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

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## Highlights

•Routine handling successfully habituated eland to the system and sample collection.
•Eland with heavier body masses are more nervous and difficult to handle.
•Calm animals (low temperament score) enter the handling system earlier than others.
•Eland were generally less excitable when exiting the system after repeat handling.
•Immunocastration does not affect the temperament of common eland during handling.

## 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. Immunocastration 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 overtime (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

## 1. 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; Parhama 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; Parhama 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.

## 2. Materials and methods

### *2.1. 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.

### *2.2. 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. 1; Supplementary Fig. 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 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.

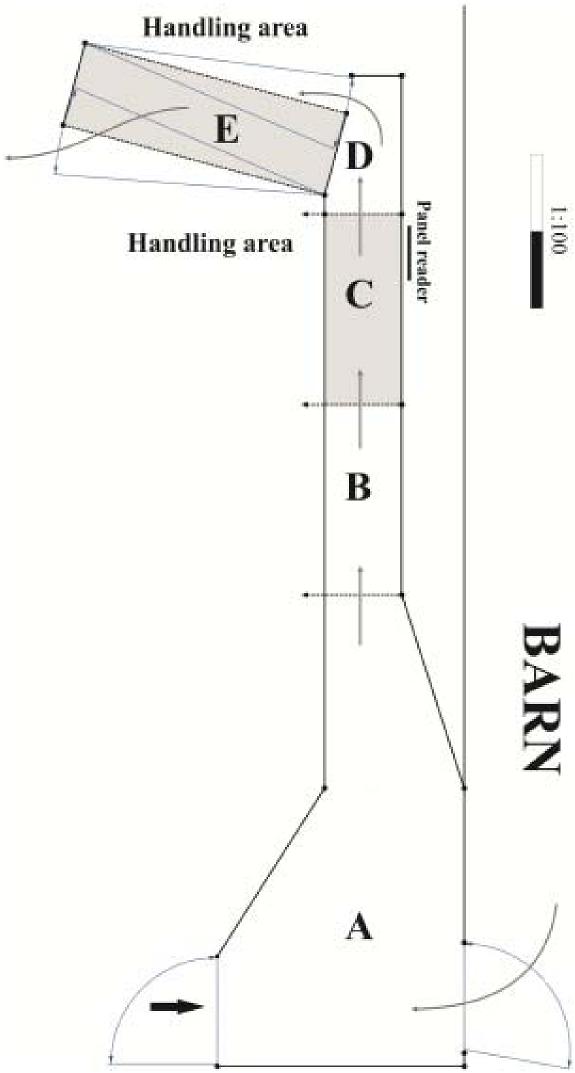


**Fig. 1.** Design of the raceway divided into four sections (A, B, C and D) and the squeeze chute system (E). Animals enter the first part of the raceway (A) from the barn, after which they are individually separated from the group into section B, using sliding galvanised doors. The animal then enters 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.

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. 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.

**Table 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 towards the raceway without the handlers' interference. | 0 |
| Empty Cell | Lured | The animal is lured with a treat or sound cue. | 1 |
| Empty Cell | Forced | The animal is forced by handler using tapping stick. | 2 |
| **State before and within the raceway** | Calm | The animal does not make frequent eye contact and is not agitated. | 0 |
| Empty Cell | Nervous | The animal is agitated and uneasy. | 1 |
| Empty Cell | 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 |
| Empty Cell | Running | The animal seems to be tense and trots from one section to another. | 1 |
| Empty Cell | Jumping | The animal is jumping and moving forwards and backwards. | 2 |
| **Vocalisation** | None | | 0 |
| Empty Cell | Low | | 1 |
| Empty Cell | Medium | | 2 |
| Empty Cell | High | | 3 |
| **State in the squeeze chute** | Calm | The animal remains calm while being measured and biological samples are being collected. | 0 |
| Empty Cell | Nervous | The animal is tense, uneasy and trying to lie down. | 1 |
| Empty Cell | Panicked | The animal is trying to jump, or pushing its head/horns towards the handler. | 2 |
| **Chute exit** | Walking | The animal calmly walks out of the chute. | 0 |
| Empty Cell | Running | The animal trots off out of the chute. | 1 |
| Empty Cell | Jumping | The animal jumps out and trots off with high speed. | 2 |

The behaviour of each animal was recorded as they moved through the system, according to the parameters specified in Table 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 (Parhama et al., 2019). The temperament scoring methodology was adapted from Schütz et al. (2016), as used for red deer, and Parhama 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 1). Exit velocity, which has been a primary parameter for measuring temperament by Curley et al. (2006) and Parhama 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 indicators recorded (Table 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.

