CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Animal Science and Food Processing

Diet quality and mineral status of large herbivores in West African savanna ecosystems

DOCTORAL THESIS

Author: Ing. Lucie Stoklasová

Supervisor: prof. RNDr. Pavla Hejcmanová, Ph.D.

Prague 2021

Declaration

I, Lucie Stoklasová, hereby declare that I have done this thesis entitled “Diet quality and mineral status of large herbivores in West African Savanna ecosystems” 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, February 15, 2021

…….

Ing. Lucie Stoklasová

Acknowledgements

I would like to express my utmost gratitude to my supervisor prof. RNDr. Pavla Hejcmanová, Ph.D. for her encouragement, patience, guidance and conveying her scientific experience during my doctorate studies. I would like to thank her for being with me and showing me direction in difficult times.
I would like to thank everyone who helped me with samples collection and assist me during their processing. Moreover, I would like to thank to the members of our Department of Animal Science and Food Processing at the FTA CZU for enabling me to finish my doctoral studies. Finally, I would like to convey my great thanks to my family, especially my sister Míša Stoklasová for her constant care and never-ending patience, without these, completion of my doctoral study would not be possible, as well as I thank my close friends for their support.

This research was financially supported by the Czech University of Life Sciences Prague, namely by projects CIGA 20175004 and IGA 20205015.

Abstract

The knowledge of adequate nutritional requirements and health status of large free ranging herbivores is limited, especially in the West African region. Many studies are focused to South Africa and diet quality and mineral status of herbivores in other regions are not investigated in depth. Lack of these essential information are burning issue primarily for endangered species. The present dissertation thesis aimed to fill this gap by expanding our understanding of the diet quality and mineral status of large herbivores in West African savanna ecosystems, i.e. in semi-arid and sub-humid savannas, with special focus on the critically endangered Western Derby eland (WDE). An integral partial aim was to explore the flexibility and clarify the position of Common eland regarding its classification as "mixed feeder" feeding type, as this species was introduced into the community of large herbivores in two wildlife reserves in Senegal, regardless its South African origin. To determine mineral status and diet quality, samples of blood serum, faeces and hair were collected from Western Derby eland from semi-arid and sub-humid areas. To test whether the flexibility of Common eland to browse vs. grass diet is linked to a 'cattle-type' or a "moose-type" forestomach physiology by measuring the mean retention time of different digesta phases in a standard experiment on a Common eland breeding farm was done. Faecal samples for diet quality determination were collected altogether from buffalo, zebra, common eland, roan antelope and Wester Derby eland (seasonal data collection) from the same semi-arid and sub-humid areas.

Mineral status of Western Derby eland was determined mainly from the blood serum since there was not found any correlation between mineral concentration in hair and faecal samples. Therefore, analysis of faeces and fur cannot stand alone for the assessment of the mineral status and the determination mineral deficiencies but may serve as useful supporting evidence. Blood mineral profile of Western Derby eland was therefore compared to other Tragelaphineae and appeared lower in almost all elements, which may reflect most likely the limited mineral background in the local environment. Nevertheless, tested individuals of WDE did not show clinical problems, which may be due to dietary adaptation of WDE to such mineral limited environment. Common eland was, based on results of our experiment, considered as 'moose-type'. Analysis of mineral concentrations and diet quality indicators (ADF, NDF, lignin, cellulose, hemicellulose, nitrogen) from faeces of five herbivore species in two localities provided information about herbivores adaptation to semi-arid and sub-humid environment. Mixed feeders and grazers maintained the quality of their diet in most of the principal parameters, especially in the content of nitrogen and fibres, regardless the savanna ecosystem type, while browser showed significantly lower nitrogen and fibres in the semi-arid environment in comparison to the sub-humid one. The diet quality indicated by the concentration of nitrogen was higher in Derby and common elands in comparison to zebra and buffaloes at both sites. Despite the fact that all species maintained their diet quality above level of deficiency, semi-arid conditions required feeding behaviour adaptation of all species across feeding types. From conservation perspective, all feeding guilds may become threatened by changing ecosystems, even if each one by different way, if they are forced to change food resources, and a conservation-oriented 'exit-strategy' for species should be conceived to address these threats, including the strengthening the ecological connectivity in savanna landscapes.

Key words: Antelope conservation; Blood serum; Faeces; Feeding type; Hair; Large herbivores; Mineral profile; Savanna ecosystem; Wildlife management

Abstrakt

Znalosti nutričních požadavků a zdravotního stavu velkých volně žijících býložravců jsou omezené, zejména pro oblast západní Afriky. Řada studií se zaměřuje na jižní Afriku a kvalita potravy a minerální status býložravců v ostatních regionech nejsou dostatečně podrobně prozkoumány. Nedostatek těchto zásadních informací je palčivým problémem zejména pro ohrožené druhy zvířat. Cílem této disertační práce bylo tyto zásadní nedostatky doplnit prohloubením znalostí o kvalitě potravy a minerálním statusu velkých přežvýkavců v savanových ekosystémech západní Afriky, a to v semi-aridní a sub-humidní savaně (ve dvou rezervacích), se zaměřením na kriticky ohrožený západní poddruh antilopy Derbyho. Důležitým dílčím cílem bylo popsat potravní flexibilitu antilopy losí a ujasnit její pozici v klasifikaci potravních typů kde je pokládána za potravního oportunistu (mixed feeder). Původem je tento druh v jižní Africe, ale byl introdukován do dvou rezervací v Senegalu, kde je součástí společenstva velkých býložravců.

Pro stanovení minerálního statusu a kvality potravy byly v obou rezervacích sebrány vzorky krevního séra, trusu a srsti antilopy Derbyho. S antilopou losí byl za účelem určení fyziologické stránky potravního typu (tzv. 'cattle-type' nebo "moose-type") proveden standardní stájový experiment na měření průměrného retenčního času tráveniny. Ke stanovení kvality potravy velkých býložravců (buvola, zebry, antilopy losí, antilopy koňské, antilopy Derbyho) a porovnání mezi semi-aridní a sub-humidní oblastí byly získány vzorky trusu sběrem v průběhu sezon v obou rezervacích.

Minerální status antilopy Derbyho byl určen zejména ze vzorků krevního séra, protože nebyla nalezena žádná korelace mezi koncentracemi minerálů ve vzorcích srsti a trusu. Analýza trusu a srsti proto nemůže být použita samostatně pro analýzu minerálního statusu a určení případného minerálního deficitu, ale může sloužit jako užitečná podpůrná informace. Krevní minerální profil antilopy Derbyho byl porovnán s ostatními zástupci kmene Tragelaphineae. Hodnoty u antilopy Derbyho byly nižší téměř ve všech ukazatelích, což pravděpodobně odráží nízký obsah minerálů v prostředí. Nicméně, testovaní jedinci nevykazovali žádné klinické příznaky nedostatku minerálů, což může být způsobeno adaptací antilopy Derbyho na takto chudé prostředí. Antilopa losí byla na základě výsledku experimentu zařazena k 'moose-type' býložravcům. Analýza koncentrací minerálů a indikátorů kvality potravy (ADF, NDF, lignin, celulóza, hemicelulóza, dusík) pěti druhů býložravců ve dvou oblastech poskytla informace o adaptacích těchto býložravců na prostředí semi-aridní a sub-humidní savany. Potravní oportunisté a spásači udrželi kvalitu své potravy ve většině základních parametrů, zejména obsah dusíku a vlákniny, bez ohledu na typ savany, zatímco okusovači vykázali signifikantně nižší koncentrace dusíku a vlákniny v semi-aridní savaně v porovnání se sub-humidní. Kvalita potravy indikovaná obsahem dusíku byla vyšší u antilopy Derbyho a antilopy losí v porovnání se zebrou a buvolem v obou oblastech. Navzdory schopnosti všech druhů udržet kvalitu potravy nad hranicí nedostatku vyžadovalo prostředí semi-aridní savany adaptaci potravního chování všech druhů napříč potravními typy. Z pohledu ochrany přírody mohou být všechny potravní typy býložravců ohroženy změnou ekosystému, i když každý jiným způsobem, pokud jsou nuceny změnit potravní zdroje. Aktéři ochrany přírody by měli předložit strategii, jak těmto hrozbám čelit, včetně posilování ekologické konektivity savanové krajiny.

Klíčová slova: Ochrana antilop; Krevní sérum; trus; Potravní typ; Srst; Velcí býložravci; Minerální profil; Savanový ekosystém; Management volně žijící zvěře

Contents

1. Introduction 1
2. Aims 2
3. Review 4
3.1 Diet quality 4
3.2 Forage quality 4
3.3 Mineral status of animals 5
3.4 How to assess mineral status of animals 5
3.4.1 Faecal samples 6
3.4.2 Blood samples 6
3.4.3 Hair samples 8
3.5 Feeding types 8
3.7 Herbivorous species 11
3.7.1 Western Derby eland (*Taurotragus derbianus derbianus*) 12
3.7.2 Roan antelope (*Hippotragus equinus*) 12
3.7.3 African buffalo (*Syncerus caffer*) 13
3.7.4 Burchell's zebra (*Equus quagga burchellii*) 14
3.7.5 Common eland (*Taurotragus oryx*) 15
3.8 Study site 15
3.8.1 The Bandia reserve 16
3.8.2 The Fathala reserve 16
4. Minerals in the blood, hair, and faeces of the critically endangered Western Derby eland under human care in two wildlife reserves in Senegal 18
5. Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal 25
6. Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet 32
7. Resilience in diet-quality strategies of mixed native and non-native herbivore communities in semi-arid and sub-humid savanna ecosystem 49
8. Conclusion 63
9. References 65
10. Appendices I

List of figures

Fig. 1: Schema of pathophysiological changes in mineral-deprived livestock……**7**
Fig. 2: Western Derby eland (*Taurotragus derbianus derbianus*) ……....**12**
Fig. 3: Roan antelope (*Hippotragus equinus*)……**13**
Fig. 4: African buffalo (*Syncerus caffer*)……..….**14**
Fig. 5: Burchell's zebra (*Equus quagga burchellii*)……..….**14**
Fig. 6: Common eland (*Taurotragus oryx*)…….....**15**
Fig. 7: Map of location of two wildlife reserves in Senegal……..…**16**
Fig. 8: Passage marker excretion pattern for cobalt (Co, solutes), chromium (Cr, particles < 2 mm) and cerium (Ce, particles < 10 mm) in a common eland (*Taurotragus oryx*) ……..…...…**36**
Fig. 9: Relationship of the mean retention time (MRT) of small particles (<2mm) in the reticulorumen (RR)…….....**38**
Fig. 10: Relationship between the average percentage of grass in the natural diet (species <15%, grey circles; species >20%, white circles) and the selectivity factor….…….…...**39**
Fig. 11: Relationship between the selectivity factor (SF, a measure for how distinctly the retention of small particles and fluid differ, i.e. a measure for the 'washing mechanism') in the reticulorumen……...**41**
Fig. 12: Constrained redundancy analysis ordination diagram……**64**

List of tables

Table 1. Feeding strategies of herbivores……..…..……**9**
Table 2. Investigated individuals of the Western Derby eland……...…….2**3**
Table 3. Mean concentrations of the principal elements in the blood, hair and faeces of the Western Derby elands, soil and preferred browsed plant species…….…...**24**
Table 4. Concentration of minerals in the blood serum of Western Derby elands in the Bandia and Fathala reserves…….....**30**
Table 5. Overview of blood (serum/plasma) mineral concentrations (mean, range) of different ruminant species……...**31**
Table 6. Nutrient concentration in the grass hay and the lucerne haylage used in the present study…….**46**
Table 7. Mean (±SD) intake of feed as fed, dry matter (DM), the relative dry matter intake (rDMI), the mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of three passage markers……...**47**
Table 8. Mean (±SD) apparent digestibility values and dry matter gut fill in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage…….…...**47**
Table 9. Nonparametric correlations (n=12) between relative dry matter intake (rDMI, $g/kg^{0.85}/d$) and various measures of mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR)..……..**48**
Table 10. Concentrations (mean ± SE) of macroelements and fiber fractions in faeces of large herbivores in Bandia and Fathala reserves…….**62**
Table 11. Concentrations (mean ± SE) of microelements in faeces of large herbivores in Bandia and Fathala reserves……..…..……**63**

1. Introduction

To protect nature and endangered species of mammals, conservationists have to work with actual and accurate information and data. The scientific knowledge on wildlife nutrition and mineral status has a lot of gaps. Reference values of mineral concentrations for many selected species are completely missing, especially for species inhabiting region of West Africa. Situation of trace minerals is even worse, and beside nutrition of livestock for production, trace elements do not have a lot of scientific attention, despite they are involved in many essential metabolic, physiological, and reproduction processes, thus in fitness. That is why, mineral status determination becomes very important. For some species, mostly domestic, there are known limits of mineral status evaluation from blood serum, hair and faeces. However, authors differ in their opinion in using different samples at different species (e.g. Dlugaszek & Kopczyński 2014; Soest 1982; Herdt & Hoff 2011). Consequently, assessment of mineral status determination means in term of efficiency, accuracy, and availability in concrete species and environment is essential. Maintenance of certain mineral levels in animal bodies depend on concentrations of these minerals in the environment where animal lives and feeds. Therefore, forage quality is essential for maintaining animals in good health condition when they can also reproduce, it means overall fitness of the individual. However, forage quality and quantity changes with changing biomes (Martens et al. 2020). Recent meta-analyses have already shown species´ range shifts because of climate change during the last century for both animal and plant taxa (Parmesan & Yohe 2003; Root et al. 2003). Those shifts are going to continue also on levels of ecosystems and biomes (Engelbrecht & Engelbrecht 2016). The influence of changing climate, especially rainfall conditions, on mammal distributions and abundances has been widely documented in Africa (Owen-Smith 1990; Mills et al. 1995; Owen-Smith & Ogutu 2003, Martens et al. 2020). For instance, in the Kruger Park in South Africa, almost all of the ungulate species seem extremely sensitive to lack of rainfall during the dry season, which is projected to be exacerbated into the future (Hulme et al. 2001). Dry season rainfall may be directly important by influencing the retention of some green forage during this critical period when malnutrition takes hold (Owen-Smith & Ogutu 2003). Another aspect of global climate change is warming, which has impact on temperatures around the world and specifically, African continent is warmer than it was 100 year ago (Hulme et al. 2001). In this perspective, the global climate change processes, which increase incidence and severity of droughts (Lyon 2004; Knapp et al. 2008) significantly impact the African savanna ecosystems, namely in their vegetation structure and fire regimes, hence in their overall ecological functionality and availability of food resources for herbivores (Hulme et al. 2001; Thuiller et al. 2006). Under droughts, grass biomass significantly declines, grasses become dormant and unavailable, while adult trees as primary resource for browsers seem more variable in response and may retain a certain level of availability (Ryan et al. 2016; Augustine 2010; Walker et al. 1987). Altered plant growth interacts with changes in fire regimes, and direct enhancement of tree seedling growth rates under elevated CO_2 increases the likelihood that young trees survive fires. This might lead to a positive feedback, where initial increase in woody vegetation supresses grasses, thereby reduces fire activity, which in turn benefits woody plants (Midgley & Bond 2015). Savannas and grasslands, as open biomes, are particularly vulnerable to biome changes. Woody

encroachment into grasslands and savannas in Africa is a major threat for their biodiversity (Bond 2016) and change soil carbon storage, ground water recharge and grazing potential (Stevens et al. 2017).

Such changes in environment conditions activate behaviours that secure the resilience of herbivores communities across feeding guilds. Mixed feeders cope with drought by widening their diet breath and eating more browse, whereas grazers tend to shift their range of distribution by tracking moving climate zones (Parmesan & Yohe 2003; Root et al. 2003) and migrate to less severely affected areas (Abraham et al. 2019; Staver 2018). Migration to more suitable areas is constrained strongly by human settlements, agricultural fields, roads, fences etc. Thereby, animals may also be forced to stay where they are and use a delimited space only, for instance within protected areas. The competition for changing resources may become critical and may lead to population declines and extinctions (Augustine 2010; Thuiller 2006; Craigie et al. 2010). Let us note at this point that most of findings related to African wildlife ecology, management, and conservation are dominated by experience from southern and southeastern parts of Africa (Bauer et al. 2020), while West African region has started relatively recently to fill these gaps and the present dissertation thesis is part of that. We expect the present dissertation thesis to provide a part of missing information critical for the conservation of mammalian herbivores in face of potential habitat changes resulting from global climate change in the West Africa. For instance, roan antelope interactions with and adaptations to the diverse environmental conditions throughout its geographic range remain largely unknown. Most studies have taken place in southern Africa, near the range limit for the species; few studies have been conducted on the historically large Central and West African populations (Havemann 2014).

The conservation and management of wildlife in the resultant 'island' ecosystems in the context of global warming is challenging due to the isolation and reduced size of the ecosystems and hence the scale over which ecosystems processes can operate (Ogutu et al. 2012).

2. Aims

The aim of the thesis was to provide an insight in the diet quality and mineral status of large herbivores in West African savanna ecosystems with special focus on the critically endangered Western Derby eland (abbreviate hereafter also as WDE) and to reveal potential deficiencies in mineral nutrition and/or quality of diet. To achieve this aim, following objectives have been formulated:

1) To evaluate the means to determine the mineral status in large herbivore bodies, taking specifically Western Derby eland as a model species, in order to identify appropriate methods with minimal effects on the animals in the wild during any future conservation action. This objective was divided into two parts: a) to determine the concentrations of macro and selected microelements in the blood serum, hair and faeces of WDEs bred in the reserves to provide the baseline data for further management, and b) to explore the correlations among the concentrations of elements in blood serum, hair and faeces.
2) To determine mineral status/profile of Western Derby eland through the mineral profiles (Ca, P, Mg, Cu, Fe, Zn, Se, K, S) in blood serum, to compare them with available

reference values from closely related (i.e. Eastern Derby/ Giant eland) and other large herbivore species (e.g. Common eland, kudu), and to identify potential mineral deficiencies, if any, which might assist to interpretation of differences in reproduction in two wildlife reserves.

3) To expand our understanding of the flexibility of Common eland regarding the "mixed feeder" feeding type classification, as this species was introduced into the community of large herbivores in two wildlife reserves in Senegal, regardless its South African origin. Specific objective was to test whether the flexibility of Common eland to browse vs. grass diet is linked to a 'cattle-type' or a "moose-type" forestomach physiology by measuring the mean retention time of different digesta phases in a standard experiment on a Common eland breeding farm.
4) To evaluate the diet quality of five large herbivore species in two wildlife reserves representing two West African savanna ecosystems, specifically in semi-arid Sahelian and sub-humid Sudano-Guinean savannas, those as a proxy for a potential extreme shift of habitats under changing climate. We examined specifically whether herbivores of different feeding guilds demonstrate distinct or the same diet quality in semi-arid and sub-humid savannas, which implicitly differ in plant species composition and abundance. Namely, we tested the differences in fibre fractions, macro- and microelements among five large ungulates belonging to grazers, mixed feeders, and browsers and then we compared their diet quality in these two savanna types in wet, dry, and late dry seasons of the year. Regarding that both reserves a food supplement in the late dry season, we expected therefore to depict the contribution of food supplement in the overall quality of diet during the period of most limited resources.

3. Review

In African savannas, superabundance of green vegetation at one time of the year changes sharply with the sparse dry remnants of fibrous plants in later stage of dry season. In savanna regions of Africa, this is represented mostly by the annual or twice annual alternation of wet and dry seasons. Herbivore populations are usually regulated by the amount of forage retained in key resource areas providing adequate forage through the period of plant dormancy (Yoganand & Owen-Smith 2014). Animals persist by responding to this variability in numerous ways: adjusting what they eat, the habitats they occupy, and when they reproduce (Watson & Owen-Smith 2002). The way how herbivorous species evolved unique feeding strategies to be able to coexist together are of high scientific interest. This review provides description and explanation of those feeding strategies and feeding guilds. Basis of all feeding differentiation among herbivorous species is a need to maintain certain level of nutrient intake. That is why diet quality and forage quality are essential.

3.1 Diet quality

Diet quality is quite complex indicator of animal nutrition. Food and its quality can be evaluated from aspect of energy intake, mineral content and fibre content. As well as from perspective of animal need, what is high quality food for one species can be a low quality diet for another one, and even it is specific at individual level according to physiological state, e.g lactating female has higher requirements than nonlactating one.

Foods supply energy and essential nutrients in the form of protein, vitamins and minerals. Essential nutrients include energy, minerals, vitamins, and amino acids, which are usually considered under the general nitrogen requirement (as crude protein) because the rumen bacteria are capable of synthesizing them.

Most common way of diet quality evaluation is nutritive value of food, which explores food contribution to the nutrient content of the diet. Diet quality depends on amount of essential nutrients (protein, fat, carbohydrate, minerals, vitamins) which it contains (Van Soest 1994).

Nutritive value is conventionally classified into three general components: digestibility, i.e. balance of matter lost in passage through the digestive tract, feed consumption, and energetic efficiency. Vegetation quality is commonly expressed in terms of percent crude protein (mg g^{-1}), or nitrogen (mg g^{-1}) (Beeri et al. 2007).

3.2 Forage quality

Quality of forage is greatly influenced by the amount of dietary fibre (plant cell wall) it contains, because the lignified part of fibre is indigestible. The primary plant cell wall is a complex structure composed of lignin, cellulose, hemicellulose, pectin, structural and other proteins, lignified nitrogenous substances, waxes, cutin, mineral components (Soest 1982), mixed linkage ß-glucans, heteroglucans, glucuronarabinoxylans and heteroxylans. During the maturation process of plant, secondary wall deposition and lignification begin and cellulose is the major polysaccharide deposited in the secondary wall (Cooper & Owen-Smith 1985). As maturation process reaches different parts of plant body diversely, similarly different nutrients vary in their concentrations within plant tissues. The digestive release of all of the nutrients

contained within cells is constrained to some extent by their encapsulation within the cell walls (complex polysaccharides, lignin etc.). A high proportion of cell wall components ultimately control the food ingestion rate, and hence the rates of assimilation of all nutrients (Van Soest 1967).

The fibre component of grasses contains particularly high percentages of hemicellulose and cellulose. Browse typically has a higher lignin content but also contains rapidly fermentable fibre such as pectins (Claus et al. 2010a). Consequently, grass has fundamentally different fermentation characteristics than browse with a slower fermentation rate but potentially higher total digestibility, which means that grass can profitably be retained in the fermentation chamber longer than browse. Browse often contains plant secondary metabolites that require neutralization/detoxification. However, grasses are physically more demanding to chew than browse (Archer & Sanson 2002; Clauss et al. 2008; Kaiser et al. 2010).

The nutritional value of consumed herbage depends on the proportion of cell wall fibre relative to cell contents and on the concentrations of protein, soluble carbohydrates and minerals in the cell contents (Robbins 1993; Van Soest 1994). For grazers, nitrogen derived the breakdown of plant protein can be especially limiting, both in relation to the protein requirements of the herbivores and by restricting microbial fermentation of the cellulose content of plant cell walls (Owen-Smith 1982). On certain soil types, phosphorus (P) may also become a limiting nutrient (Robbins 1993). Nutrient concentrations in plant tissues decline during the dry season as plants become dormant and green leaves become brown (Georgiadis & McNaughton 1990; Owen-Smith 2008). Hence, for large herbivores, the late dry season is typically the critical period for survival.

3.3 Mineral status of animals

Mineral status of animal is concentration of mineral in the body of the animal, which can be measured by concentrations of mineral in the blood, faeces, hair, liver and other tissues (Kincaid 1999; Herdt et al. 2000; Mills 1987). All animal and plant tissues contain widely varying amounts and proportions of mineral elements. Finite amounts of all minerals vary for different elements and species and can therefore change with time and by husbandry change. The clinical and pathological abnormalities associated with most mineral imbalances are rarely specific and often bear a clinical similarity to other nutritional disorders and parasitic or microbial infections of the gut (Suttle 2010). Determination of mineral status serves as indicator of presence or prevalence of nutrient deficiencies (or toxicities) within a population (Kincaid 1999). The biologic availability of dietary minerals is variable and difficult to predict because mineral availability can be affected by mineral source, chemical form of the mineral and interactions among dietary constituents (Herdt & Hoff 2011)

3.4 How to assess mineral status of animals

Evaluation of diet quality by chemical analysis of available standing forage plants is complicated method since nutrient content differs not just on levels of plant species, but particular parts of plant bodies differ in their nutrient content and mineral availability as well (Hanley 1982). Determination of diet quality by method of chemical analysis of forage plants in condition of large open space of natural reserve and elusive animal species is extremely

difficult. Another limitation of plant mineral analysis is, that it is not sufficient indicator of diet quality. Huge attention must be given to anti-nutritional compounds or secondary plant compounds like tannins, lignin, silica etc. which reduce nutrient utilization or make them completely unavailable to the metabolism and may decrease food intake of plant foods. That is why different methods of diet quality have to be involved. Another indirect method of animal mineral status determination is e.g. investigation of soil mineral concentrations. However, many factors can interrupt the flow of mineral from soil into the animal: a) accessibility – the potential access of the plant mineral to the absorptive mucosa; b) absorbability – the potential transfer of absorbable mineral across the mucosa; right chemical form of the mineral to be able to absorb c) retainability – the potential retention of the transferred mineral; d) functionality – the potential for incorporation of retained mineral into functional forms. The limited diagnostic value of measures of soil and plant mineral status means that focus must be placed on the animal (Suttle, 2010).

3.4.1 Faecal samples

Assessment of diet quality based on minerals in faeces is widely used and studied in terms of assisting in understanding animal's nutritional ecology (Wrench et al. 1997). Chemical characteristics of faeces are related to the quality or quantity of ingested diets (Holechek et al. 1982). Researchers have used faecal nutrient levels for different species e.g. elk (Leslie & Starkey 1985), black-tailed deer (Jenks et al. 1989); duiker, eland, hartebeest, mule deer, Thomson's gazelle (Robbins 1983).

Faecal endogenous losses are almost entirely of salivary origin and thus related to dietary intake. The faeces contain not only the undigested diet but also metabolic products including bacteria and endogenous wastes from animal metabolism (Soest 1982).

The quantity of a mineral excreted in the faeces is the sum of the unavailable dietary mineral, available but unabsorbed dietary mineral and endogenous mineral. The dietary mineral available for absorption is the amount released during the processes of digestion and appears to be in the same pool as the endogenous mineral at the absorptive sites in the gastro-intestinal tract (Primary Industries Standing Committee 2007).

3.4.2 Blood samples

Blood measures are frequently used in assessment of animal response because they are significantly correlated to nutritional status of some trace elements such as Cu, Co and Zn (Levander 1986; Mills 1987, Herdt et al. 2000). Analysis of blood calcium concentration, however, is generally an ineffective means of assessing calcium status, probably because of the sensitive homeostatic mechanism for regulation of blood calcium concentration and the large store of calcium in bone. Blood serum phosphorus concentration is a good tool for nutritional monitoring, although there are several confounding variables that must be considered. The characteristics of magnesium metabolism make serum magnesium concentration a relatively good indicator of nutritional status (Herdt et al. 2000).

Blood is less invasive to sample than liver or other internal tissues but it is subject of many limitations and results must be interpreted with care (Herdt & Hoff 2011). Whole blood, serum, and plasma are widely sampled, and serum is usually chosen for analytical analysis because it is easy to obtain and it avoids possible analytical complications of adding an anticoagulant (as

to the plasma) and gives a more stable form for transportation as long as it is free of products of hemolysis. However, blood sample collection requires training to prevent hemolysis and contamination of plasma/serum and sample storage outside the laboratory may be problematic (Sach et al. 2020). Results for plasma and serum have been assumed to be the same, but serum invariably contains less copper than plasma (Laven & Livesey 2006). Results for serum or plasma samples generally reflect the mineral status of the transport pool of the element, and low values indicate onset of the deficiency. Blood mineral level often does not correspond to their contents in the whole body, because the composition of plasma results from supplementation of deficiencies by different homeostatic mechanisms (Bland 1984). Those homeostatic mechanisms control mineral concentrations in the body, even when intake is insufficient (Kincaid 1999). And fluctuation in dietary intake may affect plasma mineral levels too slowly or too rapidly to demonstrate true nutritional status in the animal (Herdt et al. 2000; Mills 1987). Homeostatic control mechanisms can limit changes in concentrations of some trace minerals in plasma until endogenous reserves are substantially depleted (Miller 1975). Other factors than nutrition and homeostatic forces are known to affect serum mineral levels, as physiological state (pregnancy, lactation, etc.), inflammation etc. may influence serum trace mineral concentrations (Herdt & Hoff 2011). Moreover, the blood mineral concentration is relatively low and depends on the current diet, thus the diagnostic value of the analytical results may be fairly small (Bland 1984). During periods of inadequate dietary intake depletion of storage pools and transport forms of trace elements occur before the development of measurable dysfunction and/or disease. Furthermore, some elements do not have recognizable storage pool and for several minerals the transport and functional pools overlap (Fig. 1.).

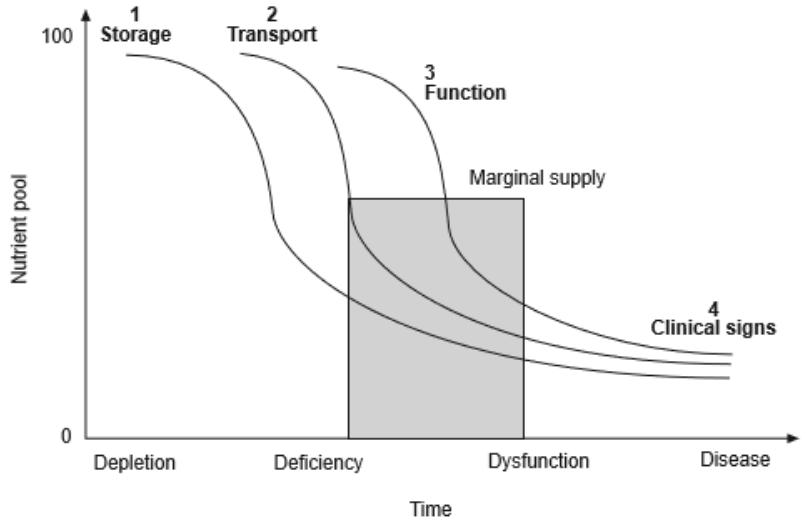


Fig. 1. Schema of pathophysiological changes in mineral-deprived livestock. There are four possible phases, beginning with depletion and ending with disease, related to changes in body pools of mineral that serve storage (e.g. liver), transport (e.g. plasma) or functional (e.g. muscle enzyme) purposes. There is usually a zone of marginal area (shaded) where stores are all but exhausted and mineral-dependent functions begin to fail, but the animal remains outwardly healthy (upper limit on the y axis represents maximum or normal attainable pool size) Source: Underwood & Suttle 1999.

3.4.3 Hair samples

Usage of animal hair as indicator of animal mineral status is still question of scientific debate. Beside fact that there are very limited data on the elemental composition of wild animal fur (Dlugaszek & Kopczyński 2014), suitability of such material for determination of mineral levels in animal body is controversial. Some authors (Dlugaszek & Kopczyński 2014; Passwater & Cranton 1983; Bland 1984) assume that the fur of wild animals can provide an information on the bioavailability of elements and environmental exposure and consider hair as useful biomarker in animals and environmental studies. While others (Combs et al. 1982; Combs 1987; Fisher et al. 1985) consider hair analyses as not precise indicator of the mineral status of animals. And recommend its application as supportive mean for detection of severe deficiencies of some required minerals or exposure to some heavy metals. There are arguments for both groups of scientists. Advantages of hair analysis are: a) that hair samples reflect longer term patterns of dietary intake, over weeks or months (Sponheimer et al. 2003) and hair collection is less invasive than for e. g. blood samples; b) animal and human hair is a stable, biologically and chemically inactive tissue, made up from a protein (keratin) and significant amounts of sulphur amino acids. Sulfhydryl groups of these compounds bind metals, including toxic elements (Combs 1987). During formation of hair, it is exposed to circulating blood, lymph and extracellular fluid. As the hair approaches the skin surface, it is removed from sites of metabolic activity and undergoes keratinization (Hinners et al. 1974; Hopps 1977); c) mineral concentrations in hair reflect dietary intake of these minerals (Miller et al. 1966; Miller 1970; Anke 1996).

Disadvantages of hair analysis are: a) the content of elements in fur can be affected by such factors as species, season, sex, age, colour, diet and health state and the environment in which animal exist (Combs 1987; Wren 1986); b) the concentration of elements in animal hair is often much higher than found in body fluids and some tissues (Dlugaszek & Kopczyński 2014); c) there is effect of body location on mineral content of hair. That may be due to differences in surface contamination (faeces, sweat, feed, airborne matters), differences in hair growth cycles and texture of the hair; d) concentrations of minerals in hair are not affected by dietary intake of these minerals (Combs et al. 1982).

3.5 Feeding types

Herbivorous mammals are sensitive to the quality and quantity of their forage and show different dietary specializations (Janis et al. 2000). Herbivores in general are morphologically classified as foregut fermenters (primary fermentation chamber proximal to the small intestine) and hindgut fermenters (primary fermentation chamber distal to the small intestine) (Clauss et al. 2010a). That classification is based on two rules: 1) fermentative digestion of fibre requires more time than fermentative digestion of easily digestible substrates; 2) auto-enzymatic digestion of easily digestible substrates is energetically more efficient than fermentative digestion of these substrates: hindgut fermenters can pursue both strategies, either high-intake/low-efficiency or low-intake/high-efficiency. Non-ruminant foregut fermenters, however, cannot adopt the high-intake/low-efficiency strategy, because they lose the easily digestible nutrients to the foregut microflora but would not achieve thorough fibre fermentation, having only the disadvantages of both ways (Clauss et al. 2008).

Foregut fermenters subsequently divide to ruminants and non-ruminant. The selection of natural diet of herbivores can be described in botanical terms (browser/grazer) or in terms of diet quality (selective/unselective) (Clauss et al. 2010a).

There are two main groups along a feeding type continuum: grazers and browsers. Grazers mainly eat _>90% the foliage of monocotyledons (such as grasses and sedges) whereas browsers mainly eat the foliage of dicotyledons (shrubs, forbs and woody plants) and portion of consumed grass is _<10% (du Toit & Olff 2014). Woody food plants have a plenty of physical and chemical defences against herbivory, towards which grasses are comparatively tolerant (Gordon & Prins 2008). Some mammals such as bovids, cervids and rhinos are represented in both groups, and some such as equids (grazers) and giraffids (browsers) are exclusive to either one group. Some species known as "mixed feeders" switch between groups, mainly grazing when green grass is available and browsing when the grass turns brown or disappear in the winter or dry season. The numerous large herbivore species that are specialized for either grazing or browsing represent two groups recognized as being behaviourally and functionally distinct within their ecosystems (Gordon & Prins 2008). The digestion of fibre by ruminants is constrained by two factors: (i) the efficiency of the ruminal flora in digestion fibre; (ii) the time that the fibre is exposed to the chemical action of the ruminal flora (Van Soest 1994).

First ground-breaking classification of herbivores was done by Hofmann (1973,1989) who classified herbivorous mammals into three major classes based on their feeding preferences (Table 1.).

Table 1.: Feeding strategies of herbivores

Class	Ruminants	Nonruminants
Concentrate selectors		
Fruit and foliage selectors	Duikers	Rabbits
Tree and shrub browsers	Deer, giraffes, kudus	Sumatran and black Rhinoceros
Intermediate feeders		
Prefer forbs or browsing	Moose, goats, elands	
Prefer grass	Sheep, impalas	
Bulk and roughage eaters		
Fresh grass grazers	Buffaloes, cattle, kobs, oribis	Hippopotamus
Roughage grazers	Harebeests, topis	Horses, elephants, zebras
Dry region grazers	Oryxes, camels, roan antelope, sable antelope	Kangaroos

Source: Adapted and extended from Hofmann 1973, 1989; Hansen et al. 1985.

Hofmann (1973,1989) based his classification on morphological differences. However, there are several limitations of Hofmann's approach. Hofmann (1973, 1989) did not consider real body weights for most individuals and gave just data from the literature, furthermore he did not provide a scheme by which the classification of a ruminant species into a feeding type could be deduced from the recorded data. Also, Hofmann's classification could not be supported by

statistical analyses that correlated morphometric data with feeding type (Clauss et al. 2003). Many authors (Gordon & Illius 1996; Robbins et al. 1995; Clauss et al. 2003) disproved Hofmann's classification and brought new findings, and that is why this thesis will follow those recent classifications (Clauss et al. 2003; Clauss et al. 2010a).

Clauss et al. (2001) observed that that the rumen contents of a free-ranging browsers were not stratified in contrast to grazers rumen contents (Clauss & Lechner-Doll 2001). Such stratification in rumen is caused by tendency of grass to fractionate into longish fibre-like particles that form a fibrous raft. Browsers have smaller rumens and are therefore not capable to handle longish, fibre like particles such as grass or lucerne. Due to the potentially faster passage from the reticulorumen, browsers absorb more nutrients from the small intestine that are, in grazers, fermented in the reticulorumen (Rowell-Schäfer et al. 2001). The presence or absence of stratified reticulorumen contents is decisive for the evolutionary adaptation of ruminant feeding types to their forages. Browsers, who rarely in nature face a situation where their reticulorumen contents stratify, did not need to evolve a particularly strong reticulorumen muscles, whereas intermediate feeder and grazers, who have to deal with stratified contents and the fibrous raft, needed stronger reticulorumen walls (Clauss et al. 2003). The tendency of grass to form a fibrous raft, and thus have longer particle retention and more thorough fibre fermentation, allows grazers to rely nearly exclusively on reticulorumen fermentation for their energy requirements. On the other hand, browsers cannot meet their energy requirements only from reticulorumen fermentation and therefore have to rely on additional strategies (Prins et al. 1984; Gordon & Illius 1994) like compensatory hindgut fermentation (Hofmann 1989; Prins & Kreulen 1991), and digestion of nutrients directly in the small intestine (Rowell-Schäfer et al. 2001).

