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Influence of short- and long-term stressors on the blood biochemistry profile of captive common eland (*Taurotragus oryx*)

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled **Influence of short- and long-term stressors on the blood biochemistry profile of captive 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 and according to Citation rules of the FTA.

In Prague

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Abstract

Stress is considered a disturbance to homeostasis, and anything that generates a stress response is a stressor. A stress response can modify blood biochemistry parameters as observed in many animal species but the effects of stressors on blood biochemistry parameters are not studied previously in common eland. Short- and long-term stressors such as handling and social rank, respectively, were studied on a total of 12 healthy common eland (135.1±2.25kg body weight, 3.8±.0.1 BCS, 9.2±1.5 months), females (n=6), and males (n=6) over a 12-month period. Handling was performed once a month in a specialised squeeze chute system and the data of their temperament was recorded in the system followed by the collection of blood samples. Behaviour data for social interactions in the barn was recorded on the following days after the handling. Blood samples were analysed for 14 blood biochemistry parameters bimonthly using a VetTest® Chemistry Analyzer. The final temperament score was obtained through the summation of the total score of the stress response of each animal which ranges from 0 to 17, while the social interactions were analysed in DomiCalc. Generalised Linear Mixed Models were designed to test the effects of temperament and social rank on the blood biochemistry parameters while considering sex, age, body condition score, and body weight as covariates with a significant level taken at 0.05. All data were analysed using the IBM SPSS software (version 28). The results showed that handling influences blood biochemistry parameters including ALB, ALKP, BUN, GLU, TBIL, and TP but social rank did not have an influence on blood biochemistry. The changes in the levels of the blood chemical parameters due to handling were still in their reference intervals implying the absence of any major pathology or adverse effects on normal physiology. Hence, it can be suggested that periodical handling of common eland can be performed in captivity. This can help towards the domestication of common eland and improve their welfare and management to a great extent in captivity.

Keywords: Stress, Handling, Social rank, Blood biochemistry, Temperament

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List of the abbreviations used in the thesis

ACTH- Adrenocorticotropic hormone

ALB- Albumin

ALKP- Alkaline phosphatase

ALP- Alkaline phosphatase

ALT- Alanine aminotransferase

BCS- Body condition score

BHB- Beta-hydroxybutyrate

BHV- 1- Bovine herpes virus-1

BUN- Blood urea nitrogen

Ca- Calcium

CHOL- Cholesterol

CRH- Corticoid releasing hormone

GAS- General adaptation syndrome

GH- Growth hormones

GLMM- Generalised linear mixed model

HPA- Hypothalamic pituitary axis

LDH- Lactate dehydrogenase

LDH- Lactate dehydrogenase

PCV- Packed cell volume

P- Phosphorus

PTH- Parathyroid hormone

TBIL- Total bilirubin

TP- Total protein

1. Introduction

Recurrent evaluation of the health status of a herd is an inevitable part of animal husbandry, and it is critical when the population is confined to an area that is not its natural habitat. Routine collection of blood samples and analysis of blood parameters is advisable to track the health record of an individual animal and the overall health status of the herd. For this purpose, handling the animals is necessary even though it is a short-term stressor. It is noted to provoke a stress response which can modify the normal physiology of the animals. Another factor that can produce stress over a period of time (long-term) in animals is their place in the hierarchy which is reported in red deer hinds (Ceacero et al. 2018). Social rank is crucial for some of the fundamental behaviours of social animals such as food resource procurement and mating opportunities not only in the wild but in captivity as well. Maintenance of the social rank requires social interactions which serve as a stressor in subordinate animals with low rank in the social hierarchy in virtually ubiquitous among all mammalian species (Blanchard et al. 2001).

The stress response is considered to be regulated mainly by nervous and endocrine systems as it is physiologically explained by an increase in glucocorticoid secretion by the hypothalamic-pituitary axis (HPA; Carlstead & Brown 2005). The stress of animal restraint while handling has been reported to affect the concentrations of different blood constituents (Spencer 1980; Panepinto et al. 1983; Farmer et al. 1991) whereas the social rank is also reported to affect the heamatological profile (Ceacero et al. 2018). For example, an increase in packed cell volume (PCV), and increased leukocytes in response to stress due to the actions of glucocorticoids such as cortisol. The biological responses, such as behavioural, immunological, and haematological changes due to stress factors are mostly used as indicators of stress in animals by complementing each other (Kumar et al. 2012). With that said, there is still a critical need to establish a connection between the stress response and the blood biochemistry parameters. These parameters might be changing alongside the haematological values as the chemical composition of blood is considerably under homeostatic control (Manston & Allen 1981) which is perturbed by stressors.

The clinical blood biochemistry profile is a valuable diagnostic tool that can be utilised to evaluate several body systems at a time. When used in conjunction with the history, and physical examination, the chemistry panel can be useful for confirming a diagnosis, determining the prognosis, planning therapeutic options, and monitoring response to treatment. The reference interval is based on a large number of samples acquired from healthy animals (the reference population) and is calculated theoretically to include 95% of the healthy population. Values obtained for blood biochemistry parameters are compared with the reference interval established for the species and interpreted accordingly by the veterinarian. The correct interpretation of the blood biochemistry test is crucial. Apparently, a value different from the normal value range does not have significant importance if it is not evaluated with specific clinical observations and combinations of other values. Nevertheless, it can be used to change the direction of the clinical diagnosis and also pinpoint the area, organ, or system of interest. Most often than not, it is a trial and error method to reach a conclusion that the deviation (either increase or decrease in the relative amount of a blood constituent) is responsible for the disturbance in the equilibrium of health of the animal.

The common eland (*Taurotragus oryx*) has been described as an ideal antelope for domestication due to its large body mass and ease of habituation to routine handling (Pennington et al. 2013; Musa et al. 2021), with current management practices similar to those of cattle (Scherf 2000). In captivity, they are handled fairly regularly for biological sample collection which is a short-term stressor. In addition, they have a complex dynamic social hierarchy as a long-term stressor. Both stressors can affect the blood chemistry profile as explained before. Unfortunately, only a limited number of studies have been performed on the blood and metabolic profile of wild animals and subsequent reference values for different species available are only a few. As it is quite challenging under free-ranging conditions with the use of chemical immobilisation, utilisation of captive herd allows us to control and/or describe many confounding factors for the experiments. So far, not many published studies have focused on the blood biochemistry of common eland (Pospisil et al. 1984; Vahala et al. 1989; Silberova et al. 2010). In addition, the studies performed had different testing methods, conditions, and small sample sizes. This leads to discrepancies in the available data for reference. Hence, there is a critical need to establish and standardize reference intervals (normal reference value range) of the blood biochemistry parameters for common elands in captivity. Also, the effects of stressors, such as handling for the short term and social rank for longer periods of time, on the blood chemistry profile have not been assessed in common elands till now.

In captivity, it is necessary to assess and monitor the standard of animal welfare which can indirectly suggest the assessment and monitoring of stress and stressors that affect the animals. The proposed study identifies the effects of short- and long-term stressors on the blood biochemistry profile of common eland as these stressors help understand the efficiency of domestication and put light on the areas of improvement in the clinical management aspect in captivity.

2. Literature review

2.1. Stress and stress physiology

All living organisms maintain a complex dynamic equilibrium or homeostasis which is essential for life and well-being (Chrousos 2009). This homeostasis is continuously challenged by internal or external adverse means collectively called stress. Stress is an external condition or event that puts a strain on a biological system, leading to many adverse outcomes, ranging from a little discomfort to even mortality (Kumar et al. 2012). It covers the behavioural and biological responses to a wide range of insults to homeostasis that originate from various types of sources. It also refers to behavioural and physiological changes, such as elevated cortisol levels associated with negative emotions (Terlouw 2015).

Previously, several general aspects of animal stress physiology have been established (Selye 1946; Bijlsma & Loeschcke 2005; Boonstra 2013) and are being continuously tested. It is generally believed that stress response is mainly aided by hormones and the nervous system. Scientists have discovered that the response to stressful stimuli is elaborated and triggered by the stress system (Figure 1.). This system is made up of a wide diversity of brain structures that, collectively, are capable of detecting events and interpreting them as either real or potential threats (Dedovic et al. 2009). The amygdala is a highly conserved brain structure that is fundamental to identifying potential danger (Janak & Tye 2015), while the hippocampus delivers support to encoding environmental information associated with the stressor (Herman et al. 2005). In addition, the pre-frontal cortex provides links between cues and stressors (Milad & Quirk 2012).

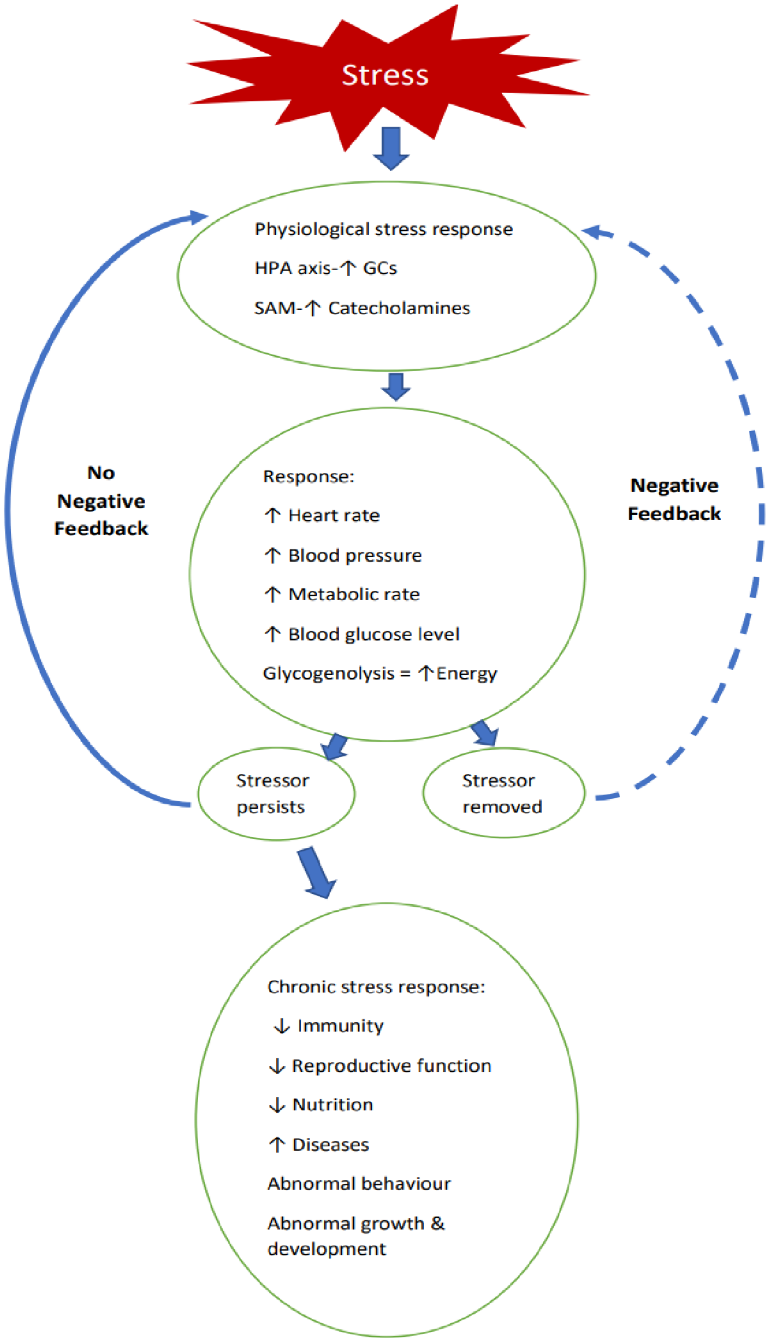

Figure 1. Schematic presentation of stress response system working in the presence of acute and chronic stress. Arrows are showing a feedback loop for the HPA-axis deactivation (Adapted from Narayan 2019).

Hormonal changes due to stress are well-accepted to assess stress in humans as well as veterinary endocrinology as they include various endocrinal responses to a stimulus that causes stress. Initiation of activation of the hypothalamic-pituitary-adrenal

(HPA) axis happens due to disruption of an animal's homeostasis (Figure 1.), which generally results in some form of exertion by the body (Whirledge & Cidlowski 2010; Hing et al. 2014). The hypothalamus releases corticotrophin-releasing hormone (CRH; Vale et al. 1981), thereby signalling the anterior pituitary to release adrenocorticotrophic hormone (ACTH; Whirledge & Cidlowski 2010) which circulates in the blood resulting in an increased output of glucocorticoids from the adrenal cortex. Glucocorticoids, cortisol in particular, are critical for larger vertebrates to act for the liberation of energy through gluconeogenesis. Cortisol essentially acts by diverting the storage of glucose (GLU) away from glycogen or fat and mobilising GLU from stored glycogen. Thus, this available energy is used in preparation for a physical challenge and is used for regaining homeostasis (Narayan 2019). Following the stress response, cortisol acts to bring about the pH balance, as a blocker within a negative feedback process to CRH secretion and induces the animal to restock the energy stores and restore homeostasis (Romero 2004).

If the stressors are not removed as it is the case for chronic stress, it leads to deleterious downstream effects due to extended activation of the stress response. It can result in reduced immunity, impaired normal reproductive function, tissue atrophy, and abnormal growth rate (Figure 1.). It can also lead to abnormal behaviours such as stereotypies (Meehan et al. 2004). Selye (1946) while working on the biochemical and pathological effects produced by noxious agents of any nature in an organism, found that the body manifests itself with a few nonspecific systemic reactions towards the agents, which are termed General Adaptation Syndrome (GAS). For example, the body develops musculature following a prolonged period of physical exercise. Furthermore, he noted that if the organism was continuously bombed by the same noxious stimuli, which is chronic stress, the resulting GAS developed in three distinct stages, namely the alarm reaction, the resistance stage, and the exhaustion stage.

2.2. Stressors

A stressor can be any physical or psychological factor that perturbs or threatens to perturb homeostasis (Sapolsky 2004). Physiologically, an event such as negative emotions or an object that can produce elevated cortisol secretion above the normal basal level can be recognized as a stressor. Stressors can have a physical or psychological origin, such as food and water deprivation, fatigue due to transport, or pain due to collisions with equipment, threat from other animals within the same social group, or human interventions (Terlouw 2015). They can also initiate from within an individual (endogenous) or the environment (exogenous). Most but not all the stimuli that cause stress are essentially painful but the psychological states, such as fear, or anxiety also can activate an array of physiological responses. It can be psychological, for example, moving to new surroundings. Depending on the context, responses to a stressor may be managed by either physiological or behavioural modulation (Johnstone et al. 2012). In this regard, stressors can generally be categorised into short- and long-term stressors based on the duration they acquire to generate a stress response.

2.2.1. Short-term stressors

Short-term stressors can also be called acute stressors. They are sudden events or effects that disrupt the normal state of well-being of the animals and generate the phenomenon of stress. These stressors act for minutes to hours on animals and acute stress responses may last from a few minutes after the beginning of the stress to a few days (Horowitz 2001). They result in increased cardiovascular tone and spleen contraction to release more red blood cells in animals for transportation of more oxygen to the organs in an effort to respond to the stimuli and balance the homeostasis. Almost but not every time, increased respiration adjoined with high blood pressure is measured and with significant peripheral vasoconstriction. The acute stress response consists of a combination of physiological and behavioural changes that are thought to help an animal survive in the wild (Sapolsky et al. 2000).