### *2.3. 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 (1 mm 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.

### *2.4. 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.

### *2.5. 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 c*hute 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.

## 3. 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 ($\beta = -0.031$, $t = -4.959$, $P < 0.001$; Fig. 2), decreasing with time (*i.e.*, the animals were calmer after repeated handling).

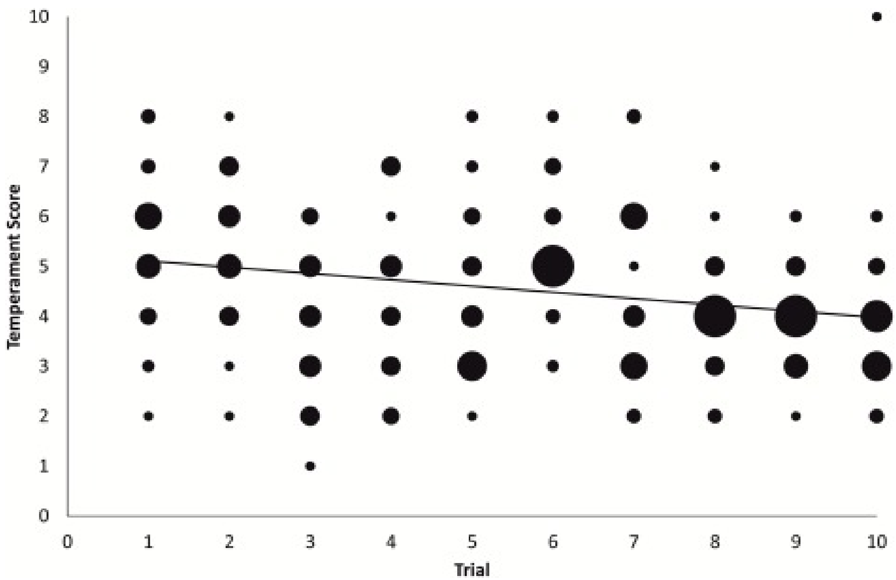


**Fig. 2.** Influence of routine handling on the temperament score of common eland (n = 29) over time. Each trial was performed in two-week intervals, for a total of 10 trials. The size of the dots is proportionate to the number of observations for a particular score within each trial period.

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 ($\beta = -0.001$, $t = -3.071$, $P = 0.002$).

## 4. 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. 2, where they already had relatively low scores 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. 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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**Appendix A. Supplementary data**

**Supplementary Figure 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.

**References**

Allan, M.R., 2015. The use of ketamine-xylazine and ketamine-medetomidine with and without their antagonists, yohimbine and atipamezole hydrochloride to immobilize Raccoons (*Procyon lotor*) in Ontario, *Canada. Can. Field-naturalist* 129, 84–89. https://doi.org/10.22621/cfn.v129i1.1672.

Appleby, M.C., 1983. The probability of linearity in hierarchies. *Anim. Behav*. 31, 600–608. https://doi.org/10.1016/S0003-3472(83)80084-0.

Bergvall, U.A., J ̈aderberg, L., Kjellander, P., 2017. The use of box-traps for wild roe deer: behaviour, injuries and recaptures. *Eur. J. Wildl. Res*. 63 (67) https://doi.org/10.1007/s10344-017-1120-1127.

Blanco, M., Casasu, I., Palacio, J., 2009. Effect of age at weaning on the physiological stress response and temperament of two beef cattle breeds. *Animal* 3, 108–117. https://doi.org/10.1017/S1751731108002978.

Bordes, C.N.M., Ceacero, F., Kotrba, R., 2018. Cues and mechanisms for lateral exposure preference in the common eland (*Taurotragus oryx*). *Behav. Eco. Socio-biol.* 72 (120) https://doi.org/10.1007/s00265-018-2535-1.

Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A., Lundström, K., 2011. Early immunocastration of male pigs with Improvac®–Effect on boar taint, hormones and reproductive organs. *Vaccin*e 29, 9514–9520. https://doi.org/10.1016/j.vacci ne.2011.10.014.