Free-ranging browsers avoid, on average, grasses to a much higher degree than grazers avoid browse not only for its different digestion mechanisms but also for physical characteristics of grass. Although grazers are limited in their ability to cope with the allelochemicals in browse (Owen-Smith 1982, 1997), there is no mechanical problem for its gut in processing browse material; but for browsers to process grass, there is (Clauss et al. 2003).

And therefore Clauss et al. (2008) came up with new classification of wild ruminant feeding types and changed classification of Hofmann (1973, 1989). Wild ruminants can be divided to "moose type" ruminants (with little stratification in the rumen and are mostly restricted to browse niche) and „cattle-type" ruminants (with high degree of stratification into gas, particle and fuid layers in rumen and are more flexible as intermediate feeders and grazers) Clauss et al. (2008). "Moose-type" ruminants can be characterised as non-grazers, whereas "cattle-type" might add varying proportions of browse to their natural diet of grass (Clauss et al. 2003). Feeding types of herbivorous (strict browsers and strict grazers) mammals evolved from ancestral intermediate-type ruminants (Codron et al. 2008; DeMiguel et al. 2008). According that, we consider the "moose-type" and the "cattle-type" both as extremes of a range of extant ruminant digestion types.

Classification of herbivores feeding type should not be taken as strict boundaries with no chance to incorporate other feeding stuff into animal diet. Feeding type categories are more as continuum (Gagnon & Chew 2000) as demonstrated in study of Clauss et al. (2010b) on free-ranging muskoxen (*Ovibos moschatus*) where captive animals performed different feeding behaviour than free-ranging ones. Muskoxen are classified as grazers (Hofmann 2000; Clauss

et al. 2006), however population of muskoxen in Canadian Arctic had to be considered as intermediate feeders (Clauss et al. 2010b).

3.7 Herbivorous species

The African savannas support high species richness and biomass of mammalian herbivores (Prins & Olff 1998; Olff et al. 2002). Assemblages of large mammalian herbivores share limited resources (i.e. space and forage) and niche partitioning facilitate their coexistence (Kartzinel et al. 2015). Resource partitioning is defined as the differential use of resources such as food and space (Begon et al. 1990). Among native herbivores, overlap in resource use is not expected based on evolutionary segregation. In a native assemblage to which an exotic species has been introduced, however, overlap in resource use can occur under food-limited conditions and consequently implies competition (Voeten & Prins 1999). Competition is considered to be the major selective force causing this differential use of resources, although processes like predation or different responses of species to environmental gradients may also lead to resource partitioning (Schoener 1986). Several complementary mechanisms have been identified, including differential consumption of grasses versus nongrasses and spatiotemporal stratification in use of different parts of the same plant. Thus, herbivores can achieve dietary separation along a spectrum from pure grazers to pure browsers (Kartzinel et al. 2015). In other words, grazing succession involves herbivores of different size feeding in the same sward at different times, whereas in browsing stratification the different-sized herbivores feed at different heights at the same time (Farnsworth et al. 2002; du Toit & Olff 2014).

A second set of insights links dietary niche structure to body size, morphology, and digestive strategy (i.e., ruminant vs. nonruminant). The Jarman–Bell Principle holds that larger species and hindgut-fermenting nonruminants subsist on larger quantities of lower quality forage than do smaller species and ruminants (Kleynhans et al. 2011; du Toit & Olff 2014; Arsenault & Owen-Smith 2008). A further complication of using body size alone to predict resource partitioning is that resources within and ecosystem are not static but vary both spatially and temporally. Grass and browse plant species encompass enormous taxonomic, phylogenetic, and trait diversity, yet few studies have evaluated resource partitioning at the plant-species level (Kleynhans et al. 2011; de Iongh et al. 2011); habitat choice (Jarman & Sinclair 1979); selection for plant heigh (Arsenault & Owen-Smith 2008) and plant part or growth stage (Murray & Illius 1996).

For large grazers such as wildebeest, zebra and cattle, the grass layer does not consist of easily distinguishable items and selection for individual grass leaves is difficult. However, grass has several characteristics related to quantity (such as biomass and grass height) and quality (such as mineral concentrations and digestibility) for which large grazers can select (Prins 1996, Heitkönig & Owen-Smith 1998). Differences in digestive system and forage selection and differences in the morphology of the animal's mouth will affect feeding site selection. Problem may occur, when species (grazer) of similar body weight and size inhabit same area, because differences in feeding site selection related to allometric relationship are not expected (Voeten & Prins 1999; Kartzinel et al. 2015). That is why five large herbivore species with comparable weights across feeding type spectrum were investigated. Each species is shortly described in following chapters in term of its feeding strategy and feeding habits.

3.7.1 Western Derby eland (*Taurotragus derbianus derbianus*)

Our study was conducted on the Western Derby eland (*Taurotragus derbianus derbianus*), which is a critically endangered antelope (IUCN 2017) with the current distribution restricted only to the Niokolo Koba National Park (wild population) and two wildlife reserves hosting a small managed population for conservation breeding purposes in Senegal.

The Western Derby eland (WDE) is a large West-African savannah-dwelling antelope with fewer than 200 remaining individuals in their last refuge in Senegal. Apart from the wild population in the Niokolo Koba National Park, there is a small semi captive population (around 100 individuals) held in the Bandia and the Fathala wildlife reserves as part of a conservation breeding programme (Brandlová et al. 2017). WDE is a browser and its diverse diet composes mainly of leaves, shoots of woody plants and fruits. These food items form 98.8% of WDE's diet in the wild, while in the Bandia reserve it represents 77.5% of diet volume, because of supplementary feeding by *Acacia albida* pods, groundnut hay, and livestock feed. Animals in the Bandia reserve are fed regularly and in larger amounts compared to the Fathala reserve (Hejcmanová et al. 2010). Despite WDE's browsing feeding strategy, animals usually collect dry, senescent leaves of *Terminalia* spp. from the ground. Other plant species preferentially selected by WDEs in their diet do not stand out for content of any macroelement (Hejcmanová et al. 2019).

Fig. 2: Western Derby eland (*Taurotragus derbianus derbianus*). Photo by Luděk Čulík.

3.7.2 Roan antelope (*Hippotragus equinus*)

Roan antelope are most abundant in moist or dystrophic (dark gray) savannas where soils are predominantly infertile. They prefer lightly wooded savannah with open areas and avoid closed-canopy woodland (Schuette et al. 1998; Martin 2003). It is able to tolerate low-quality food and use plant parts not used by other ruminants (Heitkönig & Owen-Smith 1998). Roan antelope is considered as mixed-feeder (Schuette et al. 1998; Dery 2016), with wide range of distribution in Africa and different foraging strategies through the continent. Vegetation preference of roan antelope are influenced by local conditions as topography, vegetation type, water availability and the presence of competitors (Havemann 2014). Structure of roan antelope's diet is so

diverse that Gagnon and Chew (2000) classified it as variable feeder. Example of roan antelope's wide diet spectrum is that Martin (2003) identified roan antelopes as highly selective feeders, with two or three grass species in their diet in Namibia, while Tyowua et al. (2013) recorded 26 grass species and 19 species of woody plants consumed by roan antelopes in Nigeria. Schuette et al. (1998) observed roan antelopes in Burkina Faso, which were able to switch from strict grazing (>95% grass) strategy to mixed feeding (<50% grass) strategy in case of low quality of grazing forage, and South African populations were determined by Heitkönig and Owen-Smith (1998) as pure grazers, with no browsing during any season. Roan antelope are considered to be delicate feeders, utilising the higher grass parts from 15 to 150 cm above ground. Therefore, they are sensitive to habitat changes caused by natural disturbance and anthropogenic disturbance (Martin 2003).

Fig. 3: Roan antelope (*Hippotragus equinus*). Photo by Luděk Čulík.

3.7.3 African buffalo (*Syncerus caffer*)

African buffalo is grazing ruminant dependent on water, and it is considered as bulky feeder which prefers green grasses even when they are fibrous (Muposhi et al. 2014). Buffalo select grass species different from grasses preferred by other ungulates (Sinclair 1977) and avoid grass species high in secondary plant compounds (Owen-Smith 1982). It's feeding habit appear to vary in different environments. Variability of feeding habit of buffalos goes beyond being just a grazer. According to Gagnon and Chew (2000) buffalo belongs to variable grazers (60–90% of their diet consist of monocots, but with seasonal and geographic variability) with 0 % of fruit, 22.5 % of dicots and 77.5 % of monocots in its diet. However, population of buffalos in Tanzania (Beekman 1989) consumed only 5% browse. Muposhi et al. (2014) recognised 42 grass species in buffalo diet and observed that buffaloes spend 20% of feeding time browsing. Macandza et al. (2004) however, did not observed any browsing at buffaloes nor in dry season. Animals rather selected different plant species, than start to browse. Buffalo prefers green leaf, while browsing and new growth after fire help compensate for the low nutritive value of pasture during the dry season (Gagnon & Chew 2000).

Fig. 4: African buffalo (*Syncerus caffer*). Photo by Luděk Čulík

3.7.4 Burchell's zebra (*Equus quagga burchellii*)

Plains zebra is a non-selective grazing hind-gut fermenter (Duncan et al. 1990; Hack et al. 2002) and it is pure grazer, consuming 100% of grass (Mandlate et al. 2019). Zebra feeds on taller grass, and Mandlate et al. (2019) recorded 24 plant species in diet of plains zebra in Mozambique. Therefore, zebra is strongly associated with grasslands and savanna woodlands. Zebra can as hind-gut fermenter consume poor quality grass and is able to occupy a more extensive geographical range, a larger variety of habitats, and reach higher densities in some of the poorest grasslands than most other ruminants. Plains zebra usually move into a grassland ahead of other grazers and, by removing the older growth layer of lignified stems, sheaths, and seed heads, open it up to grazing by the more selective ruminants (Hack et al. 2002).

Fig.5: Burchell's zebra (*Equus quagga burchellii*). Photo by Luděk Čulík.

3.7.5 Common eland (*Taurotragus oryx*)

Common eland is a species with questionable feeding type determination. Hofmann and Stewart (1972) characterized common eland as a selective mixed feeder inhabiting savannah, open woodland or grassland plains. Gagnon and Chew (2000) characterized common eland as browser-grazer intermediate (30-70% monocots and dicots but always less than 20% fruits). Contrarily Lamprey (1963) observed common eland grazing from 70% in Tanzania. Common eland was often observed to graze mainly during wet season when the grass is young and green (Buys 1990; Watson & Owen-Smith 2000). However, this is not the rule for whole range of distribution, e.g. in South Africa Posselt (1963) and van Zyl (1965) observed almost no grazing at common eland during wet season. Similarly, other studies state common eland as almost pure browser (e.g. Sponheimer et al 2003; Wallington et al. 2007; Parrini et al. 2019). There is no clear reason for the wide variation in common eland's diet, although grazing appears to be more pronounced in East African populations (Gagnon & Chew 2000; Sponheimer et al. 2003)
Usual trees and shrubs in common eland's diet are *Acacia* spp., *Combretum* spp., *Commiphora* spp. and *Diospyros* spp. and grasses include *Setaria* spp. and *Themeda* spp. (Hillman 1979).

Fig. 6: Common eland (*Taurotragus oryx*). Photo by Luděk Čulík.

3.8 Study site

Our research was conducted in savanna areas of West Africa, particularly in Senegal, where scientific knowledge about mineral status and diet quality of herbivores is scarce. Beside that, Senegal is also home of critically endangered western subspecies of Derby eland (*Taurotragus derbianus derbianus*) and investigation of these unique species led us to two wildlife reserves described below (Fig.7).

3.8.1 The Bandia reserve

The Bandia reserve was established in 1990 for protecting natural vegetation and animals were placed gradually later. The reserve hosts large herbivores, native to Senegal such as African buffalo (*Syncerus caffer brachyceros*), defassa waterbuck (*Kobus ellipsiprymnus defassa*), roan antelope (*Hippotragus equinus koba*), and Western Derby eland (*Taurotragus derbianus derbianus*), and non-native species which have been introduced from South Africa such as giraffe (*Giraffa camelopardalis giraffa*), greater kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), common eland (*Tragelaphus oryx oryx*), Burchell's zebra (*Equus quagga burchelli)* and white rhino (*Ceratotherium simum simum*) (for more details see Chapter 3.7). In these days reserve works with enclosed breeding facility for large animals. The Bandia reserve is located 65 km east from Dakar (Fig. 7) and it is part of large nature reserve CF Bandia (classified forest) (Hejcmanová et al. 2010) and its area is approximately 3500 ha. Bandia´s environment has Sahel-Sudanese characteristics with rainy season from July to October and dry season which starts in November and ends in April. The annual average of precipitation is 350-742.4 mm. The temperature reflects seasons and average is 25 ˚C in cold dry season and can raise up to 30 ˚C in hot rainy season. These conditions support growth of *Acacia* spp. and *Balanites aegyptiaca,* which dominate in the reserve (Hejcmanová et al. 2010).

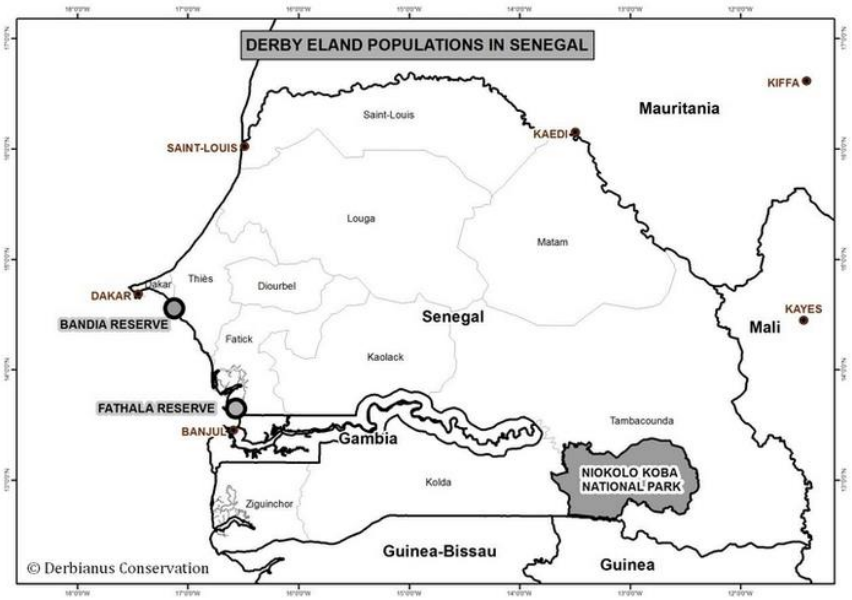


Fig. 7: Map of location of two wildlife reserves in Senegal (Bandia reserve, Fathala reserve).

Source: Derbianus Conservation 2020

3.8.2 The Fathala reserve

The Fathala reserve is enclosed area established for protection of the thick tree savannah with the fauna. It is located 250 km southward from Dakar (Fig. 7) and it occupies area of 6000 ha from 'Foret de Fathala' in the area of the Delta du Saloum National park. The Fathala lies in the sudano-guinean savanna with annual average of precipitations 839 mm. The rainy season comes in July and ends in October and the dry season occurs from November to May. Maximum

temperatures can reach up to 31.2 ˚C in May and the minimal temperature is 26 ˚C in January (Nežerková-Hejcmanová et al. 2005). Fathala´s environment support dominance of *Acacia* spp., *Combretaceae* family trees, and *Danielia oliveri* (Hejcmanová et al. 2010).
There are native herbivores such as bushbuck (*Tragelaphus scriptus*) and warthog (*Phacochoerus africanus*), and several introduced wildlife species from Senegal, such as African buffalo, defassa waterbuck, roan antelope, Western Derby eland, and non-native species from South Africa, such as giraffe, white rhino, zebra, and common eland (for more details see Chapter 3.7).

4. Minerals in the blood, hair, and faeces of the critically endangered Western Derby eland under human care in two wildlife reserves in Senegal

Adopted from: Stoklasová L, Váhala J, Hejcmanová P. 2019. Minerals in the blood, hair, and faeces of the critically endangered Western Derby eland under human care in two wildlife reserves in Senegal. Biological Trace Element Research 195:105-109.

Lucie Stoklasová participated in samples collection in Senegal, performed analyses of blood serum (P, Ca, Mg) on the IDEXX VetTest Chemistry Analyzer in the Animal Science laboratory at the Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, run statistical analyses of the data and wrote substantial part of the text.

Introduction

Minerals are involved in many physiological processes; thus, knowledge of their concentrations to assess and detect potential deficiencies is crucial before clinical signs appear. The main means for investigating animal mineral profiles are blood serum, plasma, and/or internal organ tissues. Such samples are, however, scarcely accessible in some cases because they require handled, immobilized or dead animals. This factor becomes a key obstacle in collecting samples from wild, i.e., free-ranging, elusive or endangered species. Therefore, non-invasive methods for mineral status assessment are sought. One non-invasive method is the analysis of animal hair. Fur is a suitable material that has been used mostly for the evaluation of diet quality through carbon and nitrogen isotopes (e.g., Sponheimer et al. 2003), but data on the elemental composition of wild animal hair are rare. A hair analysis is considered a useful biomarker in animal studies, which may infer about the bioavailability of elements and about the environmental exposure of animals (Gabryszuk et al. 2010). The content of elements in fur can additionally be affected by its location on the body of the animal, the colour of the hair, or the environment in which the animal lives and what its hair is exposed to (Combs 1982). The concentration of elements in animal hair is often much higher than that found in body fluids and other tissues, and trace elements accumulate in hair at concentrations that are at least 10 times higher than those present in blood serum and urine (Maugh 1978).

Another non-invasive way to obtain information about the mineral status of the animals is faeces, which have been commonly used to assess animal nutrition for decades (Emmet & Grindley 1909; Stapelberg et al. 2008). Nutrients present in high or variable concentrations in faeces of animals indicate that they are also present in adequate or excessive amounts in their diet. In addition, nutrients present in low and/or non-variable concentrations in faeces are most likely present in minimal or inadequate concentrations in the diet (Studier et al. 1994).

Our study was conducted on the Western Derby eland (*Taurotragus derbianus derbianus*, WDE), which is a critically endangered antelope with a current distribution restricted only to the Niokolo Koba National Park (wild population) and two wildlife reserves hosting a small managed population for conservation breeding purposes in Senegal (IUCN 2017). Due to the rarity of the species, minimal handling and disturbances are required. The aim of our investigation was therefore 1) to determine the concentrations of macro and selected microelements in the blood serum, hair and faeces of WDEs bred in the reserves to provide the baseline data for further management and 2) to explore the correlations among the concentrations of elements in blood serum, hair and faeces. The ultimate aim of the study was to evaluate the means to determine the WDEs' mineral status in order to use appropriate methods with minimal effects on the animals in the wild during any future conservation action.

Materials and methods

Research site

Our study was conducted in two wildlife reserves where conservation programme has been conducted since 2002: the Bandia reserve located 65 km south of Dakar in the Sahel-Sudanese savannah (Hejcmanová et al. 2010) and the Fathala reserve, located 250 km south of Dakar in the Sudano-Guinean savannah (Nežerková-Hejcmanová et al. 2005). Both reserves have seasonal dry and wet climates with an annual rainfall of approx. 350 mm (Bandia reserve) and 800 mm (Fathala reserve), respectively.

Sample collection and processing

Blood, hair and faecal samples from 11 young animals (6 males and 5 females, Table 2) were collected during the translocations for breeding management purposes in the dry season in March 2017. The blood samples were taken from the *vena saphena lateralis* in the morning by a veterinarian from immobilized animals before the application of the antidote (approx. 15 – 20 min after darting the animal). The samples were manually centrifuged after 1 h of settling, and the clear serum was placed in a deep freezer. The mineral concentrations in the samples were examined at the State Veterinary Institute in Prague 16 days after sampling. The concentrations of P, Ca, and Mg were determined by an IDEXX VetTest Chemistry Analyzer, the concentrations of S, Se, Fe, Zn were determined by an ICP-OES (Thermo Scientific™ iCAP 6000 Series, Thermo Fisher Scientific), the concentration of K was determined by AAS-flame (AA240, Varian), and the concentration of Cu was determined by GF-AAS (Spectra AA220Z, Varian) with a Zeeman correction.

The hair samples were collected from the mane and the tail during immobilization. Hairs were cut by stainless steel scissors as close to the skin as possible. The faeces were taken straight from the rectum of the immobilized animal and dried on site. The hair and faecal sample analyses were conducted as follows: concentrations of K, Fe, Zn, S, Cu, Se, P, Ca, Mg were tested by ICP-OES (IRIS Intrepid II XSP Duo, THERMO Elemental, USA), and Se was analysed by the AAS technique.

The correlations of mineral concentrations among the different types of samples, i.e., hair, blood and faeces, were performed using non-parametric Spearman rank correlation analyses in the Statistica 13 package (StatSoft, Tulsa).

Results and discussion

The mean concentrations of the elements in WDE blood, hair and faeces are given in Table 3. When compared to the reference levels of Ca, P, and K concentrations in the blood serum of Derby/Giant eland (ZIMS, 2013) and other bovid species (Váhala et. al. 1989; Dermauw et. al. 2013), our values were within or slightly below the clinical range. The mineral concentrations in WDE hair were within the range or slightly exceeded the values reported for domestic cattle (Puls 1994), 1-25 g/kg for Ca, 130-455 mg/kg for Mg, 6.7-32 mg/kg for Cu, 59-200 mg/kg for Fe, 0.5-1.32 mg/kg for Se and 100-150 mg/kg for Zn. The highest concentrations of all elements, except Zn and Se, among the collected types of samples were found in the faeces. The faecal P concentrations in the WDEs were within the range of the values reported in other antelopes in various African savannah habitats (Stapelberg et al. 2008) and were above the critical faecal P concentration of 2.0 g/kg (0.2%) identified for most herbivore species (Wrench et al. 1997). This result should indicate that the P concentration in the WDE diet in the reserves is sufficient, despite the fact that the mean P concentrations in the browsed plants, which were part of the WDE diet in the Fathala reserve, were rather low (Table 3, Hejcmanová, unpublished data). The additional information about the concentrations of minerals in the environment, particularly the P concentrations in the soils of both reserves (Table 3, Stoklasová 2016), which are low compared to those in other savannah areas (e.g., Stephenson et al. 2010), allows us to suggest that the WDEs seem to be adapted to cope with the environmental conditions and a diet poor in phosphorus. The other elements investigated in the blood profiles have usually been

omitted from faecal sample analyses in nutritional studies and have no reference values. The Ca:P ratio deserves attention. In our study, the mean Ca:P ratio was 0.88±0.13 SD in the blood serum, 7.3±0.11 SD in the hair, and 12.8±3.03 SD in the faeces. Thus, the Ca:P ratio in the blood serum was inverted to values recommended for ruminants (Ca:P ~ 1-2, Bock & Polach 1994) but agreed with findings from non-domesticated bovids bred in captivity (Váhala et al. 1989). However, the Ca:P ratios in the hair and faeces were opposite and at levels that mostly exceeded the recommended ratio, as reported by Gabryszuk et al. (2010), where the Ca:P ratio in the hair was 15.42. The inverse Ca:P ratios in the blood and high Ca:P in faeces indicate a metabolic inability of the animals to absorb Ca from the diet, which was rich in Ca but contained low P browsed plants (the mean Ca:P ratio in the browsed plants in the Fathala reserve was 12.37±0.41 SD, Hejcmanová, unpublished data). This result suggests that Ca is either in an inaccessible form or is present in excessive amounts, which in combination with low P, causes Ca to be unavailable to the animal and to leave the body without utilization.

The concentrations of the elements in the blood, hair and faecal samples were not correlated (all $p>0.05$), with the only exception being Fe. The iron concentration in the faeces was positively correlated with the Fe concentration in the hair ($r=0.64$, $P<0.05$) and blood ($r=0.69$) ($P< 0.05$). The lack of correlations among the different types of samples does not correspond with the results by McDowell (1982), who described positive correlations between plasma Cu and faecal Cu and between hair Cu and faecal Cu and a negative correlation between plasma Cu and hair Cu. Positive correlations were also reported between blood Fe and Zn concentrations and hair Fe and Zn concentrations, respectively (Patra et al. 2006).

Conclusion

We could not confirm any correlations as previous authors (McDowell 1982; Patra et al. 2006) in our samples, which may be given to the low number of samples; hence, we recommend conducting further research with a broader dataset. Our findings, however, currently indicate that faecal and fur analyses cannot stand alone to assess the mineral status and determine the potential mineral deficiencies in Western Derby elands or in large herbivores in general.

References

Bock U, Polach. 1994. Směrné hodnoty důležitých laboratorních vyšetření pro domácí zvířata: pes, kočka, kůň, tele, skot, prase, ovce. Translation to czech Eduard Jiran. Vetpres-vydavatelství a.s. Biopharm – VÚBVL, Czech Republic.

Brandlová K, Kubátová A, Fedorova T, Štochlová K, Grúňová M, Hejcmanová P. 2017. African Studbook. Western Derby Eland, *Taurotragus derbianus derbianus* (Gray 1847), volume 10, Czech University of Life Sciences Prague, Prague.

Combs DK, Goodrich RD, Meiske JC. 1982. Mineral concentrations in hair as indicators of mineral status: a review. Journal of animal science 54(2):391-398.

Dermauw V, Yisehak K, Belay D, Van Hecke T, Laing GD, Duchateau L, Janssens GPJ. 2013. Mineral deficiency status of ranging zebu (*Bos indicus*) cattle around the Gilgel Gibe catchment, Ethiopia. Tropical Animal Health Production 45:1139-1147.

Emmet AD, Grindley HS. 1909. The chemistry of animal feces. First paper – A comparison of the fresh and air-dried feces. Journal of American Chemical Society 31(5):569-579.

Gabryszuk M, Sloniewski K, Metera E, Sakowski T. 2010. Content of mineral elements in milk and hair of cows from organic farms. Journal of Elementology 15(2):259-267.

Hejcmanová P, Homolka M, Antonínová M, Hejcman M, Podhájecká V. 2010. Diet Composition of Western Derby eland (*Taurotragus Derbianus Derbianus*) in the Dry Season in a Natural and a Managed Habitat in Senegal using Faecal Analyses. South African Journal of Wildlife Research 40:27-34

Maugh TH. 1978. Hair: A diagnostic tool to complement blood serum and urine. Science 202:1271-1273.

McDowell LR, Kiatoko M., Bertrand JE, Chapman HL, Pate FM, Martin FG, Conrad JH. 1982. Evaluating the nutritional status of beef cattle herds from four soil order regions of Florida. II. Trace minerals. Journal of animal science 55(1):38-47.

Nežerková-Hejcmanová P, Hejcman M, Camara AA, Antonínová M, Pavlů V, Černý T, BÂ AT. 2005. Analysis of the herbaceous undergrowth of the woody savanna in the Fathala Reserve, Delta du Saloum National Park (Senegal). Belgian Journal of Botany 138:119-128.

Patra RC, Swarup D, Sharma MC, Naresh R. 2006. Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. Journal of Veterinary Medicine Series A banner 53:511-517.

Puls R. 1994. Mineral levels in animal health. 2nd ed. Diagnostic Data. Sherpa Int. Clearbrook, Canada.

Sponheimer M, Lee-Thorp JA, DeRuiter DJ, Smith JM, Van Der Merwe NJ, Reed K, Marcus W. 2003. Diets of southern African Bovidae: stable isotope evidence. Journal of Mammalogy 84(2):471-479.

Stapelberg FH, van Rooyen MW, Bothma JP. 2008. Spatial and temporal variation in nitrogen and phosphorus concentrations in faeces from springbok in the Kalahari. South African Journal of Wildlife Research 38(1):82-87.

Stephenson JD, Mills A, Eksteen JJ, Milewski AV, Myburgh JG. 2010. Geochemistry of mineral licks at Loskop Dam Nature Reserve, Mpumalanaga, South Africa. Environmental Geochemistry Health 33:49-53.

Stoklasová L. 2016. Soils in Africa as source of mineral nutrition for large herbivores [Msc. Thesis]. Czech University of Life Sciences Prague, Prague.

Studier EH, Sevick SH, Ridley DM, Wilson DE. 1994. Mineral and nitrogen concentrations in feces of some neotropical bats. Journal of Mammalogy 75(3):674-680.

Váhala J, Pospíšil J, Špála P, Kaše 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.

Wrench JM, Meissner HH, Grant CC. 1997. Assessing diet quality of African ungulates from faecal analyses: the effect of forage quality, intake and herbivore species. Koedoe 40:125-136.

ZIMS. 2013. Species 360 Physiological Reference Intervals for Captive Wildlife. Giant Eland *(Taurotragus derbianus)*. Available from https://zims.species360.org/Main.aspx (accessed 13 March 2017).

Table 2. Investigated individuals of the Western Derby eland. For more details, see the studbook data in Brandlová et al. (2017).

ID in PCA	Studbook ID	Animal name	Sex	Date of birth	Age	Location
1-M2B	1141	Docteur	Male	2014-11-28	2 years	Bandia
2-F2B	1144	Felicia	Female	2014-12-10	2 years	Bandia
3-F2B	1139	MSoukeina	Female	2014-11-21	2 years	Bandia
4-F2B	1147	Safíra	Female	2014-12-21	2 years	Bandia
5-F1B	1154	Damaye-Niane	Female	2015-11-25	1 year	Bandia
6-M1B	1163	Dayo	Male	2016-02-25	1 year	Bandia
7-F1B	1148	Driankee	Female	2015-01-04	1 year	Bandia
8-M1F	1159	Fredy	Male	2016-01-01	1 year	Fathala
9-M2F	1151	Fode	Male	2015-03-25	2 years	Fathala
10-M2F	1150	Fadel	Male	2015-03-05	2 years	Fathala
11-M3F	1137	Falco	Male	2014-04-01	3 years	Fathala

Table 3. Mean concentrations of the principal elements in the blood, hair and faeces of the Western Derby elands, soil and preferred browsed plant species.

Elements	**Blood serum** (mmol/L) *Mean ± SE*	**Hair** (g/kg) *Mean ± SE*	**Faeces** (g/kg) *Mean ± SE*	**Soil*** (mg/kg) *Mean ± SE*		**Browsed plants Fathala**** (g/kg) *Mean± SE*
				Bandia reserve	Fathala reserve	
Ca	1.87 ± 0.143	3.59 ± 0.189	35.818 ± 5.999	1.204±0.717	0.182±0.178	15.42±0.78
P	2.12 ± 0.130	0.49 ± 0.023	2.818 ± 0.077	0.142±0.068	0.054±0.024	1.34±0.04
Mg	1.55 ± 0.098	0.699 ± 0.027	4.436 ± 0.59	-	-	3.5±0.16
K	5.21 ± 0.109	3.59 ± 0.189	7.818 ± 0.408	0.122±0.034	0.012±0.01	6.74±0.31
Fe	0.022 ± 1.214	0.527 ± 0.018	0.879 ± 0.105	2.510±0.540	0.916±0.355	-
Cu	0.011 ± 2.781	0.008 ± 0.000	0.011 ± 0.000	0.003±0.000	0.002±0.000	-
Zn	0.012 ± 0.400	0.11 ± 0.003	0.042 ± 0.001	0.004±0.000	0.002±0.000	-
Se	0.002 ± 0.166	0.0005 ± 0.000	0.0004 ± 0.000	-	-	-
S	27.2 ± 0.504	-	-	-	-	-

**Mean concentrations of the elements in most of the preferred plants browsed by the Western Derby elands in the Fathala reserve (*Acacia ataxacantha, A. macroptera, Combretum glutinosum, C. micranthum, C. paniculatum, Saba senegalensis, Terminalia laxiflora*, and *T. macroptera*), (Hejcmanová, unpublished data). *Source: (Stoklasová 2016)

5. Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal

Adopted from: Stoklasová L, Váhala J, Hejcmanová P. 2020. Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal. Animal Physiology and Animal Nutrition 00:1-4.

Lucie Stoklasová participated in samples collection in Senegal, performed analyses of blood serum (P, Ca, Mg) on the IDEXX VetTest Chemistry Analyzer in the Animal Science laboratory at the Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, run statistical analyses of the data and wrote substantial part of the text.

Introduction

The Western Derby eland (WDE) (*Taurotragus derbianus derbianus*) is a large West-African savannah-dwelling antelope with fewer than 200 remaining individuals in their last refuge in Senegal. Apart from the wild population in the Niokolo Koba National Park, there is a small semi-captive population (101 individuals) held in the Bandia and the Fathala wildlife reserves as part of a conservation breeding programme (Brandlová et al. 2017). WDE is a browser, and its diet composes mainly of leaves, shoots of woody plants and fruits. These food items form 98.8% of WDE's diet in the wild, while in the Bandia reserve it represents 77.5% of diet volume, because of supplementary feeding of *Acacia albida* pods, groundnut hay and livestock feed. Animals in the Bandia reserve are fed regularly and in larger amounts compared to the Fathala reserve (Hejcmanová et al. 2010). Supplementary feeding is provided by rangers mainly in dry and hot dry seasons. There is also occasional provision of mineral licks (of unknown mineral composition) on the Fathala reserve only. The knowledge of adequate nutritional requirements and health status is important for the conservation breeding programme. Knowing reference intervals for minerals in serum is relevant for monitoring population health, and for investigating the lower rate or reproduction in the Fathala reserve compared to the Bandia reserve (Brandlová et al. 2017). The only relevant information is reference values for the eastern subspecies of Giant eland from ZIMS (2013). The aim of the present study was therefore to describe the mineral status of WDEs through the mineral profiles (Ca, P, Mg, Cu, Fe, Zn, Se, K, S) in blood serum.

Materials and methods

Research site

Our study was conducted on WDEs in two breeding reserves in Senegal. The Bandia reserve is located 65 km southward from Dakar in the Sahel-Sudanese savannah, which is dominated by Acacia spp. and Balanites aegyptiaca. The Fathala reserve is located 250 km southward from Dakar in the Sudano-Guinean savannah, which is dominated by Acacia spp., Combretaceae family trees, and Danielia oliveri (Hejcmanová et al. 2010). Both reserves have a dry and wet seasonal climate, receiving approx. 350 mm and 800 mm of rainfall annually in the Bandia and Fathala reserves, respectively.

Sample collection and processing

Blood was sampled from 11 young animals (1–3 years old) in total (4 males from the Fathala reserve; 2 males and 5 females from the Bandia reserve) during translocations in the dry season in March 2017. Individual animals were selected on basis of the genetic kinship (to avoid inbreeding of related individuals) under the supervision of conservationists rather than the need of this research. Animals were immobilized with a combination of medetomidine (Medetomidine 10 mg/ml; Kyron Laboratories), butorphanolum (Nalgosed 10 mg/ ml; Bioveta, a.s.), azaperone (Stresnil 40 mg/ml; Janssen Animal Health) and ketamine (Narkamon 100 mg/ml; Bioveta, a.s.), and blood was taken from the Vena saphena lateralis 18–33 min after application of drugs and before the i.v. application of antidotes atipamezole (Revertor 5 mg/ml; CP-Pharma Handelsges, GmbH) and naloxone (Naloxone WZT Polfa 0.4 mg/ml; Warszawskie Zaklady Farmaceutyczne Polfa S.A.). The blood was collected in 5 ml blood serum plastic tubes with a separating gel (VACUETTE; Dialab). Animals were immobilized by one dart shot from

distance in open space of boma in the early morning hours. Detailed health status of individual animals was not known before darting; however, no clinical signs of diseases or health problems, for example poor growth, hair loss or discoloration, weakness and swollen joints, were observed. The samples were centrifuged by portable manual centrifuge after 1 hr of settling. The centrifuged, clear serum was placed in a deep freeze in Eppendorf tubes. Serum samples were transported to the Czech Republic in cooling box filled with gel cooling packs. Temperature could not be controlled during 12 hr of transportation from Senegal to Czech Republic. The samples were frozen immediately after transportation to Czech Republic again. The samples were examined in accredited laboratory at the State Veterinary Institute in Prague 16 days after sampling. The concentrations of P, Ca and Mg were determined by an IDEXX VetTest Chemistry Analyzer, the concentrations of S, Se, Fe and Zn were determined by an ICP-OES (Thermo Scientific™ iCAP 6000 Series; Thermo Fisher Scientific), the concentration of K was determined by an AAS-flame (AA240; Varian), and the concentration of Cu was determined by a GF-AAS (Spectra AA220Z; Varian) with a Zeeman correction. Statistical analyses were performed using the tibco® statistica™ package (StatSoft). The small sample size suggested a non-parametric approach, without need of normality testing. A comparison of mineral concentrations between two localities was performed using the non-parametric Mann-Whitney U test. Serum mineral relationships were tested by Spearman's correlation.