Handling animals is an example of short-term stress. Indications for the handling of elands may include veterinary care, application of reproduction techniques, sample collection such as blood, faeces, and hair, moving them from one pen to another for cleaning or feeding, transportation, human presence, and slaughter (Hemsworth 2003). This ignites a short but very strong and measurable reaction leading to stimulation of HPA (Collier & Gebremedhin 2015). Previously, researchers have found that the value of the heamatological and chemical parameters of wild animals depends in part on the physiological effects of capture and handling techniques (Franzmann & Thorn 1970; Franzmann 1972; LeResche et al. 1974; Seal 1974). For example, sodium levels in the blood were reported to be affected by handling in beef cattle (Gartner et al. 1965) and in reindeer (Hyvarinen et al. 1976). In addition, the short term changes in GLU and phosphorus (P) values are indicative of the initial handling stress followed by a return to normal state in woodland caribou (Karns & Chrichton 1978).

Common eland are characteristically calm animals, except in situations when poorly handled or exposed to novel situations, then they tend to be flighty in response to the influence of such stressors (Pennington et al. 2013). While farming eland in captivity, it is thus necessary to habituate them to handling. Being careful that forceful handling can result in a serious injury (Bergvall et al. 2017), their handling should be done in a specialized system such as a handling chute. Producing animals with docile temperaments on the farm and in captivity is necessary as it affects important production parameters including average daily gain (Voisinet et al. 1997), and milk production (Sutherland & Dowling 2014), meat quality (Fordyce et al. 1988), and their overall welfare. Hence, it becomes important to understand the variations in the blood biochemistry profile, which is less studied till date, to examine the effects of handling to better manage the common elands in the captivity.

Temperament is an essential qualitative management parameter, particularly for large ungulates such as common eland, which are challenging to handle in comparison to small ungulates (Rice et al. 2016). Fordyce et al. (1982) defined temperament as the behavioural, stress and physiological responses of an animal to being handled by humans, while Koolhaas et al. (2010) described temperament as the response patterns in reaction to a stressor such as handling. Generally, temperament covers the idea that individual behavioural patterns are repeatable over time and across situations, and thus includes numerous traits, such as sociality, aggressiveness, avoidance of novelty, and aversion to taking risks such as entering inside the chute (Reale et al. 2007). Recording the temperament when being handled is useful to assess the ease of handling (Pennington et al. 2013; Schütz et al. 2016; Parhama et al. 2019) which indirectly assesses the level of stress and the degree of habituation. In the study of Musa et al. (2021) on common eland, habituation was found to positively influence the temperament and ease of handling. Typically used parameters for temperament recording include escape velocity, flight distance, and chute scores during handling. These parameters have produced 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). Although there are some factors that may affect the strength of the reaction of the animal body to handling, such as intensity of handling, duration of handling, time of handling, familiarization of the personnel and equipment used, etc. The animal factors that might affect handling can be age, sex and body condition score (BCS).

2.2.2. Long-term stressors

Long-term stressors are also known as chronic stressors. As the name suggests, they are present for a long time to exert their effects and trigger a response that is measurable. The chronic response to stress is controlled by the endocrine system and is related to an altered population of receptors, changing the sensitivity of tissues leading to homeostatic signals, and resulting in a completely new physiologic state (Bligh 1976; Bauman & Currie 1980). Kiley-Worthington (1978) and Hillman (1987) suggested elands are not social animals. As per their observations, apart from mating and mother-infant interactions, elands rarely interact with herd mates. But in the wild, they were observed to get together to collectively defend against predators (Kingdon 1982) and eland cows were seldom seen alone (Kingdon 1982; Spinage 1986; Hillman 1987). Wirtu et al. (2004) confirmed in their studies that there is a higher level of interaction between female eland herd mates than was previously estimated.

In animal societies individuals often engage in aggressive interactions with each other. With identifications of winners and losers, such interactions are usually referred to as dominant–subordinate interactions. Based on who wins against whom, ranks can be established for all or most individuals 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). Here, linearity essentially means that the top ranked individual is dominant over all other individuals, second-highest ranked individual is dominant over all individuals besides the top ranked individual and so on, with the least-ranked individual being dominated by (i.e., subordinate to) all others (Schmid & de Vries, 2013). Gregarious animals such as common eland, engage in social interactions, which leads to the establishment of a dominance hierarchy. Presence of a dominance hierarchy in a captive eland herd was noted by Kiley-Worthington (1978) but was indicated as not a “rigid” one. But after a few years, a study on elands that were kept in a reserve alongside five different species of antelopes showed clear intraspecific dominance hierarchies (Cransac & Aulagnier 1996). Wirtu et al. 2004 confirmed a predominantly linear but complex and dynamic dominance hierarchy in the elands. Access to food sources, space, and mating opportunities is determined by the place of animals in the hierarchy (Wirtu et al. 2004). Subordinate animals with lower ranks can be under stress because of the aggressive interactions leading to the establishment and maintenance of hierarchies (Thompson 1993; Clarke & Faulkes 1997; Grandin 1999). Biochemical, physiological, behavioural, and anatomical parameters including reproductive performance and overall health can be affected by social rank. For example, in red deer hinds, social rank influences heamatological parameters such as white blood cell counts, haemoglobin and haematocrit (Ceacero et al. 2018). An animal's social rank within the group can also affect stress levels. This can be reflected in the changes in the normal physiological activities and hence in the values of the blood chemistry parameters. Interestingly, is also noted to affect overall health and reproductive performance in many mammals (Arave & Albright 1976; Clarke & Faulkes 1997; Ellis 1995; Morell 1996; Flint et al. 1997a, b; Pedersen et al. 2003; Phillips & Rind 2002).

2.2.3. Effect of stress on animals

Since early 2000s, many researchers have investigated the impact of stress on the physiology of animals. Successful attempts have been made to find a relation between stress and growth, production, and reproduction of various species of animals. Das et al. (2001) reported physiological changes such as electrolyte imbalances, increased breathing and heart rate, dehydration, energy deficits, and related catabolism during transportation that resulted in activation of stress hormones and decreased production. Kannan et al. (2002) in their research on meat goats found that stress such as transportation adversely affects body weight. Increased glucocorticoids stimulate the hypothalamic secretion of somatostatin in response to stress and inhibit the secretion of growth hormones (GH) from the anterior pituitary gland. Lower levels of GH were found to lead to reduced growth.

Another factor that affects growth and production is heat stress. Overall milk production in lactating cows and protein content in the milk decreases in the presence of heat stress (Ravagnolo & Mitzal 2000). A study in beef cattle by Mitlöhner et al. (2001) found that high ambient air temperatures and sunlight have a negative impact on the intake of dry matter, the average daily gain, the weight of the carcass, and the thickness of fat. In heat-stressed pigs, the milk yield of the sow decreases due to reduced feed intake; hence, the growth, viability and survival of the piglets also decline (Renaudeau et al. 2004). Exploring the meat production aspect, a study showed a decrease in growth, fat content and bacon content in pigs living at temperatures above the thermoneutral zone (White et al. 2008).

The effects of stress on reproduction depend on the type of stress, animal genetic predisposition, time, and duration of stress (Kumar et al. 2012). Reproductive pathologies such as infertility, reduced conception rates and defective oocytes have been reported to have occurred as a result of stress such as malnutrition, exertion, and infection. For example, lactating dairy cows experience lowered conception rates from 40-60% to 10-20% due to heat stress in summer (Wolfenson et al. 2000). Wilson et al. (1998) noted that the ovarian follicle in cattle can get damaged by heat stress, and it disrupts the oestradiol synthesis. Similarly, to humans, where maternal prenatal stress may cause excess activity and/or dysregulation of the HPA-system in offspring (Mulder et al. 2002), it results in an increased incidence of spontaneous abortion, preterm foetus expulsion and low birth weight in livestock (Kumar et al. 2012). In bulls, stress causes interference with sperm production, reduces the fertility parameters of the sperm, and interferes with follicle growth because of the interference of transportation of gametes and reduced blood flow by the catecholamines (Breen et al. 2007).

Presence of an infectious organism in the body is considered as stress as it disturbs homeostasis is to a great extent and renders animals susceptible to other infections. In many species, the risk of fatal respiratory bacterial infections is increased following primary viral infections (Hodgson et al. 2005). For example, bovine respiratory disease is usually caused by primary viral infections (BHV-1, commonly known as bovine herpes virus), and secondary bacterial infections with *Mannheimia haemolytica* (Palok et al. 2009). Pigs are susceptible to three different but closely related stress syndromes namely, porcine stress syndrome, malignant hyperthermia, and black muscle necrosis (Kumar et al. 2012). In addition, the work by Veasey (2006) on elephants suggests that there are other factors and phenomena associated with the response, such as the source and the duration of stress.

2.2.4. Biochemical markers indicating stress

Various biochemical indicators give clues about the effects of short- and long- term stress on the animals. Increase in non-esterified fatty acids, beta-hydroxybutyrate (BHB), urea, and a decrease in glucose levels which indicate food deprivation are noted due to acute stress (Sapolsky et al. 2000; Broom 2003). Broom (2003) also noted an increase in packed cell volume (PCV), total protein (TP) and albumin (ALB) which suggest dehydration with or without the presence of haemoconcentration. Furthermore, Lay and Wilson (2004) and Broom (2003) reported elevated creatine kinase (CK), lactate and lactate dehydrogenase (LDH) due to physical exertion for a short period of time. Interestingly fear and arousal also produces higher PCV, glucose, BHB and urea levels in cattle (Lay & Wilson 2004; Broom 2003).

Chronic stress can produce prolonged negative energy balance and increased glycated proteins such as haemoglobin and fructosamine (Tahara & Shima 1995; Kelly et al. 1997). Various studies (Colditz 2002; Arthington et al. 2003, 2005; Murata et al. 2004; Gruys et al. 2005; Bionaz et al. 2007; Piñeiro et al. 2007; Bertoni et al. 2008; Lomborg et al. 2008) noted an increase in the disease markers such as positive acute phase proteins (e.g. haptoglobin, ceruloplasmin) and a decrease in the negative acute phase proteins (e.g. albumin, lipoprotein) due to long term stress. Furthermore, a decline in serum potassium levels (Trevisi et al. 1992; Halperin & Kamel 1998) and an elevation in alkaline phosphatase (ALP) levels (Vazhapilly et al. 1992; Tuchscherer et al. 1998; Calamari et al. 2003; Abeni et al. 2007) was also reported due to chronic stress.

2.3. Blood biochemistry profile

Generally, it is challenging to obtain blood biochemical values for wild animals, because capture stress and the drugs administered for the restraining and capture can potentially change or influence the values (Chao et al. 1984; Kock et al. 1987). Due to this difficulty, only a limited number of studies have been performed on the blood and metabolic profile of wild animals and subsequent reference values for different species available are only a few. On the contrary, a good amount of data is available from the zoo animals although without clear demarcation of the methods of immobilisation. Common eland are quite large animals and are hard to capture and restrain in the wild. They mostly require darting in the field for their capture to avoid any incidental injuries to personnel and themselves. In captivity, they maintain their natural prey instincts and are still considered undomesticated. Wirtu et al. (2005) and Allan (2015) pointed toward chemical immobilization of common eland for routine management procedures and biological sample collection. Although extremely useful, chemical immobilization requires specific expertise and is not cost-effective. Most importantly, it affects normal physiology and can even lead to mortalities (Loskutoff & Betteridge 1992). Musa et al. (2021) in their study used a combination of the squeeze chute system and behavioural training successfully, which avoids the problems while the routine collection of biological samples such as blood and faeces for further evaluation of their health status.

Blood chemistry holds importance in the diagnosis, treatment, and prognosis of diseases. Blood parameters are usually studied in domesticated ruminants including cattle, goats, and sheep (Kida 2003; Caldeira et al. 2007a, b; Watanabe 2010). Most studies on blood biochemistry are concentrated on dairy cattle and they are established as tests to diagnose the metabolic status of a dairy animal (Kida 2003), which are also known as blood metabolic/chemistry profiles. Many factors such as species, breed, sex, age, malnutrition, illness, reproductive status, and seasonal variations (i.e., pregnancy), can affect the blood chemistry profile (Pernthaner et al. 1993; Nazifi et al. 2003; Swanson et al. 2004) that can be identified in the laboratory. For example, variation in protein metabolism can be identified with the measurement of blood metabolites such as Albumin (ALB) and Blood Urea Nitrogen (BUN). Furthermore, blood metabolites such as cholesterol (CHOL), P, triglycerides, and globulin (GLB) can be utilized to predict and interpret the variability of animal feeding (Adachi et al. 1997; Kida 2003).

Not many published studies have focused on the blood biochemistry of common elands (Pospisil et al. 1984; Vahala et al. 1989; Sliberova et al. 2010). In addition, the studies performed had different testing methods, conditions, and minimal numbers of animals. For example, Vahala et al. (1989) used chemicals to immobilise the animals in their study, and the animals selected were over one year of age, but the age range or body weight was not specified.

To interpret biochemical data correctly, the results obtained in the laboratory must be compared with values corresponding to that of healthy animals, the so-called reference values. Clinical pathology reference intervals are one of the most valuable diagnostic tools in veterinary medicine and are used to help differentiate diseased from healthy individuals (Solberg & Petitclerc 1987; Petitclerc & Solberg 1988). Although care should be taken while interpreting the values as the blood parameter reference is influenced by various factors such as nutrition, age, method of capture, season, and current health status of the animals (Marco & Lavin 1999).

2.3.1. Total Proteins, Albumin, and Globulin

Proteins account for a large amount of the dissolved substances in plasma/serum. It has been suggested (Anderson & Anderson 2002) that virtually all diseases affect the proteins found in serum. Total protein and Albumin (ALB) are utilized for metabolic profile tests measuring protein metabolism and determining conditions such as hepatic and renal function, and degree of hydration (Russell & Roussel 2007; IDEXX 2014). Globulins (GLOB) and albumin to globulin ratio (A: G) are calculated from ALB and TP values. Total protein levels can be affected by various causes which are listed in Table 1.

Table 1. Different causes of dysproteinaemias in animals.

Hyperproteinaemia	Hypoproteinaemia
Haemoconcentration	Blood loss
Inflammatory diseases:	Protein-losing nephropathy
Infectious: bacterial, protozoal, viral, fungal	Protein-losing enteropathy
Non-infectious: immune-mediated diseases, neoplasia	Hepatic insufficiency
	Malabsorption and maldigestion

Albumin is a globular protein synthesized in the liver. It is catabolized in various tissues where it is taken up by pinocytosis. It is 35-50% of total plasma proteins by weight which is the largest proportion of all the plasma proteins. ALB makes a large contribution to plasma colloid osmotic pressure and in transporting endogenous and exogenous compounds (Russell & Roussel 2007; IDEXX 2014). Plasma half-life of ALB varies among species (e.g., in humans it is 19 days (Merlot et al. 2014) and tends to increase with body size. Being more anionic (negatively charged at physiological pH), albumin also transports positively charged minerals, such as calcium, magnesium, zinc, and copper. (eClinpath 2020a) addition to being a carrier for molecules, albumin is also believed to be an antioxidant protein by scavenging reactive oxygen species, protecting bound substances from oxidant injury, and binding free copper, which acts as an oxidant (Merlot et al. 2014). Relative value changes of ALB are of clinical significance for animal health. An increased albumin concentration (hyperalbuminemia) is most observed due to dehydration or secondary blood volume concentration due to fluid loss. The pathophysiological causes of the increase in ALB can include adrenal dysfunction (Cooper et al. 2009) and hepatocellular carcinoma (Cortright et al. 2014) whereas hypoalbuminemia occurs mostly due to excessive fluid administration. In humans, ALB has been advocated as a highly sensitive marker for an individual patient's nutritional status (Chang & Holcomb 2016).