Burdick, N.C., Carroll, J.A., Hulbert, L.E., Dailey, J.W., Willard, S.T., Vann, R.C., Welsh Jr., N.C., Randel, R.D., 2010. Relationships between temperament and transportation with rectal temperature and serum concentration of cortisol and epinephrine in bulls. *Livest. Sci*. 129, 166–172. https://doi.org/10.1016/j.livsci.2010.01.020.

Burrow, H.M., Corbet, N.J., 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. *Aust. J. Agric. Res*. 51, 155–162. https://doi.org/10.1071/ar99053.

Ceacero, F., García, A.J., Landete-Castillejos, T., Bartošová, J., Bartoš, L., Gallego, L., 2012. Benefits for dominant red deer hinds under a competitive feeding system: food access behaviour, diet and nutrient selection. PLoS One 7, e32780. https://doi.org/10.1371/journal.pone.0032780.

Ceacero, F., Landete-Castillejos, T., Bartošová, J., García, A.J., Bartoš, L., Komárková, M., Gallego, L., 2014. Habituating to handling: factors affecting preorbital gland opening in red deer calves. *J. Anim. Sci*. 92, 4130–4136. https://doi.org/10.2527/jas2014-7716.

Core, S., Widowski, T., Mason, G., Miller, S., 2009. Eye white percentage as a predictor of temperament in beef cattle. *J. Anim. Sci*. 87, 2168–2174. https://doi.org/10.2527/jas.2008-1554.

Curley Jr., K.O., Paschal, J.C., Welsh, Randel, T.H., R. D, 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. *J. Anim. Sci*. 84, 3100–3103. https://doi.org/10.2527/jas.2006-055.

Della Rosa, M.M., Pavan, E., Maresca, S., Spetter, m., Ramiro, F., 2018. Performance, carcass and meat quality traits of grazing cattle with different exit velocity. *Anim. Prod. Sci.* 59, 1752–1761. https://doi.org/10.1071/AN18064.

Elder, J.K., Kearnan, J.F., Waters, K.S., Dunwell, G.H., Emmerson, F.R., Knott, S.G., Morris, R.S., 1980. A survey concerning cattle tick control in Queensland. 4. Use of resistant cattle and pasture spelling. *Aust. Vet. J*. 56, 219–223. https://doi.org/10.1111/j.17510813.1980.tb15976.x.

Fordyce, G., Goddard, M.E., Seifert, G.W., 1982. The measurement of temperament in cattle and the effect of experience and genotype. In: *Proceedings of the Australian Society of Animal Production*, 4, pp. 329–332.

Fordyce, G., Goddard, M.E., Tyler, R., Williams, C., Toleman, M.A., 1985. Temperament and bruising in Bos indicus cattle. Aust. J. Exp. Agric. 25, 283–288.

Fordyce, G., Wythes, J.R., Shorthose, W.R., Underwood, D.W., Shepherd, R.K., 1988. Cattle temperaments in extensive beef herds in northern Queensland. 2. Effect of temperament on carcass and meat quality. *Aust. J. Exp. Agric*. 28, 689–693. https://doi.org/10.1071/EA9880689.

Ganswindt, A., Heistermann, M., Borragan, S., Hodges, J.K., 2002. Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. *Zoo Biol*. 21, 27–36. https://doi.org/10.1002/zoo.10034.

Grandin, T., 1997. Assessment of stress during handling and transport. *J. Anim. Sci*. 75, 249–257. https://doi.org/10.2527/1997.751249x.

Hearnshaw, H., Morris, C.A., 1984. Genetic and environmental effects on a temperament score in beef cattle. *Aust. J. Agric. Res*. 35, 723–733. https://doi.org/10.1071/ar9840723.

Hemsworth, P.H., 2003. Human-animal interactions in livestock production. *Appl. Anim. Behav. Sci*. 81, 185–198. https://doi.org/10.1016/S01681591(02)00280-0.

Janett, F., Gerig, T., Tschuor, A.C., Amatayakul-Chantler, S., Walker, J., Howard, R., Bollwein, H., Thun, R., 2012. Vaccination against gonadotropin-releasing factor (GnRF) with Bopriva significantly decreases testicular development, serum testosterone levels and physical activity in pubertal bulls. *Theriogenology* 78, 182–188. https://doi.org/10.1016/j.theriogenology.2012.01.035.

Kamgang, V.W., Bennett, N.C., Hart, D.W., van der Goot, A.C., Ganswindt, A., 2020. Reproductive events and respective faecal androgen metabolite concentrations in captive male roan antelope (*Hippotragus equinus*). *PLoS One* 15 (12), e0243277. https://doi.org/10.1371/journal.pone.0243277.