Results and discussion

The values of the WDE serum mineral concentrations (Table 4) were lower in almost all elements compared to the blood parameters of other Tragelaphineae, zebu or cattle (Table 5). The only detected correlations between minerals were those of Ca with Mg ($r_s = .96$, $p = .05$) and Ca with P ($r_s = .96$, $p = .05$) in Bandia reserve animals. The serum mineral levels were comparable for males and females with the exception of Mg concentrations, which were lower in males ($U = 3$, $p = .035$) without any obvious reason, which was similar to the findings in the plasma of captive zebu (Bos indicus) in Ethiopia (Dermauw et al., 2013). We recorded higher serum levels of Fe ($U = 1.5$, $p = .023$) and K ($U = 1$, $p = .018$) in the Bandia reserve compared to the Fathala reserve. Higher serum level of S ($U = 3$, $p = .046$) was recorded in the Fathala reserve compared to the Bandia reserve. Higher serum levels of Fe and K in the animals living in the Bandia reserve correspond to the higher concentrations of these minerals in the soil in the Bandia reserve (Stoklasová, 2016). Other tested minerals in soil (Ca, P, Cu, Fe, Zn, K) were higher on the Bandia reserve compared to the Fathala reserve as well. Animals from the Fathala reserve had slightly, but not significantly higher concentrations of Ca, Cu and Zn in the blood, thus not corresponding to soils in reserves. Soils in both reserves are generally very low in mineral concentrations compared to other localities (Stoklasová, 2016). Regarding the animals from the Bandia reserve, there was more mineral content variability in the blood serum among individual animals with no specific pattern. Predominantly low concentrations of minerals in WDE's blood serum might indicate mineral deficiency; however, the animals were all in a good condition without any obvious clinical issues. Recorded mineral levels may reflect most likely an adaptation of the WDE to a specific environment that is poor in minerals. Considerably low concentrations were recorded for Cu, Fe and Zn. The low Cu concentration could be explained by copper-containing serum proteins that are incorporated into the clotting during the serum

extraction, making the Cu levels rather variable (Laven & Smith 2008). The low Zn could be linked to either low Zn levels in the mature forages or to an antagonistic relation to the P in phytates (Suttle 2010). Cu and Zn are susceptible to so called "herd effect" which influences the concentrations of minerals in serum independently on diet, and more animals must be tested for convincing results (Herdt & Hoff 2011). Regarding low number of individuals involved in the study, our results might be partly affected by a small sample size. A low concentration of Cu matches a low concentration of Fe, since Cu is essential for absorption and transport of Fe (Tuormaa 2000). The serum Ca:P ratio of WDEs in the reserves (mean 0.88 ± 0.13 SD) was inverted compared to the ratio values for ruminants considered clinically normal (1.5–2 for domestic cattle, see Gizachew et al. 2002; for wild antelopes, see Drevemo et al. 1974). This shows the same phenomenon commonly observed in non-domesticated bovids bred in captivity, for example greater kudu (Tragelaphus strepsiceros) and bongo (Tragelaphus eurycerus) (Table 5) (Miller et al. 2010; Váhala et al. 1989).

Conclusion

Considering diet selection of WDE, the Fathala reserve appears unfavourable for WDE in terms of macro-elements' content and fibre fractions of local plants (Hejcmanová et al. 2019), and similar conditions in the Bandia reserve can be expected. Combined findings of soil and our serum analyses suggest that WDEs might benefit from mineral supplementation, for example mineral licks. Effects of such a supplementation would have to be evaluated.

References

Brandlová K, Kubátová A, Fedorová T, Štochlová K, Grúňová M, Hejcmanová P. 2017. African Studbook. Western Derby Eland, *Taurotragus derbianus derbianus* (Gray 1847), volume 10, Prague: Czech University of Life Sciences Prague.

Dermauw V, Yisehak K, Belay D, Van Hecke T, Du Laing G, Duchateau L, Janssens GPJ. 2013. Mineral deficiency status of ranging zebu *(Bos indicus)* cattle around the Gilgel Gibe catchment, Ethiopia. Tropical Animal Health and Production 45:1139-1147.

Drevemo S, Grootenhuis JG, Karstad L. 1974. Blood parameters in Wild Ruminants in Kenya. Journal of Wildlife Diseases 10:327-334.

Gizachew L, Hirpha A, Jalata F, Smit GN. 2002. Mineral element status of soils, native pastures and cattle blood serum in the mid-altitude of western Ethiopia. African Journal of Range & Forage Sicence 19:147–155.

Hejcmanová P, Homolka M, Antonínová M, Hejcman M, Podhájecká V. 2010. Diet composition of Western Derby eland (*Taurotragus Derbianus Derbianus*) in the dry season in a natural and a managed habitat in Senegal using faecal analyses. South African Journal of Wildlife Research 40:27-34.

Hejcmanová P, Miřejovská M, Homolka P, Hejcman M. 2019. Drivers of diet selection of critically endangered Western Derby eland during the food shortage period within conservation breeding in Senegal. Scientific Reports 9:1-7.

Herdt T H, Hoff B. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics: Food Animal Practice 27:255-283.

Laven R, Smith S. 2008. Copper deficiency in sheep: an assessment of relationship between concentrations of copper in serum and plasma. New Zealand Veterinary Journal, 56:334-338.

Miller M, Weber M, Valdes EV, Neiffer D, Fontenot D, Fleming G, Stetter M. 2010. Changes in serum calcium, phosphorus, and magnesium levels in captive ruminants affected by diet manipulation. Journal of Zoo and Wildlife Medicine 41:404-408.

Stoklasová L. 2016. Soils in Africa as source of mineral nutrition for large herbivores [Msc. Thesis]. Czech University of Life Sciences Prague, Prague.

Suttle NF. 2010. Mineral nutrition of livestock (4th ed.). Wallingford, UK.

Tuormaa TE. 2000. Chromium, selenium, copper and other trace minerals in health and reproduction. Journal of Orthomolecular Medicine 15:145-157.

Váhala J, Pospíšil J, Špála P, Kaše 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.

ZIMS. 2013. Species 360 Physiological Reference Intervals for Captive Wildlife. Giant Eland *(Taurotragus derbianus)*. Available from https://zims.species360.org/Main.aspx (accessed 13 March 2017).

Table 4. Concentration of minerals (mean ±SE, range) in the blood serum of Western Derby elands (n=11) in the Bandia and Fathala reserves, Senegal.

	Total n=11	**Bandia n=7**	**Fathala n=4**	**Males n=6**	**Females n=5**
Ca, mmol l^{-1}	1.87±0.14 (0.95-2.39)	1.77±0.54 (0.95-2.39)	2.03±0.34 (1.54-2.27)	1.75±0.53 (0.95-2.27)	2.00±0.41 (1.31-2.39)
P, mmol l^{-1}	2.12±0.13 (1.36-2.76)	2.17±0.52 (1.36-2.76)	2.04±0.27 (1.77-2.38)	1.96±0.37 (1.36-2.38)	2.31±0.46 (1.55-2.76)
Mg, mmol l^{-1}	1.55±0.10 (1.09-2.05)	1.68±0.33 (1.09-2.05)	1.32±0.18 (1.15-1.49)	1.36±0.26* (1.09-1.75)	1.78±0.24* (1.39-2.05)
Cu, µmol l^{-1}	10.87±2.78 (6.55-38.50)	12.14±11.65 (6.86-38.50)	8.66±1.47 (6.55-9.95)	8.58±1.20 (6.55-9.95)	13.62±13.91 (6.86-38.50)
Fe, µmol l^{-1}	22.35±1.21 (16.80-31.30)	24.2±3.73* (19.2-31.30)	19.13±2.11* (16.8-21.9)	20.92±3.33 (16.80-25.80)	24.08±4.46 (19.20-31.30)
Zn, µmol l^{-1}	11.51±0.40 (10.60-14.90)	11.34±0.84 (10.70-13.10)	11.80±2.07 (10.60-14.90)	11.55±1.67 (10.60-14.90)	11.46±0.97 (10.70-13.10)
Se, µmol l^{-1}	1.75±0.17 (0.87-2.92)	1.62±0.49 (0.87-2.34)	1.97±0.66 (1.43-2.92)	1.73±0.68 (0.87-2.92)	1.78±0.43 (1.26-2.34)
K, mmol l^{-1}	5.21±0.11 (4.70-5.75)	5.40±0.26* (5.11-5.75)	4.87±0.23* (4.70-5.20)	5.00±0.27 (4.70-5.26)	5.46±0.30 (5.11-5.75)
S, mmol l^{-1}	27.23±0.50 (24.00-30.00)	26.54±1.61* (24.00-29.30)	28.43±1.05* (27.90-30.00)	27.90±1.15 (26.70-30.00)	26.42±1.95 (24.00-29.30)

*Significant ($p<0.05$) differences tested by Mann-Whitney U test according to locality and sex.

Table 5. Overview of blood (serum/plasma) mineral concentrations (mean, range) of different ruminant species.

	Cape eland	Greater kudu	Lesser kudu	Bongo	Zebu	Horro cattle	Cape eland (free)
Ca, mmol l^{-1}	2.44 (1.91-2.81)	1.84 (0.99-2.23)	2.27 (2.09-2.62)	1.73 (1.14-2.47)	5.40 (4.10-12.30)	3.82	2.87
P, mmol l^{-1}	2.44 (1.91-2.81)	2.70 (1.36-4.60)	2.32 (1.24-3.37)	2.21 (1.94-2.74)	3.30 (1.80-4.60)	4.56	1.29
Mg, mmol l^{-1}	0.84 (0.50-1.06)	0.53 (0.30-1.07)	0.75 (0.62-0.95)	0.71 (0.33-1.12)	1.90 (1.40-2.30)	0.71	0.78
Cu, µmol l^{-1}	22.55 (17.20-30.80)	27.80 (17.20-46.90)	21.35 (19.60-23.10)	25.5 (7.60-34.40)	6.00 (0.80-18)	10.38	14.10
Fe, µmol l^{-1}	39.60 (15.90-50.50)	35.48 (8.40-56.60)	53.94 (39.60-69.80)	39.55 (23.90-58.60)	143 (75-1200)	24.35	.
Zn, µmol l^{-1}	103.3 (29.20-198)	91.36 (48.60-137)	71.15 (53.70-86.60)	117.60 (114.50-120.70)	46 (24-107)	13.92	.
K, mmol l^{-1}	5.86 (5.39-6.25)	4.55 (4.01-5.24)	5.72 (4.34-8.35)	6.19 (4.88-7.40)	4.40 (2.70-6.60)	.	.
S, mmol l^{-1}	.		.	.	37 (22-47)	.	.

Source: For captive Cape eland (n=8-10), Greater kudu (n=3-20), Bongo (n=4-8) and Lesser kudu (n=2-8) (Váhala et al. 1989), domestic zebu (n=90) (Dermauw et al. 2013), domestic Horro cattle (n=10-14) (Gizachew et al. 2002), and free ranging Cape eland (n=4-12) (Drevemo et al. 1974).

6. Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet

Adopted from: Hejcmanová P, Ortmann S, Stoklasová L, Clauss M. 2020. Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet. Comparative Biochemistry and Physiology, Part A 246: 110720.

Lucie Stoklasová carried out the substantial part of samples collection during the experiment at the University Farm Estate in Lány, CZU and of samples processing for the ICP-OES analyses.

Introduction

Ruminants vary widely in feeding habits and morphophysiology of the digestive tract. There is a long-standing tradition of linking the two in the sense of convergent adaptations to either browse- or grass-dominated diets (Hofmann 1973, 1988; Clauss et al. 2008; Codron et al. 2019), and while a series of correlations between diet and morphological or physiological measures have been documented, these relationships comprise a relevant degree of data scatter (Ehrlich et al. 2019). An outstanding feature of rumen physiology, the separation of fluids and small particles in their passage through the rumen (Hummel et al. 2005; Dittmann et al. 2015a), is no exception. On the one hand, there is a general pattern of animals mainly consuming browse, with a 'moose-type' physiology, to have fluids and particles to flow out of the rumen in close association and comparatively unstratified rumen contents, and of animals that are no strict browsers, with a 'cattle-type' physiology, to have a distinctively higher fluid than particle throughput through the rumen and comparatively stratified rumen contents. On the other hand, outliers to the pattern exist, not only, but particularly among the Bovinae – the bovini and the tragelaphini (Przybyło et al. 2019b). The current interpretation is that 'moose-type' ruminants defend themselves against secondary plant compounds in browse by salivary proteins and are hence limited in the amount of protein-rich saliva they can produce, whereas 'cattle-type' ruminants are not constrained in saliva production by a requirement for high salivary protein contents (Clauss et al. 2010). 'Cattle-type' ruminants can thus putatively use a higher fluid throughput through the rumen to increase the harvest of rumen microbes (Clauss & Hummel 2017). This scenario does not exclude 'moose-type' ruminants from grass diets, but should just make them less competitive in the grazing niche. On the other hand, 'cattletype' ruminants may develop other, hitherto unknown strategies to cope with secondary plant compounds of browse-dominated diets (Przybyło et al. 2019b). The common eland (*Taurotragus oryx*) is an interesting species in terms of dietary adaptations. It belongs to a ruminant tribe (tragelaphini) that comprises some species that have been identified as outliers to common patterns, in terms of salivary gland size (Robbins et al. 1995; Hofmann et al. 2008) and in the retention pattern for fluids and particles (Przybyło et al. 2019b). However, the digesta passage kinetics of eland have not been investigated so far. The eland is also an example of the difficulty to definitely ascribe a feeding type to a ruminant species. Traditionally, the eland has been considered a prime example of an intermediate feeder (Hofmann & Stewart 1972; Hofmann 1989; Gagnon & Chew 2000), based on studies that reported a mixed diet of grass and browse (Kerr et al. 1970; Nge'the & Box 1976; Abdullahi 1980; Buys 1990) (older reports reviewed by Littlejohn 1968), or even a mixed diet with a predominance of grass (Lamprey 1963). However, a larger number of studies have demonstrated that eland are predominantly browsers (e.g., van Zyl 1965; Cerling et al. 2003; Sponheimer et al. 2003; Codron et al. 2007; Steuer et al. 2014; Venter & Kalule-Sabiti 2016). Increased reported proportions of grass in eland diets are considered either due to methodological problems (Watson & Owen-Smith 2000), due to seasonal effects when green grass is taken in the rainy season (Parrini et al. 2019), or exceptions due to specific circumstances (D'Ammando et al. 2015). Similarly, the closely related Derby eland (*Taurotragus derbianus*) is a browser (Hejcmanová et al. 2010; Galat-Luong et al. 2011), and even fossil eland were found to be more browsers than intermediate feeders (Stynder 2009). Taken together, these results suggest a highly flexible species that, in contrast to some other mixed feeders, mostly prefers browse, but is able to thrive on grass nevertheless. We aimed to

test whether this flexibility is linked to a 'cattle-type' forestomach physiology, similar to other Bovinae that can use mixed diets (Przybyło et al. 2019b), by measuring the mean retention time of different digesta phases in eland. In order to account for the different diets naturally eaten by eland, we tested them separately on a monocot (grass hay) and a dicot (lucerne haylage) diet. We expected differences in intake level between the diets, and intake level to affect absolute measures of digesta retention – because higher food intakes are typically associated with shorter retention times (Müller et al. 2013). In contrast, the ratio of small particle to fluid passage has been shown to be species-specific across diets and experiments, with no effect of the intake level and only slight differences due to diet that are of a much lower magnitude than differences between species (Renecker & Hudson 1990; Lechner et al. 2010; Dittmann et al. 2015a; Przybyło et al. 2019b). Therefore, regardless of whether this ratio would classify the eland as 'cattle-type' or 'moose-type' ruminants, we expected no effect of diet on this classification.

Materials and Methods

The experiment was conducted from May to November 2017 at the University Farm Estate in Lány (Czech University of Life Sciences Prague, Czech Republic), which is accredited as research facility according to European and Czech laws for ethical use of animals in research (recent permission no. 63479_2016-MZE-17214 valid until 10th October 2021). For a description of the usual husbandry regime of elands at this farm, see Hejcmanová et al. (2011). In the year of the present study, the animals usually had access to a grass pasture, meadow hay, lucerne haylage, and wheat bran, which they ingested at individually varying proportions, and received this combination before and in between experiments. The experimental proposal was approved by the Institutional Animal Care and Use Committee of the Czech University of Life Sciences Prague as the experimental design did not require use of animals above standard husbandry procedures or extended intervention. Six adult male common eland (411 ± 90 kg, range 278–535 kg) were chosen for the experiment. This was done to exclude reproducing (pregnant) animals; the animals were picked randomly from among the available males in the herd. They were subjected to two diet treatments each. The animals were part of a larger breeding herd that was usually kept together in one large freestall barn without compartments. Treatments consisted of adapting animals on a monocot (grass hay)- only and a dicot (lucerne haylage)-only diet for at least 2 weeks before each trial (adaptation period), followed by a 1 week trial phase. For a nutritional characterisation of the diets, see Table 1. These two forages were from the same batches as those used for the regular feeding of the herd. For the adaptation period and during the entire trial week, each animal was kept individually in a separate compartment (20 m^2), to facilitate measuring of individual food intake and collection of faeces. Compartments were provided with a straw bedding, and animals were not observed to ingest the bedding. Between treatments, each animal returned for at least 1 month into the general herd. Food intake was measured by weighing food offered and leftovers each day in the morning during the experiment. Grass hay was offered for ad libitum consumption, with leftovers averaging 29 ± 13% of the total amount offered. Lucerne haylage was always consumed completely, and therefore did not meet the definition for ad libitum consumption. Samples of the forages offered and the grass hay leftovers were taken on a daily basis and pooled for each individual for nutrient analyses. During the adaptation period, the animals were accustomed to receive a handful of wheat bran every day. On the first day of the trial week, they received a

dose of three passage markers in a handful of wheat bran, which was ingested completely within 15 min. The markers were cobalt (Co)-EDTA as a solute marker (at a dose of 5–6 g/animal), and chromium (Cr)-mordanted fibre (particle size < 2 mm; 40–50 g/ animal) a well as cerium (Ce)-mordanted fibre (particle size < 10 mm; 30–40 g/animal) as particle markers, prepared according to Udén et al. (1980). Co-EDTA was mixed in the wheat bran after being dissolved in water. Faeces were collected at least twice before marker feeding for background levels, and every 4 h on trial days 1 and 2, every 6 h on day 3, every 8 h on days 4 and 5, and every 12 h on days 6 to 8. All faeces defecated in a respective interval were collected, pooled, weighed, and a representative subsample was stored frozen until drying at 60 °C for 72 h and grinding. The eland usually defecated in one or two piles in their enclosures, making total collection easily feasible. For the calculation of digestibility, faeces weights were used from consecutive days for which total collection data was available. Of the 12 trials, the number of total collection considered reliable was 4 times for 7 consecutive days, 4 times for 6 consecutive days, and 4 times for three consecutive days. Individual faecal samples were used for passage marker analysis, and a representative faecal pool sample per animal and trial was composed of all available individual samples and used for nutrient analysis. Analyses of marker concentrations was made by inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany) according to Frei et al. (2015). Forage samples were subjected to standard nutrient analyses (AOAC 1995) for dry matter (DM) and total ash (AOAC no. 942.05), crude protein (AOAC no. 977.02), neutral detergent fibre (NDF, AOAC no. 2002.04; corrected for residual ash), acid detergent fibre and acid detergent lignin (ADF, ADL, AOAC no. 973.18). The pooled faecal samples were only analysed for total ash, crude protein and NDF. The MRT in the whole gastrointestinal tract (GIT) was calculated according to Thielemans et al. (1978) as

$$\text{MRT} = \frac{\Sigma t_i C_i dt_i}{\Sigma C_i dt_i}$$

with Ci = marker concentration in the faecal samples from the interval represented by time ti (h after marker administration, using the midpoint of the sampling interval) and dti = the interval (h) of the respective sample

$$\text{dt}_\text{i} = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}$$

Complete excretion of the markers was assumed once the faecal marker concentrations were similar to the background levels determined in pre-dose faecal samples. Mean retention time in the reticulorumen (RR) was estimated following Lechner-Doll et al. (1990). The MRTsoluteRR is determined by estimating the rate constant of the descending part of the marker excretion curve via an exponential equation:

$$y = A * e^{-k*t}$$

with y = faecal marker concentration at time t (mg/kg DM), A = a constant, k = rate-constant (h−1) and t = time after marker dosing (h); the reciprocal of k represents the MRT for the RR. The MRTparticleRR is calculated based on the assumption that fluid and particles do not differ in passage characteristics distal to the RR (Mambrini & Peyraud 1997):

$$\text{MRT}_\text{particle}\text{RR} = \text{MRT}_\text{particle}\text{GIT} - (\text{MRT}_\text{solute}\text{GIT} - \text{MRT}_\text{solute}\text{RR}).$$

The ‘selectivity factor’ (SF, the ratio of two MRT of different markers) was calculated for both the total GIT and the RR. The relative daily dry matter intake was expressed on the basis of body mass$^{0.85}$ (Hackmann & Spain 2010; Müller et al. 2013). Apparent digestibilities were

calculated as (Intake – Faecal excretion)/Intake*100. Using the dry matter intake, large particle MRT, and apparent dry matter digestibility, the dry matter gut fill was calculated by the linear approach of Holleman and White (1989). Statistical comparisons between treatments were made by paired tests (paired t-test, Wilcoxon signed rank test), depending on normal distribution of data (as assessed by Kolmogorov-Smirnov test). To compare MRTs of the markers within either the GIT or the RR, a repeated-measures ANOVA was performed with Sidak post hoc test. Correlations between the relative dry matter intake and retention measurements were tested by Spearman's correlation, and subsequently by General Linear Models (assessing normal distribution of residuals) with individuum as a random factor (to account for repeated measures) and the relative dry matter intake as the independent variable. Tests were performed using SPSS v. 25.0 (IBM), with the significance level set to 0.05. For a comparison with other ruminant species, a species average was compared to the data compilation from Przybyło et al. (2019b).

Results

The animals always ingested the lucerne haylage completely, and ingested significantly more absolute (P = .002) and relative (P = .002) dry matter of the lucerne haylage than of the grass hay (Table 7). Both absolute and relative dry matter intake were doubled on lucerne haylage compared to the grass hay. When feeding on grass hay, leftovers were significantly lower in protein and higher in acid detergent fibre than the diet offered, indicating a certain degree of feeding selectivity (Table 6). The marker excretion patterns indicated a nearly parallel movement of solutes and small particles, with an increase in the difference between small and large particles on lucerne haylage (Fig. 8).

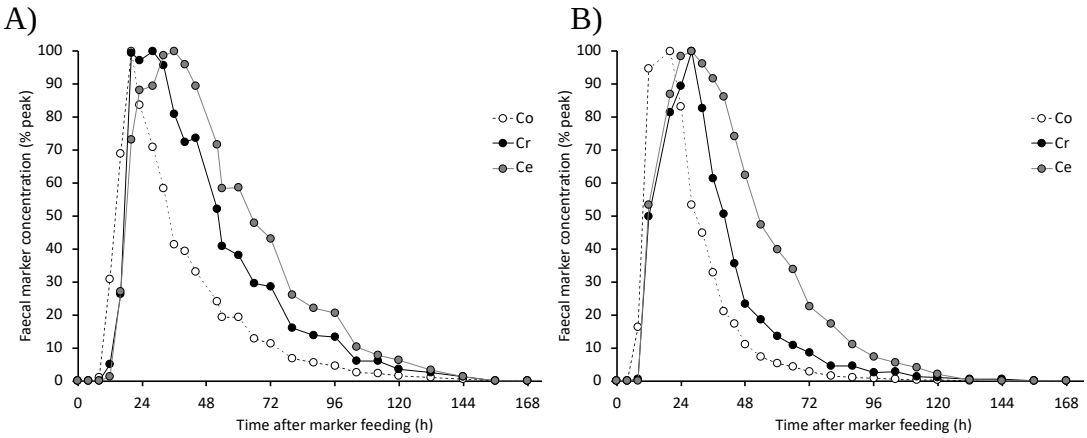


Figure 8. Passage marker excretion pattern for cobalt (Co, solutes), chromium (Cr, particles < 2 mm) and cerium (Ce, particles < 10 mm) in a common eland (*Taurotragus oryx*) on (A) grass hay and (B) lucerne haylage.

All MRT measures were significantly shorter on lucerne silage (Table 7); however, the SF measures for particles vs. solutes did not differ between the diets. Only the SF of large to small particles were significantly higher on lucerne haylage, both for the GIT (P = .038) and for the RR (P = .024). In particular, the largest animal, a 10 year-old, 535 kg bull, showed an extreme difference between the treatments, with a very low dry matter intake on the grass hay (13

g/kg$^{0.85}$/d vs. 45 g/kg$^{0.85}$/d on the lucerne haylage) and the longest MRT (MRT GIT for solutes, small and large particles 46, 72 and 71 h on the grass hay vs. 36, 46 and 53 h on the lucerne haylage). The apparent digestibility of dry matter and organic matter of the grass hay was lower than that of the lucerne haylage (Table 8). Similar to intake, the calculated dry matter gut fill was nearly double on the lucerne haylage compared to the grass hay (Table 8). Using Spearman's correlation, we observed significant, negative correlations between the relative dry matter intake and the MRT GIT of all markers (Table 9). For MRT in the RR, this was only the case for the small particle marker. The SFs were not correlated with intake, except for the SF of the large vs. small particles, with increased sorting of particles at the higher intake (Table 8). When assessing the effect of intake on passage kinetics in a GLM (accounting for repeated measures by including individual as a random factor), there were significant, negative relationships between the relative dry matter intake and the $MRT_{solute}GIT$ ($F_{1,5}$ = 19.424, P = .007) and also the $MRT_{solute}RR$ ($F_{1,5}$ = 11.142, P = .021), but not with any other MRT measure (P always > 0.05). Both SF for particles vs. solutes, for the GIT and the RR, also had no significant relationships with the relative dry matter intake (P always > 0.05), but again the SF of large vs. small particles did, both for the GIT ($F_{1,5}$ = 7.128, P = .044) and the RR ($F_{1,5}$ = 13.679, P = .014)

Discussion

The results of our study clearly indicate digesta kinetics in eland with a relatively closely related passage of solutes and small particles from the reticulorumen, with a nearly identical 'selectivity factor' (SF) as recently determined in muntjac (Muntiacus reevesi, Przybyło et al. 2019a) (Fig. 9A). Given findings in other members of the tragelaphini that had shown more distinct differences between the digesta phases (Przybyło et al. 2019b), this result was unexpected. Yet, it was robust across individuals and across two different diets (that also triggered different intake levels). In this respect, the present study adds to the existing evidence that neither diet nor intake has an effect on the difference between solute and small particle retention that is of similar magnitude as the species-specific differences themselves (Renecker & Hudson 1990; Lechner et al. 2010; Dittmann et al. 2015a; Przybyło et al. 2019b).Typical relationships between different measures known from other ruminants were demonstrated in the eland of the present study as well. There was a clear effect of intake level on retention times for particles and for solutes in the GIT, but not clearly for solutes in the reticulorumen (Table 9) (Dittmann et al. 2015a; Grandl et al. 2018). As most herbivores, eland select among the available plants and plant parts for low fibre content (Watson &Owen-Smith 2002), which was also evident on the grass hay diet (Table 6). The forage with the higher neutral detergent fibre was ingested at lower quantities, as is typical for most herbivores (Meyer et al. 2010) (Table 7), and the digestibility increased with forage quality (Van Soest 1994) (Table 8). The selective retention of larger particles when compared to the smaller ones was within the lower range reported for other ruminants (Fig. 9B).

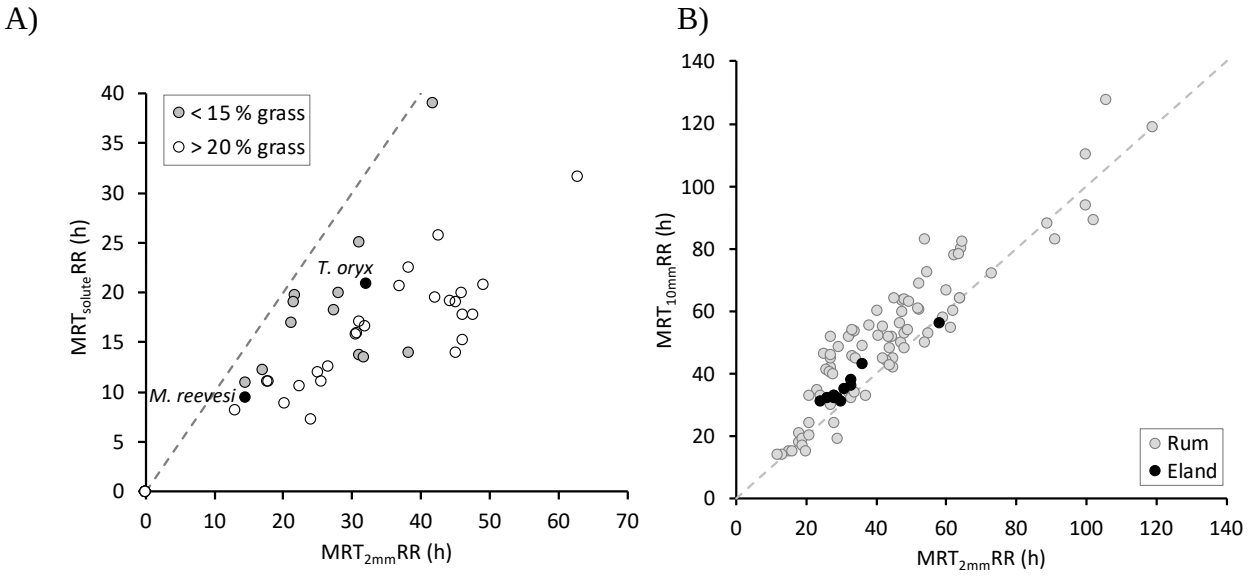


Figure 9. Relationship of the mean retention time (MRT) of small particles (<2mm) in the reticulorumen (RR) with **(A)** the MRT of a solute marker in the RR in various ruminant species (RUM; data collection from Przybyło et al. 2019b; one value per species) and **(B)** the MRT of a large particle (10mm) marker in the RR in various ruminant species (data collection from Dittmann et al. 2015b; multiple values per species), including the eland (*Taurotragus oryx*) of the present study and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a).

The selective retention of large vs. small particles (the SF Ce/Cr in Tables 7 and 9) showed a distinct correlation with the intake level (Table 9). In other words, when intake was high, and capacity in the reticulorumen potentially constraining, the reticulorumen sorting mechanism led to a clear difference between large and small particles (Fig. 8B). When intake was low, with a lesser gut fill and less space constraints on the reticulorumen, the increase in small particle retention was higher (on average, 9 h; Table 7) than that of large particle retention (on average, 7 h), as there was less need for expeditious reticulorumen clearance. In contrast to other members of tragelaphini investigated so far, eland are thus classified as 'moose-type' ruminants. The findings add to the existing evidence that digesta kinetics are species-specific and can vary even within taxonomic ruminant clades. For example, among the cervinae (old world deer), both 'cattle-type' (Cervus elaphus, Renecker & Hudson 1990; Elaphurus davidianus, Derix et al. 2019) and 'moosetype' (Muntiacus reevesi, Przybyło et al. 2019a) exist. The same is true for the bovidae in general (Dittmann et al. 2015a), with 'cattle-types' in bovini, alcelaphini, hippotragini, and all caprinae investigated so far, and 'moose-types' in the cephalophini, but with both types represented among the antilopini. This variation begs for an explanation. So far, the main explanation has focussed on the benefits of having a distinct difference in the digesta phases in 'cattle-types', which should facilitate a more efficient harvest of microbes from the reticulorumen by 'digesta washing', where the faster-moving fluid washes microbes out of the particulate digesta and inadvertently selects for fastergrowing strains of microbes, thus increasing microbial protein yield (Hummel et al. 2008; Hummel et al. 2015; Clauss & Hummel 2017). In this scenario, 'moose-types' are considered dependent on salivary defences against tannins; the necessity to enrich saliva with tanninbinding proteins is thought to constrain absolute saliva production and fluid flow through the reticulorumen (Hofmann et

al. 2008), and to also lead to more viscous rumen fluid (Clauss et al. 2009b; Lechner et al. 2010) in which particles do not separate as easily from the fluid as in a less viscous environment. To date, including the findings of the present study, ruminant species classified as 'moose-type' based on their reticulorumen morphophysiology (i.e., a low SF RR) appear constrained to a dietary niche of browsing (Codron & Clauss 2010), whereas 'cattle-type' ruminants apparently (with a high SF RR) exist across the whole browser-grazer spectrum (Fig. 10).

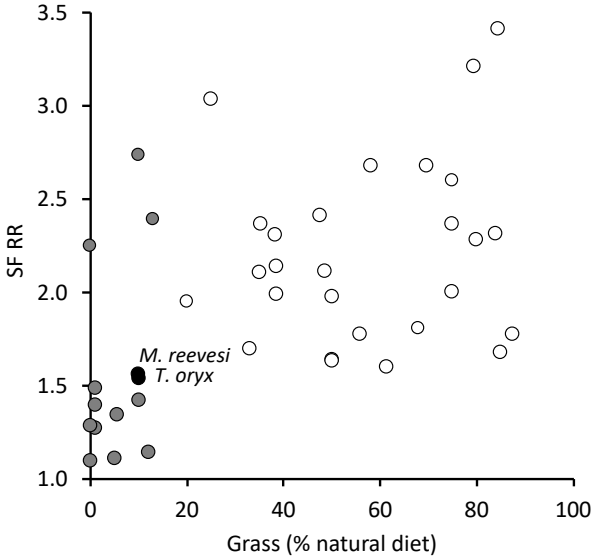


Figure 10. Relationship between the average percentage of grass in the natural diet (species <15%, grey circles; species >20%, white circles) and the selectivity factor (SF, a measure for how distinctly the retention of small particles and fluid differ) in the reticulorumen (RR) of various ruminant species (data collection from Przybyło et al. 2019b; one value per species), including the eland (*Taurotragus oryx*) of the present study and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a). Species with a SF up to ~ 1.5 would be considered 'moose-type' ruminants, and species above that as 'cattle-type'. Note that 'moose-type' ruminants occur only among browsers, whereas 'cattle-type' cover the whole dietary spectrum. The 'cattle-type' browsers (grey circles with SF RR > 2) are the European bison (*Bison bonasus*), gerenuk (*Litocranius walleri*) and the bongo (*Tragelaphus eurycerus*).

The major argument proposed for the absence of 'moose-types' among grazers is the competitive disadvantage of not using an optimal microbial harvesting in the reticulorumen. Pfau et al. (2019) suggested that due to the increased microbial harvest in ruminants with distinct digesta washing, more metabolic faecal nitrogen (a measure for microbial protein) should be excreted in these animals, and measures of apparent nitrogen digestibility might correspondingly be lower compared to species with less distinct digesta washing. In line with this concept, our present findings of a very low degree of digesta washing in eland matches reports of Arman et al. 1975 of particularly low metabolic nitrogen, and particularly high apparent protein digestibility, in eland compared to other ruminant species (Arman & Hopcraft 1975; Arman et al. 1975). More experimental passage studies, such as the present one, or more investigations on physical characteristics of reticulorumen contents (Sauer et al. 2017), can add to the catalogue of species considered 'cattle-types' or 'moose-types', and potentially

strengthen the relationships between the measures of digesta kinetics and anatomy, such as the intraruminal papillation pattern or omasum size (Przybyło et al. 2019b). These data collection indicate that on the one hand, the concept of comparative forestomach physiology in ruminants follows general overall patterns (Codron et al. 2019; Ehrlich et al. 2019), but that on the other hand, there is a large scatter in the patterns, suggesting that different species evolved different combinations of adaptations. For eland, measures of rumen contents, and more detailed investigations on their intraruminal papillation pattern, would be interesting in this respect. However, adding more species to the pattern will not replace more detailed investigations into the presence of tannin-binding proteins in saliva, or more detailed quantifications of the effect of digesta washing. More recently, another possible adaptive value of the rumen 'washing mechanism' has received some attention: in the process of sorting particles for regurgitation and rumination, they are inadvertently washed, by the rumen fluid, from adhering siliceous contaminations such as grit or dust (Hatt et al. 2019; Hatt et al. 2020). This mechanism could explain various differences between ruminants and nonruminants, for example the observation that ruminants generally do not achieve the same degree of hypsodonty as nonruminant herbivores (Hatt et al. 2019). This mechanism is subject to various physical principles, among them Stokes' law (Stokes, 1851), which implies that the washing effect will be the more efficient the less viscous the washing fluid is. If we accept that the difference between small particle and fluid retention (quantified as the selectivity factor SF) depends on the viscosity of the rumen fluid, with higher viscosities measured in species that have lower SF (Clauss et al. 2009a; Clauss et al. 2009b; Hummel et al. 2009; Lechner et al. 2010), then one might predict a relationship between high SF and a high degree of hypsodonty as combined evolutionary adaptations to habitats or feeding methods in which ruminants are exposed to high dust or grit loads on their food. The expected relationship appears evident (Fig. 11), but it is of course compromised by the fact that both hypsodonty (Damuth & Janis 2011) and the SF (Fig. 10) are related to the percentage of grass in the natural diet. Comparative data on the presence of dust and grit in the digestive tract of ruminant species would be ideal to further address this question. For eland, the combination of a low SF and an intermediate hypsodonty index would suggest a diet with some possible dust/grit contamination and relevant amounts of tannins. Consistent with these predictions, tannins do not appear to have a major influence on eland foraging decisions (Watson & Owen-Smith 2002), and eland are known to be able to use plants that cattle do not utilize (Hofmeyer 1970; Retief 1971; Lightfoot & Posselt 1977), possibly due to a higher tolerance against secondary plant compounds. More detailed studies on the tolerance of eland against tannins, and comparative data on the size of elands' salivary glands and saliva composition would be particularly welcome in this respect. The classification of eland as browsers (see Introduction) apparently matches the distinctively lower food intake on the grass hay. A reluctance to ingest grass or grass hay has been reported for several browsing ruminants (Clauss et al. 2003), and also directly for eland (Hofmann 1973 p. 40; Miller et al. 2010). While the results of the present study are compromised in this respect by the difference in fibre levels, which would suffice to explain a lower intake on the higher-fibre diet (Meyer et al. 2010), it is remarkable that this reluctance cannot be explained by a gut capacity constraint, as the animals showed significantly higher gut fill on the lucerne haylage diet (Table 8). Whether eland could be maintained over longer periods of time on grass or grass hay remains to be tested. In conclusion, we show that eland have patterns of digesta kinetics typical for 'moose-type'

ruminants. Given the ease with which eland can be kept as farm animals (Hansen et al. 1985), they appear as ideal model animals to investigate the consequences of being a 'moose-type' ruminant.