Globulins (GLB) value on the chemistry panel is calculated by the following equation: GLBs = TP - ALB (considering all other proteins than ALB as globulins; IDEXX, 2014). Electrophoretic mobility classifies globulins into α (α-1, α-2), β (β-1, β-2) and γ-GLBs. γ-GLBs mostly consist of immunoglobulins that are synthesized by lymphoid cells, and other GLBs are synthesized by the liver. The increased concentration of GLBs is known as hyperglobulinemia which is commonly caused by chronic antigenic stimulation and hepatic disease (Russell & Roussel 2007). An increase in α and β globulins with a particular increase in α-2 GLBs occurs as they are acute phase reactants following various types of tissue injuries such as physical trauma, inflammation, or infection of a bacterial or viral etiology. An elevated γ-GLBs level is of particular significance when there is an active immune response leading to a polyclonal gammopathy (eClinpath 2020b). In addition, neoplasia and nephrotic syndrome are frequently associated with hyperglobulinemia. Whereas hypoglobulinemia (decreased level of GLBs) can be a result of inherited or acquired immunodeficiency in many cases.

2.3.2. Blood Urea Nitrogen / Urea

BUN or urea is the main form in which nitrogen is eliminated from the body in mammals (Kaneko et al. 2008). It is a nitrogenous waste formed in the liver as the end product of protein breakdown (Figure 2.). After synthesis, it is distributed into the blood for transportation to the kidneys (Schloerb 1960; Dunegan et al. 1978). This is the sample point where BUN concentration is measured in the blood sample. Elimination of urea from the body in urine is completed after glomerular filtration in the kidneys (Russell & Roussel 2007; IDEXX, 2014). Production of urea is influenced by diet and hepatic function (Russell & Roussel 2007) as the liver is the main production site. Also, in hepatopathies, hyperammonaemia is observed as it cannot be converted to urea for excretion. On the other hand, urea concentration in the kidneys is elevated during water insufficiency or dehydration (Barboza et al. 2009). Hence, it can indirectly suggest glomerular filtration rate or renal efficiency.

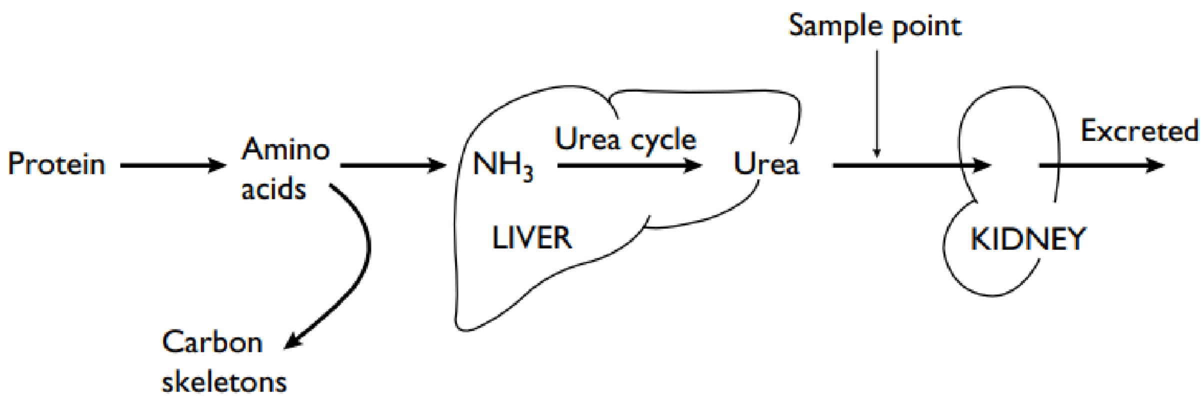

Figure 2. Process of blood urea production in kidneys by protein catabolism (Kerr 2002).

2.3.3. Creatinine

Creatinine, similar to BUN is also a nitrogenous waste product. Although it is a product of the breakdown of creatine. Present largely in skeletal muscles (Perrone et al. 1992; Wyss & Kaddurah-Daouk 2000; Braun et al. 2003), creatine is a substance involved in energy metabolism for stabilizing high-energy phosphate bonds (Adenosine triphosphate or ATP) which are not required for immediate utilisation. Creatine is synthesized in the liver and then taken up by the muscles where it is reversibly converted into creatine-phosphate by creatine-kinase (CK) with phosphorylation (Figure 3).

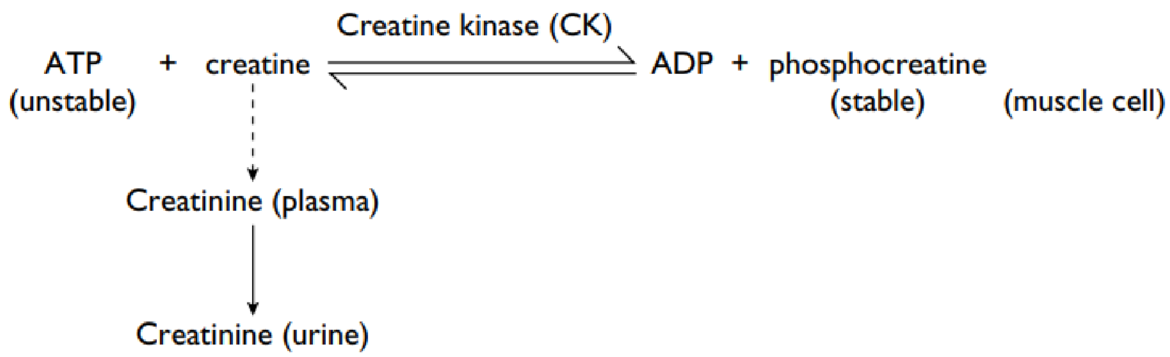

Figure 3. Schematic presentation of reversible process of CREA production (Kerr 2002).

2.3.4. Enzymes

A group of proteins that catalyses the chemical reactions are known as enzymes. Most of the enzymes are exogenous to serum because they are of cellular origin. They are present in different cell components such as mitochondria, cytoplasm, or cell membrane. Upon the breaking of the protective membrane, they enter the serum circulation. Their presence in the serum of healthy animals does not always show a pathology; rather, it is a normal physiological process. The purpose of the study by Boyd (1983) was to identify the mechanisms related to deviations in isoenzymes and plasma enzymes in different diseases in animals. The results indicated that the increase in serum activity is the result of the imbalance between the levels of enzymes entering plasma and their removal or inactivation.

2.3.4.1. Alanine aminotransferase

Alanine aminotransferase, formerly known as the serum glutamate pyruvate transaminase or SGPT, acts as a catalyst for the transamination of pyruvate and L-glutamate from 2-oxoglutarate and L-alanine, which is a reversible process. Hepatocellular inflammation and necrosis present with the highest serum ALT increase (Center 2007). Despite being found in various organs, the magnitude of activity of ALT varies significantly even within species. According to various studies, (Clampitt & Hart 1978; Keller 1981; Zinkl et al. 1971), the liver of dogs showed almost 4 times higher ALT activities per gram than other body organs, although both the skeletal and heart muscles had significant ALT activity. Interestingly, cats present similar findings as dogs but large animals such as horses and ruminants such as cattle and pigs had different liver and muscle ALT values. (eClinpath 2020c) This led to the conclusion that, based on tissue ALT concentration, increased serum ALT is not an indication of liver injury in horses and cattle, but it fairly indicates liver damage in domestic canines and felines. Interestingly, some animals with severe hepatic diseases show apparently normal serum ALT levels (Kaneko et al. 2008). Following vigorous exercise in dogs, transaminase such as ALT showed increased serum activity (Valentine et al. 1990), but the origin of the enzyme is not identified (Loegering & Critz 1971).

2.3.4.2. Alkaline phosphatase

Alkaline phosphatase contains a group of heterogeneous enzymes that work at alkaline pH to catalyze the hydrolysis of monophosphate esters (Syakalima et al. 1998). Most of the ALP in apparently healthy animals is derived from the liver and bone (Hoffmann & Dorner 1975; Rogers 1976) and is widely distributed in mammalian cells. In serum, it is presented as a soluble form but is also available in cellular membranes throughout the body (Fernandez & Beverly 2007). Different isoenzymes are encoded by different loci of genes. Two isoenzymes are available in domestic animals, namely, tissue-specific isoenzyme and intestinal isoenzyme, which are encoded by two loci of genes (Hank et al. 1993). An increase in serum ALP concentration is generally attributed to a liver disorder such as cholestasis which can be due to frequently identified hepatopathies including but not limited to tumors, inflammation, fibrosis, endocrine disorders, and choleliths. For example, various canine cholestatic liver diseases show increased serum AP, which has been confirmed (Abdelkader & Hauge 1986; Center et al. 1985; Hoe & Jabara 1967; Solter & Hoffmann 1995, 1999). Another important factor for elevated serum ALP has significantly increased osteoblast activity. Noonan and Meyer (1979) found that serum ALP increases modestly with hepatic necrosis. Anecdotally, in cholestasis, increases in serum ALP are followed by increases in bilirubin. The diagnostic importance of serum ALP value with respect to liver diseases is limited in large domestic animals such as cattle, due to its wide distribution in various tissues such as the kidneys and intestines and the rare occurrence of cholestasis in large animals (eClinpath 2020d). Hence, it must only be considered significant along with the presence of other specific indicators of liver damage in large animals.

2.3.4.3. Lactate Dehydrogenase

LDH has been the most thoroughly investigated among isoenzymes. It catalyses the process of reversible interconversion of lactate and pyruvate. Structurally a tetramer, LDH is made up of two subunits which are H (heart) and M(muscle). Various combinations of these two subunits result in five different isoenzymes namely, LDH1, LDH2, LDH3, LDH4, and LDH5 which are separated by electrophoresis. LDH5 has the slowest electrophoretic mobility while LDH1 has the fastest (Kerr 2002). Many studies have emphasized on LDH isoenzymes in the tissues of several other species including cattle (Keller & Stanbridge 1972), sheep (Beatty & Doxey 1983), horses (Thornton & Lohni 1979), and cats (Nilkumhang & Thornton 1979). The immense diagnostic importance of LDH and its isoenzymes is acknowledged in liver and heart-related disorders (Satyanarayana & Chakrapani 2007). LDH1 is considered a useful tool when diagnosing heart disease (Kerr 2002). LDH1 and LDH2 are increased in cattle and sheep in a hepatocellular injury and whereas LDH5 elevates in horses and small animals and is considered one of the indicators of liver disease for them (eClinpath 2020e). Hemolysis must be prevented before measuring the LDH as RBCs have almost 80-100 times higher activity of LDH than serum LDH and it can cause false positives. Also, routine biochemistry panels only offer the total LDH value and not differentiated isoenzyme values rendering the test with less specificity for either skeletal muscle or hepatic disease (Kerr 2002). In summary, LDH can point toward a muscle or hepatic problem but cannot lead to the origin.

2.3.4.4. Amylase

Amylase is responsible for the dietary breakdown of starch and glycogen to a simple sugar molecule, maltose. The main sites for amylase include the pancreas and salivary glands. Normal serum amylase activity can be mostly contributed directly by the amylase production from the liver and intestine (Murtaugh & Jacobs 1985; Nothman & Callow 1971). But Simpson et al. (1991) in their experiments on dogs found a 50% decrease in serum amylase when pancreatectomy was performed. Hence, it proved the assumption of non-pancreatic sources of some serum amylase. Routine serum amylase testing has significance as a screening test for acute pancreatitis mostly in dogs. But on the contrary in cats, Kitchell et al. (1996) noted that in experimentally induced pancreatitis the serum amylase activity decreases, and it renders the testing less important in diagnosis.

2.3.5. Minerals

2.3.5.1. Calcium and Phosphorus

Total serum Ca exists in three forms which are namely ionized, protein-bound, and chelated. Ionized Ca is the most important and biologically active fraction which is around 50% of total serum Ca. Protein-bound Ca represents approximately 40% of total serum Ca. Albumin is the major protein to which Ca is bound in serum. Chelated or complexed Ca is formed largely with phosphate, sulphate, citrate, bicarbonate, and lactate anions, and accounts for approximately 10% of total serum Ca (Ferguson 2011). Calcium is of major importance in transmission at the neuromuscular junction and in the propagation of contraction impulses within the muscle. It is an essential element that is involved in many body systems including the skeleton, enzyme activation, muscle metabolism, blood coagulation, and osmoregulation (IDEXX 2014). Three hormones namely, calcitriol, parathyroid hormone (PTH) and calcitonin are the major factors that regulate blood/plasma Ca (Figure 4.; Satyanarayana & Chakrapani 2007). The main organs involved in calcium homeostasis are the intestines and kidneys.

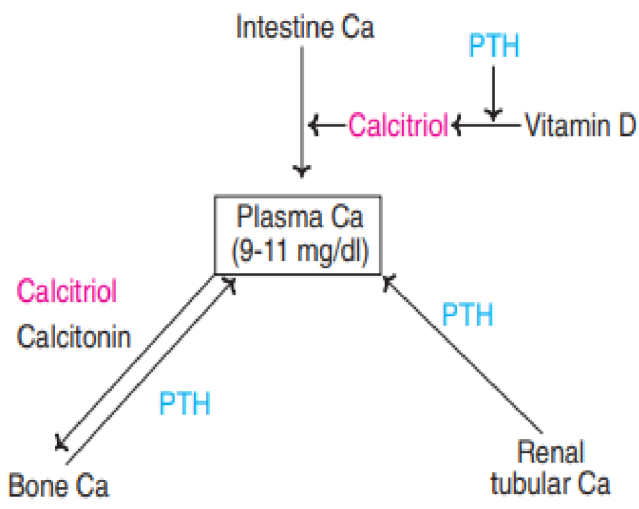

Figure 4. Overview of homeostasis of Ca (extracted from Satyanarayana & Chakrapani 2007), PTH- Parathyroid hormone.

Serum or plasma inorganic P only represents only a tiny portion of phosphate in the body. Most of the body's P is stored in bone in the form of hydroxyapatite and calcium phosphate. Hence, serum or plasma inorganic phosphate cannot be considered a good indicator of total body stores, but it is considered a good marker of extracellular phosphate (i.e., that is immediately available to cells), which only comprises approximately 1% of total body phosphate (the rest being in soft tissues, such as skeletal muscle). Phosphate is used in many of cellular processes. It is a fundamental component of phospholipid membranes, and metabolic proteins (e.g., ATP, glycolytic pathways; eClinpath 2020f). Phosphorus and Ca are absorbed in the small intestine. Absorption is influenced by the presence of other minerals, nutrients, vitamins, and intestinal pH. Calcium and P metabolisms are interdependent (IDEXX 2014).

