (playing, wrestling and grooming, Figure 5). These social activities decreased immediately after the first vaccination until the second vaccination, thereafter it gradually increases again and equalises with that of the controls. Thus, this perhaps might be attributed to diminishing in the suppressive effects of immunocastration which needs to be examine physiologically. This agrees with Rydhmer et al. (2010), who also reported that the social behaviour of immunocastrates male pigs decreases immediately after the second injection.

6. Conclusions and Recommendations

Reducing aggressive behaviour may increase the welfare and economic return by reducing the incidence of injury to both animals and their handlers, while conserving energy to enhance growth. The use of immunocastration in the present study, has been effective in reducing the aggressive behaviour of male common eland without negatively influencing their daily activity. This achievement will help in the effort toward breeding and taming common eland and other related antelopes, and thus supports the use of immunocastration for future management protocol. However, for a long-term management beyond the duration of this study, a booster vaccine should be considered. Furthermore, the effects of immunocastration on the reproductive functioning and meat quality in male common eland should be established.

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List of the Appendices:

Appendix l

Animal identification number	Sex	Age class	Birth Year	Initial Weight (Kg)	Treatment	Initial weight (m±SD)
225	M	SA	2017	118.0	IC	
202	M	SA	2016	248.0	IC	
208	M	SA	2017	237.0	IC	218.8 ± 63.67
203	M	SA	2016	204.0	IC	
198	M	SA	2016	287.0	IC	
221	M	SA		165.5	E	
206	M	SA	2017	186.5	E	
199	M	SA	2016	262.0	E	194.1 ± 46.21
209	M	SA	2017	142.5	E	
222	M	SA	2017	214.0	E	
228	M	C	2018	99.0	IC	
230	M	C	2018	103.5	IC	
226	M	C	2018	95.0	IC	99.8 ± 41.78
T2	M	C	2018	115.0	IC	
T4	M	C	2018	86.5	IC	
229	M	C	2018	117.0	E	
232	M	C	2018	134.0	E	89.88 ± 41.78
T1	M	C	2018	57.0	E	
T3	M	C	2018	51.5	E	
205	F	SA	2016	191.0	F	
207	F	SA		129.0	F	
219	F	SA	2017	79.0	F	135.9 ± 40.25
224	F	SA		132.5	F	
218	F	SA		148	F	
B	F	C	2018	108.5	F	
C	F	C	2018	65.0	F	
A	F	C	2018	79.5	F	92.0 ± 22.55
227	F	C	2018	121.0	F	
231	F	C	2018	86.0	F	
233	F	C	2018	93	F	

Appendix ll Animal restrained in the squeeze of the chute system

Date ____________________________

Time started ____________________

Time finished ____________________

Observer ________________________

Appendix III: Table for recording of behavioural observation of social interaction

Time	Winner	Loser	Interaction	Remark