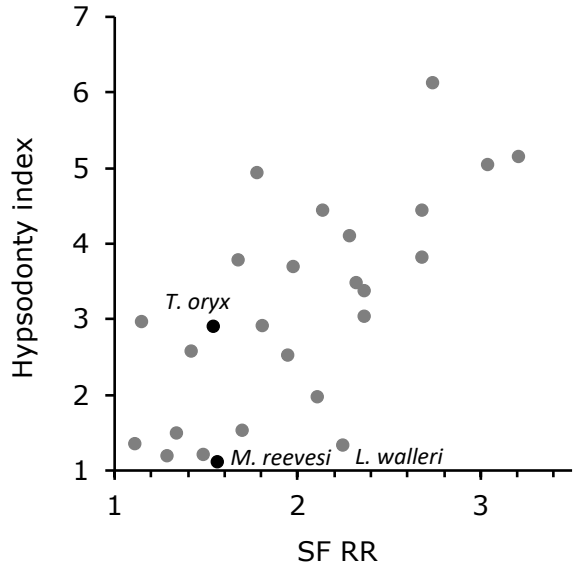


Figure 11. Relationship between the selectivity factor (SF, a measure for how distinctly the retention of small particles and fluid differ, i.e. a measure for the 'washing mechanism') in the reticulorumen (RR; data collection from Przybyło et al. (2019b; one value per species), including the eland (*Taurotragus oryx*) of the present study and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a)) and the hypsodonty index (a measure how high-crowned the cheek teeth of a species are, from Mendoza and Palmqvist (2008)).

References

Abdullahi AN. 1980. Forage Preference of Cattle and Eland on a Desert Grassland in Southern Arizona [PhD Thesis]. University of Arizona, Arizona.

AOAC. 1995. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Arlington VA.

Arman P, Hopcraft D. 1975. Nutritional studies on east African herbivores. 1. Digestibilities of dry matter, crude fibre and crude protein in antelope, cattle and sheep. Br. J. Nutr. 33:255-264.

Arman P, Hopcraft D, McDonald I. 1975. Nutritional studies on east African herbivores. 2. Losses of nitrogen in the faeces. Br. J. Nutr. 33:265-276.

Buys D. 1990. Food selection by eland in the western Transvaal. S. Afr. J. Wildl. Res. 20:16–20.

Cerling TE, Harris JM, Passey BH. 2003. Diets of east African Bovidae based on stable isotope analysis. J. Mammal. 84:456-470.

Clauss M, Hummel J. 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Rev. Bras. Zootec. 46:606–613.

Clauss M, Kienzle E, Hatt JM. 2003. Feeding practice in captive wild ruminants: Peculiarities in the nutrition of browsers/concentrate selectors and intermediate feeders. A review. Pages 27-

52 in Fidgett A, Clauss M, Ganslosser U, Hatt JM, Nijboer J. (Eds.), Zoo animal nutrition. vol. 2. Filander, Fuerth, Germany.

Clauss M, Kaiser T, Hummel J. 2008. The morphophysiological adaptations of browsing and grazing mammals. Pages 47-88 in Gordon IJ, Prins HHT. (Eds.), The Ecology of Browsing and Grazing. Springer, Heidelberg.

Clauss M, Fritz J, Bayer D, Hummel J, Streich WJ, Südekum KH, Hatt JM. 2009a. Physical characteristics of rumen contents in two small ruminants of different feeding type, the mouflon (*Ovis ammon musimon*) and the roe deer (*Capreolus capreolus*). Zoology 112:195-205.

Clauss M, Fritz J, Bayer D, Nygren K, Hammer S, Hatt JM, Südekum KH, Hummel J. 2009b. Physical characteristics of rumen contents in four large ruminants of different feeding type, the addax (*Addax nasomaculatus*), bison (*Bison bison*), red deer (*Cervus elaphus*) and moose (*Alces alces*). Comp. Biochem. Physiol. A 152:398-406.

Clauss M, Hume ID, Hummel J. 2010. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. Animal 4:979-992.

Codron D, Clauss M. 2010. Rumen physiology constrains diet niche: linking digestive physiology and food selection across wild ruminant species. Can. J. Zool. 88:1129-1138.

Codron D, Codron J, Lee-Thorp JA, Sponheimer M, De Ruiter D, Sealy J, Grant R, Fourie N. 2007. Diets of savanna ungulates from stable carbon isotope composition of faeces. J. Zool. 273:21-29.

Codron D, Hofmann RR, Clauss M. 2019. Morphological and physiological adaptations for browsing and grazing. Pages 81-125 in Gordon IJ, Prins HHT. (Eds.), The Ecology of Browsing and Grazing II. Springer, Berlin, Germany.

D'Ammando G, Parrini F, Attorre F, Boitani L. 2015. Observations on dry season grazing by eland in a Magaliesberg nature reserve, South Africa. Afr. J. Ecol. 53:112-115.

Damuth J, Janis CM. 2011. On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. Biol. Rev. 86:733-758.

Derix J, Ortmann S, Wiegmann L, Lawrenz A, Janssens GPJ, Clauss M. 2019. Retention of solutes and particles in the gastrointestinal tract of a grazing cervid: Père David's deer (*Elaphurus davidianus*). Eur. J. Wildl. Res. 65:46.

Dittmann MT, Hummel J, Hammer S, Arif A, Hebel C, Müller DWH, Fritz J, Steuer P, Schwarm A, Kreuzer M, Clauss M. 2015a. Digesta retention in gazelles in comparison to other ruminants: evidence for taxon-specific rumen fluid throughput to adjust digesta washing to the natural diet. Comp. Biochem. Physiol. A 185:58-68.

Dittmann MT, Runge U, Ortmann S, Lang RA, Moser D, Galeffi C, Schwarm A, Kreuzer M, Clauss M. 2015b. Digesta retention patterns of solutes and differentsized particles in camelids compared with ruminants and other foregut fermenters. J. Comp. Physiol. B. 185:559-573.

Ehrlich C, Codron D, Hofmann RR, Hummel J, Clauss M. 2019. Comparative omasum anatomy in ruminants: relationships with natural diet, digestive physiology, and general considerations on allometric investigations. J. Morphol. 280:259-277.

Frei S, Ortmann S, Reutlinger C, Kreuzer M, Hatt JM, Clauss M. 2015. Comparative digesta retention patterns in ratites. The Auk: Ornithol. Adv. 132:119-131.

Gagnon M, Chew AE. 2000. Dietary preferences in extant African Bovidae. J. Mammal. 81:490-511.

Galat-Luong A, Nizinski JJ, Galat G. 2011. Diet preferences of a Western giant's (Lord Derby's) eland group in a Sahelian dry habitat. Anim. Biol. 61:485-492.

Grandl F, Schwarm A, Ortmann S, Furger M, Kreuzer M, Clauss M. 2018. Kinetics of solutes and particles of different size in the digestive tract of cattle of 0.5 to 10 years of age, and relationships with methane production. J. Anim. Physiol. Anim. Nutr. 102:639-651.

Hackmann TJ, Spain JN. 2010. Ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. J. Dairy Sci. 93:1320-1334.

Hansen RM, Skovlin JM, Chimwani DM. 1985. Ability of eland and cattle to rumen digest forage. East African Agricult. Forest. J. 51:63-65.

Hatt JM, Codron D, Müller DWH, Ackermans NL, Martin LF, Kircher PR, Hummel J, Clauss M. 2019. The rumen washes off abrasives before heavy-duty chewing in ruminants. Mamm. Biol. 97:104-111.

Hatt JM, Codron D, Ackermans NL, Martin LF, Richter H, Kircher PR, Gerspach C, Hummel J, Clauss M. 2020. Differences in the rumen washing mechanism in sheep fed diets with abrasives of various concentrations and sizes. Palaeogeogr. Palaeoclimatol. Palaeoecol. 550:109728.

Hejcmanová P, Homolka M, Antonínová M, Hejcman M, Podhájecká V. 2010. Diet composition of western Derby eland (*Taurotragus derbianus derbianus*) in the dry season in a natural and a managed habitat in Senegal using faecal analyses. S. Afr. J. Wildl. Res. 40:27-34.

Hejcmanová P, Vymyslická P, Koláčková K, Antonínová M, Havlíková B, Stejskalová M, Policht R, Hejcman M. 2011. Suckling behavior of eland antelopes (Taurotragus spp.) under semi-captive and farm conditions. J. Ethol. 29:161-168.

Hofmann RR. 1973. The Ruminant Stomach. East African Literature Bureau, Nairobi. Hofmann RR. 1988. Morphophysiological evolutionary adaptations of the ruminant digestive system. Pages 1-20 in Dobson, A., Dobson, M.J. (Eds.), Aspects of Digestive Physiology in Ruminants. Cornell University Press, Ithaca, NY.

Hofmann RR. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78:443-457.

Hofmann RR, Stewart DRM. 1972. Grazer or browser: a classification based on the stomach-structure and feeding habit of east African ruminants. Mammalia 36:226-240.

Hofmann RR, Streich WJ, Fickel J, Hummel J, Clauss M. 2008. Convergent evolution in feeding types: salivary gland mass differences in wild ruminant species. J. Morphol. 269:240-257.

Hofmeyer JM. 1970. A review of the food preferences and feeding habits of some indigenous herbivores in the Ethiopian faunal region and some studies on animal plant relationships. Proc. South African Soc. Anim. Prod. 9:89-99.

Holleman DF, White RG. 1989. Determination of digesta fill and passage rate from non absorbed particulate phase markers using the single dosing method. Can. J. Zool. 67:488-494.

Hummel J, Clauss M, Zimmermann W, Johanson K, Norgaard C, Pfeffer E. 2005. Fluid and particle retention in captive okapi (*Okapia johnstoni*). Comp. Biochem. Physiol. A 140:436-444.

Hummel J, Steuer P, Südekum KH, Hammer S, Hammer C, Streich WJ, Clauss M. 2008. Fluid and particle retention in the digestive tract of the addax antelope (*Addax nasomaculatus*) – adaptations of a grazing desert ruminant. Comp. Biochem. Physiol. A 149:142-149.

Hummel J, Südekum KH, Bayer D, Ortmann S, Hatt JM, Streich WJ, Clauss M. 2009. Physical characteristics of reticuloruminal contents of cattle in relation to forage type and time after feeding. J. Anim. Physiol. Anim. Nutr. 93:209-220.

Hummel J, Hammer S, Hammer C, Ruf J, Lechenne M, Clauss M. 2015. Solute and particle retention in a small grazing antelope, the blackbuck (*Antilope cervicapra*). Comp. Biochem. Physiol. A 182:22-26.

Kerr MA, Wilson VJ, Roth HH. 1970. Studies on the agricultural utilization of semidomesticated eland (*Taurotragus oryx*) in Rhodesia. 2. Feeding habits and food preferences. Rhodesian J. Agricult. Res. 8:71-77.

Lamprey HF. 1963. Ecological separation of the large mammal species in the Tarangire game reserve, Tanganyika. East African Wildlife J. 1:63-92.

Lechner I, Barboza P, Collins W, Fritz J, Günther D, Hattendorf B, Hummel J, Südekum KH, Clauss M. 2010. Differential passage of fluids and different-sized particles in fistulated oxen (*Bos primigenius f. taurus*), muskoxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*) and moose (*Alces alces*): rumen particle size discrimination is independent from contents stratification. Comp. Biochem. Physiol. A 155:211-222.

Lechner-Doll M, Rutagwenda T, Schwartz HJ, Schultka W, von Engelhardt W. 1990. Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing in a thornbush savanna pasture in Kenya. J. Agric. Sci. (Camb.) 115:409-420.

Lightfoot CJ, Posselt J. 1977. Eland (*Taurotragus oryx*) as a ranching animal complementary to cattle in Rhodesia. 2. Habitat and diet selection. Rhod. Agric. J. 74, 53-61.

Littlejohn A. 1968. The feeding behaviour of eland. Br. Vet. J. 124:335-341.

Mambrini M, Peyraud JL. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculation based on fecal collection. Reprod. Nutr. Dev. 37:427-442.

Mendoza M, Palmqvist P. 2008. Hypsodonty in ungulates: an adaptation for grass consumption or for foraging in open habitat? J. Zool. 274:134-142.

Meyer K, Hummel J, Clauss M. 2010. The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. Mammal Rev. 40:221-245.

Miller M, Weber M, Valdes EV, Neiffer D, Fontenot D, Fleming G, Stetter M. 2010. Changes in serum calcium, phosphorus, and magnesium levels in captive ruminants affected by diet manipulation. J. Zoo Wildlife Med. 41:404-408.

Müller DWH, Codron D, Meloro C, Munn A, Schwarm A, Hummel J, Clauss M. 2013. Assessing the Jarman-Bell principle: scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. Comp. Biochem. Physiol. A 164:129-140.

Nge'the JC, Box FW. 1976. Botanical composition of eland and goat diets on an acacia grassland community in Kenya. J. Range Manag. 30:290-293.

Parrini F, D'Ammando G, Marshal JP. 2019. Seasonal shifts in habitat use and diet by eland confined in a small fenced reserve. Afr. J. Ecol. **57**:394-403.

Pfau F, Clauss M, Hummel J. 2019. Cattle and sheep – a hypothesis for a basic difference in digestive physiology. Adv. Anim. Biosci. 10:486.

Przybyło M, Clauss M, Ortmann S, Kowalski ZM, Górka P. 2019a. The effect of fructose supplementation on feed intake, nutrient digestibility and digesta retention time in Reeves's muntjac (*Muntiacus reevesi*). J. Anim. Physiol. Anim. Nutr. 103:1684-1693.

Przybyło M, Hummel J, Ortmann S, Codron D, Kohlschein GM, Kilga D, Smithyman J, Przybyło U, Świerk S, Hammer S, Hatt JM, Górka P, Clauss M. 2019b. Digesta passage in nondomestic ruminants: separation mechanisms in 'moose-type' and 'cattle-type' species, and seemingly atypical browsers. Comp. Biochem. Physiol. A 235:180-192.

Renecker LA, Hudson RJ. 1990. Digestive kinetics of moose, wapiti and cattle. Anim. Prod. 50:51-61.

Retief GP. 1971. The potential of game domestication in Africa with special reference to Botswana. J. S. Afr. Vet. Assoc. 42:119-127.

Robbins CT, Spalinger DE, Van Hoven W. 1995. Adaptations of ruminants to browse and grass diets: are anatomical-based browser-grazer interpretations valid? Oecologia 103:208–213.

Sauer C, Clauss M, Bertelsen MF, Weisbjerg MR, Lund P. 2017. Rumen content stratification in the giraffe (*Giraffa camelopardalis*). Comp. Biochem. Physiol. A 203:69-76.

Sponheimer M, Lee-Thorp JA, DeRuiter DJ, Smith JM, Van der Merwe J, Grant CC, Ayliffe LK, Robinson TF, Heidelberger C, Marcus W. 2003. Diets of southern African Bovidae: stable isotope evidence. J. Mammal. 84:471.

Steuer P, Südekum KH, Tütken T, Müller DWH, Kaandorp J, Bucher M, Clauss M, Hummel J. 2014. Does body mass convey a digestive advantage for large herbivores? Funct. Ecol. 28:1127-1134.

Stokes GG. 1851. On the effect of internal friction of fluids on the motion of pendulums. Transac. Cambridge Phil. Soc. 9 (ii):8-106.

Stynder DD. 2009. The diets of ungulates from the hominid fossil-bearing site of Elandsfontein, Western cape, South Africa. Quat. Res. 71:62-70.

Thielemans MF, François E, Bodart C, Thewis A. 1978. Mesure du transit gastrointestinal chez le porc a l'aide des radiolanthanides. Comparaison avec le mouton. Annales de Biologie Animale Biochimie Biophysique 18:237-247.

Udén P, Colucci PE, Van Soest PJ. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31:625-632.

Van Soest PJ. 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca.

van Zyl JHM. 1965. The vegetation of the S.a. Lombard nature reserve and its utilization by certain antelopes. Zool. Afr. 1:55-71.

Venter JA, Kalule-Sabiti MJ. 2016. Diet composition of the large herbivores in Mkambati nature reserve, eastern cape, South Africa. S. Afr. J. Wildl. Res. 46:49-56.

Watson LH, Owen-Smith N. 2000. Diet composition and habitat selection of eland in semi-arid shrubland. Afr. J. Ecol. 38:130-137.

Watson LH, Owen-Smith N. 2002. Phenological influences on the utilization of woody plants by eland in semi-arid shrubland. Afr. J. Ecol. 40:65-75.

Table 6. Nutrient concentration (in % dry matter, except where indicated) in the grass hay and the lucerne haylage used in the present study. Data represent means (±SD) of six pool samples.

Nutrient	Lucerne haylage	Grass hay		
		Offered	Leftover	Ingested
Dry matter (% as fed)	40.7 ±1.5	95.4 ±1.8	-	95.4 ±1.8
Total ash	12.1 ±0.2	7.0 ±0.8^{A}	6.1 ±1.1^{B}	7.4 ±1.0
Organic matter	87.9 ±0.2	93.0 ±0.8^{B}	93.9 ±1.1^{A}	92.6 ±1.0
Crude protein	23.4 ±0.7	5.7 ±0.4^{A}	4.2 ±0.6^{B}	6.3 ±0.6
Neutral detergent fibre	38.1 ±1.7	72.0 ±3.8	73.9 ±3.7	71.4 ±4.5
Acid detergent fibre	26.4 ±1.0	40.4 ±3.3^{B}	45.1 ±2.9^{A}	38.4 ±4.2
Acid detergent lignin	6.7 ±0.4	6.0 ±1.5	6.9 ±0.9	5.6 ±1.8

^{A,B} different letters within a row for grass hay indicate significant differences ($P < 0.05$) in paired tests between the offered diet and the leftovers; absence of letters in these columns indicates no significant difference

Table 7. Mean (±SD) intake of feed as fed, dry matter (DM), the relative dry matter intake (rDMI), the mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of three passage markers (Co – solutes, Cr – small particles, Ce – large particles) in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage

Diet	Intake	Intake	rDMI	MRT GIT			MRT RR			SF GIT			SF RR		
	as fed	DM		Co	Cr	Ce	Co	Cr	Ce	Cr/Co	Ce/Co	Ce/Cr	Cr/Co	Ce/Co	Ce/Cr
	kg	kg	g/kg$^{0.85}$/d		h			h							
Grass hay	4.05	3.87	24	35	47	50	23	36	38	1.35	1.43	1.07	1.54	1.66	1.09
	±1.36^{B}	±1.31^{B}	±7^{B}	±6^{Ab}	±12^{Aa}	±11^{Aa}	±5^{Ab}	±11^{Aa}	±9^{a}	±0.15	±0.10	±0.05^{B}	±0.27	±0.18	±0.07^{B}
Lucerne haylage	20.49	8.34	51	28	38	43	19	29	34	1.36	1.55	1.14	1.54	1.84	1.20
	±2.25^{A}	±1.07^{A}	±8^{A}	±5^{Bc}	±5^{Bb}	±5^{Ba}	±4^{Bc}	±4^{Bb}	±4^{a}	±0.09	±0.11	±0.05^{A}	±0.18	±0.23	±0.06^{A}

^{A,B} *different letters within columns indicate significant differences (P < 0.05) in paired tests*

^{a,b,c} *no common letters within rows indicate significant differences (RM-ANOVA, Sidak post hoc) between markers for a MRT or SF measure*

Table 8. Mean (±SD) apparent digestibility values and dry matter gut fill in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage

Diet	Apparent digestibility				Dry matter gut fill
	Dry matter	Organic matter	Protein	Neutral detergent fibre	
	%				kg
Grass hay	76 ±8^{B}	77 ±8^{B}	65 ±14^{B}	77 ±7	4.89 ±1.50^{B}
Lucerne haylage	89 ±5^{A}	89 ±4^{A}	91 ±4^{A}	85 ±6	8.43 ±2.17^{A}

^{A,B} *different letters within columns indicate significant differences (P < 0.05) in paired tests; absence of letters indicates no significant difference*

Table 9. Nonparametric correlations (n=12) between relative dry matter intake (rDMI, g/kg$^{0.85}$/d) and various measures of mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of three passage markers (Co – solutes, Cr – small particles, Ce – large particles) in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage

MRT GIT			MRT RR			SF GIT			SF RR		
Co	Cr	Ce	Co	Cr	Ce	Cr/Co	Ce/Co	Ce/Cr	Cr/Co	Ce/Co	Ce/Cr
***R* = -0.63**	***R* = -0.66**	***R* = -0.59**	*R* = -0.53	***R* = -0.63**	*R* = -0.33	*R* = -0.02	*R* = 0.39	***R* = 0.66**	*R* = -0.15	*R* = 0.19	***R* = 0.67**
***P* = 0.028**	***P* = 0.019**	***P* = 0.043**	*P* = 0.075	***P* = 0.029**	*P* = 0.291	*P* = 0.957	*P* = 0.217	***P* = 0.018**	*P* = 0.640	*P* = 0.556	***P* = 0.017**

*significant correlations in **bold***

7. Resilience in diet-quality strategies of mixed native and non-native herbivore communities in semi-arid and sub-humid savanna ecosystem

Adopted from: Stoklasová L, Hejcmanová P. 2021. Resilience in diet-quality strategies of mixed native and non-native herbivore communities in semi-arid and sub-humid savanna ecosystem.

Lucie Stoklasová participated in samples processing for the analyses of microelements by ICP-OES in the Laboratory of environmental chemistry at the Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, in statistical analyses of the data and in manuscript writing.

Introduction

Dietary choices of large mammalian herbivores are complex and animals use a wide variety of foraging strategies, based basically on their evolutionary adaptations and ecological processes, i.e. competition, facilitation, and habitat selection, to acquire and utilize foodstuffs (McNaughton & Georgiadis 1986; Kleynhans et al. 2011). While the diets are consistent at the plant functional level within feeding guilds (Kleynhans et al. 2011; Abraham et al. 2019), there is evidence on dietary richness at plant-species levels within guilds which underpins their coexistence, particularly in African savannas (Prins et al. 2006; Kleynhans et al. 2011; Kartzinel et al. 2015). The seasonal character of African savanna climate plays the primer role in these processes, specifically shaping the competition forces to strengthen during the dry season when the high-quality resources are scarce (McNaughton 1985; Voeten & Prins 1999). Coexistence of herbivores is therefore driven by resource partitioning, which enables species consuming the same source of food to acquire sufficient amount of forage for survival (Kartzinel et al. 2015). Among native herbivores, overlap in resource use is not expected due to evolutionary segregation. In a native assemblage to which an exotic species has been introduced, however, overlap in resource use can occur under food-limited conditions and consequently implies competition (Voeten & Prins 1999). This is case of many wildlife reserves, where non-native species are introduced into delimited fenced areas. Animals, moreover, have to face also to the global climate change processes which are expected to increase incidence and severity of droughts (Lyon 2004; Knapp et al. 2008) and to significantly impact availability of food resources for herbivores (Hulme et al. 2001; Thuiller et al. 2006). When climate changes trigger changes in habitats, it activates animal behaviours that secure the adequate nutrition through a mechanism of resilience which may involve either shift in amount and selection of available forage or physiological processes. Animal communities thus become vulnerable as on islands surrounded by a fence, similarly as may happen to protected areas isolated in the anthropogenic matrix. The competition for changing resources may become critical and may lead to population declines and extinctions (Thuiller et al. 2006; Augustine 2010; Craigie et al. 2010). Specifically, in areas constrained by real or virtual barriers, the competition for limited resources and dietary partitioning represents crucial mechanisms determining the decisions for effective conservation management of large herbivore communities' diversity. The understanding of resilience of herbivore communities in fragmented landscapes to changing climatic patterns are therefore of vital concern for conservation.

To understand the feeding responses of large savanna herbivores placed to space-constrained and two environmentally distinct (semi-arid *versus* sub-humid savanna) conditions, we investigated the diet quality of five species living in a mixed community of species native and non-native for the West African savanna and compared them between the two sites as a proxy for a potential ecosystem shift due to climate change. The species were namely the Western Derby eland (native browser), common eland (non-native mixed feeder/browser), roan antelope (native mixed feeder), buffalo (native grazer) and zebra (non-native grazer). We predicted that maintaining the diet quality at nutrient level represents for large herbivores a significant adaptation mechanism in satisfying species-specific nutritional requirements in divergent habitats. We examined specifically whether herbivores of different feeding guilds demonstrate distinct or the same diet quality in semi-arid and sub-humid savannas, which implicitly differ in plant species composition and abundance. Namely, we tested the differences in fibre

fractions, macro- and microelements among five large ungulates belonging to grazers, mixed feeders, and browsers and then we compared their diet quality in these two savanna types in wet, dry, and late dry seasons of the year. The both sites were fenced private managed nature reserves where animals receive food supplement in the late dry season to ensure the adequate animal nutrition and to prevent animal losses. We expected therefore to depict the contribution of food supplement in the overall quality of diet during the period of most limited resources. The ultimate aim was to provide a critical missing information for the conservation of mammalian herbivores in face of potential habitat changes resulting from global climate change.

Materials and methods

Study sites

Study was conducted in two fenced wildlife reserves in Senegal, Bandia and Fathala reserves, managed by private operators for habitat and wildlife conservation and safari-tourism. The Bandia reserve (14°35'N, 17°00'W; 3000 ha), located 65 km southward from Dakar, has a semi-arid hot climate with annual average temperature 26°C and mean precipitation 450 - 530 mm divided into wet (July – October), dry (November – April) and hot dry (May – June) seasons. Vegetation is Sahelo-Sudanese savanna dominated by baobab trees (*Adansonia digitata*), various species of acacia (*Acacia* spp., *Faidherbia albida*), *Balanites aegyptiaca*, *Boscia senegalensis* and *Cassia obtusifolia* (Hejcmanová et al. 2010). The reserve hosts large herbivores, native to Senegal such as African buffalo (*Syncerus caffer brachyceros*), defassa waterbuck (*Kobus ellipsiprymnus defassa*), roan antelope (*Hippotragus equinus koba*), and Western Derby eland (*Taurotragus derbianus derbianus*), and non-native species which have been introduced from South Africa such as giraffe (*Giraffa camelopardalis giraffa*), greater kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), common eland (*Tragelaphus oryx oryx*), Burchell's zebra (*Equus quagga burchelli)* and white rhino (*Ceratotherium simum simum*).

The Fathala reserve (13°39'N, 16°30'W; 2000 ha) is located 250 km southward from Dakar, near the northern border of the Gambia. The area has a sub-humid climate, with annual average temperature 27°C and mean precipitation range 770 – 1000 mm divided into wet (July – November), dry (December – March), and hot dry (April – May) seasons. Vegetation belongs to the Sudano-Guinean savannah with dominants of *Acacia macrostachya*, *Combretum* spp., *Danielia olliveri, Piliostigma thonningii, Pterocarpus erinaceus, Terminalia avicennoides*, *T. macroptera*, and *Andropogon gayanus* grass (Hejcmanová et al. 2019). There are native herbivores such as bushbuck (*Tragelaphus scriptus*) and warthog (*Phacochoerus africanus*), and several introduced wildlife species from Senegal, such as African buffalo, defassa waterbuck, roan antelope, Western Derby eland, and non-native species from South Africa, such as giraffe, white rhino, zebra, and common eland.

Herbivore diet evaluation

Five herbivore species were selected as model species for diet evaluation. Grazers were represented by buffalo (Sponheimer et al. 2003; Codron et al. 2007) and zebras (Codron et al. 2007; Mandlate et al. 2019), and browsers by Derby eland (Hejcmanová et al. 2010, 2019). Mixed feeders were represented by roan antelope which tends more to grazing (Schuette et al.

1998; Tyowua et al. 2013) and by common eland which tends more to browsing (Sponheimer et al. 2003; Parrini et al. 2019; Hejcmanová et al. 2020). To evaluate the diet quality, we sampled fresh dung three times of which each represented the specific season, i.e. in December 2011 (dry season), April 2012 (hot dry season), and August 2012 (wet season) in both reserves. Sample collection was combined with observation of animals while defecating to associate correctly the dung to species. Samples were from adult males and non-lactating females. From each reserve 4 -5 samples from each species at each sampling date were taken. A total of 120 samples were collected in the Bandia reserve (57 for Derby eland; 14 for common eland; 17 for roan antelope; 12 for buffalo; 20 for zebra) and total of 84 samples were collected in the Fathala reserve (36 for Derby eland; 10 for common eland; 17 for roan antelope; 7 for buffalo; 14 for zebra).

Samples were oven-dried at 60°C for at least 72 hours, then ground and homogenized. The samples were subjected to standard nutrient analyses (AOAC, 1995) for dry matter (DM), neutral detergent fibre (NDF, AOAC no. 2002.04), acid detergent fibre and acid detergent lignin (ADF, ADL, AOAC no. 973.18). The content of hemicellulose was calculated as the difference between NDF and ADF content; the content of cellulose was calculated as the difference between ADF and lignin, both for each sample.

The N concentration was determined using an automated analyser TruSpec (LECO Corporation, USA) by combustion with oxygen in an oven at 950 °C. Combustion products were mixed with oxygen and the mixture passed through an infrared CO2 detector and through a circuit for aliquot ratio where carbon is measured as CO2. Gases in the aliquot circuit were transferred into helium as a carrying gas, conducted through hot copper and converted to N.

Concentrations of K, S, Co, P, Ca, Mg, and Na were tested by ICP-OES (IRIS Intrepid II XSP Duo, THERMO Elemental, USA), and Se was analysed by hydride generation atomic absorption spectroscopy technique (HG-AAS) using Analyst 100 spectrometer (Perkin Elmer, USA). For ICP-OES measurements samples were mineralized in the mixture of nitric and hydrochloric acid in 6:1 ratio using a closed microwave digestion system. Then standard ICP-OES measurements followed.

Other group of elements Mn, Fe, Cu, Zn and Mo was analysed by ICP – MS. Fine-grained, dry and homogenized faecal samples were acid digested in 5 ml HNO_3 (Analpure®, Analytika, Czech Republic) for 20 min at 180 °C in closed microwave digestion system (Discover SP-D, CEM Corp., USA). Final digest was diluted by water (Milli-Q purification system; Millipore, SAS, France) till volume of 45 ml, aliquot proportion (1 ml) was removed from this volume and diluted again by H_2O to the final volume of 4 ml. In such diluted digests concentration of S, Mn, Fe, Co, Mn, Zn, Se, Mo were determined by technique of inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA).

Data analyses

Data, i.e. concentrations of all elements and fibre fractions, were first tested by Kolmogorov-Smirnov test of normality. We tested for differences in concentration of elements and fibre fractions between animal species for each reserve separately using a series of one-way ANOVAs. In case of significant differences, we applied Tukey *post hoc* comparison test. Then, we tested the differences in concentrations of each element and fibre fractions between the

reserves separately for each animal species using a series of Student's t-tests. These analyses were performed using the TIBCO® Statistica™ package (StatSoft, Palo Alto, CA, USA).
The multivariate constrained Redundancy Analysis (RDA) in the Canoco 5 package (Ter Braak & Šmilauer 2012) was applied to examine the relationships among the nutrients (macro-, microelements and fibre fractions) and effects of explanatory environmental variables at each wildlife reserve separately. Explanatory environmental variables were animal species and season. The data were log-transformed and standardized during the analysis. To test the significance of our constrained ordination model, unrestricted Monte Carlo permutation test (permutations n=999) was applied. Results of the analyses were visualised in the form of ordination diagrams.

Results

Overall, mixed feeders and grazers maintained the quality of their diet in most of the principal parameters, especially in the content of nitrogen and fibres, regardless the savanna ecosystem type, while browser showed significantly lower nitrogen and fibres in the semi-arid environment in comparison to the sub-humid one (Table 1). The diet of all ruminants, i.e. all species except zebra, contained consistently lower proportion of indigestible lignin and higher concentration of calcium in the semi-arid environment (Table 1). The concentrations of phosphorus in diet of buffalo, roan, and Derby eland were lower in the semi-arid environment. Regarding the inter – species comparisons, the diet quality indicated by the concentration of nitrogen was higher in Derby and common elands in comparison to zebra and buffaloes at both sites, while roan antelope was similar to both eland species in the semi-arid and similar to zebra and buffaloes in the sub-humid savanna. Hemicellulose, the most digestible fibre fraction in the diet, followed the opposite patterns, being the highest in zebra and the lowest in Derby elands at both sites, conversely to the proportion to indigestible fibres. Phosphorus concentrations in diets did not differ between species in the semi-arid savanna, whereas in the sub humid savanna Derby eland and Common eland showed the highest values. Lignin which is the indicator of browse plants in herbivore's diet, its proportion and calcium were the highest in Derby and common elands at both sites, significantly different from the other species (Table 1).
Microelements reflect the mineral background of the environment and animal physiological state, rather than direct indicators of herbivores diet quality. There was no consistent pattern in response to the savanna type, neither differences among species (Table 2). In the semi-arid savanna, the concentration of Zn was the highest in buffalo and concentration of Se was the highest in buffalo and roan antelope, while at the sub-humid site concentration of those microelements were highest in browsers, i.e. Zn in Common eland and Se in Derby eland. Highest concentrations among microminerals were recorded for Mn.
In terms of nutrient landscape pointing out the mutual relationships among nutrients, the multivariate RDA analysis showed significant effects of herbivore species and seasons at both sites; on first axis $F=2.4$, $P=0.001$, on all axes $F=10.4$, $P=0.001$ in the semi-arid savanna, and on first axis $F=2.7$, $P= 0.001$, on all axes $F=7.1$, $P=0.001$ in the sub-humid savanna.
At semi-arid site, in the Bandia reserve, the first axis of the RDA explained 20.5%, the second axis 18.1%, the third axis 6.7%, and all four axes together show 50% of the data variability. The first (x) axis represented the content of N, Mg, P, S, and Cu which were positively and negatively related to the wet and dry seasons, respectively. The second axis represented the

content of lignin, Ca and Mn with positive relation of Derby and common elands there on one side, and represented hemicellulose, K, and Mo to which roan and buffalo were related closer (Fig 1a).

At sub-humid site, in the Fathala reserve, the first axis of the RDA explained 24.3%, the second axis 11.1%, the third axis 5.4%, and all four axes together show 44% of the data variability. The first (x) axis predominantly represented the content of Ca, P, Mg together with Cu, Zn, and Mo which was positively related to wet season and Common eland on one side, and hemicellulose related to buffalo on the other side. The second axis represented mostly lignin and Mn, which were more positively related to Derby eland. On another side second axis also represented K and Co which were not closely related to any animal species (Fig 1b). Zebra as a pure grazer stands a side of all other species and elements in both reserves and is in perfect opposite position to pure browser Derby eland.

Discussion

Our findings suggest that the mixed feeders and grazers were resilient to the change of habitat by maintaining the level of diet quality in both, sub-humid and semi-arid savanna ecosystems, even if those substantially differ in the vegetation structure and plant species composition. Few differences found in zebra and buffalo in the sub-humid ecosystem with higher NDF and ADF fibre content indicated a higher proportion of fresh grass biomass in grazers' diet. Similar proportion of lignin in zebra's diet in semi-arid and sub-humid conditions indicated that zebra did not incorporate any higher proportion of browse to its diet despite grass limited environment, similarly as reported by (Landman & Kerley 2001). Such feeding strategy put other grazing herbivores, here namely roan antelope and buffalo, under a strong competition. Higher proportions of lignin in the diet of roan, and buffalos in the sub-humid savanna suggest that they include browse in their diet as elsewhere, specifically for buffalo see Jarman (1971), Mloszewski (1983), Gagnon & Chew (2000). It seems contradictory as buffalos are primarily grazers and roan antelopes switch their diet to browsing especially during dry seasons when resources are scarce, switching from strict grazing (>95% grass) to mixed feeding (<50% grass) (Schuette et al. 1998), whereas in our study, we expect there were more resources in the sub-humid savanna. Our results suggest that the vegetation structure of the sub-humid savanna that is composed by denser woody plants canopy and tall grasses (*Andropogon gayanus*, *Schizachyrium sanguineum*) and herbs in the undergrowth (Nežerková-Hejcmanová et al. 2005) does not offer for grazers adequate grass resources, i.e. appropriate leaf-stem ratio (Heitkönig & Owen-Smith 1998). Roan antelope are sensitive to habitat change and competition from sympatric grazers and are susceptible to nutritional deficiencies (Wilson & Hirst 1977). Therefore, facing the spatial limitation, i.e. no option to migrate, and competition from other bulky feeders, i.e., zebras, the species that are physiologically able to switch from grazing to browsing on trees, shrubs and herbs (Clauss et al. 2003), do so and maintain their diet quality.