2.3.6. Glucose

Glucose is the principal source of energy in animals (IDEXX 2014) which is obtained from food exogenously and from liver glycogen storage, fat, and proteins endogenously by conversion. (Figure 5.). The circulating concentration in the healthy animal is maintained within narrow limits with the help of various hormones including insulin, glucocorticoids, glucagon, adrenaline, and growth hormone. The concentration of cortisol after psychological stress correlates with the concentration of glucose in the blood, indicating that the response to stress is enhanced by an increased concentration of glucose (Kerr 2002). Kidneys primarily deal with blood glucose levels by allowing all the plasma glucose to be filtered and then reabsorbed by proximal tubules.

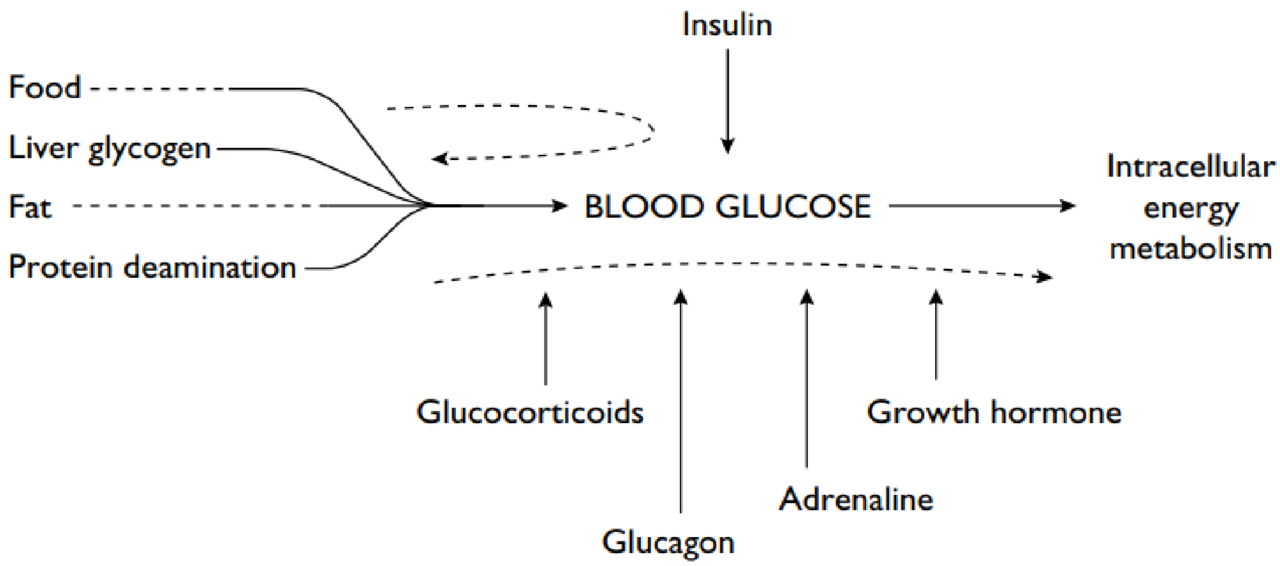

Figure 5. Schematic diagram of different hormones regulating blood glucose levels (Kerr 2002).

2.3.7. Total bilirubin

Bilirubin is a by-product of haem breakdown. In its initial form in the plasma, it is not water-soluble and is bound to albumin. It is further transported to the liver by the reticuloendothelial system where it is rendered soluble by conjugation with glucuronic acid. The bilirubin conjugate is then eliminated in bile. The brown colour of faeces is due to the pigments (mainly stercobilin) present in the bile. Conjugated and unconjugated bilirubin sum up together to make TBIL value (Kerr 2002). It is widely used as a diagnostic marker for hepatic function and cholestasis. During intravascular haemolysis, large numbers of erythrocytes get destroyed instantly and the conjugation mechanism in the liver becomes overloaded so that high concentrations of free bilirubin are found in the blood. Hence, it is also useful to check the presence of intravascular haemolysis (IDEXX 2014).

2.3.8. Cholesterol

Cholesterol is found only in animals (Satyanarayana & Chakrapani 2007) and is not present in plants or other microorganisms. Cholesterol is the precursor of steroid hormones, vitamin D, and bile acids. It is also a constituent of cell membranes. Cholesterol can be gained from the diet if it contains animal products, or it can be synthesized in the liver which is the main organ for CHOL synthesis and catabolism (Bruss 2008). Absorption site for CHOL is the intestines. Steroidogenic endocrine organs (adrenal cortex, testis, ovary, placenta) can also produce small amounts of cholesterol; however, these organs utilize hepatically synthesized cholesterol for most of their steroid synthesis (Pedersen 1988). Cholesterol is present in abundance in nervous tissues, and it functions as an insulating cover for the transmission of electrical impulses in the nervous tissue (Satyanarayana & Chakrapani 2007).

3. Aims of the thesis

The goal of this thesis was to describe the influence of short- and long-term stressors on the blood biochemistry of common elands (*Taurotragus oryx)*.

Few studies have explored the blood biochemistry profile of common elands in an effort to determine and standardise reference intervals of various parameters. This study was aimed at examining the changes in blood biochemistry parameters of common eland in relation to their exposure to short- and long-term stressors.

The specific objectives of this study were:

1. To standardise available reference values of blood biochemistry parameters for common eland.
2. To examine the effects of short-term stressor on the blood biochemistry profile of common eland.
3. To examine the effects of long-term stressor on the blood biochemistry profile of common eland.

Hypothesis:

H_1: The short-term stressor will influence blood biochemistry parameters.

H_1: The long-term stressor will influence blood biochemistry parameters.

4. Methods and materials

4.1. Description of the experimental site

The research was conducted at the Czech University of Life Sciences Prague (CZU) Common Eland Research Facilities located at Lány (Central Bohemia region, Czech Republic, 13°57´ E, 50°07´ N). The farm consists of two separated open paddocks (grass area of 2.5 ha) and two enclosed barns with solid walls which were divided by a feeding alley. The study location was at an altitude of 421 m above sea level and had a temperate cold climate, which was characterised as fully humid with cool summers (Köppen–Geiger classification; updated by Rubel & Kottek 2010). Animals are housed inside the barn from December to April during which the environmental temperature was below the thermoneutral zone of eland (Kotrba et al. 2007). The internal temperature of the barn ranged from 2° to 10 °C. Though, animals are given free access to the paddock when there were abrupt changes in temperature as well as the remaining part of the year. The animals were always provided complete feed mixture diet consisting of 60% corn silage, 30% lucerne haylage, 7% meadow hay and 3% barley straw as well as mineral supplement (Barton et al. 2014), this were all provided *ad libitum* inside the barn throughout the year. A concentrate pelleted cattle feed (19% crude protein) was given routinely as supplement (Nutritional information in Needham et al. 2020). Automatic drinkers with unlimited access were also placed inside the barn.

Figure 6. Two covered barns separated by a feeding alley.

4.2. Data Collection

The Czech Republic Ministry of Agriculture accredited (clearance no. 63479_2016-MZE-17214) all experimental procedures involving husbandry, handling and treatment of the experimental 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 study was conducted from January 2020 to November 2020. Routine handling of animals was performed once a month for research and management purposes and the present study was planned in connection to the routine handling program. It involved blood collection, recording of the temperament, and the assessment of the social rank during the following days. A total of 12 animals (135.1±2.25kg initial body weight, 3.8±0.1 BCS, 9.2±1.5 months), females (n=6), and males (n=6) were chosen for the experiment. All 12 animals were accustomed to the routine handling since birth, and they were from the same age group and housed in the same pen. All the animals selected were regularly observed throughout the period of the experiment, and they were noted as healthy. During blood sample collection and social interaction observation, all animals were checked for any visible injuries that could potentially affect their behaviour as well as their handling. No animal was found to have any clinical signs of diseases or health problems, for example, a fractured limb, a bleeding wound, skin problem with lesions such as hair loss or discoloration, weakness, and swollen joints. None of the animals were being treated before and during the course of the experiment with any medicines that can alter the results of the experiment. They were kept under visual observation at least for one minute after their exit from the squeeze chute into the paddock and no abnormality was noted.

4.2.1. Temperament assessment and blood collection

A specially designed squeeze chute system with a raceway developed for routine handling and sample collection from eland and other large antelopes was utilized for temperament assessment and blood sample collection. The raceway was 11.60 m long ×1.05 m wide ×1.85 m high, and it was divided into four sections (A–D) by three galvanized sliding doors that ended in a squeeze chute (E; Figure 9). The entire raceway was covered by a roof to provide animals with limited light in the corridors to stop them from jumping out of the system. A slit was cut out to observe the animals without disturbing them on the sides of the corridors that was also utilized to insert a "touching stick" made from a bamboo stem (10 mm in diameter) by gently touching the animal to trigger the forward movement if they refrained to move to the consecutive corridor after opening the door approximately for 10 s. The first section of corridor (A) could be accessed through a rotating door that was directly attached to the barn. Animals were herded in batches of three to five from the barn and led into the first part of the raceway (A) and only one animal was allowed to move to the second section (B). This second section was designed to briefly contain a single animal while waiting for the weight of the animal in the third section (C) to be recorded. Section (C) was a weighing area with a platform on load bars, and an ear-tag panel reader (TW-1 Weigh Scale G02601, Gallagher, Hamilton 3240, New Zealand).

Figure 7. A complete view of the squeeze chute system with a sliding door opened for the exit of the animal.

Figure 8. Animal restrained in the squeeze chute system with its head elevated.

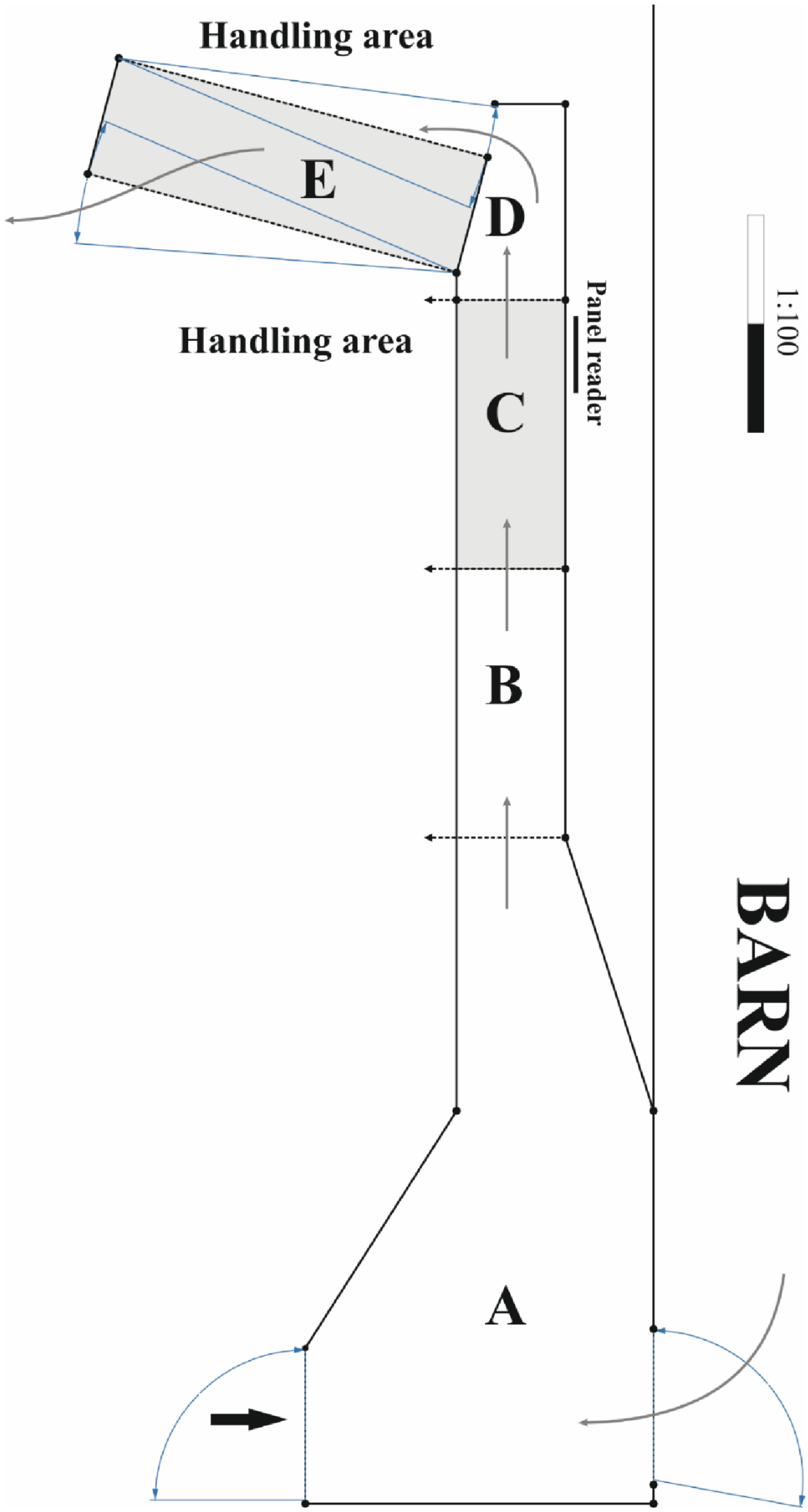

Figure 9. Design of the raceway divided into four corridors (A, B, C, and D) and the squeeze chute system (E) (Musa et al. 2021)

After their body weight was recorded, the animal moved to section (D) and then directly into the squeeze chute. The squeeze restraint chute (E) consists of two padded, vertically adjustable, wheeled, rotating doors (1.50 m long). The doors can be adjusted such that the upper part of its neck and head are accessible and the top of the door is above the shoulder of the animal. To prevent the animals from jumping out, the chute has been equipped with two thick plastic curtains which are kept close till the animal is confirmed inside the chute. Once the animal has moved into the chute, both the doors are wheeled towards the animal. Fine adjustments were done with the help of serrated clips and the animal was squeezed and restrained. The curtains are lifted up once the animal is confirmed restrained, and the head is lifted to facilitate normal respiration. Both eyes are covered with a cloth to avoid startling the animal while collecting the blood sample by a specialist. The horns are held tightly to avoid any injury to the personnel and to the animal itself (Figure 10). Blood is collected from the *vena jugularis* on either side of the neck (Figure 11). Subsequently, blood is collected using an 18-gauge needle attached to a vacutainer. With the aid of the vacuum in the heparinised tube. After the blood is collected, the pressure is released, and the needle is removed. Finally, the area is gently massaged to preserve continuous blood flow and prevent inflammation.

Figure 10. Horns are held tight to avoid injury to personnel and to animals.

Figure 11. Collection of blood from right *vena jugularis*

Besides blood samples, other biological samples such as faeces were also collected for the purpose of another study and routine coprological analysis for examining their health status. Morphometric measurements of different body parts and horns were also being conducted. Before the animal finally exited the chute, its body condition was examined using a five-point scale (1-5), with one being the poorest condition and five being the best. This scoring system was an adaptation of the methodology used for deer (Audigé 1998).