Kiley-Worthington, M., 1977. The causation, evolution and function of the visual displays of the Eland (*Taurotragus Oryx*). *Behaviour* 66, 179–222. https://doi.org/10.1163/156853978X00116.

Kiley-Worthington, M., 1978. The social organization of small captive group of eland, oryx and roan antelope with an analysis of personality profiles. *Behaviour* 66, 32–55.

King, D.A., Pfeiffer, C.E.S., Randel, R.D., welsh Jr., T.H., Oliphint, R.A., Baird, B.E., Curley Jr., K.O., Vann, R.C., Hale, D.S., Savell, J.W., 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. *Meat Sci*. 74, 546–556. https://doi.org/10.1016/j.meatsci.2006.05.004.

Koolhaas, J.M., Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Front. Neuroendocrinol*. 31, 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001.

Lansade, L., Bouissou, M., Erhard, H.W., 2008. Fearfulness in horses: a temperament trait stable across time and situations. *Appl. Anim. Behav. Sci*. 115, 182–200. https://doi.org/10.1016/j.applanim.2008.06.011.

Loskutoff, N.M., Betteridge, K.J., 1992. Embryo technology in pets and endangered species. In: Lauria, A., Gandolfi, F. (Eds.), *Embryonic Development and Manipulation in Animal Production: Trends in Research and Applications*. Portland Press, London, U.K, pp. 235–248.

Mondal, M., Rajkhowa, C., Prakash, B.S., 2006. Relationships between plasma growth hormone concentrations and temperament in mithuns (*Bos frontalis*). *Hormone Behav*. 49, 190–196. https://doi.org/10.1016/j.yhbeh.2005.06.006.

Moreira, A.D., Siqueira, G.R., Lage, J.F., Benatti, J.M.B., Moretti, M.H., Miguel, G.Z., de Oliveira, I.M., de Resende, F.D., 2016. Castration methods in crossbred cattle raised on tropical pasture. *Anim. Prod. Sci*. 58, 1307–1315. https://doi.org/10.1071/AN16580.

Needham, T., Lambrechts, H., Hoffman, L.C., 2017. Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: invited Review. *South African J. Anim. Sci*. 47, 731–742. https://doi.org/10.4314/sajas.v47i6.1.

Needham, T., Lambrechts, H., Hoffman, L., 2019. Extending the interval between second vaccination and slaughter: I. Effects on growth, scrotal size, and stress responses of immunocastrated ram lambs. *Animal* 13, 1952–1961. https://doi.org/10.1017/S1751731118003592.

Needham, T., Kotrba, R., Hoffman, L.C., Bureš, D., 2020. Ante-and post-mortem strategies to improve the meat quality of high-value muscles harvested from farmed male common eland (*Taurotragus oryx*). *Meat Sci*. 168, 108183 https://doi.org/10.1016/j.meatsci.2020.108183.

Noya, A., Ripoll, G., Casasús, I., Sanz, A., 2019. Effects of immunocastration performed at two live weights on the growth physiology, temperament and testicular development of feral beef bulls. *Anim. Sci. J*. 00, 1–13. https://doi.org/10.1111/asj.13307.

Palme, R., Möstl, E., 1993. Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. In: *Proc. of the 5th Symp. On the Analysis of Steroids Szomnathely. Hungary.*

Parhama, J.T., Tanner, A.E., Wahlberg, M.L., Grandin, T., Lewisa, R.M., 2019. Subjective methods to quantify temperament in beef cattle are insensitive to the number and biases of observers. *Appl. Anim. Behav. Sci*. 212, 30–35. https://doi.org/10.1016/j.applanim.2019.01.005.

Pelletier, F., Festa-Bianchet, M., 2006. Sexual selection and social rank in bighorn rams. *Anim. Behav*. 71, 649–655. https://doi.org/10.1016/j.anbehav.2005.07.008.

Pennington, P.M., Pope, C.E., MacLean, R.A., Galigius, J., Gentry, L.R., Godke, R.A., Wirtu, G., 2013. Daily or thrice weekly handling of eland antelope (*Taurotragus oryx*). *Res. Vet. Sci.* 94, 711–716. https://doi.org/10.1016/j.rvsc.2012.12.006.