Browsers are considered most resilient, not changing the diet, nor the use of space during drought events because trees are more resistant to drought and provide forage resources more or less continuously (Abraham et al. 2019; Craigie et al. 2010). Derby elands browse on a wide variety of woody species and are not particularly selective (Hejcmanová et al. 2013, 2019), therefore we expected the animals to find adequate food resources in both environments, despite different vegetation. Their diet, however, differed in quality, specifically the animals' diet was

lower in macroelements and fibres in the semi-arid savanna in comparison to the sub-humid one. The levels of nutrients, however, did not fall under critical limits which could lead animals to nutritional deficiency. For large African herbivores, suggested critical faecal nitrogen concentration lies within the range 13 – 16 g/kg of dry matter (1.3-1.6 % of dry matter) and critical faecal phosphorus concentration is estimated to 2 g/kg (0.2% of dry matter) (Wrench et al. 1997; Grant et al. 2000). Especially pronounced difference in the diet quality parameters between the two sites for browsers, i.e. for Derby eland, but also for common eland, was found in the content of lignin and its proportion as indigestible component in the diet. The sub-humid savanna offers broad-leaved tree species, for instance *Terminalia* spp., *Combretum* spp., *Saba senegalensis* (Žáčková et al. 2013), these species thus provide more biomass, while they also contain more lignin, tannins, and/or other secondary metabolites which simultaneously increase nitrogen content irrelevant as nutritive element due to its non-protein origin. On the other hand, the semi-arid savanna vegetation is composed dominantly by *Acacia* spp. with more narrow leaves, less biomass, with less antinutritive compounds. The consumption of *Acacia* spp. by browsing animals is indirectly confirmed by very high concentration of Ca and strongly biased Ca:P ratio, especially in Derby eland diet, as legume, including *Acacia* spp., and other tree and shrub species alike are rich source of Ca (Topps 1992; Tefera & Mlambo 2017). Elands having Ca rich and P poor diet are likely unable to absorb Ca from the diet and then Ca leave the body without utilization (Stoklasová et al. 2019).

There was no specific consistent pattern in the microelement concentrations in relation to feeding type, species, or savanna type. In tropical regions, soils often have limiting supplies of trace elements (Hacker 1982). This environmental background translates into plant food and as a consequence, domestic ungulates in tropical regions feeding on natural resources were reported as mineral deficient (McDowell et al. 1983). Data from wild large savanna herbivores on microelements are virtually inexistent. In our study, we have not found any low values of microelements which would indicate any deficiency. Nevertheless, Mn concentration at all species at both localities are very high in comparison to, for instance, 4 μg/kg maintenance requirements of ewes (Zhang et al. 2018). High concentrations of Mn are even sharply increased at roan antelopes and buffaloes in the sub-humid savanna. On the other hand, high Mn concentrations can be toxic, leading to depressed iron status and hematologic changes or damage of rumen microbial flora. Despite the fact, that Mn is one of the least toxic of the essential elements (Herdt & Hoff 2011) concentrations of Mn, as well as of other microelements, and their sources from environment should be investigated further.

We must pay a close attention to the supplementary feeding and its role in the diet quality during the hot dry season when the supplement is supplied to animals. The food supplement is composed by *Acacia albida* (synonymous to *Faidherbia albida*) pods and peanut hay (*Arachis hypogea*). Both are legume species, rich in nitrogen, phosphorus, and calcium (Hassan et al. 2007; Mokgolodi et al. 2011; Khan et al. 2013) and supplied to maintain the animals' fitness during the most critical period of scarce natural resources, especially in regard to conservation program of critically endangered western subspecies of Derby eland. The supplement may change the interplay of all diet components and prevents a meaningful comparison to free ranging wildlife. For instance, faecal nitrogen and phosphorus concentrations in the animals' diet in our study were higher at both sites and for all species in comparison to the same and related species during the dry season in Kruger national park (Macandza et al. 2013). Similarly,

the Ca:P ratio in diet of browsing species, i.e. both browsers and intermediate, was higher in the semi-arid savanna reserve where the supplementing management is more regular and intensive in comparison to the reserve in sub-humid area. The multivariate analysis in the sub-humid savanna showed the importance of ongoing season from the wet season on one side, through dry season with low explanatory value, to the hot dry on the other side, and this pattern suggests that a supplementary feeding was complementary only, without any substantial impact on natural dietary associations. Two species of mixed feeders, roan antelope and common eland, showed opposite association to nutrients and seasons, confirming thus their distinct features, thus incomparability despite their 'mixed feeders' designation. The position of roan antelope shows its similarity to buffalo and previous consideration of buffalo's partial browsing. Derby eland related especially to lignin and nitrogen, and zebra appear independent on the season, each species on the opposite side. In contrast, the multivariate analysis of nutrients, species and seasons in the semi-arid savanna revealed the importance of wet and dry seasons, while the hot dry season appeared to be of low importance for the nutrients in the animal diet. We associate this to the effect of supplementary feeding which changes the relationships of nutrients in animal diets and similarities among animals. Under these conditions, common eland diet was more similar to Derby eland's diet, and their relation to Ca suggest that they benefit from supplementary feeding, alike buffalo was more similar to zebra, all these species independent of season. Diet of roan antelope was more distinct from other species and remained on the axis of seasons.

Conclusion

Overall, our results showed that despite differences in aggregate rainfall and vegetation structure (including plant species composition) between semi-arid and sub-humid savannas at two sites, grazers and mixed feeders maintained the diet quality in most nutrients at similar levels. Our findings suggest that to maintain the level of diet quality, the conditions of different ecosystems with no possibility to leave the area imposed changes in feeding behaviour across feeding types, most pronounced in mixed feeders. Based on proportion of indigestible lignin in faeces, we infer that buffalos and roan antelopes escaped the competition with non-native zebras for grasses in sub-humid savanna where denser canopy and mostly herbs in the undergrowth make grasses a scarce resource, by switching to browse. Therefore, introduction of not native species into native communities may have a big impact on behaviour and may force them to go to the limits of their feeding resilience. In terms of diet quality, the species differing the most between the two ecosystems was Derby eland, the pure browser, showing consistently lower levels of all macroelements and fibres in the semi-arid environment. Therefore, browsers may experience intensifying impacts in terms of diet quality and available nutrients, despite they have been considered not to be affected behaviourally by drier conditions, for instance by drought events, based on assumption that woody plant resources remain available and browsers thus appeared more resilient (Abraham et al. 2019). From conservation perspective, all feeding guilds may become threatened by changing ecosystems, even if each one by different way, e.g. by increased competition or inadequate nutrient levels if they are forced to change food resources, and a conservation-oriented 'exit-strategy' for species should be conceived to address these threats, including the strengthening the ecological connectivity in savanna landscapes.

References

Abraham JO, Hempson GP, Staver AC. 2019. Drought-response strategies of savanna herbivores. Ecology and Evolution 9:7047–7056. DOI: 10.1002/ece3.5270

AOAC International. 1995. Official methods of analysis of AOAC International. Volume 1. 16th edition. Arlington, USA, Association of Analytical Communities. ISBN:0935584544

Augustine DJ. 2010. Response of native ungulates to drought in semi-arid Kenyan rangeland. African Journal of Ecology 48: 1009-1020. DOI: 10.1111/j.1365-2028.2010.01207.x

Clauss M, Lechner-Doll M, Streich WJ. 2003. Ruminant diversification as an adaptation to the physicomechanical characteristics of forage. A reevaluation of an old debate and a new hypothesis. Oikos 102:253-262. DOI: 10.1034/j.1600-0706.2003.12406.x

Codron D, Lee-Thorp JA, Sponheimer M, Codron J. 2007. Nutritional content of savanna plant foods: implications for browser/grazer models of ungulate diversification. Eur J Wildl Res 53:100-111. DOI: 10.1007/s10344-006-0071-1

Craigie ID, Baillie JE, Balmford A, Carbone C, Collen B, Green RE, Hutton JM. 2010. Large mammal population declines in Africa's protected areas. Biological Conservation 143: 2221-2228. DOI: 10.1016/j.biocon.2010.06.007

Gagnon M, Chew AE. 2000. Dietary preferences in extant african bovidae. Journal of Mammalogy 81(2):490-511. DOI: 10.1093/jmammal/81.2.490

Grant CC, Peel MJS, Zambatis N, Van Ryssen JBJ. 2000. Nitrogen and phosphorus concentration in faeces: an indicator of range quality as a practical adjunct to existing range evaluation methods. Afr. J. Range Forage Sci. 17: 81–92. DOI: 10.2989/10220110009485743

Hacker JB ed. 1982. Nutritional Limits to Animal Production From Pastures. Slough: Commonwealth Agric. Bur. 548 pp. IBSN: 0851984924

Hassan LG, Umar KJ, Yuguda H. 2007. Nutritional evaluation of Faidherbia albida seeds and pulp as source of feeds for livestock. African Journal of Food, Agriculture, Nutrition and Development, 7(5).

Heitkönig IMA, Owen-Smith N. 1998. Seasonal selection of soil types and grass swards by roan antelope in a South African savanna. African Journal of Ecology 36: 57-70.

Hejcmanová P, Homolka M, Antonínová M, Hejcman M, Podhájecká V. 2010. Diet composition of western Derby eland (*Taurotragus derbianus derbianus*) in the dry season in natural and managed habitat in Senegal using faecal analysis. South African Journal of Wildlife Research 40(1):27-34. DOI: 10.3957/056.040.0105

Hejcmanová P, Vymyslická P, Žáčková M, Hejcman M. 2013. Does supplemental feeding affect behaviour and foraging of critically endangered western giant eland in an ex situ conservation site? African Zoology 48(2):250-258. DOI: 10.3377/044.048.0205

Hejcmanová P, Mířejovská M, Homolka P, Hejcman M. 2019. Drivers of diet selection of critically endangered Western Derby eland during the food shortage period within conservation breeding in Senegal. Scientific reports: 8712. DOI: 10.1038/s41598-019-45035-z

Hejcmanová P, Ortmann S, Stoklasová L, Clauss M. 2020. Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet. Comparative Biochemistry and Physiology, Part A 246. 110720. DOI: 10.1016/j.cbpa.2020.110720

Herdt T, Hoff B. 2011. The use of blood analysis to evaluate trace mineral status in Ruminant livestock. Vet Clin Food Anim 27: 255-283. DOI: 10.1016/j.cvfa.2011.02.004

Hulme M, Doherty R, Ngara TNM, Lister D. 2001. African climate change: 1900 – 2100. Climate Research 17: 145 – 168. DOI: 10.3354/cr017145

Jarman PJ. 1971. Diets of Large Mammals in the Woodlands around Lake Kariba, Rhodesia. Oecologia 8: 157-178.

Kartzinel TR, Chen PA, Coverdale TC, Erickson DL, Kress WJ, Kuzmina ML, Rubenstein DI, Wang W, Pringle RM. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. PNAS 26:8019-8024 DOI: 10. 1073/pnas.1503283112

Khan MT, Khan NA, Bezabih M, Qureshi MS, Rahman A. 2013. The nutritional value of peanut hay (*Arachis hypogaea L.)* as an alternate forage source for sheep. Tropical Animal Health and Production 45(3):849-853. DOI: 10.1007/s11250-012-0297-8

Kleynhans EJ, Jolles AE, Marjan RE, Olff BH. 2011. Resource partitioning along multiple niche dimensions in differently sized African savanna grazers. Oikos 120: 591-600. DOI: 10.1111/j.1600-0706.2010.18712.x

Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, et al. 2008. Consequences of more extreme precipita- tion regimes for terrestrial ecosystems. BioScience, 58(9):811–821. DOI: 10.1641/B580908

Landman M, Kerley GIH. 2001. Dietary shifts: do grazers become browsers in the Thicket Biome? Koedoe 44: 31-36.

Lyon B. 2004. The strength of El Niño and the spatial extent of trop- ical drought. Geophysical Research Letters 31:L21204. DOI: 10.1029./2004GL020901

Macandza V, Owen-Smith N, Le Roux E. 2013. Faecal nutritional indicators in relation to the comparative population performance of sable antelope and other grazers. African Journal of Ecology 52:300-307. DOI: 10.1111/aje.12119

Mandlate JRLC, Arsenault R, Rodrigues FHG. 2019. Grass greenness and grass height promote the resource partitioning among reintroduced Burchell´s zebra and blue wildebeest in southern Mozambique. Austral Ecology 44:648-657. DOI: 10.1111/aec.12708

McDowell LR, Conrad JH, Ellis GL. 1983. Mineral Deficiencies and Imbalances and Their Diagnosis. Proc. Symp. Herbivore Nutrition in the subtropics and tropics, Pretoria pp.67-88.

McNaughton SJ, Georgiadis NJ. 1986. Ecology of African grazing and browsing mammals. Annual Review of Ecology and Systematics 17:39-65. DOI: 10.1146/annurev.es.17.110186.000351

McNaughton. 1985. Ecology of a Grazing Ecosystem: The Serengeti. Ecological Monographs 55:259-294. DOI: 10.2307/1942578

Mloszewski MJ. 1983. The Behavior and Ecology of the African Buffalo**.** Cambridge University Press,Cambridge. DOI: 10.2307/2405076

Mokgolodi NC, Setshogo MP, Shi LL, Liu YJ, Ma C. 2011. Achieving food and nutritional security through agroforestry: a case of Faidherbia albida in sub-Saharan Africa. Forestry Studies in China 13(2):123-131. DOI: 10.1007/s11632-011-0202-y

Nežerková-Hejcmanová P, Hejcman M, Camara AA, Antonínová M, Pavlů V, Černý T, Bâ AT. 2005. Analysis of herbaceous undergrowth in woody savanna in Fathala Reserve, Delta du Saloum National Park, Senegal. *Belgian Journal of Botany* 138:119–128.

Parrini F, D'Ammando G, Marshal JP. 2019. Seasonal shifts in habitat use and diet by eland confined in a small fenced reserve. African Journal of Ecology 57:394-403. DOI: 10.1111/aje.12618

Prins HHT, Boer de WF, Oeveren van H, Correia A, Mafuca J, Olff H. 2006. Co-existence and niche segregation of three small bovid species in southern Mozambique. African Journal of Ecology 44:186-198. DOI: 10.1111/j.1365-2028.2006.00619.x

Schuette JR, Leslie DM Jr, Lochmiller RL, Jenks JA. 1998. Diets of heartebeest and roan anelope in Burkina Faso: support of the long-faced hypothesis. Journal of Mammalogy 79: 426-436.

Sponheimer M, Lee-Thorp JA, DeRuiter DJ, Smith JM, Van der Merwe J, Grant CC, Ayliffe LK, Robinson TF, Heidelberger C, Marcus W. 2003. Diets of southern African Bovidae: stable isotope evidence. Journal of Mammalogy 84:471-479. DOI: 10.1644/1545-1542

Stoklasová L, Váhala J, Hejcmanová P. 2019. Minerals in the Blood, Hair, and Faeces of the Critically Endangered Western Derby Eland Under Human Care in Two Wildlife Reserves in Senegal. Biological Trace Element Research 195:105-109. DOI: 10.1007/s12011-019-01839-8

Tefera S, Mlambo V. 2017. Macro and trace elements of 26 browse species growing in semi-arid grazing lands of Eastern Cape, South Africa. African Journal of Range and Forage Science 34:209-217. DOI: 10.2989/10220119.2017.1412354

Ter Braak CJF, Šmilauer P. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA, 496 pp.

Thuiller W, Broennimann O, Hughes G, Alkemade JRM, Midgley GF, Corsi F. 2006. Vulnerability of African mammals to anthropogenic climate change under conservative land transformation assumptions. Global Change Biology 12: 424-440. DOI: 10.1111/j.1365-2486.2006.01115.x

Topps JH. 1992. Potential, composition and use of legume shrubs and trees as fodders for livestock in the tropics. Journal of Agricultural Science 118:1-8. DOI: 10.1017/S0021859600067940

Tyowua BT, Agbelusi EA, Orsar JT. 2013. Roan antelope (*Hippotragus equinus*, Desmarest 1804) food plants and feeding habits in Kainji Lake National Park, Nigeria. Journal of Research in Forestry, Wildlife and Environment 4:22-29.

Voeten MM, Prins HHT. 1999. Resource partitioning between sympatric wild and domestic herbivores in the Tarangire region of Tanzania. Oecologia 120:287-294. DOI: 10.1007/s004420050860

Wilson DE, Hirst SM. 1977. Ecology and factors limiting roan and sable antelope populations in South Africa. Wildlife Monographs 54: 3-111.

Wrench JM. 1997. The use of faecal analyses to predict dietary composition in wild herbivores. M.Sc. thesis. University of Pretoria, Pretoria.

Žáčková M, Hejcmanová P, Hejcman M. 2013. Does a change in land use affect woody vegetation in sub-humid sudanian savanna in Senegal? Scientia Agriculturae Bohemica, 44:209-2017. DOI: 10.7160/sab.2013.440403

Zhang H, Nie H, Wang Z, Wang F. 2018. The net iron, manganese, copper, and zinc requirements for maintenance and growth of Dorper x Hu ewe lambs. Italian Journal of Animal Science 17:941-949. DOI: 10.1080/1828051X.2018.1431964

RESULTS

Table 10. Concentrations (mean ± SE) of macroelements and fiber fractions in faeces of large herbivores in Bandia and Fathala reserves.

	Derby eland	Common eland	Roan antelope	Buffalo	Zebra	F; p-value
BANDIA	**n = 57**	**n = 14**	**n= 17**	**n = 12**	**n = 20**	
Nitrogen g/kg	21.1 ± 0.39 $^{b/***}$	24.10 ±1.27 $^{b/NS}$	21.47 ±1.05 $^{b/NS}$	16.39 ±1.28 $^{a/NS}$	17.1 ± 1.26 $^{a/NS}$	9.76; <0.001
Phosphorus g/kg	4.5 ± 0.18 $^{NS/*}$	4.95 ±0.62 $^{NS/NS}$	4.75 ±0.39 $^{NS/**}$	4.93 ±0.42 $^{NS/**}$	4.1 ± 0.41 $^{NS/NS}$	0.95; 0.43
Potassium g/kg	6.7 ± 0.16 $^{a/*}$	6.99 ±0.39 $^{a/NS}$	6.56 ±0.57 $^{a/NS}$	8.14 ± 0.68 $^{a/NS}$	11.7 ±0.84 $^{b/NS}$	22.8; <0.001
Calcium g/kg	40.9 ± 0.78 $^{d/***}$	35.42 ±1.71 $^{c/**}$	23.96 ±2.59 $^{b/***}$	17.80 ± 1.73 $^{b/*}$	8.7 ± 0.67 $^{a/NS}$	110; <0.001
Magnesium g/kg	6.7 ± 0.35 $^{b/NS}$	7.49 ±0.87 $^{b/NS}$	5.54 ±0.42 $^{b/NS}$	6.26 ±0.53 $^{b/NS}$	3.4 ± 0.16 $^{a/***}$	9.01; <0.001
Sulphur mg/g	2.2 ± 0.082 $^{b/NS}$	2.38 ± 0.089 $^{b/NS}$	2.40 ± 0.10 $^{b/***}$	2.0 ± 0.23 $^{ab/NS}$	1.65 ± 0.08 $^{a/***}$	9.24; <0.001
NDF g/kg	525 ± 8.14 $^{b/***}$	499 ±29.21 $^{ab/NS}$	492 ±19.88 $^{ab/NS}$	426 ±31.21 $^{a/**}$	535 ± 23.53 $^{b/**}$	4.15; 0.004
ADF g/kg	495 ± 7.40 $^{c/***}$	448 ±25.32 $^{bc/NS}$	432 ±17.74 $^{b/NS}$	344 ±26.89 $^{a/**}$	390 ± 18.17 $^{ab/**}$	15.6; <0.001
Cellulose	224 ± 4.85 $^{a/NS}$	213 ±14.26 $^{a/**}$	237 ±14.03 $^{ab/NS}$	202 ±17.82 $^{a/*}$	263 ± 15.34 $^{b/**}$	3.62; 0.008
Hemicellulose	30 ± 2.89 $^{a/***}$	51 ±10.04 $^{ab/NS}$	60 ±9.12 $^{b/NS}$	82 ±8.84 $^{b/NS}$	145 ± 10.49 $^{c/NS}$	48.1; <0.001
Lignin g/kg	271 ± 5.27 $^{d/***}$	235 ±14.13 $^{c/NS}$	195 ±8.82 $^{b/NS}$	142 ±10.34 $^{a/***}$	128 ± 6.24 $^{a/NS}$	66.5; <0.001
Lignin proportion %	54.7 ± 0.6 $^{c/***}$	52.6 ±1.4 $^{c/***}$	45.6 ±1.6 $^{b/*}$	41.9 ±1.3 $^{b/**}$	33.2 ± 1.3 $^{a/NS}$	66.8; <0.001
Ca:P	11.18 ± 1.0 $^{c/***}$	8.1 ± 0.89 $^{b/***}$	5.19 ± 0.74 $^{ab/NS}$	3.5 ± 0.34 $^{a/**}$	2.7 ± 0.38 $^{a/NS}$	23.7; <0.001
FATHALA	**n = 36**	**n = 10**	**n = 17**	**n = 7**	**n = 14**	
Nitrogen g/kg	25.6 ± 0.88 ^{b}	25.18 ±2.08 ^{b}	18.31 ±1.47 ^{a}	18.03 ±0.69 ^{a}	15.33± 0.82 ^{a}	14.5; <0.001
Phosphorus g/kg	5.5 ± 0.47 ^{b}	5.90 ±0.77 ^{b}	2.99 ±0.32 ^{a}	2.61 ±0.34 ^{a}	4.10± 0.60 ^{ab}	5.43; <0.001
Potassium g/kg	7.6 ± 0.43 ^{ab}	7.60 ±1.30 ^{ab}	5.54 ±0.43 ^{a}	8.11 ±0.65 ^{ab}	9.74± 0.77 ^{b}	4.88; 0.0014
Calcium g/kg	23.2 ± 1.77 ^{bc}	26.57 ±3.40 ^{c}	10.46 ±1.13 ^{a}	12.80 ±0.52 ^{ab}	12.66± 2.90 ^{a}	9.32; <0.001
Magnesium g/kg	5.7 ± 0.47 ^{ab}	7.75 ±1.37 ^{b}	4.63 ±0.54 ^{a}	5.53 ±0.40 ^{ab}	5.00± 0.29 ^{ab}	2.43; 0.054
Sulphur mg/g	2.33 ± 0.17 ^{c}	2.14 ± 0.14 ^{bc}	1.84 ± 0.11 ^{b}	1.56 ± 0.05 ^{ab}	1.22 ± 0.05 ^{a}	13.1; <0.001
NDF g/kg	575 ± 13.7 ^{b}	433 ±55.5 ^{a}	525 ±43.9 ^{ab}	579 ±19.5 ^{ab}	622± 18.3 ^{b}	4.32; 0.003
ADF g/kg	574 ± 19.1 ^{b}	459 ±67.4 ^{ab}	454 ±40.8 ^{a}	505 ±31.7 ^{ab}	479± 28.6 ^{ab}	3.11; 0.020
Cellulose	219 ± 9.82 ^{ab}	154 ±15.2 ^{a}	230 ±24.6 ^{ab}	266 ±17.1 ^{bc}	334± 17.9 ^{c}	11.4; <0.001
Hemicellulose	55.5 ± 6.84 ^{a}	36.9 ±9.6 ^{a}	71 ±9.9 ^{a}	74 ±15.6 ^{a}	143± 15.5 ^{b}	12.3; <0.001
Lignin g/kg	355 ± 14.4 ^{c}	305 ±54.1 ^{bc}	224 ±18.8 ^{ab}	239 ±15.2 ^{ab}	145± 12.2 ^{a}	15.9; <0.001
Lignin proportion %	61.6 ± 1.4 ^{c}	63.3 ±2.8 ^{c}	52.0 ±2.4 ^{b}	47.3 ±0.6 ^{b}	29.7± 1.2 ^{a}	47.3; <0.001
Ca:P	4.25 ± 0.2 ^{a}	4.58 ± 0.2 ^{a}	4.02 ± 0.4 ^{ab}	5.17 ± 0.7 ^{a}	2.75 ± 0.34 ^{b}	5.76; <0.001

First mark explore concentration (mean ± standard error of mean) of macroelements and fibre fractions calculated by one-way ANOVA. Using Tukey *post-hoc* comparison test, species with the same letter were not significantly different. Second mark explore differences of elements concentrations between two reserves tested by two-sample T-test significant results have: * if p≤0.05, ** if p≤0.01 and *** if p≤0.001.

Table 11. Concentrations (mean ± SE) of microelements in faeces of large herbivores in Bandia and Fathala reserves.

	Derby eland	**Common eland**	**Roan antelope**	**Buffalo**	**Zebra**	**F; p-value**
BANDIA	**n = 15**	**n = 11**	**n= 13**	**n = 8**	**n = 15**	
Mn µg/g	$162.7 \pm 16.94^{ab/NS}$	$135 \pm 9.81^{a/NS}$	$84.56 \pm 3.98^{ac/***}$	$100,4 \pm 10,35^{ac/***}$	$117.4 \pm 13.62^{a/NS}$	5.90; 0.001
Fe mg/g	$1.89 \pm 0.14^{NS/***}$	$1.79 \pm 0.28^{NS/NS}$	$1.63 \pm 0.1^{NS/NS}$	$2,38 \pm 0,4^{NS/NS}$	$1.79 \pm 0.2^{NS/NS}$	1.39; 0.251
Co µg/g	$0.84 \pm 0.06^{NS/NS}$	$0.82 \pm 0.11^{NS/***}$	$0.72 \pm 0.06^{NS/NS}$	$0,93 \pm 0,10^{NS/*}$	$0.85 \pm 0.12^{NS/NS}$	0.56; 0.696
Cu µg/g	$13.41 \pm 1.25^{a/*}$	$17.4 \pm 2.84^{ab/NS}$	$15.23 \pm 1.68^{a/NS}$	$8,31 \pm 0,63^{ac/NS}$	$12.36 \pm 1.64^{a/NS}$	2.81; 0.034
Zn µg/g	$63.74 \pm 3.44^{ac/NS}$	$66.9 \pm 6.77^{a/*}$	$67.47 \pm 3.22^{a/***}$	$85,48 \pm 8,08^{ab/**}$	$60.40 \pm 2.78^{ac/NS}$	3.50; 0.013
Se µg/g	$0.46 \pm 0.036^{ac/NS}$	$0.71 \pm 0.07^{a/**}$	$0.87 \pm 0.10^{ab/***}$	$0,9 \pm 0,09^{ab/***}$	$0.72 \pm 0.07^{a/***}$	5.72; 0.001
Mo µg/g	$0.94 \pm 0.06^{b/NS}$	$1.66 \pm 0.23^{ab/NS}$	$2.36 \pm 0.26^{a/***}$	$2,33 \pm 0,27^{a/***}$	$1.82 \pm 0.18^{a/***}$	8.96; <0.001
FATHALA	**n = 14**	**n = 10**	**n = 15**	**n = 6**	**n = 14**	
Mn µg/g	178.1 ± 16.4^{ac}	157.9 ± 11.04^{a}	209.7 ± 13.96^{ac}	197.4 ± 13.75^{a}	128.5 ± 6.91^{ab}	6.15; <0.001
Fe mg/g	0.94 ± 0.11^{ac}	2.02 ± 0.33^{ab}	1.38 ± 0.35^{a}	1.47 ± 0.1^{a}	1.47 ± 0.17^{a}	2.10; 0.095
Co µg/g	1.00 ± 0.12^{NS}	1.6 ± 0.17^{NS}	1.02 ± 0.28^{NS}	1.84 ± 0.54^{NS}	1.33 ± 0.24^{NS}	1.53; 0.208
Cu µg/g	18.18 ± 1.74^{bc}	22.08 ± 2.3^{c}	12.75 ± 1.33^{ab}	8.82 ± 0.24^{a}	12.3 ± 0.84^{a}	8.24; <0.001
Zn µg/g	66.57 ± 4.86^{ab}	90.22 ± 8.42^{b}	50.54 ± 3.04^{a}	51.57 ± 2.62^{a}	64.7 ± 7.86^{a}	5.57; 0.0008
Se µg/g	0.61 ± 0.07^{c}	0.45 ± 0.08^{bcd}	0.33 ± 0.02^{ab}	0.26 ± 0.02^{ad}	0.17 ± 0.02^{a}	12.66; <0.001
Mo µg/g	1.03 ± 0.17^{a}	1.69 ± 0.38^{ab}	0.65 ± 0.13^{ac}	0.40 ± 0.02^{ac}	0.94 ± 0.12^{a}	4.39; 0.004

First mark explore concentration (mean ± standard error of mean) of microelements calculated by one-way ANOVA. Using Tukey *post-hoc* comparison test, species with the same letter were not significantly different. Second mark explore differences of elements concentrations between two reserves tested by two-sample T-test significant results have: * if p≤0.05,
** if p≤0.01 and *** if p≤0.001.

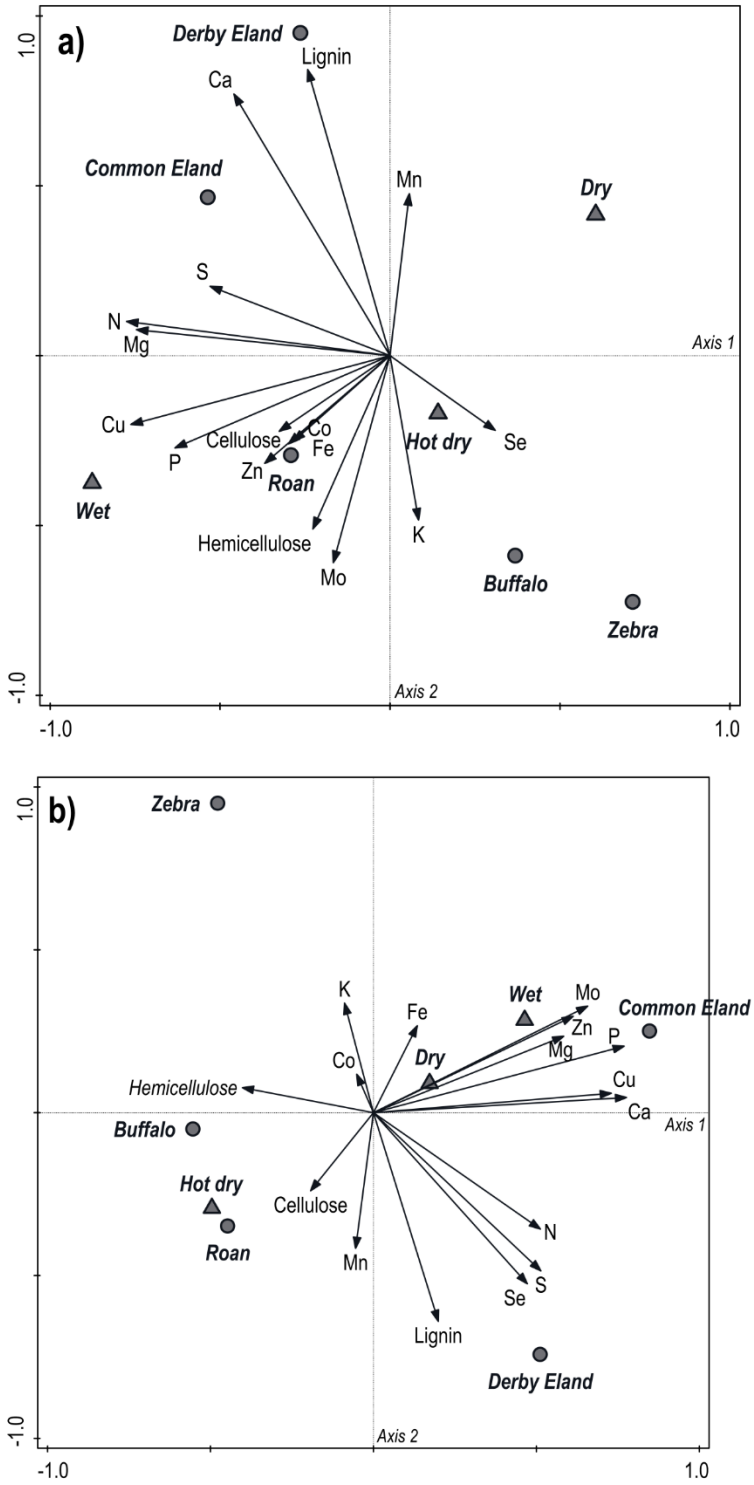


Figure 12. Constrained redundancy analysis ordination diagram showing mutual relationships of nutrients (macro-, microelements and fibre fractions) and effects of animal species and seasons in two localities (the Bandia reserve and the Fathala reserve) separately.

8. Conclusion

Main contribution of the present thesis is bringing the mineral profile of critically endangered Western Derby eland and its diet quality indicators in semi-captive conditions in two savanna types. The present study also brings valuable information about concentration of diet quality indicators and faecal mineral concentrations for other four herbivore species. Then, this study determined mineral concentrations in different sample materials with consideration of their suitability of collection and handling in particular condition of field work in harsh African conditions. Lack of published mineral reference values for semi-captive animals (e.g. serum mineral reference values for WDE do not exist) limits assessment of our results in comparison to concentrations of minerals with other studies. However, our data are exclusive since they can serve as first reference values for further research, which could be conducted in concrete locality of Senegal.

First part of this research was focused on testing of different materials suitability for mineral status determination. The highest concentrations of almost all minerals among the collected types of samples were found in the faeces. That can be explained by fact that the quantity of a mineral excreted in the faeces is the sum of the unavailable dietary mineral, available but unabsorbed dietary mineral and endogenous mineral (Primary Industries Standing Committee 2007). The concentrations of the elements in the blood, hair, and faecal samples were not correlated, and therefore our findings currently indicate that faecal and fur analyses cannot stand alone to assess the mineral status and determine the potential mineral deficiencies in Western Derby elands or in large herbivores in general. As the best approach we recommend to combine at least two materials for mineral status evaluation. Different materials for mineral concentration determination differ also in availability and processability. Blood serum samples were the most complicated samples among collected materials in term of availability and processing. Animals had to be anaesthetized, which brings high risk of animal death. Blood samples have to be processed on place (centrifugation, separation of serum, storage in deep freezer) which can be problematic in condition of field work. Acquiring hair samples require proximity to the animal and cutting hair from the same spot on the body of each animal. Further manipulation and storage of hair sample is not difficult. Faecal samples seem the easiest to collect. There is no need of direct contact with the animal. Storage and manipulation of faeces is after drying of fresh samples also easy. However, caution in faecal mineral concentration interpretation is needed.

Comparison of serum mineral concentrations of WDE showed, that values of WDE were lower in almost all elements compared to relative species. Predominantly low concentrations of minerals in WDE's blood serum might indicate mineral deficiency; however, the animals were all in a good condition without any obvious clinical issues. Recorded mineral levels may reflect most likely an adaptation of the WDE to a specific environment that is poor in minerals. Certain type of adaptation confirmed also analysis of faecal samples of 5 herbivore species from two localities. WDE adapted by its feeding behaviour to more harsh condition of semi-arid savanna and is able to handle decreased diet quality. Also inverted Ca:P ratio in the blood serum and very high Ca:P ratios in the hair and faeces indicate a metabolic inability of the animals to absorb Ca from the diet. High Ca:P of other four herbivore species from the same areas support

hypothesis that Ca is probably present in excessive amounts in the diet, which in combination with low P, causes Ca to be unavailable to the animal and to leave the body without utilisation. High concentrations of ingested Ca can be caused by supplementary feeding by *Acacia albida* pods since majority of browse plant species have Ca concentrations beyond the maximum tolerable limits. Other herbivore species showed adaptation to different condition as well. Mixed feeders and grazers were resilient to the change of habitat by maintaining the level of diet quality in both, sub-humid and semi-arid savanna ecosystems. However, buffalos and roan antelopes had to escape competition with zebras for grasses in sub-humid savanna by switching to browse. Common eland classified as browser, under competition pressure from WDE, broadened its diet by significant amount of grass, that had to be classified as mixed feeder anyway. Our research showed that not only vulnerable grazer species might be affected by climate change. Therefore, despite browsers are considered not to be affected behaviourally by drier conditions, for instance by drought events, as woody plant resources may remain available, thus browsers appear more resilient, they may experience intensifying impacts in terms of diet quality and available nutrients. From conservation perspective, all feeding guilds are threatened by changing ecosystems, even if each one by different way, e.g. by increased competition or inadequate nutrient levels if they are forced to change food resources, and a conservation-oriented 'exit-strategy' for species should be conceived to address these threats, including the strengthening the ecological connectivity in savanna landscapes. Regarding low number of individuals involved in the study, our results might be partly affected by a small sample size and further research with larger number of animals is recommended.

9. References

Anke M. 1966. Major and trace elements in cattle hair as an indicator of Ca, Mg, P, K, Na, Fe, Zn, Mn, Cu, Mo and Co. 3. Effect of additional supplements on mineral composition of cattle hair. Arch. Tierzucht.16: 57.

Archer D, Sanson G. 2002. Form and function of the selenodont molar in southern African ruminants in relation to their feeding habits. Journal of Zoology 257:13–26.

Arsenault R, Owen-Smith N. 2008. Resource partitioning by grass height among grazing ungulates does not follow body size relation. Oikos 117:1711-1717.

Bauer H, Chardonnet B, Scholte P, Kamgang SA, Tiomoko DA, Tehou AC, Sinsin B, et al. 2020. Consider divergent regional perspective to enhance wildlife conservation across Africa. Nature ecology and evolution. DOI: 10.1038/s41559-020-01343-6

Begon M, Harper JL, Townsend CR. 1990. Ecology: individuals, populations and communities. Blackwell, Oxford.

Beeri O, Phillips R, Hendrickson J, Frank AB, Kronberg S. 2007. Estimating forage quantity and quality using aerial hyperspectral imagery for northern mixed-grass prairie. Remote Sensing of Environment 110:216-225.