A temperament scoring system approved for elands was utilized to record data for temperament assessment (Table 2.; Musa et al. 2021). The scoring system included recoding of the temperament of animals from their entry into the raceway till their exit from the squeeze chute. The scoring of the temperament and ease of handling started with the entry of the animal into the system from the barn (lured with pelleted feed, voluntary, or forced). The overall state of the animal while passing through the raceway (calm, nervous, panicked), its speed or type of movement in the raceway (walking, running, jumping), presence and intensity of vocalization (low, medium or high level), the necessity to use the tapping stick to trigger the animal to move forward in each section, the overall state in the squeeze chute (calm, nervous, panicked) and finally, its exit from the squeeze chute system after handling was noted (Table 2.). A primary parameter for measuring temperament by Curley et al. (2006) and Parhama et al. (2019) which is exit velocity, was judged as running, walking, or jumping out of the squeeze chute. Any specific behaviors such as clicking of joints or unusual behaviors such as shivering are also recorded. The final temperament score was the sum of the score for each parameter ranging between 0 and 17.

Table 2: Temperament indicators used to assess the temperament of farmed eland during routine handling procedures (Musa et al. 2021).

Parameters	**Categories**	**Description**	**Score**
Entrance in the system	Voluntary	The animal walks toward the raceway without the handlers' interference	0
	Lured	The animal is lured with a treat or sound cue	1
	Forced	The animal is forced by the handler using a tapping stick or by rising up and clapping hands	2
Overall state before and along the raceway	Calm	The animal does not make frequent eye contact and is not agitated	0
	Nervous	The animal is agitated, uneasy	1
	Panicked	Jumping, backward and forward movement, aggressive toward the handler	2
Use of tapping sticks	Yes/No	If a stick was used in each of the four areas of the corridor to touch/tap the animals to move to the following corridor	1/0
Nature of the movement through the system	Walking	The animal walks calmly along the raceway	0
	Running	The animal seems to be tensed and trots from one corridor to another	1
	Jumping	The animal is jumping and moving forward and backward	2
Presence and intensity of vocalisation	None		0
	Low		1
	Medium		2
	High		3
Overall state in the squeeze chute	Calm	The animal remains mildly calm while measured and biological samples are being collected	0
	Nervous	The animal is tense, uneasy, and lying down	1
	Panicked	The animal is trying to jump, pushing its head/horns toward the handler	2
Exit from the chute	Walking	The animal calmly walks out of the chute with mild human interference	0
	Running	The animal runs away from the chute	1
	Jumping	The animal jumps out and trots off at high speed	2

4.3. Social interaction, Linearity, and Dominance index

The experimental animals were routinely observed for social interaction throughout the period of the study. While recording the data for social interaction, and through the experimental period, the studied animals were put together in the same group. The total number of animals in the group was 14 in January 2020 and it gradually increased up to 20 in November 2020 due to management needs. The behavioural observation was done in the barn to increase the frequency of agonistic interaction, as prior studies in the farm had shown poor interaction in the paddock (Kiley-Worthington 1977); as confinement increases aggression and competition for scarce resources thereby increasing social interaction which can equally reveal the true status of the resource-holding potential of each individual in the social group. *Ad libitum* sampling (Altmann 1974) was used to continuously observe agonistic interaction for a period of 5 hours (0800 – 1300) during each observation. The social interaction was done the subsequent day or within the week in which handling of the animals was carried out. Before the study, the animals were already habituated to the observer as such the presence of the observer in the barn did not confound the frequency of interaction. Moreover, observers also carried out the observation from a distance that would not influence the occurrence of interactions.

Caution was taken to make sure the identity of the animals from their ear tag or unique natural marking is recognized before an interaction was considered valid or recorded. Moreover, binoculars (Canon 10X30 IS) were used to increase the visibility of the identity of the experimental animals. Table 2. shows the ethogram of behaviours observed and recorded during the study. The ethogram was adapted from Kiley-Worthington (1977) where dyadic or triadic initiator-recipient interactions were measured by considering postural, protective, and orientation movements, geared toward establishing dominance. Simply put, a dyadic (interaction between two individuals) interaction was observed from the beginning to the end, the winner and the loser were recorded and taken note of the time during which the interaction occurred. Agonistic/aggressive interactions were ensured when one animal physically confronts or attacks another animal or makes a ritualized action that threatens another animal or group of animals to move away. Generally, agonistic interactions were categorized into contact actions (wrestling, yielding/butting, pushing) while non-contact agonistic behaviours were recorded as threatening and passing. Other behaviours (grooming, flehmen, mounting; Kiley-Worthington 1977) though affiliative but were considered as part of the dominance depending on who benefited from the interaction because in most mammals the submissive animal initiates affiliative behaviour (such as grooming the dominant animal) to avoid the threat and to gain protection either from conspecifics or other species (Vymyslická et al. 2015; Šárová et al. 2016). But in case both individuals benefited such as in reciprocal grooming it is considered a draw; this circumstance was controlled in the DomiCalc software (Schmid & de Vries 2013). Meanwhile, it has already been established in most mammals that age has a strong influence on the frequency of agonistic interaction and the linearity of interaction as well as dominance (Vymyslická et al. 2015). As such, data relating to agonistic interaction during which linearity was beginning to establish was considered in the study. The linearity of the social group was examined through two different methods using the winner-loser outcome of interactions on DomiCalc (Noldus, Wageningen, The Netherlands; Schmid & de Vries 2013). The first method was Landau's (1951) linearity index modified by de Vries (1995; *h'*) which also allows testing 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). Secondly, the linearity was tested through triangular transitivity (Shizuka & McDonald 2012). This is because the modified Landau's linearity index does not account fully for dyadic interactions which are unknown or individuals failing to interact (Klass & Cords 2011). The triangular transitivity (*tTri*) is based on the dominance relationships among sets of three players (triads) and assumes that all interact with each other. It is more effective in considering the null or unknown dyadic interactions (Shizuka & McDonald 2012). The triangle transitivity (*tTri*) like the linearity ranges from 0 to 1, although it can sometimes be negative as described by Shizuka and McDonald (2012); the interpretation is generally more complex. Still, the method also provides an associated p-value. Both analyses were performed using the software DomiCalc (Schmid & de Vries 2013). Linearity tests (*h'*) were carried out with 10000 randomizations and the triangle transitivity (*tTri*) tests with 1000 randomizations. After evaluating the linearity of the group, the dominance indices were then computed using four different methods. These are David's score (*DS*; David 1987), Clutton-Brock index (*CBI*; Clutton-Brock et al. 1979), Proportion of dominations (*PD;* Barroso et al. 2000), and lastly the inconsistencies and strength of inconsistencies (*I&SI;* de Vries 1998; Leiva et al. 2010).

4.4. Blood Sample Processing

Data were analysed bimonthly for 6 months, including January, March, May, July, September, and November 2020. Analyses were performed at the Laboratory of Animal Science, Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, at the Czech University of Life Sciences Prague. A total of 68 blood samples were analysed (4 samples were not collected to avoid any possible injury to the animal itself and the personnel). After handling all animals for blood collection, all the samples were transferred to the laboratory in a box with ice packs. All the samples were left to settle at least 45 minutes before centrifugation at 2800 rpm for 15 minutes. After the centrifugation is complete, the supernatant plasma is pipetted into Eppendorf tubes with the help of transferring pipette. The Eppendorf tubes were then stored in the freezer at -18°C (0°F).

On the day of processing, frozen blood plasma samples were thawed at room temperature for at least 1 hour. Then they were mixed by inversion followed by centrifugation at 12000 RCF for 2 minutes. Centrifugation ensured homogeneity and removed fibrin particles that may have formed during the storage. Prepared plasma samples were then analysed for the concentration of blood biochemistry parameters such as albumin (ALB), globulin (GLOB), total proteins (TP), creatinine (CREA), blood urea nitrogen (BUN), total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP) , lactate dehydrogenase (LDH), amylase (AMYL), glucose (GLU), calcium (Ca), phosphorus (P), and cholesterol (CHOL), total bilirubin (TBIL) using VetTest® Chemistry Analyzer (IDEXX Laboratories, Westbrook, Maine, USA) with a standard commercial kit (IDEXX, Czech Republic). All the samples were processed following the procedure described in IDEXX VetTest® Chemistry Analyzer, Operator’s manual. The values obtained for blood biochemistry were compared with the values available for common elands from ZIMS (2022).

4.5. Statistical Analysis

Kolmogorov-Smirnov and visual examination of the histograms and the Q-Q plots was used for examining the normality of the variables analysed. All of them were normally distributed except the four social rank indices.

Non-parametric Spearman´s ranked correlation was used to examine the degree of correlation between the two temperament variables and the four social rank indices. As expected, it was strong for the two temperament variables ($\rho=0.412$, $p<0.001$) and very strong for the four social rank indices (all $\rho>0.802$ and $p<0.001$). For that reason, Principal Component Analysis was used for both groups of variables to obtain a single factor for each one. Varimax rotation was used since this method is recommended when it is desired to obtain a low number of factors. For temperament, the first factor explained 70.4% of the variance in the original variables. For social rank, the first factor explained 74.8% of the variance in the original variables. These factors were used in further analyses as short-term (temperament) and long-term (social rank) explanatory variables for the studied blood biochemistry markers. The new variables were both normally distributed.

Generalized Linear Mixed Models were used to test the effect of temperament and social rank (as short- and long-term stressors) in the values obtained for the 14 blood biochemistry markers studied. Repeated measures structure was used, with individuals as subjects and months as a repeated measure. Sex, body weight, body condition, and age in days were also included in the models as covariates. All models were designed with normal distribution and identity link function, given the normality of all the variables used. Collinearity was confirmed in the 5 continuous variables (temperament, social rank, body weight, body condition, and age) through the Variance Inflation Factor, which was always less than 5, as suggested (Kock & Lynn 2012); thus, ensuring their validity as independent variables. All statistical analyses were performed using the software SPSS ver. 28.0 (IBM, Armonk, NY, USA).

5. Results

The results obtained are within the expected ranges (ZIMS 2022) for all the animals (Table 3.). However, four to five samples were removed as they had extreme values which might be a result of improper handling of samples in the laboratory as a human error. As expected, most of the blood biochemical markers studied were in a certain degree affected by general characteristics like sex, age, body weight, and body condition (Table 4.). Moreover, our first hypothesis was confirmed and temperament during handling, as a proxy of short-term stress, affected several blood biochemical markers. ALP and BUN increased in animals with a calm temperament, while ALB, GLU, TBIL, and TP decreased in these animals. On the contrary, second hypothesis was not confirmed since social rank, as a proxy of long-term stress, just affected ALB (higher in dominant animals). Males had a higher but not significant average rank than females ($t=-5.185$, $p=0.197$), and thus this social rank effect can be considered independent of the sex effect also found (Figure 12.).

Table 3. Descriptive statistics of the biochemistry parameters for common eland obtained in the study compared with the reference interval ZIMS (2022).

Biochemistry parameters	N	Mean	Median	Maximum – Minimum	Reference Interval (95%)	Reference Interval ZIMS (2022)
ALB (g/L)	64	34.2	35.0	30.0 – 39.0	30.0 – 39.0	13.0 – 42.9
ALP (U/L)	64	96.0	87.0	43.0 – 206.0	43.6 – 202.3	36 – 367
ALT (U/L)	64	23.5	23.0	10.0 – 38.0	10.6 – 36.1	1 – 25
AMYL (U/L)	63	455	451	301 – 600	326 – 597	30 – 750
BUN (mmol/L)	64	5.95	5.90	4.20 – 8.00	4.33 – 7.75	2.9 – 12.1
Ca (mmol/L)	63	2.36	2.41	1.59 – 2.80	1.62 – 2.67	1.7 – 2.8
CHOL (mmol/L)	64	2.12	2.05	1.52 – 3.08	1.62 – 3.02	0.65 – 2.58
CREA (μmol/L)	64	127	125	91 – 172	92 – 163	35 – 265
GLOB (g/L)	64	41.4	41.0	35.0 – 48.0	35.6– 47.38	3 – 59
GLU (mmol/L)	64	4.45	4.45	2.64 – 6.66	2.75 – 6.24	3.61– 15.65
P (mmol/L)	64	2.52	2.27	1.25 – 5.96	1.31 – 5.48	0.94 – 3.88
TBIL (μmol/L)	64	4.08	4.00	1.00 – 8-00	1.00 – 8.00	1.7 – 23
TP (g/L)	64	75.6	76.0	67.0 – 85.0	67.0 – 83.75	47 – 93
LDH (u/L)	63	3994	3673	2137 – 8720	2164 – 8259	352 – 1794

Table 4. GLMMs. Factors affecting each biochemical marker. Shown β coefficient and p-value in brackets. Full models are shown. P-values over 0.1 are indicated as not significant.

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

[1] Male as category of reference.

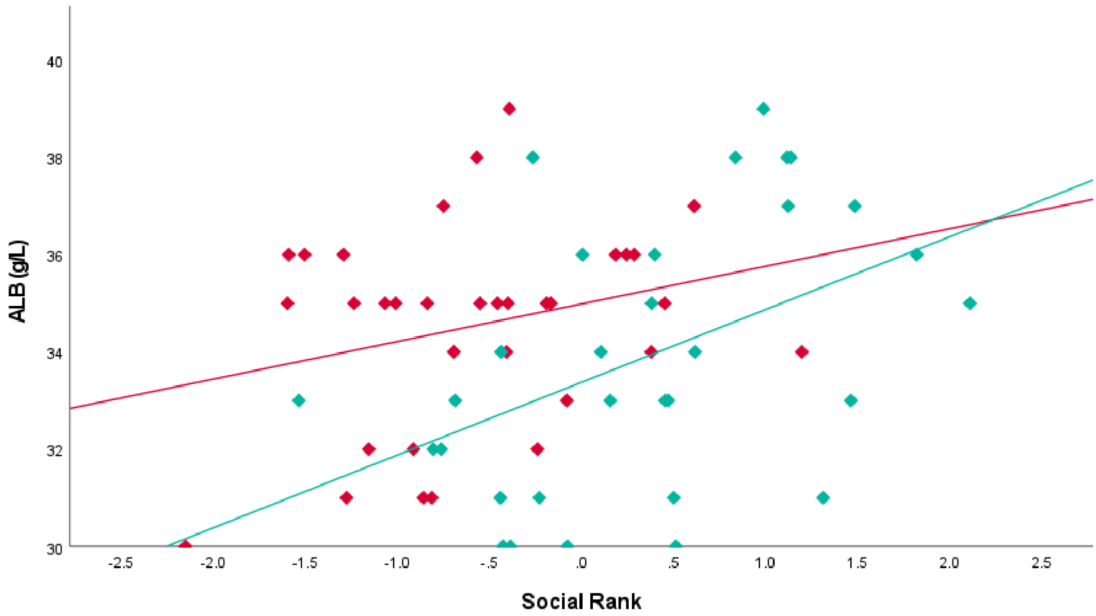

Figure 12: Changes in ALB concentrations (g/L) due to the effect of social rank on both sexes of common eland in captivity (Males in blue, Females in Red).