Pereira, R.J.G., José Duarte, J.M.B., Negrao, J.A., 2005. Seasonal changes in fecal testosterone concentrations and their relationship to the reproductive behavior, antler cycle and grouping patterns in free-ranging male Pampas deer (*Ozotoceros bezoarticus bezoarticus*). *Theriogenology* 63, 2113–2125. https://doi.org/10.1016/j.theriogenology.2004.08.014.

Petherick, J.C., Doogan, V.J., Holroyd, R.G., Olsson, P., Venus, B.K., 2009. Quality of handling and holding yard environment, and beef cattle temperament: 1. Relationships with flight speed and fear of humans. *Appl. Anim. Behav. Sci.* 120, 18–27. https://doi.org/10.1016/j.applanim.2009.05.008.

Phillips, M., Grandin, T., Graffam, W., Irlbeck, N.A., Cambre, R.C., 1998. Crate conditioning of bongo (*Tragelaphus eurycerus*) for veterinary and husbandry procedures at the Denver Zoological Gardens. *Zoo Biol.* 17, 25–32. https://doi.org/10.1002/(SICI)1098-2361(1998)17:1<25::AID-ZOO3>3.0.CO;2-C.

Price, E.O., Adams, T.E., Huxsoll, C.C., Borgwardt, R.E., 2003. Aggressive behaviour is reduced in bulls actively immunized against gonadotropin-releasing hormone. *J. Anim. Sci.* 81, 411–415. https://doi.org/10.2527/2003.812411x.

Reale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. *Biol. Rev.* 82, 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x.

Rice, M., Jongman, E.C., Butler, K.L., Hemsworth, P.H., 2016. Relationships between temperament, feeding behaviour, social interactions, and stress in lambs adapting to a feedlot environment. *Appl. Anim. Behav. Sci.* 183, 42–50 dx.doi.org/10.1016/j.applanim.2016.07.006.

Sarmah, J., Chaya, R., Hazarika, C.R., Berkeley, E.V., Ganswindt, S.B., Ganswindt, A., 2017. Non-invasive assessment of adrenocortical function as a measure of stress in the endangered golden langur. *Zoo Biol.* 36, 278–283. https://doi.org/10.1002/zoo.21369.

Šárová, R., Gutmann, A.K., Špinka, M., Stěhulová, I., Winckler, A., 2016. Important role of dominance in allogrooming behaviour in beef cattle. *Appl. Anim. Behav. Sci.* 18, 41–48. https://doi.org/10.1016/j.applanim.2016.05.017.

Šárová, R., Špinka, M., Ceacero, F., 2017. Higher dominance position does not result in higher reproductive success in female beef cattle. *J. Anim. Sci.* 95, 3301–3309. https://doi.org/10.2527/jas.2017.1415.

Schütz, K.E., Ward, J.F., Coxa, N.R., Asher, G.W., 2016. Development and evaluation of a temperament-scoring system for farmed deer: genetic and environmental components. In: *Proceedings of the New Zealand Society of Animal Production*, 76, pp. 109–113.

Sutherland, M.A., Dowling, S.K., 2014. The relationship between responsiveness of first lactation to humans and the behavioral response to milking and milk production measures. *J. Vet. Behav.* 9, 30–33. https://doi.org/10.1016/j.jveb.2013.09.001.

Valenchon, M., Levy, F., Moussu, C., Lansade, L., 2017. Stress affects instrumental learning based on positive or negative reinforcement in interaction with personality in domestic horses. *PLoS One* 12, e0170783.

Voisinet, B.D., Grandin, T., Connor, S.F.O., Tatum, J.D., Deesing, M.J., 1997. Bos indicus cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Sci*. 46, 367–377. https://doi.org/10.1016/S0309-1740(97)00031-00034.

Weerasekera, D.S., Perera, S.J., Nanayakkara, D.K., Herath, H.M., Rathnasekara, A.N., Ranawana, K.B., 2020. The antler cycle and fecal testosterone of male sambar deer Rusa unicolor unicolor at the Horton Plains National Park in Sri Lanka. *Biomed Res. Int.* 1–7. https://doi.org/10.1155/2020/6903407.

Wirtu, G., Cole, A., Pope, C.E., Short, C.R., Godke, R.A., Dresser, B.L., 2005. Behavioural training and hydraulic chute restraint enables handling of eland antelope (*Taurotragus oryx*) without general anesthesia. *J. Zoo Wildl. Med.,* 36 (2005), pp. 1-11, 10.1638/04-007