Bland J. 1984. Hair Tissue Mineral Analysis, An Emergent Diagnostic Technique, Thorsons Publishing, New York.

Bond WJ. 2016. Ancien grasslands at risk. Science 351:120-122.

Clauss M, Hume ID, Hummel J. 2010a. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. Animal 4:7:979-992.

Clauss M, Adamczewski J, Hofmann RR. 2010b. Surface enlargement in the rumen of free-ranging muskoxen (*Ovibos moschatus*). European Journal of Wildlife Research 56:181-185.

Clauss M, Hofmann RR, Streich WJ, Fickel J and Hummel J. 2008. Higher masseter mass in grazing than in browsing ruminants. Oecologia 157:377–385.

Clauss M, Hofmann RR, Hummel J, Adamczewski J, Nygren K, Pitra C, Reese S. 2006. The macroscopic anatomy of the omasum of free-ranging moose (*Alces alces*) an muskoxen (*Ovibos muschatus*) and a comparison of the omasal laminal surface area in 34 ruminant species. Journal of Zoology (Lond) 270:346-358.

Clauss M, Lechner-Doll M. 2001. Differences in selective particle retention as a key factor in the diversification of ruminants. Oecologia 129:321-327.

Clauss M, Lechner-Doll M, Behrend A, et al. 2001. Particle retention in the forestomach of a browsing ruminant, the roe deer. Acta Theriol. 46:103–107.

Codron D, Brink JS, Rossouw L, Clauss M. 2008. The evolution of ecological specialization in southern African ungulates: competition of physical environmental turnover? Oikos 117:344-353.

Combs DK. 1987. Hair analysis as an indicator of mineral status of livestock. Journal of Animal Science 65:1753-1758.

Cooper SM, Owen-Smith N. 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. Oecologia 67:142-146.

de longh HH, de Jong CB, van Goethem J, Klop E, Brunsting AMH, Loth PE, Prins HHT. 2011. Resource partitioning among African savanna herbivores in North Cameroon: The importance of diet composition, food quality, and body mass. Journal of Tropical Ecology 27(5):503–513.

DeMiguel D, Fortelius M, Azanza B, Morales J. 2008. Ancestral feeding state of ruminants reconsidered: earliest grazing adaptation claims a mixed condition for Cervidae. BMC Evolutionary Biology 8:13.

Dery G. 2016. Feeding Habits of the Roan Antelope *Hippotragus Equinus* (Desmarest, 1804) in Gbele Resource Reserve. International journal of innovative research and development 1:360-377.

Duncan P, Foose TJ, Gordon IJ, Gakahu CG, Lloyd M. 1990. Comparative nutrient extraction from forages by grazing bovids and equids: a test of the nutritional model of equid/bovid competition and coexistence. Oecologia 84:411–8.

du Toit JT, Olff H. 2014. Generalities in grazing and browsing ecology: using across-guild comparisons to control contingencies. Oecologia 174(4):1075-1083.

Engelbrecht CJ, Engelbrecht FA. 2016. Shifts in Köppen-Geiger climate zones over southern Africa in relation to key global temperature goals. Theoretical and Applied Climatology 123:247-261.

Fisher DD, Wilson LL, Leach RM, Scholz RW. 1985. Switch hair as an indicator of magnesium and copper status of beef cows. American Journal of Veterinary Research 46:2235-2240.

Farnsworth KD, Focardi S, Beecham JA. 2002. Grassland-herbivore interactions: How do grazers coexist? Am Nat 159(1):24–39.

Georgiadis NJ, McNaughton SJ. 1990. Elemental and fibre contents of savanna grasses: variation with grazing, soil type, season and species. Journal of Applied Ecology 27:623–634.

Gordon IJ, Prins HHT. 2008. The ecology of browsing and grazing. Springer, Berlin.

Gordon IJ, Illius AW. 1994. The functional significance of the browser-grazer dichotomy in African ruminants. Oecologia 98:167–175.

Gordon IJ, Illius AW. 1996. The nutritional ecology of African ruminants: a reinterpretation. Journal of Aniaml Ecology 65:18-28.

Hack MA, East R, Rubenstein DI. 2002. Status and action plan for the plains zebra (Equus burchelli). In: Equids: Zebras, Asses and Horses. Status Survey and Conservation Action Plan. IUCN/SSC Equid specialist group (ed. P. D. Moehlman) pp. 43–60. IUCN, Gland & Cambridge.

Hanley TA. 1982. The nutritional basis for food Selection by ungulates. Journal of range management 35:146-151.

Havemann CP. 2014. Population Dynamics and Foraging Ecology of Roan Antelope in Northern Botswana [MSc. thesis]. University of Pretoria, Pretoria, South Africa.

Heitkönig IMA, Owen-Smith N. 1998. Seasonal selection of soil types and grass swards by roan antelope in a South African savanna. African Journal of Ecology 36:57-70.

Herdt T, Rumbeiha W, Braselton W. 2000. The use of blood analyses to evaluate mineral status of livestock. Veterinary Clinics of North America: Food Animal Practice 16:423-444.

Hinners TA, WJ, Terrill JL, Colucci AV. 1974. Hairmetal binding. Environ. Health Perspectives 8:191.

Hillman JC.1979. The biology of the eland (Taurotragus oryx) in the wild [Ph.D. thesis]. University of Nairobi, Kenya.

Holechek JL, Vavra M, Pieper RD. 1982. Methods for determining the nutritive quality of range ruminant diets: A review. Journal of Animal Sicence 54:263-375.

Hopps HC. 1977. The biologic basis for usifig hair and nail for analysis of trace elements. Science of the Total Environment 7:71.

Hofmann RR. 2000. Functional and comparative digestive system anatomy of Arctic ungulates. Rangifer 20:71-81.

Hulme M, Doherty R, Ngara T, New M, Lister D. 2001. African climate change: 1900-2100. Climate research 17:145-168.

IUCN SSC Antelope Specialist Group. 2017. Tragelaphus derbianus. The IUCN Red List of Threatened Species 2017. Available from https://doi.org/10.2305/IUCN.UK.2017-2.RLTS.T44172A50197518.en (accessed June 2019).

Jarman PJ, Sinclair ARE. 1979. Feeding strategy and the pattern of resource partitioning in ungulates. Pages 130-163 in Sinclair ARE, Norton-Griffiths M (eds), Serengeti: dynamics of an ecosystem. University of Chicago Press, USA.

Janis CHM, Damuth J, Theodor JM. 2000. Miocene ungulates and terrestrial primary productivity: Where have all the browsers gone? Proc Natl Acad Sci USA 97(14):7899-7904.

Jenks JA, Leslie DM, Lochmiller RL, Melchiors MA, Warde WD. 1989. Effect of compositing samples on analyses of faecal nitrogen. Journal of Wildlife Management 53:213-215.

Kaiser TM, Fickel J, Streich WJ, Hummel J and Clauss M. 2010. Enamel ridge alignment in upper molars of ruminants in relation to their natural diet. Journal of Zoology 281:12-25 DOI:10.1111/j.1469-7998.2009.00674.

Kartzinel TR, Chen PA, Coverdale TC, Erickson DL, Kress WJ, Kuzmina ML, Rubenstein DI, Wang W, Pringle RM. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proc Natl Acad Sci USA 112:7873-8154.

Kincaid RL. 1999. Assessment of trace mineral status of ruminants. A review. Journal of Animal Science 77(1):1-10.

Kleynhans EJ, Jolles AE, Boss MRE, Olff H. 2011. Resource partitioning along multiple niche dimensions in differently sized African savanna grazers. Oikos 120: 591-600.

Laven RA, Livesey CT. An evaluation of the effect of clotting and processing of blood samples on the recovery of copper from bovine blood. Vet J 2006; 171(2):295–300.

Leslie DM, Starkey EE. 1985. Fecal indices to dietary quality of cervids in old growth forests. Journal of Wildlife Management 49:142-146.

Levander, O. A. 1986. Selenium. In: W. Mertz (Ed.) Trace Elements in Human and Animal Nutrition 5th edition. Academic Press, New York.

Dlugaszek M, Kopczyński K. 2014. Correlations Between Elements in the Fur of Wild Animals. Bulletin of Environmental Contamination and Toxicology 93:25-30.

Macandza VA, Owen-Smith N, Cross PC. 2004. Forage selection by African buffalo in the late dry season in two landscapes. South African Journal of Wildlife Research 34(2):113-121.

Martens C, Hickler T, Davis-Reddy C, Engelbrecht F, Higgins SI, von Maltitz GP, Midgley GF, Pfeiffer M, Scheiter S. 2020. Large uncertainties in future biome changes in Africa call for flexible climate adaptation strategies. Global Change Biology 27:340-358.

Martin RB. 2003. Species Report for Roan Hippotragus equinus, Sable Hippotragus niger and Tsessebe Damaliscus lunatus. Ministry of Environment and Tourism, Windhoek, Namibia.

Available from http://www.rarespecies.org.na/library/speciesreports.htm (accessed January 2021).
McNaughton SJ, Georgiadis NJ. 1986. Ecology of African Grazing and Browsing Mammals). Annual Reviews of Ecological Systems 17:39-65.
Midgley GF, Bond WJ. 2015. Future of African terrestrial biodiversity and ecoystems under anthropogenic climate change. Nature Climate Change 5:823-829.
Miller WJ. 1970. Zinc nutrition of cattle: A review. Journal of Dairy Sciences 53:1123.
Miller WJ. 1975. New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle. A review. Journal of Dairy Sciences 58:1549-1560.
Miller WJ, Blackmon DM, Gentry RP, Powell GW, Perkins HF. 1966. Influence of zinc deficiency on zinc and dry matter content of ruminant tissues and on excretion of zinc. Journal of Dairy Sciences 49:1453.
Mills C. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. Journal of Animal Science 65:1702-11.
Mills MGL, Biggs HC, Whyte IJ. 1995. The relationship between rainfall, lion predation and population trends in African herbivores. Wildlife Research, 22:75–88.
Muposhi VK, Chanyandura A, Gandiwa E, Muvengwi J, Muboko N, Taru P, Kupika OL. 2014. Post-release monitoring of diet profile and diet quality of reintroduced African buffalo (Syncerus caffer) in Umfurudzi Park, Zimbabwe. Tropical Conservation Science 7:440-456.
Murray MG, Illius AW. 1996. Multi-species grazing in the Serengeti. Pages 247-272 in Hodgson J, Illius AW (eds) The ecology and management of grazing systems. CAB International
Ogutu JO, Owen-Smith N, Piepho HP, Kuloba B, Edebe J. 2012. Dynamics of ungulates in relation to climatic and land use changes in an insularized African savanna ecosystem. Biodiversity Conservation 21:1033-1053
Olff H, Ritchie ME, Prins HHT. 2002. Global environmental controls of diversity in large herbivores. Nature 415:901-904.
Owen-Smith N, Mills MGM. 2008. Shifting prey selection generates contrasting herbivore dynamics within a large mammal predator-prey web. Ecology 89:1120–1133.
Owen-Smith N. 1982. Factors influencing the consumption of plant products by large herbivores. Pages 359-404 in Huntley, B. J. and Walker, B. H. (eds), Ecology of tropical savannas. Springer.
Owen-Smith N. 1997. Distinctive features of the nutritional ecology of browsing versus grazing ruminants. Pages 176-191in Proc. 1st Int. Symp. Physiol. Ethol. Wild and Zoo Anim., Gustav Fischer, Berlin.
Owen-Smith N. 1990. Demography of a large herbivore, the greater kudu, in relation to rainfall. Journal of Animal Ecology 59:893–913.
Owen-Smith N, Ogutu J. 2003. Rainfall influences on ungulate populations dynamics. Pages 310-330 in The Kruger Experience: Ecology and Management of Savanna Heterogeneity (eds Toit JT, Rogers KH, Biggs HC). Island Press, Washington.
Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.
Passwater R, Cranton WM. 1983. Trace Elements, Hair Analysis and Nutrition. Keats Publishing Inc., New Canaan.

Posselt J. 1963. The domestication of the eland. The Rhodesian Journal of Agricultural Research 1:81-87.

Primary Industries Standing Committee. 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Collingwood, Australia.

Prins RA, Kreulen DA. 1991. Comparative aspects of plant cell wall digestion in mammals. Pages 109-121 in Hoshino S, Onodera R, Minoto H, Itabashi H. (eds), The rumen ecosystem: the microbial metabolism and its regulation. The Japan Science Society Press, Tokyo.

Prins HHT. 1996. Ecology and behaviour of the African buffalo: social inequality and decision making. Chapman Hall, London.

Prins RA, Lankhorst A, Van Houven W. 1984. Gastrointestinal fermentation in herbivores and the extent of plant cell-wall digestion. Pages 403-434 in Gilchrist FMC, Mackie RI, (eds), Herbivore nutrition in the subtropics and tropics. Science Press, Craighall, South Africa.

Prins HHT, Olff H. 1998. Species-Richness of African grazer communities: towards a functional explanation. Dynamics of Tropical Communities, Blackwell Science, Oxford.

Ripple WJ, Newsome TM, Wolf Ch, Dirzo R, Everatt KT, Galetti M et al. 2015. Collapse of the world's largest herbivores. Science Advances 1:e1400103.

Robbins CT. 1983. Protein requirements for maintanance. Pages 148-165 in Cunha TJ. (ed.), Wildlife feeding and nutrition. Academic Press, London.

Robbins CT. 1993. Wildlife Feeding and Nutrition. Academic Press, New York.

Robbins CT, Spalinger DE, Van Houven W. 1995. Adaptations of ruminants to browse and grass diets: are anatomical-based browser-grazer interpretations valid? Oecologia 103:208-213.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA. 2003. Fingerprints of global warming on wild animals and plants. Nature 421:57–60.

Rowell-Schäfer A, Lechner-Doll M, Hofmann RR, et al. 2001. Metabolic evidence of a 'rumen bypass' or a 'ruminal escape' of nutrients in roe deer. Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology 128A: 289–298.

Sach F, Dierenfeld ES, Langley-Evans SC, Hamilton E, Lark RM, Yon L, Watts MJ. 2020. Potential bio-indicators for assessment of mineral status in elephants. Scientific Reports 10:8032.

Schoener TW. 1986. Resource partitioning. Pages 91-126 in Kikkawa J, Anderson DJ. Community ecology, pattern and process. Blackwell, Oxford.

Soest PJV. 1982. Nutritional ecology of the ruminant, second edition. Cornell university press, London.

Stevens N, Lehmann CR, Murphy B, Durigan G. 2017. Savanna woody encroachment is widespread across three continents. Global Change Biology 23:235-244.

Topps JH. 1992. Potential, composition and use of legume shrubs and trees as fodders for livestock in the tropics. Journal of Agricultural Science 118:1-8.

Tefera S, Mlambo V. 2017. Macro and trace elements of 26 browse species growing in semi-arid grazing lands of Eastern Cape, South Africa. African Journal of Range and Forage Science 34:209-217.

Underwood EJ. 1981. The Mineral Nutrition of Livestock, 2nd edn. Commonwealth Agricultural Bureaux International, UK.

Underwood EJ, Suttle NF. 1999. The mineral nutrition of livestock. 3rd Edition. CAB International, UK.

Van Soest PJ. 1967. Development of a comprehensive system of feed analyses and its application to forages. Journal of Animal Sciences 26:119-128.

Van Soest PJ. 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York.

Voeten MM, Prins HHT. 1999. Resource partitioning between sympatric wild and domestic herbivores in the Tarangire region of Tanzania. Oecologia 120:287-294.

Wren CD (1986) Mammals as biological monitors of environmental metal levels. Environ Monit Assess 6:127–144.

Wallington BP, McKechnie AE, Owen-Smith N, Woodborne S. 2007. Stable carbon isotope analysis of eland (*Taurotragus oryx*) diet in the Suikerbosrand Nature Reserve. South African Journal of Wildlife Research 37:127-131.

Watson LH, Owen-Smith N. 2000. Diet composition and habitat selection of eland in semi-arid shrubland. African Journal of Ecology 38:130-137.

Yoganand K, Owen-Smith N. 2014. Restricted habitat use by an African savanna herbivore through the seasonal cycle: key resources concept expanded. Ecography 37:969-982.

10.Appendices

List of Appendices:

Appendix 1: Minerals in the blood, hair, and faeces of the critically endangered Western Derby eland under human care in two wildlife reserves in Senegal……..……**II**

Appendix 2: Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal…...**VIII**

Appendix 3: Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet…….……..……....**XII**

Appendix 4: Author's CV……..……**XX**

Appendix 5: List of author's publications……...**XXII**

APPENDIX 1

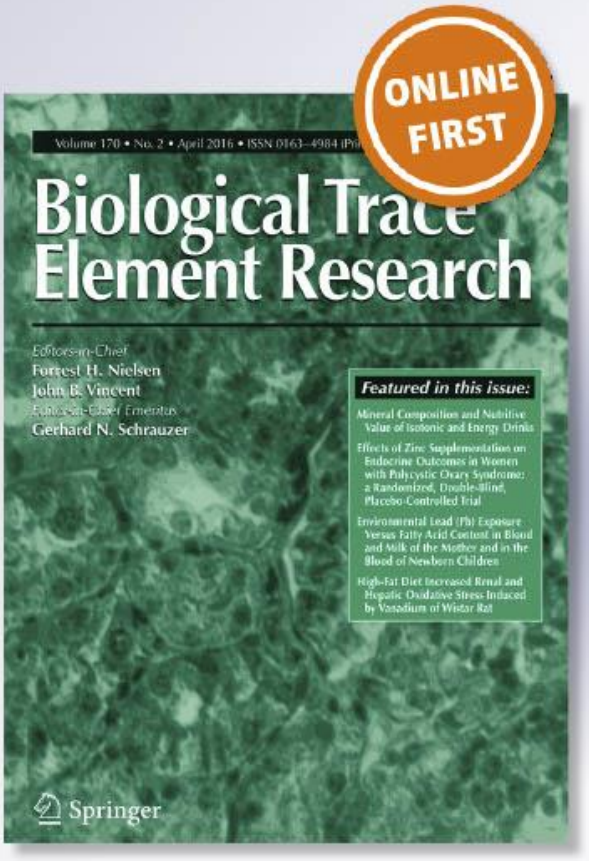
Minerals in the Blood, Hair, and Faeces of the Critically Endangered Western Derby Eland Under Human Care in Two Wildlife Reserves in Senegal

Lucie Stoklasová, Jiří Váhala & Pavla Hejcmanová

Biological Trace Element Research

ISSN 0163-4984

Biol Trace Elem Res
DOI 10.1007/s12011-019-01839-8



Springer

Author's personal copy

Biological Trace Element Research
https://doi.org/10.1007/s12011-019-01839-8

Minerals in the Blood, Hair, and Faeces of the Critically Endangered Western Derby Eland Under Human Care in Two Wildlife Reserves in Senegal

Lucie Stoklasová [1] · Jiří Váhala [2] · Pavla Hejcmanová [1]

Received: 12 March 2019 / Accepted: 16 July 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract
The widely used means of investigating animal mineral profiles are blood serum or plasma and internal organ tissues. The acquisition of these types of samples can be invasive and requires much effort. These factors become key obstacles in the case of rare and elusive species such as the Western Derby eland (*Taurotragus derbianus derbianus*, WDE), which is a critically endangered antelope with a current distribution limited to the Niokolo Koba National Park, and two wildlife reserves in Senegal. One of the solutions to this problem is to collect easily accessible samples, such as faeces or fur, which may provide valid information about animal mineral status. Our study focuses on determining the macroelement and microelement levels in animal blood serum, hair, and faeces, and analysing their correlations to evaluate whether hair and/or faeces can be used as a proxy for blood mineral levels. Samples were collected from 11 individual WDEs (6 males, 5 females) during translocations within two reserves. Correlations of mineral concentrations in the blood, hair, and faeces were not found except for Fe in the faeces, which was positively correlated with Fe in the hair ($r = 0.64$, $P < 0.05$) and blood ($r = 0.69$, $P < 0.05$). The lack of correlations among the different types of samples may be because of the low number of samples; hence, we recommend conducting further research with a broader dataset. Our findings, however, currently indicate that faeces and fur analyses cannot stand alone for the assessment of the mineral status and the determination of WDEs' potential mineral deficiencies.

Keywords Conservation translocation · Giant eland · Large antelope · Mineral profile · West Africa · Wildlife nutrition

Introduction

Minerals are involved in many physiological processes; thus, knowledge of their concentrations to assess and detect potential deficiencies is crucial before clinical signs appear. The main means for investigating animal mineral profiles are blood serum, plasma, and/or internal organ tissues. Such samples are, however, scarcely accessible in some cases because they require handled, immobilised, or dead animals. This factor becomes a key obstacle in collecting samples from wild, i.e., free-ranging, elusive, or endangered species. Therefore, non-invasive methods for mineral status assessment are sought. One non-invasive method is the analysis of animal hair. Fur is a suitable material that has been used mostly for the evaluation of diet quality through carbon and nitrogen isotopes e.g., [1], but data on the elemental composition of wild animal hair are rare. A hair analysis is considered a useful biomarker in animal studies, which may infer about the bioavailability of elements and about the environmental exposure of animals [2]. The content of elements in fur can additionally be affected by its location on the body of the animal, the colour of the hair, or the environment in which the animal lives and what its hair is exposed to [3]. The concentration of elements in animal hair is often much higher than that found in body fluids and other tissues, and trace elements accumulate in hair at concentrations that are at least 10 times higher than those present in blood serum and urine [4].

Another non-invasive way to obtain information about the mineral status of the animals is faeces, which have been commonly used to assess animal nutrition for decades [5, 6]. Nutrients present in high or variable concentrations in faeces of animals indicate that they are also present in adequate or excessive amounts in their diet. In addition, nutrients present in low

✉ Pavla Hejcmanová
hejcmanova@ftz.czu.cz

[1] Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, 16500 Prague 6—Suchdol, Czech Republic

[2] Derbianus Conservation, z.s., 16500 Kamýcká 129, Prague 6—Suchdol, Czech Republic

Published online: 24 July 2019

Author's personal copy

and/or non-variable concentrations in faeces are most likely present in minimal or inadequate concentrations in the diet [7].

Our study was conducted on the Western Derby eland (*Taurotragus derbianus derbianus*, WDE), which is a critically endangered antelope with a current distribution restricted only to the Niokolo Koba National Park (wild population) and two wildlife reserves hosting a small managed population for conservation breeding purposes in Senegal [8]. Due to the rarity of the species, minimal handling and disturbances are required. The aim of our investigation was therefore (1) to determine the concentrations of macroelements and selected microelements in the blood serum, hair, and faeces of WDEs bred in the reserves to provide the baseline data for further management and (2) to explore the correlations among the concentrations of elements in blood serum, hair, and faeces. The ultimate aim of the study was to evaluate the means to determine the WDEs' mineral status in order to use appropriate methods with minimal effects on the animals in the wild during any future conservation action.

Materials and methods

Research site

Our study was conducted in two wildlife reserves where conservation programme has been conducted since 2002: the Bandia reserve, located 65 km south of Dakar in the Sahel-Sudanese savannah [9], and the Fathala reserve, located 250 km south of Dakar in the Sudano-Guinean savannah [10]. Both reserves have seasonal dry and wet climates with an annual rainfall of approx. 350 mm (Bandia reserve) and 800 mm (Fathala reserve), respectively.

Sample collection and processing

Blood, hair, and faecal samples from 11 young animals (6 males and 5 females, Table 1) were collected during the translocations for breeding management purposes in the dry season in March 2017. The blood samples were taken from the vena saphena lateralis in the morning by a veterinarian from immobilised animals before the application of the antidote (approx. 15–20 min after darting the animal). The samples were manually centrifuged after 1 h of settling, and the clear serum was placed in a deep freezer. The mineral concentrations in the samples were examined at the State Veterinary Institute in Prague 16 days after sampling. The concentrations of P, Ca, and Mg were determined by an IDEXX VetTest Chemistry Analyzer (IDEXX Laboratories, Inc., USA); the concentrations of S, Se, Fe, and Zn were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Thermo Scientific™ iCAP 6000 Series spectrometer (Thermo Fisher Scientific, USA); the concentration of K was determined by flame atomic absorption spectroscopy (F-AAS) using a SpectrAA 240 spectrometer (Varian Inc., USA), and the concentration of Cu was determined by graphite furnace atomic absorption spectroscopy (GF-AAS) using a SpectraAA 220Z spectrometer with a Zeeman correction (Varian Inc., USA).

The hair samples were collected from the dorsal hair line and the tail (distal part) during immobilisation. Hairs were cut by stainless steel scissors as close to the skin as possible, placed to labelled plastic bags and stored like that until analysis. The faeces were taken straight from the rectum of the immobilised animal and dried on site. The hair and faecal sample analyses were conducted as follows: samples were cut or crush and homogenised (hair samples were washed according to standard procedure of the accredited laboratory before homogenisation) and concentrations of K, Fe, Zn, S, Cu, P, Ca, and Mg were tested by ICP-OES (IRIS Intrepid II XSP Duo, THERMO Elemental, USA), and Se was analysed by hydride generation atomic absorption spectroscopy technique (HG-AAS) using an Analyst 100 spectrometer (Perkin Elmer, USA).

For ICP-OES measurements, samples were mineralised in the mixture of nitric and hydrochloric acid in a 6:1 ratio using a closed microwave digestion system. Then standard ICP-OES measurements were followed [12]. Calibration curve was prepared by appropriate dilution of certified reference standard solutions for each element (Analytika s.r.o., Czech Republic). Correctness of measurements was validated by analysis of standard solution of CRM used also for calibration curve. Set limits of determination were as follows: Na, 0.001%; K, 0.03%; Ca, 0.002%; Mg, 0.01%; P, 0.008%; Fe, 0.5 mg/kg; Cu, 0.9 mg/kg; Zn, 0.9 mg/kg. Control samples with known mineral concentration were added to each measurement.

For HG-AAS method, hair samples were washed and analysed and faeces samples were analysed without a pretreatment. Decomposition of the sample was done in magnesium nitrate, and HG-AAS followed. Limit of determination was 0.002 mg/kg. Correctness of measurements was validated by analysis of standard solution of CRM used also for calibration curve (Analytika s.r.o., Czech Republic).

The correlations of mineral concentrations among the different types of samples, i.e., hair, blood, and faeces, were performed using non-parametric Spearman rank correlation analyses in the Statistica 13 package (TIBCO Software Inc. 2013, USA). All results are expressed in dry weight.

Results and discussion

The mean concentrations of the elements in WDE blood, hair, and faeces are given in Table 2. When compared with the reference levels of minerals in the blood serum of Derby/

Author's personal copy

Table 1 Investigated individuals of the Western Derby eland. For more details, see the studbook data in Brandlová et al. [11]

ID	Studbook ID	Animal name	Sex	Date of birth	Age	Location
1-M2B	1141	Docteur	Male	2014-11-28	2 years	Bandia
2-F2B	1144	Felicia	Female	2014-12-10	2 years	Bandia
3-F2B	1139	MSoukeina	Female	2014-11-21	2 years	Bandia
4-F2B	1147	Safira	Female	2014-12-21	2 years	Bandia
5-F1B	1154	Damaye-Niane	Female	2015-11-25	1 year	Bandia
6-M1B	1163	Dayo	Male	2016-02-25	1 year	Bandia
7-F1B	1148	Driankee	Female	2015-01-04	1 year	Bandia
8-M1F	1159	Fredy	Male	2016-01-01	1 year	Fathala
9-M2F	1151	Fode	Male	2015-03-25	2 years	Fathala
10-M2F	1150	Fadel	Male	2015-03-05	2 years	Fathala
11-M3F	1137	Falco	Male	2014-04-01	3 years	Fathala

Giant eland [14] and published serum concentrations of other bovid species [15, 16] (Table 3), Ca, P, and K concentrations were within or slightly below the clinical range, while Cu, Fe, and Zn were noticeable lower and similar to cattle reference levels. Only Mg had higher concentration compared with the similar species. The highest concentrations of Ca, P, Mg, and K among the collected types of samples were found in the faeces. The faecal P concentrations in the WDEs were within the range of the values reported in other antelopes in various African savannah habitats [6] and were above the critical faecal P concentration of 2.0 g/kg (0.2%) identified for most herbivore species as indicator of dietary P deficiency [18]. This result should indicate that the P concentration in the WDE diet in the reserves is sufficient, despite the fact that the mean P concentrations in the browsed plants, which were part of the WDE diet in the Fathala reserve, were rather low (Table 2, Hejcmanová, unpublished data). The same as concentration of P in the soils of both reserves [19], which is low compared with those in other savannah areas e.g., [20], allows us to suggest that the WDEs seem to be adapted to cope with the environmental conditions and a diet poor in phosphorus. The other elements investigated in the blood profiles have usually been omitted from faecal sample analyses in nutritional studies and have no reference values. The Ca:P ratio deserves attention. In our study, the mean Ca:P ratio was 0.88 ± 0.13 SD in the blood serum, 7.3 ± 0.11 SD in the hair, and 12.8 ± 3.03 SD in the faeces. Thus, the Ca:P ratio in the blood serum was inverted to values recommended for ruminants (Ca:P~1–2) [17] but agreed with findings from non-domesticated bovids bred in captivity [15]. However, the Ca:P ratios in the hair and faeces were opposite and at levels that mostly exceeded the recommended ratio, as reported by Gabryszuk et al. [2], where the Ca:P ratio in the hair was 15.32. The inverse Ca:P ratios in the blood and high Ca:P in

Table 2 Mean concentrations of the principal elements in the blood, hair, and faeces of the Western Derby elands, and preferred browsed plant species

Elements	**Blood serum (mmol/L)**	**Hair (g/kg)**	**Faeces (g/kg)**	**Browsed plants Fathala*** **(g/kg)**
Macro-	**mean ± SE**	**mean ± SE**	**mean ± SE**	**mean ± SE**
Ca	1.87 ± 0.143	3.59 ± 0.189	35.818 ± 5.999	15.42 ± 0.78
P	2.12 ± 0.130	0.49 ± 0.023	2.818 ± 0.077	1.34 ± 0.04
Mg	1.55 ± 0.098	0.699 ± 0.027	4.436 ± 0.59	3.5 ± 0.16
K	5.21 ± 0.109	3.59 ± 0.189	7.818 ± 0.408	6.74 ± 0.31
S	27.2 ± 0.504	–	–	–
	Blood serum (μmol/L)	**Hair (mg/kg)**	**Faeces (mg/kg)**	**Browsed plants Fathala*** **(g/kg)**
Micro-	**mean ± SE**	**mean ± SE**	**mean ± SE**	**mean± SE**
Fe	22.36 ± 1.214	526.7 ± 17.969	879.051 ± 104.675	–
Cu	10.87 ± 2.781	8.11 ± 0.134	10.952 ± 0.43	–
Zn	11.51 ± 0.400	110.15 ± 2.718	41.5 ± 0.95	–
Se	1.75 ± 0.166	0.548 ± 0.066	0.404 ± 0.043	–

* Mean concentrations of the elements in most of the preferred plants browsed by the Western Derby elands in the Fathala reserve (*Acacia ataxacantha*, *A. macroptera*, *Combretum glutinosum*, *C. micranthum*, *C. paniculatum*, *Saba senegalensis*, *Terminalia laxiflora*, and *T. macroptera*) [13]

Author's personal copy

Table 3 Values of blood serum mineral concentration at representative herbivore species

	Cape eland			Greater kudu			Zebu			Cattle	Derby eland (ZIMS)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Reference interval	Mean	Min	Max
Ca, mmol/L	2.44	1.91	2.81	1.84	0.99	2.23	5.4	4.1	12.3	2.1–2.8	2.28	1.30	3.50
P, mmol/L	2.44	1.91	2.81	2.70	1.36	4.60	3.3	1.8	4.6	1.6–2.27	2.93	0.48	5.39
Mg, mmol/L	0.84	0.50	1.06	0.53	0.30	1.07	1.9	1.4	2.3	1–1.3	–	–	–
K, mmol/L	5.86	5.39	6.25	4.55	4.01	5.24	4.4	2.7	6.6	4.6–6.4	4.9	3.5	7.7
S, mmol/L	–	–	–	–	–	–	37	22	47	–	–	–	–
Fe, µmol/L	39.6	15.9	50.5	35.48	8.4	56.6	143	75	1200	26.85–40.28	–	–	–
Cu, µmol/L	22.55	17.2	30.8	27.8	17.2	46.9	6.0	0.8	18	1.6–3.19	–	–	–
Zn, µmol/L	103.3	29.2	198	91.36	48.6	137	46	24	107	10.71–19.89	–	–	–
Se, µmol/L	–	–	–	–	–	–	–	–	–	–	–	–	–

Source: For Cape eland and Greater kudu [15], for zebu [16], for cattle [17], for Derby eland [14]

faeces indicate a metabolic inability of the animals to absorb Ca from the diet, which was rich in Ca but contained low P browsed plants (the mean Ca:P ratio in the browsed plants in the Fathala reserve was 12.37 ± 0.41 SD, Hejcmanová, unpublished data). This result suggests that Ca is either in an inaccessible form or is present in excessive amounts, which in combination with low P, causes Ca to be unavailable to the animal and to leave the body without utilisation.

Table 4 Spearman rank correlation coefficients (R) of mineral concentrations in hair, faeces, and blood

		N	Spearman R	$t(N-2)$	p value
Ca	Hair and faeces	10	0.56	1.93	0.09
	Hair and blood	10	− 0.01	− 0.02	0.99
	Faeces and blood	11	− 0.36	− 1.17	0.27
P	Hair and faeces	10	0.04	0.12	0.91
	Hair and blood	10	− 0.10	− 0.29	0.78
	Faeces and blood	11	− 0.31	− 0.99	0.35
Mg	Hair and faeces	10	− 0.08	− 0.22	0.83
	Hair and blood	10	− 0.06	− 0.17	0.87
	Faeces and blood	11	0.51	1.77	0.11
K	Hair and faeces	10	− 0.50	− 1.65	0.14
	Hair and blood	10	0.52	1.70	0.13
	Faeces and blood	11	− 0.44	− 1.45	0.18
Fe	Hair and faeces	10	**0.64**	**2.38**	**0.04**
	Hair and blood	10	0.18	0.53	0.61
	Faeces and blood	11	**0.69**	**2.88**	**0.02**
Cu	Hair and faeces	10	0.26	0.75	0.48
	Hair and blood	10	0.02	0.07	0.95
	Faeces and blood	11	0.19	0.58	0.57
Zn	Hair and faeces	10	− 0.10	− 0.28	0.79
	Hair and blood	10	0.24	0.69	0.51
	Faeces and blood	11	0.04	0.12	0.90
Se	Hair and faeces	10	− 0.09	− 0.26	0.80
	Hair and blood	10	− 0.41	− 1.28	0.24
	Faeces and blood	11	0.13	0.38	0.71

*Level of significance $P < 0.05$

The concentrations of the elements in the blood, hair, and faecal samples were not correlated (all $P > 0.05$), with the only exception being Fe (Table 4). The iron concentration in the faeces was positively correlated with the Fe concentration in the hair ($r = 0.64$, $P < 0.05$). The lack of correlations among the different types of samples does not correspond with the results by McDowell [21], who described positive correlations between plasma Cu and faecal Cu and between hair Cu and faecal Cu and a negative correlation between plasma Cu and hair Cu. Positive correlations were also reported between blood Fe and Zn concentrations and hair Fe and Zn concentrations, respectively [22]. We could not confirm any of these correlations in our samples, which may be given to the low number of samples; hence, we recommend conducting further research with a broader dataset. Our findings, however, currently indicate that faecal and fur analyses cannot stand alone to assess the mineral status and determine the potential mineral deficiencies in Western Derby elands or in large herbivores in general.

Acknowledgements We are grateful to the managers of the Bandia and Fathala reserves and their staff for their kind assistance with animal translocation. We are also grateful to the Zoo Dvůr Králové for their cooperation and support. The study was supported by the Czech University of Life Sciences Prague, grant CIGA 20175004.