Blood chemistry parameters including AMYL, Ca, CREA, GLOB, and P were not influenced due to the temperament or social rank, as proxies of short- and long-term stress, respectively. Although they showed changes due to the controlled factors including sex, age, body condition and body weight of animals. On the other hand, ALT, CHOL and LDH were affected by handling, social rank, and the controlled factors.

6. Discussion

The animals within the experiment were habituated to monthly handling for routine collection of biological samples. Hence, the stress hormones production and ultimately the physiological manifestations of the stress response should be minimum while handling them, as in cattle (Grandin 1997). All the results obtained for blood biochemistry parameter values were within the reference value range (ZIMS 2022), proving that the animals had no subclinical pathologies. It satisfies the first objective of the study to standardise the reference values available for common eland blood chemistry parameters. It is also important to acknowledge that the reference values may be affected by factors such as the method of capture, age, nutrition, season, and health status of the animals

(Marco & Lavin 1999; ZIMS 2022). The results confirm that the values obtained fluctuated, although were almost all within the reference range during the experiment.

The first hypothesis that a short-term stressor i.e., handling, affects the blood biochemistry parameters was confirmed as six blood biochemistry parameters displayed changes among 14 parameters analysed. The parameters that were affected due to handling include ALB, ALP, BUN, GLU, TBIL, and TP. However, the second hypothesis that long-term stressor i.e., social rank, affects the blood biochemistry parameters, was not confirmed as only one parameter ALB showed changes in its levels. These results were obtained while controlling other factors in the experimental settings which might affect the results including sex, age, body weight, and body condition score.

The temperament score shows the amount of stress response produced by animals. Animals with higher temperament scores are more stressed and vice versa. The present study shows an increase in ALB and TP with the increase in the temperament score. ALB, which contains almost 50% of TP, increased showing influence by handling. The present study's results align with those obtained by Marco and Lavin (1999) who reported that TP, ALB, and GLOB concentrations are affected by capture methods of red deer. Changes in ALB levels are counterbalanced by changes in GLOB levels (IDEXX 2014) as they sum up to make the TP value. It was noted as opposite direction changes in the ALB and GLOB levels in strong correlation with age and body weight but interestingly temperament was not correlated with GLOB. Glucose is the main energy source in animals, and it is also known that the stress response is enhanced by an increased concentration of GLU (Kerr 2002). Similarly, in the present study, GLU levels increased in stressed animals while handling showing a strong positive correlation. This indicated the increase in the stress response with the increase in the temperament score. It essentially means that the more the animal struggles in the chute, the more energy it requires for movement, and that energy is available in the form of GLU. Urea or BUN is a nitrogen waste product and is an indicator of renal efficiency. Elevated BUN is due to the high production of ammonia in the rumen and high exogenous nitrogen in the intestine (Caldeira et al. 2007a) which is to a large extent affected by protein content in the diet. Calm animals showed higher BUN levels, although not outside the reference interval, in the present study which implies increased renal function. However, the reason for the increase cannot be specified to handling as the diet was offered *ad libitum* and the amount of feed intake and water was unknown due to practical experimental limitations. Total bilirubin is used as a marker for cholestasis which mostly accompanies a liver problem. Increase in the temperament score showed an increase in the TBIL value in the present study. A potential reason for the increase can be the instantaneous breakdown of red blood cells which are released in the acute response to stress stimuli. The only enzyme that was negatively correlated to temperament was ALP. A widely studied enzyme ALP shows elevations when there is a hepatic pathology involving the biliary system (IDEXX 2014). It increased in the females with a calm temperament in the present study which was not previously observed before in common elands. The reason for this correlation is unclear due to the absence of any hepatic problem. Interestingly, in dogs, ALP production is reported to be induced by stress but the increase in other species such as common eland is debatable and requires further exploration.

The parameters that were not affected due to the influence of handling were CREA, Ca, P, ALT, AMYL, CHOL, GLOB, and LDH. A possible explanation for the normal value can be majorly attributed to the absence of any clinical pathologies in the animals that might have occurred due to the short-term stress i.e., handling. For example, significant CREA changes can be seen due to muscle damage which was not evident in the experiment. Furthermore, minerals such as Ca and P are maintained by hormonal regulation which in healthy individuals is mostly in equilibrium. Lastly, ALT, AMYL, CHOL, and GLOB levels are maintained by mostly their production by the liver (and intestine for AMYL), which remain unchanged in the absence of hepatopathy or cholestasis.

The social rank only affected ALB, out of all parameters analysed and it was not influenced by the sex of the animals as explained in the results section. Albumin levels increase in the presence of dehydration or secondary blood concentration due to fluid loss. Observations during the experiments did not record any event for potential fluid loss such as diarrhoea directly. It could have been due to their hydration status in morning hours when the fluid intake is less, and the blood samples were taken. It is generally believed that resources such as water are more accessed by animals with higher ranks (Vymyslická et al. 2015; Šárová et al. 2016), and they should not be dehydrated with increased ALB levels. Hence, it is surprising to observe that the results obtained here are contrary to the general perception.

Factors such as age, sex, body weight, and body condition score affected the blood biochemistry parameters to some extent. Russell and Roussel (2007) pointed out that CREA production is related to extent of the muscle mass in the body. In addition, alterations of CREA concentrations in stressed animals have been reported before (Knowles & Warriss 2000; Lopez-Olvera et al. 2006). Interestingly, the present study noted a strong positive effect of BCS on CREA production, aligning with the previous studies (Knowles & Warriss 2000; Lopez-Olvera et al. 2006; Russell & Roussel 2007). Minerals such as Ca and P were not affected by stress from handling. Old, heavy females showed increased Ca with no effect on temperament. On the other hand, heavy males irrespective of their age showed lower P levels. Satyanarayana & Chakrapani (2007) noted that blood Ca is regulated by three different hormones (Figure 2.) and Ca and P metabolism is interdependent (IDEXX 2014). As mentioned before, all the animals were healthy and were not having any pathology that could potentially affect the parathyroid glands responsible for the hormonal regulation of Ca and P. Hence, it can be said that the changes were purely normal physiological fluctuations and not due to the stress of handling. Interestingly, AMYL whose production site and activity is dependent on the liver and intestine was not affected by the temperament of animals but was strongly positively and strongly negatively affected by age and body weight of the males, respectively. Alanine aminotransferase, LDH, and CHOL did not show any correlations with the proposed continuous variables. It can be fairly said that in the absence of any hepatic pathology, skeletal damage, or endocrine disorder, the relative levels of AMYL, TBIL, ALT, LDH, and CHOL remain in the reference ranges. Hepatic diseases mostly manifest with some evident clinical signs such as jaundice which was not observed in any animals, and it supports the normal levels and fewer fluctuations.

Overall, handling affected females more than males as ALB, ALP, CREA, and Ca values were significantly higher in females. Males on the other hand showed higher AMYL and P levels than females. The age of the animals was noted to have strongly correlated with the increase in AMYL, CREA, and GLOB whereas it was strongly negatively correlated with ALB, Ca, and GLU. Animals with higher BCS had decreased ALP, AMYL, and Ca levels and increased BUN, CREA, and slightly increased P and TP levels. An increase in body weight influenced ALB, ALP, and Ca levels to rise and decreased AMYL, GLOB, and P levels. Total bilirubin was only influenced by the handling of the animals which decreased in calm animals and was not affected by sex, age, BCS, body weight, or rank of the animals. Alanine aminotransferase, CHOL, and LDH remained unchanged and showed no influence because of handling. Although GLU levels were strongly negatively correlated with age proving that the animals are getting habituated to handling showing stress responses of lesser intensity.

The blood biochemistry parameter values in the present study are mostly within the normal reference range after applying an outlier removal approach for the few values which were out of the normal reference range. Few values outside the reference interval are not considered to be a reason for a potential disease or a pathology rather they are normal physiological processes without any manifestation. Although, a careful approach that involves physical evaluation, and re-testing the animals for particular parameters that are out of the range is advisable.

Interpretation of the blood biochemistry profile is also crucial. Despite being a very complicated process, it has been simplified over the years with the help of pattern identification. The process starts with asking if the constituents present in the plasma are supposed to be there or not. For example, albumin and electrolytes are normally present in plasma and their presence cannot be considered abnormal. The second step of this process is to consider the functions of plasma to transport some molecules, i.e., glucose and hormones, and to clear waste products such as urea from the body. The final step involves minimizing the area of interest in a particular system or an organ, for example, a sudden increase in the ALT level will move the direction of the diagnosis towards liver damage (IDEXX 2014). Ideally, the following tests will include only specific parameters that magnify the pathology or disease process and will add one more step to the pattern. Identification of combinations of parameters that vary and the patterns they present with their deviation help in correctly choosing the next step for further examination and this is the key to reaching a definitive diagnosis. Stress has been known to affect haematological parameters and this study shows the influence of short and long-term stress on the blood biochemistry parameters as discussed above. This can be considered by veterinarians while identifying patterns for diagnosis to avoid false diagnosis. Correctly interpreted tests lead to the continuation or cessation of medicinal treatment, nutrition plans, and other husbandry measures. This can significantly affect the economic and management aspect of any captive population, be it a zoo or a research facility.

The main difficulty for this study was comparing the literature available for blood biochemistry parameters of common eland that is related to the proposed problem and the acquired methodology for the present study. To overcome these hurdles, concepts relating mostly to farm animals such as cattle and pigs were reviewed and presented. The present study gives new insights into the blood biochemistry of common eland which was not studied previously to the extent that involves the effects of short-and long-term stressors. This study also verifies the reference values (ZIMS 2022) and thereby establishes the normal reference values of 14 different blood biochemistry parameters for common eland (135.1±2.25kg body weight, 3.8±0.1 BCS, 9.2±1.5 months). Handling, which is an essential part of routine herd management, is now established to affect the blood chemistry of common eland satisfying the second objective of the study. As handling is a routine procedure for farm/captive animals, now we know the magnitude of its effect on normal physiology as a short-term stressor. This can be used more efficiently and justifiably in captivity management of common eland and other related antelopes. Also, it can be crucial for the understanding of the welfare of the animals as it is not affecting the animal to a level that can be pathologic. The last objective of the present study was examining the effects of long-term stressor i.e., social rank. Social rank did not affect the blood biochemistry profile (only affected ALB) as per the present study which can lead to a conclusion that keeping the animals in the same group will not affect their normal physiology to a large extent. This also lines with the normal behavior of common eland to be in herds in nature. Furthermore, it also suggests that common elands can be kept in captivity the same as farm animals as we know that the impact of the long term stress due to social rank is negligible.

7. Conclusions

The present study shows that short term stress through routine handling using a specialised squeeze chute affected the blood biochemistry profile of captive common eland. Thus, the first hypothesis was confirmed. On the other hand, the second hypothesis was rejected in the absence of relatively insignificant changes in the blood biochemistry parameters concluding that the social rank of animals does not have an influence on the blood biochemistry parameters. Although, the variations remained within the reference intervals suggesting that handling of the animals can be performed periodically without any major pathological consequences while maintaining animal welfare in the captivity. The results of the present study standardised the available reference intervals for blood biochemistry parameters with fairly large number of samples and established that their proper interpretation can be helpful to veterinarians in making a clinical diagnosis. In future, studies with controlled diet and water intake can be performed to identify their influence on blood biochemistry which was a limitation that might have impacted the results of this study.

8. References

Abdelkader SV, Hauge JG. 1986. Serum enzyme determination in the study of liver disease in dogs. Acta Veterinaria Scandinavica **27**: 59–70.

Abeni F, Calamari L, Stefanini L. 2007. Metabolic conditions of lactating Friesian cows during hot season in Po valley. 1. Blood indicators of heat stress. International Journal of Biometeorology **52**: 87-96.

Adachi K, Kawano H, Tsuno K, Nomura Y, Katsura N, Arikawa A, Tsuji A, Onimaru T. 1997. Values of the serum components in Japanese Black beef steers at farms with high productivity and low frequencies of disease and death in Miyazaki prefecture. Journal of Veterinary Medical Science **59**: 873-877.

Allan MR. 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. Candian Field-naturalist **129**: 84–89.

Altmann J. 1974. Observational study of behaviour: sampling methods. Behaviour **49**: 227–267.

Anderson NL, Anderson NG. 2002. The human plasma proteome. Molecular & Cellular Proteomics **1**: 845–867.

Arave CW, Albright JL. 1976. Social rank and physiological traits of dairy cows as influenced by changing group membership. Journal of Dairy Science **59**: 974–81.

Arthington JD, Eicher SD, Kunkle WE, Martin FG. 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. Journal of Animal Science **81**: 1120-1125.

Arthington JD, Spears JW, Miller DC. 2005. The effect of early weaning on feedlot performance and measures of stress in beef calves. Journal of Animal Science **83**: 933-939.

Audige L, Wilson PR, Morris RS. 1998. A body condition score system and its use for farmed red deer hinds. New Zealand Journal of Agricultural Research **41**: 545-553

Bang A, Deshpande S, Sumana A, Gadagkar R. 2010. Choosing an appropriate index to construct dominance hierarchies in animal societies: a comparison of three indices. Animal Behaviour **79**: 631–636.

Barboza PS, Parker KL, Hume ID. 2009. Integrative wildlife nutrition. Germany: Springer-Verlag Berlin Heidelberg. 342p.

Barroso FG, Alados CL, Boza J. 2000. Social hierarchy in the domestic goat: effect on food habits and production. Applied Animal Behaviour Science **69**: 35-53.

Bartoň L, Bureš D, Kotrba R, Sales J. 2014. Comparison of meat quality between eland (*Taurotragus oryx*) and cattle (*Bos taurus*) raised under similar conditions. Meat Science, **96**: 346–352.

Bauman DE, Currie WB. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science **63**: 1514–1529.

Beatty EM, Doxey DL.1983. Research in Veterinary Science **3S**: 325-330.

Bergvall UA, Jaderberg L, Kjellander P. 2017. The use of box-traps for wild roe deer: behaviour, injuries and recaptures. European Journal of Wildlife Research **63**: 67.

Bertoni G, Trevisi E, Han X, Bionaz M. 2008. Effects of Inflammatory Conditions on Liver Activity in the Puerperium and Consequences for Performance in Dairy Cows Journal of Dairy Science **91**: 3300-3310.

Bijlsma R, Loeschcke V. 2005. Environmental stress, adaptation and evolution: An overview. The Journal of Evolutionary Biology **18**: 744–749.

Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrari A, Bertoni G. 2007. Plasma Paraoxonase, Inflammatory Conditions, Liver Functionality and Health Problems in Transition Dairy Cows. Journal of Dairy Science **90**: 1740-1750.

Blanchard RJ, McKittrick CR, Blanchard DC. 2001. Animal Models of Social Stress: effect on behaviour and brain neurochemical systems. Physiology & Behaviour **73**: 261-271.

Bligh J. 1976. Introduction to acclimatory adaptation-including notes on terminology. Pages 219–229 in Environmental Physiology of Animals. J. Bligh, J. L. Cloudsley-Thompson, and A. G. Macdonald, ed. John Wiley and Sons, New York, NY.

Boonstra R. 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Functional Ecology **27**: 11–23.

Boyd JW.1983. The Mechanisms Relating to Increases in Plasma Enzymes and Isoenzymes in Diseases of Animals. Veterinary Clinical Pathology **2**: 9-24.