Compliance with ethical standards

Statement of animal rights This study was approved by the Directorate of National Parks in Senegal, the official state authority for biodiversity conservation in Senegal. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

Author's personal copy

References

1. Sponheimer M, Lee-Thorp JA, DeRuiter DJ, Smith JM, Van Der Merwe NJ, Reed K, Marcus W (2003) Diets of southern African Bovidae: stable isotope evidence. Journal of Mammalogy 84(2): 471–479
2. Gabryszuk M, Sloniewski K, Metera E, Sakowski T (2010) Content of mineral elements in milk and hair of cows from organic farms. Journal of Elementology 15(2):259–267
3. Combs DK, Goodrich RD, Meiske JC (1982) Mineral concentrations in hair as indicators of mineral status: a review. Journal of animal science 54(2):391–398
4. Maugh TH (1978) Hair: a diagnostic tool to complement blood serum and urine. Science 202:1271–1273. https://doi.org/10.1126/science.725602
5. Emmet AD, Grindley HS (1909) The chemistry of animal feces. First paper—a comparison of the fresh and air-dried feces. Journal of American Chemical Society 31(5):569–579
6. Stapelberg FH, van Rooyen MW, Bothma JP (2008) Spatial and temporal variation in nitrogen and phosphorus concentrations in faeces from springbok in the Kalahari. South African Journal of Wildlife Research 38(1):82–87
7. Studier EH, Sevick SH, Ridley DM, Wilson DE (1994) Mineral and nitrogen concentrations in feces of some neotropical bats. Journal of Mammalogy 75(3):674–680
8. IUCN SSC Antelope Specialist Group (2017) Tragelaphus derbianus. The IUCN Red List of Threatened Species 2017. https://doi.org/10.2305/IUCN.UK.2017-2.RLTS.T44172A50197518.en. Accessed 10 June 2019.
9. Hejcmanová P, Homolka M, Antonínová M, Hejcman M, Podhájecká V (2010) Diet somposition of Western Derby eland (*Taurotragus derbianus derbianus*) in the dry season in a natural and a managed habitat in Senegal using faecal analyses. South African Journal of Wildlife Research 40:27–34
10. Nežerková-Hejcmanová P, Hejcman M, Camara AA, Antonínová M, Pavlů V, Černý T, BÂ AT (2005) Analysis of the herbaceous undergrowth of the woody savanna in the Fathala Reserve, Delta du Saloum National Park (Senegal). Belgian Journal of Botany 138: 119–128
11. Brandlová K, Kubátová A, Fedorova T, Štochlová K, Grúňová M, Hejcmanová P (2017) African Studbook. Western Derby Eland, *Taurotragus derbianus derbianus* (Gray, 1847), vol 10. Czech University of Life Sciences Prague, Praha
12. Sommer L et al (1992) Optická emisní spektrometrie v indukčně vázaném plazmatu a vysokoteplotních plamenech (Academia Praha). *In:* kolektiv autorů (2014). Jednotné pracovní postupy analýza rostlinného materiálu ÚKZÚZ Brno.
13. Hejcmanová P, Mářejovská M, Homolka P, Hejcman M (2019) Drivers of diet selection of critically endangered Western Derby eland during the food shortage period within conservation breeding in Senegal. Scientific Reports 9(1):8712
14. ZIMS. Species 360 Physiological reference intervals for captive wildlife—2013. Giant Eland *(Taurotragus derbianus)*. https://zims.species360.org/Main.aspx. Accessed 13 March 2017.
15. Váhala J, Pospíšil J, Špála P, Kaše 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
16. Dermauw V, Yisehak K, Belay D, Van Hecke T, Laing GD, Duchateau L, Janssens GPJ (2013) Mineral deficiency status of ranging zebu (Bos indicus) cattle around the Gilgel Gibe catchment, Ethiopia. Tropical Animal Health Production 45:1139–1147
17. Bock U Polach (1994) Směrné hodnoty důležitých laboratorních vyšetření pro domácí zvířata: pes, kočka, kůň, tele, skot, prase, ovce. Translation to czech Eduard Jiran. Vetpres-vydavatelství a.s. Biopharm – VÚBVL, Czech Republic
18. Wrench JM, Meissner HH, Grant CC (1997) Assessing diet quality of African ungulates from faecal analyses: the effect of forage quality, intake and herbivore species. Koedoe – African Protected Area Conservation and Science 40:125–136
19. Stoklasová L (2016) Soils in Africa as source of mineral nutrition for large herbivores. Msc. Thesis. In: Wildlife Management. Czech University of Life Sciences Prague, Czech Republic
20. Stephenson JD, Mills A, Eksteen JJ, Milewski AV, Myburgh JG (2010) Geochemistry of mineral licks at Loskop Dam Nature Reserve, Mpumalanaga, South Africa. Environmental Geochemistry Health 33:49–53
21. McDowell LR, Kiatoko M, Bertrand JE, Chapman HL, Pate FM, Martin FG, Conrad JH (1982) Evaluating the nutritional status of beef cattle herds from four soil order regions of Florida. II. Trace minerals. Journal of Animal Science 55(1):38–47
22. Patra RC, Swarup D, Sharma MC, Naresh R (2006) Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. Journal of Veterinary Medicine Series A Banner 53:511–517

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

APPENDIX 2

Received: 7 January 2020 | Revised: 5 June 2020 | Accepted: 30 June 2020
DOI: 10.1111/jpn.13432

SHORT COMMUNICATION

WILEY

Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal

Lucie Stoklasová[1] | Jiří Váhala[2] | Pavla Hejcmanová[1]

[1]Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic

[2]Safari Park Dvůr Králové, Prague, Czech Republic

Correspondence
Pavla Hejcmanová, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, 16500 Prague 6 – Suchdol, Czech Republic.
Email: hejcmanova@ftz.czu.cz

Funding information
Česká Zemědělská Univerzita v Praze, Grant/Award Number: CIGA 20175004 and IGA 20205015

Abstract

For many nondomestic species, nutritional requirements and the challenges faced in their current habitats are unknown. This is the case of small semi-captive population of the critically endangered Western Derby eland held in two wildlife reserves (the Bandia and the Fathala) in Senegal. The aim of this study was to determine the mineral profile (Ca, P, Mg, Cu, Fe, Zn, Se, K, S) in blood serum and to identify potential mineral deficiencies. Serum data (11 individuals) were compared to other Tragelaphineae, where it was lower in almost all elements. Considerably low concentrations were recorded for Cu, Fe and Zn. Animals in the Bandia reserve had higher serum levels of Fe and K compared to the Fathala reserve and a higher serum level of S in the Fathala reserve compared to the Bandia reserve. Recorded mineral levels may reflect most likely the limited mineral background in the local environment. The knowledge of adequate nutritional requirements and health status of these animals is relevant for the conservation breeding programme. No other serum mineral reference values exist for Western Derby eland, neither for free-ranging nor captive animals. Therefore, the knowledge of reference intervals for minerals in serum may serve for monitoring of the population's health.

KEYWORDS
antelope, blood serum biochemistry, mineral nutrition, ruminant, West Africa

The Western Derby eland (WDE) (*Taurotragus derbianus derbianus*) is a large West-African savannah-dwelling antelope with fewer than 200 remaining individuals in their last refuge in Senegal. Apart from the wild population in the Niokolo Koba National Park, there is a small semi-captive population (101 individuals) held in the Bandia and the Fathala wildlife reserves as part of a conservation breeding programme (Brandlová et al., 2017). WDE is a browser, and its diet composes mainly of leaves, shoots of woody plants and fruits. These food items form 98.8% of WDE's diet in the wild, while in the Bandia reserve it represents 77.5% of diet volume, because of supplementary feeding of *Acacia albida* pods, groundnut hay and livestock feed. Animals in the Bandia reserve are fed regularly and in larger amounts compared to the Fathala reserve (Hejcmanová, Homolka, Antonínová, Hejcman, & Podhájecká, 2010). Supplementary feeding is provided by rangers mainly in dry and hot dry seasons. There is also occasional provision of mineral licks (of unknown mineral composition) on the Fathala reserve only. The knowledge of adequate nutritional requirements and health status is important for the conservation breeding programme. Knowing reference intervals for minerals in serum is relevant for monitoring population health, and for investigating the lower rate or reproduction in the Fathala reserve compared to the Bandia reserve (Brandlová et al., 2017). The only relevant information is reference values for the eastern subspecies of Giant eland from ZIMS (2013). The aim of the present study was therefore to describe the mineral status of WDEs through the mineral profiles (Ca, P, Mg, Cu, Fe, Zn, Se, K, S) in blood serum.

Our study was conducted on WDEs in two breeding reserves in Senegal. The Bandia reserve is located 65 km southward from Dakar

 © 2020 Blackwell Verlag GmbH

in the Sahel-Sudanese savannah, which is dominated by *Acacia* spp. and *Balanites aegyptiaca*. The Fathala reserve is located 250 km southward from Dakar in the Sudano-Guinean savannah, which is dominated by *Acacia* spp., *Combretaceae* family trees, and *Danielia oliveri* (Hejcmanová et al., 2010). Both reserves have a dry and wet seasonal climate, receiving approx. 350 mm and 800 mm of rainfall annually in the Bandia and Fathala reserves, respectively.

Blood was sampled from 11 young animals (1–3 years old) in total (4 males from the Fathala reserve; 2 males and 5 females from the Bandia reserve) during translocations in the dry season in March 2017. Individual animals were selected on basis of the genetic kinship (to avoid inbreeding of related individuals) under the supervision of conservationists rather than the need of this research. Animals were immobilized with a combination of medetomidine (Medetomidine 10 mg/ml; Kyron Laboratories), butorphanolum (Nalgosed 10 mg/ml; Bioveta, a.s.), azaperone (Stresnil 40 mg/ml; Janssen Animal Health) and ketamine (Narkamon 100 mg/ml; Bioveta, a.s.), and blood was taken from the *Vena saphena lateralis* 18–33 min after application of drugs and before the i.v. application of antidotes atipamezole (Revertor 5 mg/ml; CP-Pharma Handelsges, GmbH) and naloxone (Naloxone WZT Polfa 0.4 mg/ml; Warszawskie Zaklady Farmaceutyczne Polfa S.A.). The blood was collected in 5 ml blood serum plastic tubes with a separating gel (VACUETTE; Dialab). Animals were immobilized by one dart shot from distance in open space of boma in the early morning hours. Detailed health status of individual animals was not known before darting; however, no clinical signs of diseases or health problems, for example poor growth, hair loss or discoloration, weakness and swollen joints, were observed. The samples were centrifuged by portable manual centrifuge after 1 hr of settling. The centrifuged, clear serum was placed in a deep freeze in Eppendorf tubes. Serum samples were transported to the Czech Republic in cooling box filled with gel cooling packs. Temperature could not be controlled during 12 hr of transportation from Senegal to Czech Republic. The samples were frozen immediately after transportation to Czech Republic again.

The samples were examined in accredited laboratory at the State Veterinary Institute in Prague 16 days after sampling. The concentrations of P, Ca and Mg were determined by an IDEXX VetTest Chemistry Analyzer, the concentrations of S, Se, Fe and Zn were determined by an ICP-OES (Thermo Scientific™ iCAP 6000 Series; Thermo Fisher Scientific), the concentration of K was determined by an AAS-flame (AA240; Varian), and the concentration of Cu was determined by a GF-AAS (Spectra AA220Z; Varian) with a Zeeman correction.

Statistical analyses were performed using the TIBCO® STATISTICA™ package (StatSoft). The small sample size suggested a non-parametric approach, without need of normality testing. A comparison of mineral concentrations between two localities was performed using the non-parametric Mann-Whitney U test. Serum mineral relationships were tested by Spearman's correlation.

The values of the WDE serum mineral concentrations (Table 1) were lower in almost all elements compared to the blood parameters of other Tragelaphineae, zebu or cattle (Table 2). The only detected correlations between minerals were those of Ca with Mg ($r_s = .96$, $p = .05$) and Ca with P ($r_s = .96$, $p = .05$) in Bandia reserve animals.

The serum mineral levels were comparable for males and females with the exception of Mg concentrations, which were lower in males (U = 3, $p = .035$) without any obvious reason, which was

TABLE 1 Concentration of minerals (mean ± *SE*, range) in the blood serum of Western Derby elands ($n = 11$) in the Bandia and Fathala reserves, Senegal

	Total $n = 11$	Bandia $n = 7$	Fathala $n = 4$	Males $n = 6$	Females $n = 5$
Ca, mmol/L	1.87 ± 0.14 (0.95–2.39)	1.77 ± 0.54 (0.95–2.39)	2.03 ± 0.34 (1.54–2.27)	1.75 ± 0.53 (0.95–2.27)	2.00 ± 0.41 (1.31–2.39)
P, mmol/L	2.12 ± 0.13 (1.36–2.76)	2.17 ± 0.52 (1.36–2.76)	2.04 ± 0.27 (1.77–2.38)	1.96 ± 0.37 (1.36–2.38)	2.31 ± 0.46 (1.55–2.76)
Mg, mmol/L	1.55 ± 0.10 (1.09–2.05)	1.68 ± 0.33 (1.09–2.05)	1.32 ± 0.18 (1.15–1.49)	1.36 ± 0.26* (1.09–1.75)	1.78 ± 0.24* (1.39–2.05)
Cu, µmol/L	10.87 ± 2.78 (6.55–38.50)	12.14 ± 11.65 (6.86–38.50)	8.66 ± 1.47 (6.55–9.95)	8.58 ± 1.20 (6.55–9.95)	13.62 ± 13.91 (6.86–38.50)
Fe, µmol/L	22.35 ± 1.21 (16.80–31.30)	24.2 ± 3.73* (19.2–31.30)	19.13 ± 2.11* (16.8–21.9)	20.92 ± 3.33 (16.80–25.80)	24.08 ± 4.46 (19.20–31.30)
Zn, µmol/L	11.51 ± 0.40 (10.60–14.90)	11.34 ± 0.84 (10.70–13.10)	11.80 ± 2.07 (10.60–14.90)	11.55 ± 1.67 (10.60–14.90)	11.46 ± 0.97 (10.70–13.10)
Se, µmol/L	1.75 ± 0.17 (0.87–2.92)	1.62 ± 0.49 (0.87–2.34)	1.97 ± 0.66 (1.43–2.92)	1.73 ± 0.68 (0.87–2.92)	1.78 ± 0.43 (1.26–2.34)
K, mmol/L	5.21 ± 0.11 (4.70–5.75)	5.40 ± 0.26* (5.11–5.75)	4.87 ± 0.23* (4.70–5.20)	5.00 ± 0.27 (4.70–5.26)	5.46 ± 0.30 (5.11–5.75)
S, mmol/L	27.23 ± 0.50 (24.00–30.00)	26.54 ± 1.61* (24.00–29.30)	28.43 ± 1.05* (27.90–30.00)	27.90 ± 1.15 (26.70–30.00)	26.42 ± 1.95 (24.00–29.30)

*Significant ($p < .05$) differences tested by Mann-Whitney *U* test according to locality and sex.

TABLE 2 Overview of blood (serum/plasma) mineral concentrations (mean, range) of different ruminant species

	Cape eland	Greater kudu	Lesser kudu	Bongo	Zebu	Horro cattle	Cape eland (free)
Ca, mmol/L	2.44 (1.91–2.81)	1.84 (0.99–2.23)	2.27 (2.09–2.62)	1.73 (1.14–2.47)	5.40 (4.10–12.30)	3.82	2.87
P, mmol/L	2.44 (1.91–2.81)	2.70 (1.36–4.60)	2.32 (1.24–3.37)	2.21 (1.94–2.74)	3.30 (1.80–4.60)	4.56	1.29
Mg, mmol/L	0.84 (0.50–1.06)	0.53 (0.30–1.07)	0.75 (0.62–0.95)	0.71 (0.33–1.12)	1.90 (1.40–2.30)	0.71	0.78
Cu, µmol/L	22.55 (17.20–30.80)	27.80 (17.20–46.90)	21.35 (19.60–23.10)	25.5 (7.60–34.40)	6.00 (0.80–18)	10.38	14.10
Fe, µmol/L	39.60 (15.90–50.50)	35.48 (8.40–56.60)	53.94 (39.60–69.80)	39.55 (23.90–58.60)	143 (75–1,200)	24.35	
Zn, µmol/L	103.3 (29.20–198)	91.36 (48.60–137)	71.15 (53.70–86.60)	117.60 (114.50–120.70)	46 (24–107)	13.92	
K, mmol/L	5.86 (5.39–6.25)	4.55 (4.01–5.24)	5.72 (4.34–8.35)	6.19 (4.88–7.40)	4.40 (2.70–6.60)		
S, mmol/L					37 (22–47)		

Source: For captive Cape eland ($n = 8–10$), Greater kudu ($n = 3–20$), Bongo ($n = 4–8$) and Lesser kudu ($n = 2–8$) (Váhala et. al., 1989), domestic zebu ($n = 90$) (Dermauw et al., 2013), domestic Horro cattle ($n = 10–14$) (Gizachew et al., 2002), and free-ranging Cape eland ($n = 4–12$) (Drevemo et al., 1974).

similar to the findings in the plasma of captive zebu (*Bos indicus*) in Ethiopia (Dermauw et al., 2013). We recorded higher serum levels of Fe ($U = 1.5$, $p = .023$) and K ($U = 1$, $p = .018$) in the Bandia reserve compared to the Fathala reserve. Higher serum level of S ($U = 3$, $p = .046$) was recorded in the Fathala reserve compared to the Bandia reserve. Higher serum levels of Fe and K in the animals living in the Bandia reserve correspond to the higher concentrations of these minerals in the soil in the Bandia reserve (Stoklasová, 2016). Other tested minerals in soil (Ca, P, Cu, Fe, Zn, K) were higher on the Bandia reserve compared to the Fathala reserve as well. Animals from the Fathala reserve had slightly, but not significantly higher concentrations of Ca, Cu and Zn in the blood, thus not corresponding to soils in reserves. Soils in both reserves are generally very low in mineral concentrations compared to other localities (Stoklasová, 2016). Regarding the animals from the Bandia reserve, there was more mineral content variability in the blood serum among individual animals with no specific pattern.

Predominantly low concentrations of minerals in WDE's blood serum might indicate mineral deficiency; however, the animals were all in a good condition without any obvious clinical issues. Recorded mineral levels may reflect most likely an adaptation of the WDE to a specific environment that is poor in minerals. Considerably low concentrations were recorded for Cu, Fe and Zn. The low Cu concentration could be explained by copper-containing serum proteins that are incorporated into the clotting during the serum extraction, making the Cu levels rather variable (Laven & Smith, 2008). The low Zn could be linked to either low Zn levels in the mature forages or to an antagonistic relation to the P in phytates (Suttle, 2010). Cu and Zn are susceptible to so called "herd effect" which influences the concentrations of minerals in serum independently on diet, and more animals must be tested for convincing results (Herdt & Hoff, 2011). Regarding low number of individuals involved in the study, our results might be partly affected by a small sample size.

A low concentration of Cu matches a low concentration of Fe, since Cu is essential for absorption and transport of Fe (Tuormaa, 2000). The serum Ca:P ratio of WDEs in the reserves (mean 0.88 ± 0.13 *SD*) was inverted compared to the ratio values for ruminants considered clinically normal (1.5–2 for domestic cattle, see Gizachew, Hirpha, Jalata, & Smit, 2002; for wild antelopes, see Drevemo, Grootenhuis, & Karstad, 1974). This shows the same phenomenon commonly observed in non-domesticated bovids bred in captivity, for example greater kudu *(Tragelaphus strepsiceros)* and bongo *(Tragelaphus eurycerus)* (Table 2) (Miller et al., 2010; Váhala, Pospíšil, Špála, & Kaše, 1989). Considering diet selection of WDE, the Fathala reserve appears unfavourable for WDE in terms of macro-elements' content and fibre fractions of local plants (Hejcmanová, Mířejovská, Homolka, & Hejcman, 2019), and similar conditions in the Bandia reserve can be expected. Combined findings of soil and our serum analyses suggest that WDEs might benefit from mineral supplementation, for example mineral licks. Effects of such a supplementation would have to be evaluated.

ACKNOWLEDGEMENTS

We are grateful to Directorate of National Parks in Senegal, managers of the Bandia and Fathala reserves, and their staff for their assistance with animal translocation. We are also grateful to the Safari

Park Dvůr Králové for their cooperation and support. The study was funded by the Czech University of Life Sciences, Prague by grant CIGA 20175004 and IGA 20205015.

CONFLICT OF INTEREST

All authors state no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The authors confirm that the study was performed within the frame of conservation translocation of the animals with approval of Senegalese conservation authorities, namely the Directorate of National Parks of Senegal, and the study has followed EU standards for the protection of animals used for scientific purposes.

ORCID

Pavla Hejcmanová https://orcid.org/0000-0001-9547-4302

REFERENCES

Brandlová, K., Kubátová, A., Fedorova, T., Štochlová, K., Grúňová, M., & Hejcmanová, P. (2017). *African Studbook. Western Derby Eland, Taurotragus derbianus derbianus* (Gray, 1847), Volume *10*, Prague, Czech: Czech University of Life Sciences Prague.

Dermauw, V., Yisehak, K., Belay, D., Van Hecke, T., Du Laing, G., Duchateau, L., & Janssens, G. P. J. (2013). Mineral deficiency status of ranging zebu *(Bos indicus)* cattle around the Gilgel Gibe catchment, Ethiopia. *Tropical Animal Health and Production*, *45*, 1139–1147. https://doi.org/10.1007/s11250-012-0337-4

Drevemo, S., Grootenhuis, J. G., & Karstad, L. (1974). Blood parameters in Wild Ruminants in Kenya. *Journal of Wildlife Diseases*, *10*, 327–334. https://doi.org/10.7589/0090-3558-10.4.327

Gizachew, L., Hirpha, A., Jalata, F., & Smit, G. N. (2002). Mineral element status of soils, native pastures and cattle blood serum in the mid-altitude of western Ethiopia. *African Journal of Range & Forage Sicence*, *19*, 147–155. https://doi.org/10.2989/10220110209485787

Hejcmanová, P., Homolka, M., Antonínová, M., Hejcman, M., & Podhájecká, V. (2010). Diet composition of Western Derby eland (*Taurotragus Derbianus Derbianus*) in the dry season in a natural and a managed habitat in Senegal using faecal analyses. *South African Journal of Wildlife Research*, *40*, 27–34. https://doi.org/10.3957/056.040.0105

Hejcmanová, P., Mířejovská, M., Homolka, P., & Hejcman, M. (2019). Drivers of diet selection of critically endangered Western Derby eland during the food shortage period within conservation breeding in Senegal. *Scientific Reports*, *9*, 1–7. https://doi.org/10.1038/s41598-019-45035-z

Herdt, T. H., & Hoff, B. (2011). The use of blood analysis to evaluate trace mineral status in ruminant livestock. *Veterinary Clinics: Food Animal Practice*, *27*, 255–283. https://doi.org/10.1016/j.cvfa.2011.02.004

Laven, R., & Smith, S. (2008). Copper deficiency in sheep: An assessment of relationship between concentrations of copper in serum and plasma. *New Zealand Veterinary Journal*, *56*, 334–338. https://doi.org/10.1080/00480169.2008.36856

Miller, M., Weber, M., Valdes, E. V., Neiffer, D., Fontenot, D., Fleming, G., & Stetter, M. (2010). Changes in serum calcium, phosphorus, and magnesium levels in captive ruminants affected by diet manipulation. *Journal of Zoo and Wildlife Medicine*, *41*, 404–408. https://doi.org/10.1638/2009-0001.1

Stoklasová, L. (2016). *Soils in Africa as source of mineral nutrition for large herbivores*. Msc Thesis, Czech University of Life Sciences Prague, Czech Republic.

Suttle, N. F. (2010). *Mineral nutrition of livestock*, 4th ed. Wallingford, UK: CABI.

Tuormaa, T. E. (2000). Chromium, selenium, copper and other trace minerals in health and reproduction. *Journal of Orthomolecular Medicine*, *15*, 145–157.

Váhala, J., Pospíšil, J., Špála, P., & Kaše, 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. https://doi.org/10.2754/avb198958010017

ZIMS. (2013). *Species 360 physiological reference intervals for captive wildlife (2013) Giant Eland* (Taurotragus derbianus). Retrieved from https://zims.species360.org/Main.aspx

How to cite this article: Stoklasová L, Váhala J, Hejcmanová P. Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal. *J Anim Physiol Anim Nutr*. 2020;00:1–4. https://doi.org/10.1111/jpn.13432

APPENDIX 3

Comparative Biochemistry and Physiology, Part A 246 (2020) 110720

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet

Pavla Hejcmanová[a], Sylvia Ortmann[b], Lucie Stoklasová[a], Marcus Clauss[c,*]

[a] Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague 6 – Suchdol, Czech Republic
[b] Leibniz-Institute for Zoo and Wildlife Research (IZW) Berlin, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany
[c] Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

ARTICLE INFO

Keywords:
Grazer
Browser
Forestomach
Passage
Selectivity factor
Saliva

ABSTRACT

The way that fluids and particles move through the forestomach of a ruminant is species-specific, and can be used to classify ruminants according to their digestive physiology into 'moose-types' (with little difference in fluid and small particle passage) and 'cattle-types' (where fluids move through the forestomach much faster than small particles). So far, 'moose-types' appear limited to a dietary niche of browsing, whereas 'cattle-types' are particularly prominent in the intermediate and grazing diet niches. However, some species, including members of the spiral-horned antelopes (the Tragelaphini), have a 'cattle-type' physiology but a browse-dominated diet niche. Eland (*Taurotragus oryx*), the largest member of the Tragelaphini, are strict browsers in the wild but have been considered intermediate feeders in the past, and can seemingly be maintained on grass diets. We quantified food intake, mean retention time (MRT) in the gastrointestinal tract and the reticulorumen (RR) of a solute, a small and a large particle marker, and diet digestibility in six eland each fed a monocot (grass hay) and a dicot (lucerne silage) forage. Food intake and digestibility was lower on the diet with higher fibre content (grass hay), with corresponding longer MRT. At the higher intakes on lucerne, the difference in MRT between small and large particles was larger, indicating a greater reliance on particle sorting and clearance under this condition of potentially limiting gut capacity. Regardless of diet or intake, the ratio of small particle and solute MRT in the RR was constant and small, at a quotient of 1.54, classifying the eland as a typical 'moose-type' ruminant. This finding is consistent with previous literature reports on low faecal metabolic nitrogen and high apparent protein digestibility in eland. Given the relative ease at which eland can be maintained under farm husbandry conditions, they appear ideal model ruminants to study the effects of differences in rumen physiology compared to cattle.

1. Introduction

Ruminants vary widely in feeding habits and morphophysiology of the digestive tract. There is a long-standing tradition of linking the two in the sense of convergent adaptations to either browse- or grass-dominated diets (Hofmann, 1973, 1988; Clauss et al., 2008; Codron et al., 2019), and while a series of correlations between diet and morphological or physiological measures have been documented, these relationships comprise a relevant degree of data scatter (Ehrlich et al., 2019). An outstanding feature of rumen physiology, the separation of fluids and small particles in their passage through the rumen (Hummel et al., 2005; Dittmann et al., 2015a), is no exception. On the one hand, there is a general pattern of animals mainly consuming browse, with a 'moose-type' physiology, to have fluids and particles to flow out of the rumen in close association and comparatively unstratified rumen contents, and of animals that are no strict browsers, with a 'cattle-type' physiology, to have a distinctively higher fluid than particle throughput through the rumen and comparatively stratified rumen contents. On the other hand, outliers to the pattern exist, not only, but particularly among the Bovinae – the bovini and the tragelaphini (Przybyło et al., 2019b).

The current interpretation is that 'moose-type' ruminants defend themselves against secondary plant compounds in browse by salivary proteins and are hence limited in the amount of protein-rich saliva they can produce, whereas 'cattle-type' ruminants are not constrained in saliva production by a requirement for high salivary protein contents (Clauss et al., 2010). 'Cattle-type' ruminants can thus putatively use a higher fluid throughput through the rumen to increase the harvest of

* Corresponding author.
E-mail addresses: hejcmanova@ftz.czu.cz (P. Hejcmanová), ortmann@izw-berlin.de (S. Ortmann), stoklasoval@ftz.czu.cz (L. Stoklasová), mclauss@vetclinics.uzh.ch (M. Clauss).

https://doi.org/10.1016/j.cbpa.2020.110720
Received 26 March 2020; Received in revised form 29 April 2020
Available online 05 May 2020
1095-6433/ © 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

rumen microbes (Clauss and Hummel, 2017). This scenario does not exclude 'moose-type' ruminants from grass diets, but should just make them less competitive in the grazing niche. On the other hand, 'cattle-type' ruminants may develop other, hitherto unknown strategies to cope with secondary plant compounds of browse-dominated diets (Przybyło et al., 2019b).

The common eland (*Taurotragus oryx*) is an interesting species in terms of dietary adaptations. It belongs to a ruminant tribe (tragelaphini) that comprises some species that have been identified as outliers to common patterns, in terms of salivary gland size (Robbins et al., 1995; Hofmann et al., 2008) and in the retention pattern for fluids and particles (Przybyło et al., 2019b). However, the digesta passage kinetics of eland have not been investigated so far. The eland is also an example of the difficulty to definitely ascribe a feeding type to a ruminant species. Traditionally, the eland has been considered a prime example of an intermediate feeder (Hofmann and Stewart, 1972; Hofmann, 1989; Gagnon and Chew, 2000), based on studies that reported a mixed diet of grass and browse (Kerr et al., 1970; Nge'the and Box, 1976; Abdullahi, 1980; Buys, 1990) (older reports reviewed by Littlejohn, 1968), or even a mixed diet with a predominance of grass (Lamprey, 1963).

However, a larger number of studies have demonstrated that eland are predominantly browsers (e.g., van Zyl, 1965; Cerling et al., 2003; Sponheimer et al., 2003; Codron et al., 2007; Steuer et al., 2014; Venter and Kalule-Sabiti, 2016). Increased reported proportions of grass in eland diets are considered either due to methodological problems (Watson and Owen-Smith, 2000), due to seasonal effects when green grass is taken in the rainy season (Parrini et al., 2019), or exceptions due to specific circumstances (D'Ammando et al., 2015). Similarly, the closely related Derby eland (*Taurotragus derbianus*) is a browser (Hejcmanová et al., 2010; Galat-Luong et al., 2011), and even fossil eland were found to be more browsers than intermediate feeders (Stynder, 2009). Taken together, these results suggest a highly flexible species that, in contrast to some other mixed feeders, mostly prefers browse, but is able to thrive on grass nevertheless.

We aimed to test whether this flexibility is linked to a 'cattle-type' forestomach physiology, similar to other Bovinae that can use mixed diets (Przybyło et al., 2019b), by measuring the mean retention time of different digesta phases in eland. In order to account for the different diets naturally eaten by eland, we tested them separately on a monocot (grass hay) and a dicot (lucerne haylage) diet. We expected differences in intake level between the diets, and intake level to affect absolute measures of digesta retention – because higher food intakes are typically associated with shorter retention times (Müller et al., 2013). In contrast, the ratio of small particle to fluid passage has been shown to be species-specific across diets and experiments, with no effect of the intake level and only slight differences due to diet that are of a much lower magnitude than differences between species (Renecker and Hudson, 1990; Lechner et al., 2010; Dittmann et al., 2015a; Przybyło et al., 2019b). Therefore, regardless of whether this ratio would classify the eland as 'cattle-type' or 'moose-type' ruminants, we expected no effect of diet on this classification.

2. Methods

The experiment was conducted from May to November 2017 at the University Farm Estate in Lány (Czech University of Life Sciences Prague, Czech Republic), which is accredited as research facility according to European and Czech laws for ethical use of animals in research (recent permission no. 63479_2016-MZE-17214 valid until 10th October 2021). For a description of the usual husbandry regime of elands at this farm, see Hejcmanová et al. (2011). In the year of the present study, the animals usually had access to a grass pasture, meadow hay, lucerne haylage, and wheat bran, which they ingested at individually varying proportions, and received this combination before and in between experiments. The experimental proposal was approved by the Institutional Animal Care and Use Committee of the Czech University of Life Sciences Prague as the experimental design did not require use of animals above standard husbandry procedures or extended intervention.

Table 1
Nutrient concentration (in % dry matter, except where indicated) in the grass hay and the lucerne haylage used in the present study. Data represent means (± SD) of six pool samples.

Nutrient	Lucerne haylage	Grass hay		
		Offered	Leftover	Ingested
Dry matter (% as fed)	40.7 ± 1.5	95.4 ± 1.8	–	95.4 ± 1.8
Total ash	12.1 ± 0.2	7.0 ± 0.8^{A}	6.1 ± 1.1^{B}	7.4 ± 1.0
Organic matter	87.9 ± 0.2	93.0 ± 0.8^{B}	93.9 ± 1.1^{A}	92.6 ± 1.0
Crude protein	23.4 ± 0.7	5.7 ± 0.4^{A}	4.2 ± 0.6^{B}	6.3 ± 0.6
Neutral detergent fibre	38.1 ± 1.7	72.0 ± 3.8	73.9 ± 3.7	71.4 ± 4.5
Acid detergent fibre	26.4 ± 1.0	40.4 ± 3.3^{B}	45.1 ± 2.9^{A}	38.4 ± 4.2
Acid detergent lignin	6.7 ± 0.4	6.0 ± 1.5	6.9 ± 0.9	5.6 ± 1.8

^{A,B} different letters within a row for grass hay indicate significant differences ($P < .05$) in paired tests between the offered diet and the leftovers; absence of letters in these columns indicates no significant difference.

Six adult male common eland (411 ± 90 kg, range 278–535 kg) were chosen for the experiment. This was done to exclude reproducing (pregnant) animals; the animals were picked randomly from among the available males in the herd. They were subjected to two diet treatments each. The animals were part of a larger breeding herd that was usually kept together in one large freestall barn without compartments. Treatments consisted of adapting animals on a monocot (grass hay)-only and a dicot (lucerne haylage)-only diet for at least 2 weeks before each trial (adaptation period), followed by a 1 week trial phase. For a nutritional characterisation of the diets, see Table 1. These two forages were from the same batches as those used for the regular feeding of the herd. For the adaptation period and during the entire trial week, each animal was kept individually in a separate compartment (20 m^2), to facilitate measuring of individual food intake and collection of faeces. Compartments were provided with a straw bedding, and animals were not observed to ingest the bedding. Between treatments, each animal returned for at least 1 month into the general herd.

Food intake was measured by weighing food offered and leftovers each day in the morning during the experiment. Grass hay was offered for *ad libitum* consumption, with leftovers averaging 29 ± 13% of the total amount offered. Lucerne haylage was always consumed completely, and therefore did not meet the definition for *ad libitum* consumption. Samples of the forages offered and the grass hay leftovers were taken on a daily basis and pooled for each individual for nutrient analyses.

During the adaptation period, the animals were accustomed to receive a handful of wheat bran every day. On the first day of the trial week, they received a dose of three passage markers in a handful of wheat bran, which was ingested completely within 15 min. The markers were cobalt(Co)-EDTA as a solute marker (at a dose of 5–6 g/animal), and chromium(Cr)-mordanted fibre (particle size < 2 mm; 40–50 g/animal) a well as cerium(Ce)-mordanted fibre (particle size < 10 mm; 30–40 g/animal) as particle markers, prepared according to Udén et al. (1980). Co-EDTA was mixed in the wheat bran after being dissolved in water.

Faeces were collected at least twice before marker feeding for background levels, and every 4 h on trial days 1 and 2, every 6 h on day 3, every 8 h on days 4 and 5, and every 12 h on days 6 to 8. All faeces defecated in an respective interval were collected, pooled, weighed, and a representative subsample was stored frozen until drying at 60 °C for

72 h and grinding. The eland usually defecated in one or two piles in their enclosures, making total collection easily feasible. For the calculation of digestibility, faeces weights were used from consecutive days for which total collection data was available. Of the 12 trials, the number of total collection considered reliable was 4 times for 7 consecutive days, 4 times for 6 consecutive days, and 4 times for three consecutive days. Individual faecal samples were used for passage marker analysis, and a representative faecal pool sample per animal and trial was composed of all available individual samples and used for nutrient analysis.

Analyses of marker concentrations was made by inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany) according to Frei et al. (2015). Forage samples were subjected to standard nutrient analyses (AOAC, 1995) for dry matter (DM) and total ash (AOAC no. 942.05), crude protein (AOAC no. 977.02), neutral detergent fibre (NDF, AOAC no. 2002.04; corrected for residual ash), acid detergent fibre and acid detergent lignin (ADF, ADL, AOAC no. 973.18). The pooled faecal samples were only analysed for total ash, crude protein and NDF.

The MRT in the whole gastrointestinal tract (GIT) was calculated according to Thielemans et al. (1978) as

$$\mathrm{MRT} = \frac{\sum t_i C_i dt_i}{\sum C_i dt_i}$$

with C_i = marker concentration in the faecal samples from the interval represented by time t_i (h after marker administration, using the midpoint of the sampling interval) and dt_i = the interval (h) of the respective sample

$$dt_i = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}$$

Complete excretion of the markers was assumed once the faecal marker concentrations were similar to the background levels determined in pre-dose faecal samples. Mean retention time in the reticulorumen (RR) was estimated following Lechner-Doll et al. (1990). The $MRT_{solute}RR$ is determined by estimating the rate constant of the descending part of the marker excretion curve via an exponential equation:

$$y = A * e^{-k*t}$$

with y = faecal marker concentration at time t (mg/kg DM), A = a constant, k = rate-constant (h^{-1}) and t = time after marker dosing (h); the reciprocal of k represents the MRT for the RR. The $MRT_{particle}RR$ is calculated based on the assumption that fluid and particles do not differ in passage characteristics distal to the RR (Mambrini and Peyraud, 1997):

$MRT_{particle}RR = MRT_{particle}GIT - (MRT_{solute}GIT - MRT_{solute}RR)$.

The 'selectivity factor' (SF, the ratio of two MRT of different markers) was calculated for both the total GIT and the RR. The relative daily dry matter intake was expressed on the basis of body $mass^{0.85}$ (Hackmann and Spain, 2010; Müller et al., 2013). Apparent digestibilities were calculated as (Intake – Faecal excretion)/Intake*100. Using the dry matter intake, large particle MRT, and apparent dry matter digestibility, the dry matter gut fill was calculated by the linear approach of Holleman and White (1989).

Statistical comparisons between treatments were made by paired tests (paired *t*-test, Wilcoxon signed rank test), depending on normal distribution of data (as assessed by Kolmogorov-Smirnov test). To compare MRTs of the markers within either the GIT or the RR, a repeated-measures ANOVA was performed with Sidak post hoc test. Correlations between the relative dry matter intake and retention measurements were tested by Spearman's correlation, and subsequently by General Linear Models (assessing normal distribution of residuals) with individuum as a random factor (to account for repeated measures) and the relative dry matter intake as the independent variable. Tests were performed using SPSS v. 25.0 (IBM), with the significance level set to 0.05. For a comparison with other ruminant species, a species average was compared to the data compilation from Przybyło et al. (2019b).