Braun JP, Lefebvre HP, Watson AD. 2003. Creatinine in the dog: a review. Veterinary clinical pathology **32**: 162 – 179.

Breen KM, Oakley AE, Pytiak AV, Tilbrook AJ, Wagenmaker ER, Fred JK. 2007. Does cortisol acting via the Type II Glucocorticoid Receptor mediate suppression of pulsatile luteinizing hormone in response to psychosocial stress? Endocrinology **148**: 1882–1890.

Broom DM. 2003. Transport stress in cattle and sheep with details of physiological, ethological and other indicators. Deutsche Tierärztliche Wochenschrift **110**: 83-89.

Bruss ML. 2008. Lipids and Ketones. Pages 81-115 in Kaneko JJ, Harvey JW, Bruss ML editors, in Clinical Biochemistry of Domestic Animals, 6th Edition, Academic Press, San Diego.

Cafazzo S, Valsecchi P, Bonanni R, Natoli E. 2010. Dominance in relation to age, sex, and competitive contexts in a group of free-ranging domestic dogs. Behavioural Ecology **21**: 443-455.

Calamari L, Maianti, MG, Stefanini L. 2003. Effect of space availabilty at feed bunk and rest area on metabolic conditions and productive responses in dairy cows. Italian journal of animal science **2**: 281-283.

Caldeira RM, Belo AT, Santos CC, Vazques MI, Portugal AV. 2007a. The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Ruminant Research **68**: 233–241.

Caldeira RM, Belo AT, Santos CC, Vazques MI, Portugal AV. 2007b. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Research **68**: 242 – 255.

Carlstead K, Brown JL. 2005. Relationships between patterns of fecal corticoid excretion and behaviour, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Zoo Biology **24**: 215–232.

Ceacero F, Gaspar-López E, García AJ, Landete-Castillejos T, Gallego L. 2018. Social rank affects the hematological profile in red deer hinds. Veterinary Record **182**:436.

Center SA, Baldwin BH, Erb HN, Tennant BC. 1985. Bile acid concentrations in the diagnosis of hepatobiliary disease in the dog. Journal of the American Veterinary Medical Association **187**: 935–940.

Center SA. 2007. Interpretation of liver enzymes. Vet Clin. Small Anim. **37**: 297–333.

Chang R, Holcomb JB. 2016. Choice of Fluid Therapy in the Initial Management of Sepsis, Severe Sepsis, and Septic Shock. Shock. **46**: 17¬26.

Chao C, Brown RD, Deftos. L J. 1984. Effects of xylazine immobilization on biochemical and endocrine values in white-tailed deer. Journal of Wildlife Diseases **20**: 328-332.

Chase ID, Seitz K. 2011. Self-structuring properties of dominance hierarchies: a new perspective. Advances in Genetics **75**: 51-81.

Chase ID, Tovey C, Spangler-Martin D, Manfredonia M. 2002. Individual differences versus social dynamics in the formation of animal dominance hierarchies. Proceedings of the National Academy of Sciences **99**: 5744-5749.

Chrousos GP. 2009. Stress and disorders of the stress system. Nature Reviews Endocrinology **5**: 374–381.

Clampitt RB, Hart RJ. 1978. The tissue activities of some diagnostic enzymes in ten mammalian species. Journal of Comparative Pathobiology **88**: 607 – 621.

Clarke FM and Faulkes CG. 1997. Dominance and queen succession in captive colonies of the eusocial naked mole-rat, Heterocephalus glaber. Proceedings of the Royal Society **264**: 993–1000.

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE. 1979. The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus L.*). Animal Behaviour **27**: 211-225.

Colditz IG. 2002. Effects of the immune system on metabolism: implications for production and disease resistance in livestock. Livestock Production Science **75**: 257-268.

Collier RJ, Gebremedhin KG. 2015. Thermal biology of domestic animals. Annual Review of Animal Biosciences **3**: 513–532.

Cooper ES, Wellman ML, Carsillo ME. 2009. Hyperalbuminemia associated with hepatocellular carcinoma in a dog. Veterinary clinical pathology **38**: 516-520.

Cortright C, et al. 2014. Clinical features of progressive vacuolar hepatopathy in Scottish Terriers with and without hepatocellular carcinoma: 114 cases (1980-2013). Journal of the American Veterinary Medical Association **245**: 797-808.

Cransac N, Aulagnier S. 1996. Factors influencing hierarchy in a captive herd of eland *Taurotragus oryx*. Aggressive behavior **22**: 209–13.

Curley Jr., KO, Paschal JC, Welsh Randel TH, RD. 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. Journal of Animal Science **84**: 3100–3103.

Das KS, Srivastava BB, Das N. 2001. Standing orientation and behaviour of goats during short-haul road transportation. Small Ruminant Research **41**: 91-94.

David HA. 1987. Ranking from unbalanced paired-comparison data. Biometrika **74**: 432-436.

de Vries H. 1995. An improved test of linearity in dominance hierarchies containing unknown or tied relationships. Animal Behaviour **50**: 1375-1389.

de Vries H. 1998. Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. Animal Behaviour **55**: 827-843.

Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC. 2009. The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. Neuroimage **47**: 864–871.

Della-Rosa MM, Pavan E, Maresca S, Spetter M, Ramiro F. 2018. Performance, carcass and meat quality traits of grazing cattle with different exit velocity. Animal Production Science **59**: 1752–1761.

Dunegan LJ, Knight DC, Brennan MF, Moore FD. 1978. Urea distribution in renal failure. Journal of Surgical Research **24**: 401 – 403.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/proteins/albumin/ 2020a. Accessed 07.2022.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/proteins/globulins/ 2020b. Accessed 07.2022.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/liver/liver-injury/alanine-aminotransferase/ 2020c. Accessed 07.2022.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/liver/cholestasis/alkaline-phosphatase/ 2020d. Accessed 07.2022.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/muscle/lactate-dehydrogenase/ 2020e. Accessed 07.2022.

eClinPath.com. Cornell University. https://eclinpath.com/chemistry/minerals/phosphate/ 2020f. Accessed 07.2022.

Ellis L. 1995. Dominance and reproductive success among non-human animals. Ethology and Sociobiology **16**: 257–333.

Farmer C, Dubreuil P, Pelletier G, Petitclerc D, Brazeau P. 1990. Active immunization against somatostatin in gestating gilts and the effect of transferred immunity on piglets. Canadian Journal of Animal Sciences **70**: 211-218.

Ferguson DC. 2011. Endocrine System. Pages 295-329 Latimer KS,Malaffey EA, Prasse KW,editors in Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th Edition. Wiley-Blackwell, UK.

Fernandez NJ and Beverly A. 2007. Pages 223-233 in Alkaline phosphatase: beyond the liver in Veterinary Clinical Pathology. American Society for Veterinary Clinical Pathology.

Flint AP, Albon SD, Jafar SI. 1997a. Blastocyst development and conceptus sex selection in red deer Cervus elaphus: studies of a free-living population on the Isle of Rum. General and Comparative Endocrinology **106**: 374–83.

Flint AP, Albon SD, Loudon AS, Jabbour HN. 1997b. Behavioral dominance and corpus luteum function in red deer Cervus elaphus. Hormonal Behaviour **31**: 296–304.

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

Fordyce G, Wythes JR, Shorthose WR, Underwood DW, Shepherd RK. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 2. Effect of temperament on carcass and meat quality. Australian Journal of Experimental Agriculture **28**: 689–693.

Franzmann AW, Thorne DET. 1970. Physiologic values in wild bighorn sheep (Ovis canadensis canadensis) at capture after handling and after captivity. Journal of the American Veterinary Medical Association **157**: 647-65.

Franzmann AW. 1972. Environmental sources of variation of bighorn sheep physiologic values. Journal of Wildlife Management **36**: 924-932.

Gartner RJW, Ryley JW, Beattie AW. 1965. The influence of degree of excitation on certain blood constituents in beef cattle. Australian journal of experimental biology and medical science **43**: 713-772.

Grandin T. 1997. Assessment of stress during handling and transport. Journal of Animal Science **75**: 249–257.

Grandin T. 1999. Safe handling of large animals. Occupational Medicine **14**: 195–212.

Gruys E, Toussaint MJM, Upragarin N, Van Ederen AM, Adewuyi AA, Candiani D, Nguyen TKA, Sabeckiene J. 2005. Acute phase reactants, challenge in the near future of animal production and veterinary medicine. Journal of Zhejiang University Science **6B**:1045-1056.

Halperin ML, Kamel KS. 1998. Potassium. Lancet. **352**: 135-140.

Hank AM, Hoffmann WE, Sanecki RK, Schaeffer DJ, Dorner JL. 1993. Quantitative determination of equine alkaline phosphatase isoenzymes in foal and adult serum. Journal of Veterinary Internal Medicine **7**: 20–24.

Hemsworth PH, 2003. Human-animal interactions in livestock production. Applied Animal Behaviour Science **81**: 185–198.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H. 2005. Limbic system mechanisms of stress regulation: hypothalamopituitary-adrenocortical axis. Progress in Neuro-Psychopharmacology and Biological Psychiatry. **29**: 1201–1213.

Hillman JC. 1987. Group size and association patterns of the common eland (*Tragelaphus oryx*). Journal Zoology **213**: 641–63.

Hing S, Narayan E, Thompson A, Godfrey S. 2014. A review of factors influencing the stress response in Australian marsupials. Conservation Physiology **2**: 1-17.

Hodgson PD, Aich P, Manuja A, Hokamp K, Roche FM, Brinkman FSL, Potter A, Babiuk LA, Griebel PJ. 2005. Effect of stress on viral–bacterial synergy in bovine respiratory disease: novel mechanisms to regulate inflammation. Comparative and Functional Genomics **6**: 244-250.

Hoe CM, Jabara AG. 1967. The use of serum enzymes as diagnostic aids in the dog. Journal of Molecular Pathology **77**: 245–254.

Hoffmann WE, Dorner JL. 1975. Separation of isoenzymes of canine alkaline phosphatase by cellulose acetate electrophoresis. Journal of the American Animal Hospital Association **11**: 283–285.

Horowitz M. 2001. Heat acclimation: Phenotypic plasticity and cues underlying the molecular mechanisms. Journal of Thermal Biology **26**: 357–363.

Hyvarinen H, Helle T,Nieminen M,Vayrynen Q,Vayrenen R. 1976. Some effects of handling reindeer during gatherings on the composition of their blood. Animal Production **22**: 105-114.

IDEXX. 2014. VetTest chemistry analyzer: operator's manual. USA: IDEXX Laboratories. 132p.

Janak PH, Tye KM. 2015. From circuits to behaviour in the amygdala. Nature **517**: 284–292.

Johnstone CP, Reina RD, Lill A. 2012. Interpreting indices of physiological stress in free-living vertebrates. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology **182**: 861–879.

Kaneko JJ, Harvey JW, Bruss ML. 2008. Clinical Biochemistry of Domestic Animals. 6th Edition, Academic Press, San Diego.

Kannan G, Terril TH, Kouokou B, Gelaye S, Amoah EA. 2002. Simulated preslaughter holding and isolation effects on stress responses and liveweight shrinkage in meat goats. Journal of Animal Science **80**: 1771-1780.

Karns PD, Crichton VFJ. 1978. Effects of Handling and Physical Restraint on Blood Parameters in Woodland Caribou. The Journal of Wildlife Management 42: 904-908.

Keller P, Stanbridge TA. 1972. Schweizer Archiv fur Tierheilkunde us, 35-48.

Keller P. 1981. Enzyme activities in the dog: tissue analysis, plasma values, and intracellular distribution. American journal of veterinary research **42**: 575 – 582.

Kelly S, Hertzman C, Daniels M. 1997. Searching for the biological patways between stress and health. Annual Revision of Public Health **18**: 437-462.

Kerr MG. 2002. Clinical enzymology- plasma enzyme in diagnosis. Pages 135-147 in Veterinary Laboratory Medicine clinical biochemistry and hematology. Blackwell Publishers, UK.

Kida K. 2003. Relationships of metabolic profiles to milk production and feeding in dairy cows. Journal of Veterinary Medical Science **65**: 671–677.

Kiley-Worthington M. 1977. The causation, evolution and function of the visual displays of the eland (*Taurotragus oryx*). University of Sussex.

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.

Kingdon J. 1982. East African mammals: an atlas of evolution in Africa. Vol. III. Part C (Bovids). London: Academic Press 393.

Kitchell BE, Strombeck DR, Cullen J., Harrold D. 1986. Clinical and pathologic changes in experimentally induced acute pancreatitis in cats. American Journal of Veterinary Research **47**: 1170–1173.

Klass K, Cords M. 2011. Effect of unknown relationships on linearity, steepness and rank ordering of dominance hierarchies: simulation studies based on data from wild monkeys. Behavioural Processes **88**: 168-176.

Knowles TG, Warriss PD. 2000. Pages 385-407 in Stress physiology of animals during transport, Grandin T, editor, Livestock Handling and Transport. 2nd ed. Cambridge, MA: CABI Publishing.

Kock MD, Jessup DA, Clark RK, Franti CE. 1987. Effects of capture on biological parameters in free-ranging bighorn sheep (*Oiis curadrrisis*): evaluation of drop-net, drive-net, chemical im- mobilization and the net gun. Journal of Wildlife Diseases **3**: 641-651.

Kock N, Lynn G. 2012. Lateral collinearity and misleading results in variance-based SEM: An illustration and recommendations. Journal of the Association for information Systems **13**: 7.

Kock N, Lynn G. 2012. Lateral collinearity and misleading results in variance-based SEM: An illustration and recommendations. Journal of the Association for information Systems **13**: 7.

Koolhaas JM, Boer SF, Coppens CM, Buwalda B. 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Frontiers in Neuroendocrinology **31**: 307–321.

Kotrba R, Knizková I, Kunc P, Bartoš L. 2007. Comparison between the coat temperature of the eland and dairy cattle by infrared thermography. Journal of Thermal Biology **32**: 355–359.

Kumar B, Aich P, Manuja A. 2012. Stress and its impact on farm animals. Frontiers in Bioscience- **E4**:1759-1767.

Landau HG. 1951. On dominance relations and the structure of animal societies: I. Effect of inherent characteristics. Bulletin of Mathematical Biology **13**: 1-19.

Lay Jr. DC, Wilson ME. 2004. Considerations When Using Physiological Data in Assessing Animal Well-Being. Journal of Animal and Veterinary Advances **3**: 614-626.

LeResche RW, Seal US, Karns PD, Franzmann AW. 1974. A review of blood chemistry of moose and other cervidae with emphasis on nutritional assessment. Nature Canada **101**: 263-290.

Lo´pez-Olvera JR, Marco I, Montane J, Lavı´n S. 2006. Transport stress in Southern chamois (*Rupicapra pyrenaica*) and its modulation by acepromazine. The Veterinary Journal **172**: 347–355.

Loegering DJ, Critz JB. 1971. Effect of hypoxia and muscular activity on plasma enzyme levels in dogs. American Journal of Physiology **220**: 100-104.

Lomborg SR, Nielsen LR, Heegaard PMH, Jacobsen S. 2008. Acute phase proteins in cattle after exposure to complex stress. Veterinary Research Communications **32**: 575-582.

Loskutoff NM, Betteridge KJ. 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.235–248.

Manston R, Allen WM. 1981. The use of blood chemistry in monitoring the health of farm livestock. British Veterinary Journal **137**: 241.

Marco I, Lavin S. 1999. Effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*). Research in Veterinary Science **66**: 81- 84.

Meehan CL, Garner JP, Mench JA. 2004. Environmental enrichment and development of cage stereotypy in Orange-winged Amazon parrots (*Amazona amazonica*). Developmental Psychobiology **44**: 209-218.

Merlot AM, Kalinowski DS, Richardson DR. 2014. Unraveling the mysteries of serum albumin-more than just a serum protein. Frontiers in Physiology **5**: 299.

Milad MR, Quirk GJ. 2012. Fear extinction as a model for translational neuroscience: ten years of progress. Annual Review of Psychology. **63**:129–151.

Mitlöhner FM, Morrow JL, Dailey JW, Wilson SC, Galyean ML, Miller MF, McGlone JJ. 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. Journal of Animal Science **79**: 2327- 2335.

Morell V. 1996. Wildlife biology: life at the top: animals pay the high price of dominance. Science **271**: 292.

Mulder EJH, Robles de Medina PG, Huizink AC, Van den Bergh BRH, Buitelaar JK, Visser GHA. 2002. Prenatal maternal stress: effects on pregnancy and the (unborn) child. Early Human Development **70**: 3–14.

Murata H, Shimada N, Yoshioka M. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Veterinary Journal **168**: 28-40.

Murtaugh RJ, Jacobs RM. 1985. Serum amylase and isoamylases and their origins in healthy dogs and dogs with experimentally induced acute pancreatitis. American Journal of Veterinary Research **46**: 742–747.

Musa AS, Needham T, Kotrba R, Neradilova S, Ganswindt A, Ceacero F. 2021. Habituation of common eland (*Taurotragus oryx*) to intensive routine handling, and the effect of immunocastration thereon. Applied Animal Behaviour Science **237**: 105294.

Narayan EJ. 2019. Introductory Chapter: Applications of Stress Endocrinology in Wildlife Conservation and Livestock Science. In (Ed.), Comparative Endocrinology of Animals. IntechOpen.

Nazifi S, Saeb M, Rowghani E, Kaveh K. 2003. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine, thyroxine and cortisol concentrations. Comparative Clinical Pathology **12**: 135–139.

Needham T, Kotrba R, Hoffman CL, 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 Science **186**: 108183

Nilkumhang P, Thornton JR. 1979. Journal of Small Animal Practice **20**: 169-174.

Noonan NE, Meyer DJ. 1979. Use of plasma arginase and gamma-glutamyl transpeptidase as specific indicators of hepatocellular or hepatobiliary disease in the dog. American Journal of Veterinary Research **40**: 942–947.

Nothman MM,Callow AD. 1971. Investigations on the origin of amylase in serum and urine. Gastroenterology **60**: 82–89.

Palok A, Babiuk LA, Potter AA, Griebel P. 2009. Biomarkers for Prediction of Bovine Respiratory Disease Outcome. OMICS **13**: 199-209.

Panepinto LM, Phillips RW, Norden S. 1983. A comfortable, minimum stress method of restraint for Yucatan minieature swine. Laboratory Animal Sciences **33**: 95-97.

Parhama JT, Tanner AE, Wahlberg ML, Grandin T, Lewisa RM. 2019. Subjective methods to quantify temperament in beef cattle are insensitive to the number and biases of observers. Applied Animal Behaviour Science **212**: 30–35.

Pedersen LJ, Heiskanen T, Damm BI. 2003. Sexual motivation in relation to social rank in pair-housed sows. Animal Reproduction Science **75**: 39–53.

Pedersen RC.1988. pages 39 – 69. Cholesterol biosynthesis, storage, and mobilization in steroidogenic organs. In "Biology of Cholesterol" Yeagle PL, Ed. CRC Press, Boca Raton, FL.

Pennington PM, Pope CE, MacLean RA, Galigius J, Gentry LR, Godke RA, Wirtu G. 2013. Daily or thrice weekly handling of eland antelope (*Taurotragus oryx*). Research in Veterinary Science **94**: 711–716.

Pernthaner A, Baumgartner W, Jahn J, Plautz W, Angel T. 1993. The hematologic parameters, concentrations of minerals and metabolic products and activities of enzymes in sheep. Berliner und Munchener Tierarztliche Wochenschrift **106**: 73–79.

Perrone RD, Madias NE, Levey AS. 1992. Serum creatinine as an index of renal function: new insights into old concepts. Clinical chemistry **38**: 1933 – 1953.

Petitclerc C, Solberg HE. 1987. Approved recommendation on the theory of reference values. Part 2. Selection of individuals for the production of reference values. Journal of clinical chemistry and clinical biochemistry **25**: 639– 644.

Phillips CJ, Rind MI. 2002. The effects of social dominance on the production and behavior of grazing dairy cows offered forage supplements. Journal of Dairy Science **85**: 51–9.

Piñeiro M, Piñeiro C, Carpintero R, Morales J, Campbell FM, Eckersall PD, Toussaint, MJM, Lampreave F. 2007. Characterisation of the pig acute phase protein response to road transport. Veterinary Journal **173**: 669-674.

Pospisil J, Kase F, Vahala J, Mouchova I. 1984. Basic Hematological Values in Antelopes 4. A Comparison and Concluding Remarks. Comparative Biochemistry and Physiology **78**: 815–821.

Ravagnolo O, Miztal I. 2000. Genetic component of heat stress in dairy cattle, parameter estimation. Journal of Dairy Science **83**: 2126-2130.

Reale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews **82**: 291–318.

Renaudeau D, Mandonnet N, Tixier-Boichard M, Noblet J, Bidanel JP. 2004. Atténuer les effets de la chaleur sur les performances des porcs: la voie génétique. INRA Production Animales **17**: 93-108.

Rice M, Jongman EC, Butler KL, Hemsworth PH. 2016. Relationships between temperament, feeding behaviour, social interactions, and stress in lambs adapting to a feedlot environment. Applied Animal Behaviour Science **183**: 42–50.

Rogers WA. 1976. Source of serum alkaline phosphatase in clinically normal and diseased dogs: a clinical study. Journal of the American Veterinary Medical Association **168**: 934–937.

Romero MF. 2004. In the beginning, there was the cell: Cellular homeostasis. Advances in Physiology Education **28**:135-138.

Rubel F, Kottek M. 2010. Observed and projected climate shifts 1901–2100 depicted by world maps of the Köppen–Geiger climate classification. Meteorologische Zeitschrift **19**: 135–141.

Russell KE, Roussel AJ. 2007. Evaluation of the ruminant serum chemistry profile. Veterinary Clinics of North America: Food Animal Practice **23**: 403-426.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews **21**: 55-89.

Sapolsky RM. 2004. Social status and health in humans and other animals. Annual Review of Anthropology **33**: 393-418.

Šárová R, Gutmann AK, Špinka M, Stěhulová I, Winckler A. 2016. Important role of dominance in allogrooming behaviour in beef cattle. Applied Animal Behaviour Science **18**: 41-48.

Satyanarayana U, Chakrapani U. 2007. Biochemistry (with clinical concepts & case studies). 3rd ed. New Delhi: Elsevier Health Sciences APAC.

Scherf DB. 2000. World watch List for domestic animal diversity (3rd edition). Rome: FAQ.

Schloerb PR. 1960. Total body water distribution of creatinine and urea in nephrectomized dogs. American Journal of Physiology **199**: 661 – 665.

Schmid VS, de Vries H. 2013. Finding a dominance order most consistent with a linear hierarchy: an improved algorithm for the I&SI method. Animal Behaviour **86**: 1097-1105.

Schütz, KE, Ward JF, Coxa NR, Asher GW. 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**: 109–113.

Seal US, Ozoga JJ, Erickson AW, Verme LJ. 1972. Effects of immobilization on blood analyses of white-tailed deer. Journal of Wildlife Management **36**: 1034-1104.

Selye H. 1946. The general adaptation syndrome and the diseases of adaptation. Journal of Clinical Endocrinology and Metabolism **6**: 117–230.

Shizuka D, McDonald D. 2012 A social network perspective on measurements of dominance hierarchies. Animal Behaviour **83**: 925-934.

Silberova P, Lukesova D, Pinc L. 2010. The determination of blood profile in captive wild ungulates with focus on eland (*Taurotragus oryx*). Agricultura Tropica et Subtropica. **43**: 360-365.

Simpson KW, Simpson JW, Lake S, Morton DB, Batt RM. 1991. Effect of pancreatectomy on plasma activities of amylase, isoamylase, lipase, and trypsin-like immunoreactivity in dogs. Research in Veterinary Science **51**: 78–82.

Solberg HE, Petitclerc C. 1988. Approved recommendation on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. Journal of clinical chemistry and clinical biochemistry **26**: 593– 598.

Solter PF and Hoffmann WE. 1999. Solubilization of liver alkaline phosphatase isoenzyme during cholestasis in dogs. American journal of veterinary research **66**: 1010 – 1015.

Solter PF, Hoffmann WE. 1995. Canine corticosteroid-induced alkaline phosphatase in serum was solubilized by phospholipase activity in vivo. American Journal of Physiology **32**: G278 – G286.

Spencer GSG. 1980. Relationship between plasma somatomedin activity and the levels of cortisol and free fatty acids following stress in pigs. Journal of Endocrinology **84**: 109-114.

Spinage CA. 1986. The natural history of antelopes. New York: Facts on File Publications. 203.

Sutherland MA and Dowling SK. 2014. The relationship between responsiveness of first lactation to humans and the behavioral response to milking and milk production measures. Journal of Veterinary Behavior: Clinical Applications and Research **9**: 30–33.

Swanson KS, Kuzmuk KN, Schook LB, Fahey GC. 2004. Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. Journal of Animal Science **82**: 1713–1724.

Syakalima M, Takiguchi M, Yasuda J, Hashimoto A. 1998. The canine alkaline phosphatases: a review of the isoenzymes in serum, analytical methods and their diagnostic application. Japanese Journal of Veterinary Research **46**: 3–11.

Tahara Y, Shima K. 1995. Kinetics of HbA1c, glycated albumin and fructosamine and analysis of their weight functions against preceding plasma glucose levels. Diabetes Care **18**: 440-447.

Terlouw EMC. 2015. Stress reactivity, stress at slaughter and meat quality. CRC Press, Philadelphia, Pa, USA.

Thompson KV. 1993. Aggressive behavior and dominance hierarchies in female sable antelope, Hippotragus niger: implications for captive management. Zoo Biology **12**: 189–202.

Thornton JR and Lohni MD. 1979. Equine Veterinary Journal **11**: 235-238.

Trevisi E, Calamari L, Bertoni G, Cappa V. 1992. The possible relationship between oxytocin release and blood potassium in stress phenomena in ruminants. Page 364 in Proc. 8th Int. Conf. on Production diseases in farm animals, Berne, Switzerland.

Tuchscherer M, Puppe B, Tuchscherer A, Kanitz E. 1998. Effects of social status after mixing on immune, metabolic, and endocrine responses in pigs. Physiology and Behavior **64**: 353-360.

Vahala J, Spala P, Kase F. 1989. Biochemical Blood Serum Values of Healthy Antelopes kept in the East-Bohemian Zoological Garden at Dvůr Králové nad Labem – I. The Tragelaphinae, the Hippotraginae and the Aepycerotinae. Acta Veterinaria Brno **58**: 17–29.

Vale W, Spiess J, Rivier C, Rivier J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and α-endorphin. Science **213**: 1394–1397.

Valentine BA, Blue JT, Shelley SM. 1990. Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog. Journal of Veterinary Internal Medicine **4**: 140–143.

Vazhapilly P, Calamari L, Frazzi E, Azzoni A, Cappa V. 1992. Effect of heat stress on cheesemaking quality of milk from dairy cows raised in Po valley. pp 19-28 in Proc. 2nd Int. Sem. CIGR (International Commission of Rural Engineers) on Environmental and energy aspects of livestock housing, Polanica, Poland.

Veasey J. 2006. Concepts in the care and welfare of captive elephants. Internal Zoo Yearbook **40**: 63–79.

Voisinet BD, Grandin T, Connor SFO, Tatum JD, Deesing MJ. 1997. *Bos indicus* cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. Meat Science **46**: 367–377.

Vymyslická PJ, Brandlová K, Hozdecká K, Žáčková M, Hejcmanová P. 2015. Feeding rank in the Derby eland: lessons for management African Zoology **50**: 313-320.

Watanabe D, Ikeda H, Kazamatsuri H, Ando T, Ohtsuka H, Kobayashi S, Oikawa M, Yoshikazu S. 2010. Alterations of Growth, Blood Biochemical Components and Hormone Profiles by Intensified Nutrition in Growth Retarded Japanese Black Cattle. Journal of Veterinary Medical Science **72**: 1203-1208.

Whirledge S, Cidlowski JA. 2010. Glucocorticoids, stress, and fertility. Minerva Endocrinologica **35**: 109.

White HM, Richert BT, Schinckel AP, Burgess JR, Donkin SS, Latour MA. 2008. Effects of temperature stress on growth performance and bacon quality in grow-finish pigs housed at two densities. Journal of Animal Science **86**: 1789-1798.

Wilson SJ, Kirby CJ, Koenigsfeld AT, Keisler DH, Lucy MC. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. Journal of Dairy Science **81**: 2132-2138.

Wirtu G, Cole A, Pope CE, Short CR, Godke, RA, Dresser BL, 2005. Behavioural training and hydraulic chute restraint enables handling of eland antelope (*Taurotragus oryx*) without general anesthesia. Journal of Zoo and Wildlife Medicine **36**: 1–11.

Wirtu G, Pope CE, Vaccaro J, Sarrat E, Cole A, Godke RA, Dresser BL. 2004. Dominance Hierarchy in a Herd of Female Eland Antelope (*Taurotragus oryx*) in Captivity. Zoo Biology **23**: 323–333.

Wolfenson D, Roth Z, Meidan R. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Animal Reproduction Science **61**: 535-547.

Wyss M, Kaddurah-Daouk R. 2000. Creatine and creatinine metabolism. Physiological Reviews **80**: 1107-1213.

ZIMS Expected Test Results for *Taurotragus oryx*. 2022. Species360 Zoological Information Management System. Retrieved on March 23rd from http://zims.Species360.org.

Zinkl JB, Bush RM, Cornelius CE, Freedland RA. 1971. Comparative studies on plasma and tissue sorbitol, glutamic, lactic, and hydroxybutyric dehydrogenase and transaminase activities in the dog. Research in Veterinary Science **12**: 211–214.

Appendices

List of the Appendices:

1. Temperament observation

Date: ________________________ Observer: __________________________

	ID	General state			Use of stick				Vocalisation				Aversion to enter system			Movement through system			State in crash			Exit			Clicking
		Calm	Nervous	Panicked	Phase 1	Phase 2	Phase 3	Phase 4	None	Low	Medium	High	Voluntary	Food lure	Forced	Walking	Running	Forced	Calm	Nervous	Panicked	Walking	Running	Jump	
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2. Social interactions observation

Date ____________________________

Group ____________________________

Observers__________________________

Time	Winner	Loser	Interaction	Remarks

Time	Winner	Loser	Interaction	Remarks