3. Results

The animals always ingested the lucerne haylage completely, and ingested significantly more absolute ($P = .002$) and relative ($P = .002$) dry matter of the lucerne haylage than of the grass hay (Table 2). Both absolute and relative dry matter intake were doubled on lucerne haylage compared to the grass hay. When feeding on grass hay, leftovers were significantly lower in protein and higher in acid detergent fibre than the diet offered, indicating a certain degree of feeding selectivity (Table 1).

The marker excretion patterns indicated a nearly parallel movement of solutes and small particles, with an increase in the difference between small and large particles on lucerne haylage (Fig. 1). All MRT measures were significantly shorter on lucerne silage (Table 2); however, the SF measures for particles vs. solutes did not differ between the diets. Only the SF of large to small particles were significantly higher on lucerne haylage, both for the GIT ($P = .038$) and for the RR ($P = .024$).

In particular, the largest animal, a 10 year-old, 535 kg bull, showed an extreme difference between the treatments, with a very low dry matter intake on the grass hay (13 $g/kg^{0.85}$/d vs. 45 $g/kg^{0.85}$/d on the lucerne haylage) and the longest MRT (MRT GIT for solutes, small and large particles 46, 72 and 71 h on the grass hay vs. 36, 46 and 53 h on the lucerne haylage).

The apparent digestibility of dry matter and organic matter of the

Table 2
Mean (± SD) intake of feed as fed, dry matter (DM), the relative dry matter intake (rDMI), the mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of three passage markers (Co – solutes, Cr – small particles, Ce – large particles) in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage.

Diet	Intake	Intake	rDMI	MRT GIT			MRT RR			SF GIT			SF RR		
	as fed	DM		Co	Cr	Ce	Co	Cr	Ce	Cr/Co	Ce/Co	Ce/Cr	Cr/Co	Ce/Co	Ce/Cr
	kg	kg	$g/kg^{0.85}$/d	h			h								
Grass hay	4.05 ± 1.36^{B}	3.87 ± 1.31^{B}	24 ± 7^{B}	35 ± 6^{Ab}	47 ± 12^{Aa}	50 ± 11^{Aa}	23 ± 5^{Ab}	36 ± 11^{Aa}	38 ± 9^{a}	1.35 ± 0.15	1.43 ± 0.10	1.07 ± 0.05^{B}	1.54 ± 0.27	1.66 ± 0.18	1.09 ± 0.07^{B}
Lucerne haylage	20.49 ± 2.25^{A}	8.34 ± 1.07^{A}	51 ± 8^{A}	28 ± 5^{Bc}	38 ± 5^{Bb}	43 ± 5^{Ba}	19 ± 4^{Bc}	29 ± 4^{Bb}	34 ± 4^{a}	1.36 ± 0.09	1.55 ± 0.11	1.14 ± 0.05^{A}	1.54 ± 0.18	1.84 ± 0.23	1.20 ± 0.06^{A}

^{A,B} Different letters within columns indicate significant differences ($P < .05$) in paired tests.
^{a,b,c} No common letters within rows indicate significant differences (RM-ANOVA, Sidak post hoc) between markers for a MRT or SF measure; absence of letters indicates no significant difference.

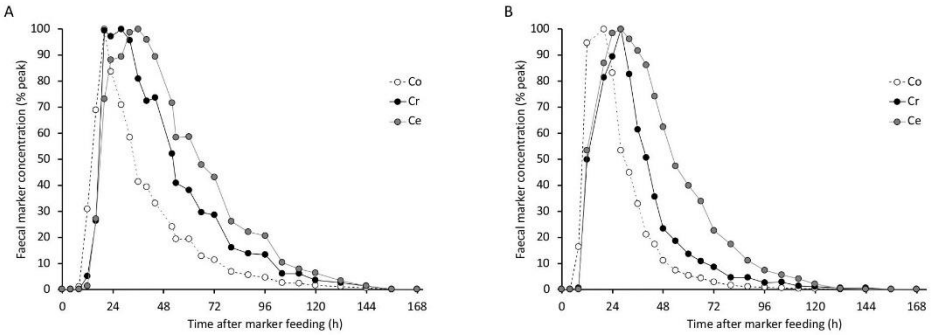


Fig. 1. Passage marker excretion pattern for cobalt (Co, solutes), chromium (Cr, particles < 2 mm) and cerium (Ce, particles < 10 mm) in a common eland (*Taurotragus oryx*) on (A) grass hay and (B) lucerne haylage.

grass hay was lower than that of the lucerne haylage (Table 3). Similar to intake, the calculated dry matter gut fill was nearly double on the lucerne haylage compared to the grass hay (Table 3).

Using Spearman's correlation, we observed significant, negative correlations between the relative dry matter intake and the MRT GIT of all markers (Table 4). For MRT in the RR, this was only the case for the small particle marker. The SFs were not correlated with intake, except for the SF of the large vs. small particles, with increased sorting of particles at the higher intake (Table 3). When assessing the effect of intake on passage kinetics in a GLM (accounting for repeated measures by including individual as a random factor), there were significant, negative relationships between the relative dry matter intake and the $MRT_{solute}GIT$ ($F_{1,5} = 19.424$, $P = .007$) and also the $MRT_{solute}RR$ ($F_{1,5} = 11.142$, $P = .021$), but not with any other MRT measure (P always > 0.05). Both SF for particles vs. solutes, for the GIT and the RR, also had no significant relationships with the relative dry matter intake (P always > 0.05), but again the SF of large vs. small particles did, both for the GIT ($F_{1,5} = 7.128$, $P = .044$) and the RR ($F_{1,5} = 13.679$, $P = .014$).

4. Discussion

The results of our study clearly indicate digesta kinetics in eland with a relatively closely related passage of solutes and small particles from the reticulorumen, with a nearly identical 'selectivity factor' (SF) as recently determined in muntjac (*Muntiacus reevesi*, Przybyło et al., 2019a) (Fig. 2A). Given findings in other members of the tragelaphini that had shown more distinct differences between the digesta phases (Przybyło et al., 2019b), this result was unexpected. Yet, it was robust across individuals and across two different diets (that also triggered different intake levels). In this respect, the present study adds to the existing evidence that neither diet nor intake has an effect on the difference between solute and small particle retention that is of similar magnitude as the species-specific differences themselves (Renecker and Hudson, 1990; Lechner et al., 2010; Dittmann et al., 2015a; Przybyło et al., 2019b).

Typical relationships between different measures known from other ruminants were demonstrated in the eland of the present study as well. There was a clear effect of intake level on retention times for particles

Table 3
Mean (± SD) apparent digestibility values and dry matter gut fill in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage.

Diet	Apparent digestibility				Dry matter gut fill
	Dry matter	Organic matter	Protein	Neutral detergent fibre	
	%				kg
Grass hay	76 ± 8^{B}	77 ± 8^{B}	65 ± 14^{B}	77 ± 7	4.89 ± 1.50^{B}
Lucerne haylage	89 ± 5^{A}	89 ± 4^{A}	91 ± 4^{A}	85 ± 6	8.43 ± 2.17^{A}

^{A,B} different letters within columns indicate significant differences (P < .05) in paired tests; absence of letters indicates no significant difference.

Table 4
Nonparametric correlations ($n = 12$) between relative dry matter intake (rDMI, g/kg$^{0.85}$/d) and various measures of mean retention times (MRT) and the selectivity factors (SF, the MRT ratios) in the gastrointestinal tract (GIT) and the reticulorumen (RR) of three passage markers (Co – solutes, Cr – small particles, Ce – large particles) in 6 common eland (*Taurotragus oryx*) fed either grass hay or lucerne haylage.

MRT GIT			MRT RR			SF GIT			SF RR		
Co	Cr	Ce	Co	Cr	Ce	Cr/Co	Ce/Co	Ce/Cr	Cr/Co	Ce/Co	Ce/Cr
***R* = −0.63**	***R* = −0.66**	***R* = −0.59**	*R* = −0.53	***R* = −0.63**	*R* = −0.33	*R* = −0.02	*R* = 0.39	***R* = 0.66**	*R* = −0.15	*R* = 0.19	***R* = 0.67**
***P* = .028**	***P* = .019**	***P* = .043**	*P* = .075	***P* = .029**	*P* = .291	*P* = .957	*P* = .217	***P* = .018**	*P* = .640	*P* = .556	***P* = .017**

Significant correlations in **bold.**

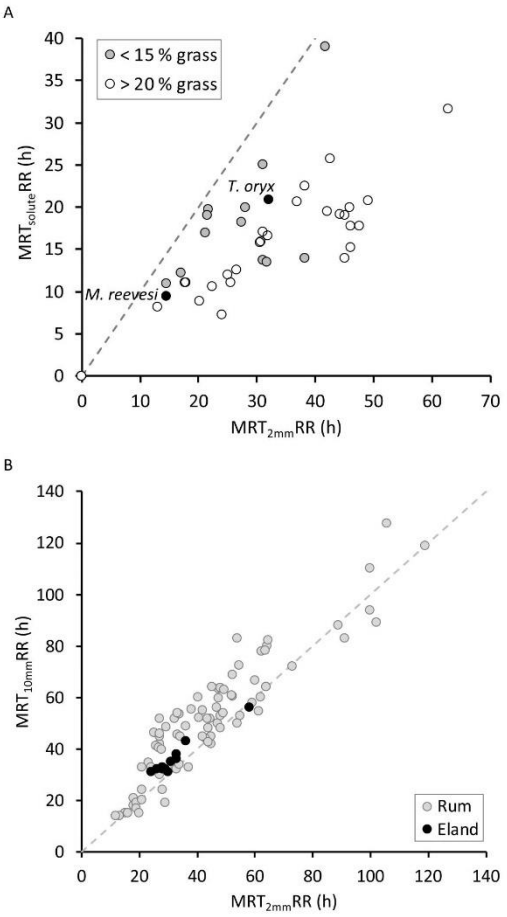


Fig. 2. Relationship of the mean retention time (MRT) of small particles (< 2 mm) in the reticulorumen (RR) with **(A)** the MRT of a solute marker in the RR in various ruminant species (RUM; data collection from Przybyło et al., 2019b; one value per species) and (B) the MRT of a large particle (10 mm) marker in the RR in various ruminant species (data collection from Dittmann et al., 2015b; multiple values per species), including the eland (*Taurotragus oryx*) of the present study, and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a) in (A).

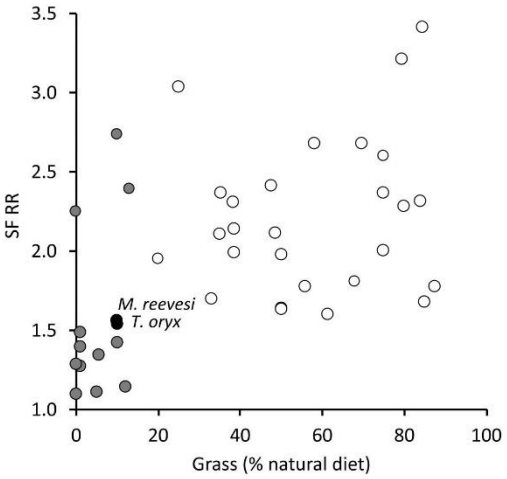


Fig. 3. Relationship between the average percentage of grass in the natural diet (species < 15%, grey circles; species > 20%, white circles) and the selectivity factor (SF, a measure for how distinctly the retention of small particles and fluid differ) in the reticulorumen (RR) of various ruminant species (data collection from Przybyło et al., 2019b; one value per species), including the eland (*Taurotragus oryx*) of the present study and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a). Species with a SF up to ~1.5 would be considered 'moose-type' ruminants, and species above that as 'cattle-type'. Note that 'moose-type' ruminants occur only among browsers, whereas 'cattle-type' cover the whole dietary spectrum. The 'cattle-type' browsers (grey circles with SF RR > 2) are the European bison (*Bison bonasus*), gerenuk (*Litocranius walleri*) and the bongo (*Tragelaphus eurycerus*).

and for solutes in the GIT, but not clearly for solutes in the reticulorumen (Table 4) (Dittmann et al., 2015a; Grandl et al., 2018). As most herbivores, eland select among the available plants and plant parts for low fibre content (Watson and Owen-Smith, 2002), which was also evident on the grass hay diet (Table 1). The forage with the higher neutral detergent fibre was ingested at lower quantities, as is typical for most herbivores (Meyer et al., 2010) (Table 2), and the digestibility increased with forage quality (Van Soest, 1994) (Table 3). The selective retention of larger particles when compared to the smaller ones was within the lower range reported for other ruminants (Fig. 2B). The selective retention of large vs. small particles (the SF Ce/Cr in Tables 2 and 4) showed a distinct correlation with the intake level (Table 4). In other words, when intake was high, and capacity in the reticulorumen potentially constraining, the reticulorumen sorting mechanism led to a clear difference between large and small particles (Fig. 1B). When intake was low, with a lesser gut fill and less space constraints on the reticulorumen, the increase in small particle retention was higher (on average, 9 h; Table 2) than that of large particle retention (on average, 7 h), as there was less need for expeditious reticulorumen clearance.

In contrast to other members of tragelaphini investigated so far, eland are thus classified as 'moose-type' ruminants. The findings add to the existing evidence that digesta kinetics are species-specific and can vary even within taxonomic ruminant clades. For example, among the cervinae (old world deer), both 'cattle-type' (*Cervus elaphus*, Renecker and Hudson, 1990; *Elaphurus davidianus*, Derix et al., 2019) and 'moose-type' (*Muntiacus reevesi*, Przybyło et al., 2019a) exist. The same is true for the bovidae in general (Dittmann et al., 2015a), with 'cattle-types' in bovini, alcelaphini, hippotragini, and all caprinae investigated so far, and 'moose-types' in the cephalophini, but with both types represented among the antilopini. This variation begs for an explanation.

So far, the main explanation has focussed on the benefits of having a distinct difference in the digesta phases in 'cattle-types', which should facilitate a more efficient harvest of microbes from the reticulorumen by 'digesta washing', where the faster-moving fluid washes microbes out of the particulate digesta and inadvertently selects for faster-growing strains of microbes, thus increasing microbial protein yield (Hummel et al., 2008; Hummel et al., 2015; Clauss and Hummel, 2017). In this scenario, 'moose-types' are considered dependent on salivary defences against tannins; the necessity to enrich saliva with tannin-binding proteins is thought to constrain absolute saliva production and fluid flow through the reticulorumen (Hofmann et al., 2008), and to also lead to more viscous rumen fluid (Clauss et al., 2009b; Lechner et al., 2010) in which particles do not separate as easily from the fluid as in a less viscous environment. To date, including the findings of the

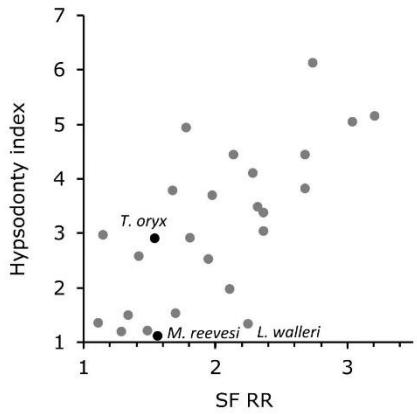


Fig. 4. Relationship between the selectivity factor (SF, a measure for how distinctly the retention of small particles and fluid differ, i.e. a measure for the 'washing mechanism') in the reticulorumen (RR; data collection from Przybyło et al. (2019b; one value per species), including the eland (*Taurotragus oryx*) of the present study and muntjac (*Muntjacus reevesi*) from Przybyło et al. (2019a)) and the hypsodonty index (a measure how high-crowned the cheek teeth of a species are, from Mendoza and Palmqvist (2008)).

present study, ruminant species classified as 'moose-type' based on their reticulorumen morphophysiology (i.e., a low SF RR) appear constrained to a dietary niche of browsing (Codron and Clauss, 2010), whereas 'cattle-type' ruminants apparently (with a high SF RR) exist across the whole browser-grazer spectrum (Fig. 3). The major argument proposed for the absence of 'moose-types' among grazers is the competitive disadvantage of not using an optimal microbial harvesting in the reticulorumen. Pfau et al. (2019) suggested that due to the increased microbial harvest in ruminants with distinct digesta washing, more metabolic faecal nitrogen (a measure for microbial protein) should be excreted in these animals, and measures of apparent nitrogen digestibility might correspondingly be lower compared to species with less distinct digesta washing. In line with this concept, our present findings of a very low degree of digesta washing in eland matches reports of Arman et al., 1975 of particularly low metabolic nitrogen, and particularly high apparent protein digestibility, in eland compared to other ruminant species (Arman and Hopcraft, 1975; Arman et al., 1975).

More experimental passage studies, such as the present one, or more investigations on physical characteristics of reticulorumen contents (Sauer et al., 2017), can add to the catalogue of species considered 'cattle-types' or 'moose-types', and potentially strengthen the relationships between the measures of digesta kinetics and anatomy, such as the intraruminal papillation pattern or omasum size (Przybyło et al., 2019b). These data collection indicate that on the one hand, the concept of comparative forestomach physiology in ruminants follows general overall patterns (Codron et al., 2019; Ehrlich et al., 2019), but that on the other hand, there is a large scatter in the patterns, suggesting that different species evolved different combinations of adaptations. For eland, measures of rumen contents, and more detailed investigations on their intraruminal papillation pattern, would be interesting in this respect. However, adding more species to the pattern will not replace more detailed investigations into the presence of tannin-binding proteins in saliva, or more detailed quantifications of the effect of digesta washing.

More recently, another possible adaptive value of the rumen 'washing mechanism' has received some attention: in the process of sorting particles for regurgitation and rumination, they are inadvertently washed, by the rumen fluid, from adhering siliceous contaminations such as grit or dust (Hatt et al., 2019; Hatt et al., 2020). This mechanism could explain various differences between ruminants and nonruminants, for example the observation that ruminants generally do not achieve the same degree of hypsodonty as nonruminant herbivores (Hatt et al., 2019). This mechanism is subject to various physical principles, among them Stokes' law (Stokes, 1851), which implies that the washing effect will be the more efficient the less viscous the washing fluid is. If we accept that the difference between small particle and fluid retention (quantified as the selectivity factor SF) depends on the viscosity of the rumen fluid, with higher viscosities measured in species that have lower SF (Clauss et al., 2009a; Clauss et al., 2009b; Hummel et al., 2009; Lechner et al., 2010), then one might predict a relationship between high SF and a high degree of hypsodonty as combined evolutionary adaptations to habitats or feeding methods in which ruminants are exposed to high dust or grit loads on their food. The expected relationship appears evident (Fig. 4), but it is of course compromised by the fact that both hypsodonty (Damuth and Janis, 2011) and the SF (Fig. 3) are related to the percentage of grass in the natural diet. Comparative data on the presence of dust and grit in the digestive tract of ruminant species would be ideal to further address this question.

For eland, the combination of a low SF and an intermediate hypsodonty index would suggest a diet with some possible dust/grit contamination and relevant amounts of tannins. Consistent with these predictions, tannins do not appear to have a major influence on eland foraging decisions (Watson and Owen-Smith, 2002), and eland are known to be able to use plants that cattle do not utilize (Hofmeyer, 1970; Retief, 1971; Lightfoot and Posselt, 1977), possibly due to a higher tolerance against secondary plant compounds. More detailed studies on the tolerance of eland against tannins, and comparative data on the size of elands' salivary glands and saliva composition would be particularly welcome in this respect.

The classification of eland as browsers (see Introduction) apparently matches the distinctively lower food intake on the grass hay. A reluctance to ingest grass or grass hay has been reported for several browsing ruminants (Clauss et al., 2003), and also directly for eland (Hofmann, 1973, p. 40; Miller et al., 2010). While the results of the present study are compromised in this respect by the difference in fibre levels, which would suffice to explain a lower intake on the higher-fibre diet (Meyer et al., 2010), it is remarkable that this reluctance cannot be explained by a gut capacity constraint, as the animals showed significantly higher gut fill on the lucerne haylage diet (Table 3). Whether eland could be maintained over longer periods of time on grass or grass hay remains to be tested.

In conclusion, we show that eland have patterns of digesta kinetics typical for 'moose-type' ruminants. Given the ease with which eland can be kept as farm animals (Hansen et al., 1985), they appear as ideal model animals to investigate the consequences of being a 'moose-type' ruminant.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Petr Beluš, Lucie Benešová and Radim Kotrba for assistance with data collection and Heidrun Barleben for laboratory analyses. The study was supported by Czech University of Life Sciences Prague, grant CIGA 20175004.

References

Abdullahi, A.N., 1980. Forage Preference of Cattle and Eland on a Desert Grassland in Southern Arizona. PhD Thesis. University of Arizona.
AOAC, 1995. Official Methods of Analysis of AOAC International. Association of Official

Analytical Chemists, Arlington VA.
Arman, P., Hopcraft, D., 1975. Nutritional studies on east African herbivores. 1. Digestibilities of dry matter, crude fibre and crude protein in antelope, cattle and sheep. Br. J. Nutr. 33, 255–264.
Arman, P., Hopcraft, D., McDonald, I., 1975. Nutritional studies on east African herbivores. 2. Losses of nitrogen in the faeces. Br. J. Nutr. 33, 265–276.
Buys, D., 1990. Food selection by eland in the western Transvaal. S. Afr. J. Wildl. Res. 20, 16–20.
Cerling, T.E., Harris, J.M., Passey, B.H., 2003. Diets of east African Bovidae based on stable isotope analysis. J. Mammal. 84, 456–470.
Clauss, M., Hummel, J., 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Rev. Bras. Zootec. 46, 606–613.
Clauss, M., Kienzle, E., Hatt, J.-M., 2003. Feeding practice in captive wild ruminants: Peculiarities in the nutrition of browsers/concentrate selectors and intermediate feeders. A review. In: Fidgett, A., Clauss, M., Ganslosser, U., Hatt, J.-M., Nijboer, J. (Eds.), Zoo animal nutrition. vol. 2. Filander, Fuerth, Germany, pp. 27–52.
Clauss, M., Kaiser, T., Hummel, J., 2008. The morphophysiological adaptations of browsing and grazing mammals. In: Gordon, I.J., Prins, H.H.T. (Eds.), The Ecology of Browsing and Grazing. Springer, Heidelberg, pp. 47–88.
Clauss, M., Fritz, J., Bayer, D., Hummel, J., Streich, W.J., Südekum, K.-H., Hatt, J.-M., 2009a. Physical characteristics of rumen contents in two small ruminants of different feeding type, the mouflon (*Ovis ammon musimon*) and the roe deer (*Capreolus capreolus*). Zoology 112, 195–205.
Clauss, M., Fritz, J., Bayer, D., Nygren, K., Hammer, S., Hatt, J.-M., Südekum, K.-H., Hummel, J., 2009b. Physical characteristics of rumen contents in four large ruminants of different feeding type, the addax (*Addax nasomaculatus*), bison (*Bison bison*), red deer (*Cervus elaphus*) and moose (*Alces alces*). Comp. Biochem. Physiol. A 152, 398–406.
Clauss, M., Hume, I.D., Hummel, J., 2010. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. Animal 4, 979–992.
Codron, D., Clauss, M., 2010. Rumen physiology constrains diet niche: linking digestive physiology and food selection across wild ruminant species. Can. J. Zool. 88, 1129–1138.
Codron, D., Codron, J., Lee-Thorp, J.A., Sponheimer, M., De Ruiter, D., Sealy, J., Grant, R., Fourie, N., 2007. Diets of savanna ungulates from stable carbon isotope composition of faeces. J. Zool. 273, 21–29.
Codron, D., Hofmann, R.R., Clauss, M., 2019. Morphological and physiological adaptations for browsing and grazing. In: Gordon, I.J., Prins, H.H.T. (Eds.), The Ecology of Browsing and Grazing II. Springer, Berlin, Germany, pp. 81–125.
D'Ammando, G., Parrini, F., Attorre, F., Boitani, L., 2015. Observations on dry season grazing by eland in a Magaliesberg nature reserve, South Africa. Afr. J. Ecol. 53, 112–115.
Damuth, J., Janis, C.M., 2011. On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. Biol. Rev. 86, 733–758.
Derix, J., Ortmann, S., Wiegmann, L., Lawrenz, A., Janssens, G.P.J., Clauss, M., 2019. Retention of solutes and particles in the gastrointestinal tract of a grazing cervid: Père David's deer (*Elaphurus davidianus*). Eur. J. Wildl. Res. 65, 46.
Dittmann, M.T., Hummel, J., Hammer, S., Arif, A., Hebel, C., Müller, D.W.H., Fritz, J., Steuer, P., Schwarm, A., Kreuzer, M., Clauss, M., 2015a. Digesta retention in gazelles in comparison to other ruminants: evidence for taxon-specific rumen fluid throughput to adjust digesta washing to the natural diet. Comp. Biochem. Physiol. A 185, 58–68.
Dittmann, M.T., Runge, U., Ortmann, S., Lang, R.A., Moser, D., Galeffi, C., Schwarm, A., Kreuzer, M., Clauss, M., 2015b. Digesta retention patterns of solutes and different-sized particles in camelids compared with ruminants and other foregut fermenters. J. Comp. Physiol. B. 185, 559–573.
Ehrlich, C., Codron, D., Hofmann, R.R., Hummel, J., Clauss, M., 2019. Comparative omasum anatomy in ruminants: relationships with natural diet, digestive physiology, and general considerations on allometric investigations. J. Morphol. 280, 259–277.
Frei, S., Ortmann, S., Reutlinger, C., Kreuzer, M., Hatt, J.-M., Clauss, M., 2015. Comparative digesta retention patterns in ratites. The Auk: Ornithol. Adv. 132, 119–131.
Gagnon, M., Chew, A.E., 2000. Dietary preferences in extant African Bovidae. J. Mammal. 81, 490–511.
Galat-Luong, A., Nizinski, J.J., Galat, G., 2011. Diet preferences of a Western giant's (Lord Derby's) eland group in a Sahelian dry habitat. Anim. Biol. 61, 485–492.
Grandl, F., Schwarm, A., Ortmann, S., Furger, M., Kreuzer, M., Clauss, M., 2018. Kinetics of solutes and particles of different size in the digestive tract of cattle of 0.5 to 10 years of age, and relationships with methane production. J. Anim. Physiol. Anim. Nutr. 102, 639–651.
Hackmann, T.J., Spain, J.N., 2010. Ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. J. Dairy Sci. 93, 1320–1334.
Hansen, R.M., Skovlin, J.M., Chimwani, D.M., 1985. Ability of eland and cattle to rumen digest forage. East African Agricult. Forest. J. 51, 63–65.
Hatt, J.-M., Codron, D., Müller, D.W.H., Ackermans, N.L., Martin, L.F., Kircher, P.R., Hummel, J., Clauss, M., 2019. The rumen washes off abrasives before heavy-duty chewing in ruminants. Mamm. Biol. 97, 104–111.
Hatt, J.-M., Codron, D., Ackermans, N.L., Martin, L.F., Richter, H., Kircher, P.R., Gerspach, C., Hummel, J., Clauss, M., 2020. Differences in the rumen washing mechanism in sheep fed diets with abrasives of various concentrations and sizes. Palaeogeogr. Palaeoclimatol. Palaeoecol. 550, 109728.
Hejcmanová, P., Homolka, M., Antonínová, M., Hejcman, M., Podhájecká, V., 2010. Diet composition of western Derby eland (*Taurotragus derbianus derbianus*) in the dry season in a natural and a managed habitat in Senegal using faecal analyses. S. Afr. J. Wildl. Res. 40, 27–34.
Hejcmanová, P., Vymyslická, P., Koláčková, K., Antonínová, M., Havlíková, B., Stejskalová, M., Policht, R., Hejcman, M., 2011. Suckling behavior of eland antelopes (*Taurotragus* spp.) under semi-captive and farm conditions. J. Ethol. 29, 161–168.
Hofmann, R.R., 1973. The Ruminant Stomach. East African Literature Bureau, Nairobi.
Hofmann, R.R., 1988. Morphophysiological evolutionary adaptations of the ruminant digestive system. In: Dobson, A., Dobson, M.J. (Eds.), Aspects of Digestive Physiology in Ruminants. Cornell University Press, Ithaca, NY, pp. 1–20.
Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78, 443–457.
Hofmann, R.R., Stewart, D.R.M., 1972. Grazer or browser: a classification based on the stomach-structure and feeding habit of east African ruminants. Mammalia 36, 226–240.
Hofmann, R.R., Streich, W.J., Fickel, J., Hummel, J., Clauss, M., 2008. Convergent evolution in feeding types: salivary gland mass differences in wild ruminant species. J. Morphol. 269, 240–257.
Hofmeyer, J.M., 1970. A review of the food preferences and feeding habits of some indigenous herbivores in the Ethiopian faunal region and some studies on animal plant relationships. Proc. South African Soc. Anim. Prod. 9, 89–99.
Holleman, D.F., White, R.G., 1989. Determination of digesta fill and passage rate from non absorbed particulate phase markers using the single dosing method. Can. J. Zool. 67, 488–494.
Hummel, J., Clauss, M., Zimmermann, W., Johanson, K., Norgaard, C., Pfeffer, E., 2005. Fluid and particle retention in captive okapi (*Okapia johnstoni*). Comp. Biochem. Physiol. A 140, 436–444.
Hummel, J., Steuer, P., Südekum, K.-H., Hammer, S., Hammer, C., Streich, W.J., Clauss, M., 2008. Fluid and particle retention in the digestive tract of the addax antelope (*Addax nasomaculatus*) – adaptations of a grazing desert ruminant. Comp. Biochem. Physiol. A 149, 142–149.
Hummel, J., Südekum, K.-H., Bayer, D., Ortmann, S., Hatt, J.-M., Streich, W.J., Clauss, M., 2009. Physical characteristics of reticuloruminal contents of cattle in relation to forage type and time after feeding. J. Anim. Physiol. Anim. Nutr. 93, 209–220.
Hummel, J., Hammer, S., Hammer, C., Ruf, J., Lechenne, M., Clauss, M., 2015. Solute and particle retention in a small grazing antelope, the blackbuck (*Antilope cervicapra*). Comp. Biochem. Physiol. A 182, 22–26.
Kerr, M.A., Wilson, V.J., Roth, H.H., 1970. Studies on the agricultural utilization of semi-domesticated eland (*Taurotragus oryx*) in Rhodesia. 2. Feeding habits and food preferences. Rhodesian J. Agricult. Res. 8, 71–77.
Lamprey, H.F., 1963. Ecological separation of the large mammal species in the Tarangire game reserve, Tanganyika. East African Wildlife J. 1, 63–92.
Lechner, I., Barboza, P., Collins, W., Fritz, J., Günther, D., Hattendorf, B., Hummel, J., Südekum, K.-H., Clauss, M., 2010. Differential passage of fluids and different-sized particles in fistulated oxen (*Bos primigenius* f. *taurus*), muskoxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*) and moose (*Alces alces*): rumen particle size discrimination is independent from contents stratification. Comp. Biochem. Physiol. A 155, 211–222.
Lechner-Doll, M., Rutagwenda, T., Schwartz, H.J., Schultka, W., von Engelhardt, W., 1990. Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing in a thornbush savanna pasture in Kenya. J. Agric. Sci. (Camb.) 115, 409–420.
Lightfoot, C.J., Posselt, J., 1977. Eland (*Taurotragus oryx*) as a ranching animal complementary to cattle in Rhodesia. 2. Habitat and diet selection. Rhod. Agric. J. 74, 53–61.
Littlejohn, A., 1968. The feeding behaviour of eland. Br. Vet. J. 124, 335–341.
Mambrini, M., Peyraud, J.L., 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculation based on fecal collection. Reprod. Nutr. Dev. 37, 427–442.
Mendoza, M., Palmqvist, P., 2008. Hypsodonty in ungulates: an adaptation for grass consumption or for foraging in open habitat? J. Zool. 274, 134–142.
Meyer, K., Hummel, J., Clauss, M., 2010. The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. Mammal Rev. 40, 221–245.
Miller, M., Weber, M., Valdes, E.V., Neiffer, D., Fontenot, D., Fleming, G., Stetter, M., 2010. Changes in serum calcium, phosphorus, and magnesium levels in captive ruminants affected by diet manipulation. J. Zoo Wildlife Med. 41, 404–408.
Müller, D.W.H., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J., Clauss, M., 2013. Assessing the Jarman-Bell principle: scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. Comp. Biochem. Physiol. A 164, 129–140.
Nge'the, J.C., Box, F.W., 1976. Botanical composition of eland and goat diets on an acacia-grassland community in Kenya. J. Range Manag. 30, 290–293.
Parrini, F., D'Ammando, G., Marshal, J.P., 2019. Seasonal shifts in habitat use and diet by eland confined in a small fenced reserve. Afr. J. Ecol. 57, 394–403.
Pfau, F., Clauss, M., Hummel, J., 2019. Cattle and sheep – a hypothesis for a basic difference in digestive physiology. Adv. Anim. Biosci. 10, 486.
Przybyło, M., Clauss, M., Ortmann, S., Kowalski, Z.M., Górka, P., 2019a. The effect of fructose supplementation on feed intake, nutrient digestibility and digesta retention time in Reeves's muntjac (*Muntiacus reevesi*). J. Anim. Physiol. Anim. Nutr. 103, 1684–1693.
Przybyło, M., Hummel, J., Ortmann, S., Codron, D., Kohlschein, G.-M., Kilga, D., Smithyman, J., Przybyło, U., Świerk, S., Hammer, S., Hatt, J.-M., Górka, P., Clauss, M., 2019b. Digesta passage in nondomestic ruminants: separation mechanisms in 'moose-type' and 'cattle-type' species, and seemingly atypical browsers. Comp. Biochem. Physiol. A 235, 180–192.
Renecker, L.A., Hudson, R.J., 1990. Digestive kinetics of moose, wapiti and cattle. Anim. Prod. 50, 51–61.

Retief, G.P., 1971. The potential of game domestication in Africa with special reference to Botswana. J. S. Afr. Vet. Assoc. 42, 119–127.
Robbins, C.T., Spalinger, D.E., Van Hoven, W., 1995. Adaptations of ruminants to browse and grass diets: are anatomical-based browser-grazer interpretations valid? Oecologia 103, 208–213.
Sauer, C., Clauss, M., Bertelsen, M.F., Weisbjerg, M.R., Lund, P., 2017. Rumen content stratification in the giraffe (*Giraffa camelopardalis*). Comp. Biochem. Physiol. A 203, 69–76.
Sponheimer, M., Lee-Thorp, J.A., DeRuiter, D.J., Smith, J.M., Van der Merwe, J., Grant, C.C., Ayliffe, L.K., Robinson, T.F., Heidelberger, C., Marcus, W., 2003. Diets of southern African Bovidae: stable isotope evidence. J. Mammal. 84, 471.
Steuer, P., Südekum, K.-H., Tütken, T., Müller, D.W.H., Kaandorp, J., Bucher, M., Clauss, M., Hummel, J., 2014. Does body mass convey a digestive advantage for large herbivores? Funct. Ecol. 28, 1127–1134.
Stokes, G.G., 1851. On the effect of internal friction of fluids on the motion of pendulums. Transac. Cambridge Phil. Soc. 9 (ii), 8–106.
Stynder, D.D., 2009. The diets of ungulates from the hominid fossil-bearing site of Elandsfontein, Western cape, South Africa. Quat. Res. 71, 62–70.
Thielemans, M.F., François, E., Bodart, C., Thewis, A., 1978. Mesure du transit gastro-intestinal chez le porc a l'aide des radiolanthanides. Comparaison avec le mouton. Annales de Biologie Animale Biochimie Biophysique 18, 237–247.
Udén, P., Colucci, P.E., Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31, 625–632.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca.
van Zyl, J.H.M., 1965. The vegetation of the S.a. Lombard nature reserve and its utilization by certain antelopes. Zool. Afr. 1, 55–71.
Venter, J.A., Kalule-Sabiti, M.J., 2016. Diet composition of the large herbivores in Mkambati nature reserve, eastern cape, South Africa. S. Afr. J. Wildl. Res. 46, 49–56.
Watson, L.H., Owen-Smith, N., 2000. Diet composition and habitat selection of eland in semi-arid shrubland. Afr. J. Ecol. 38, 130–137.
Watson, L.H., Owen-Smith, N., 2002. Phenological influences on the utilization of woody plants by eland in semi-arid shrubland. Afr. J. Ecol. 40, 65–75.

APPENDIX 4

Curriculum vitae

PERSONALIA

First name	**Lucie**
Surname	**Stoklasová**
Date of birth	29.5.1991
Address	Josefův Důl 347, 468 44 Jablonec and Nisou
Telephone number	721 290 379
E-mail	stoklasoval@ftz.czu.cz
Nationality	Czech

EDUCATION

2017- 2021 **Doctoral study**
Czech University of Life Sciences Prague
Faculty of Tropical AgriSciences
Study programme: Tropical Agrobiology and Bioresource Management
Thesis: Diet quality and mineral status of large herbivores in West African savanna ecosystems

2015-2017 **Master's degree**
Czech University of Life Sciences Prague
Faculty of Tropical AgriSciences
Study programme: Wildlife Management
Thesis: Soils in Africa as source of mineral nutrition for large herbivores

2010-2015 **Bachelor's degree**
Czech University of Life Sciences Prague
Faculty of Tropical AgriSciences
Study programme: Sustainable rural development in tropics and subtropics
Thesis: Efektivita multilaterálního přístupu pro řešení otázky dodržování lidských práv – příklad Barmy

ABRODAD EXPERIENCES

07/2017 – 08/2017 **Internship IZW Berlin in Germany**
Leibniz Institute for Zoo and Wildlife Research
Laboratory of Evolutionary ekology
- laboratory work, determination of mineral concentrations in faeces by ICP-OES

02/2017 **Sample collection, Senegal**
the Bandia reserve and the Fathala reserve

	- Derby eland translocation, samples collection (blood, hair, faeces)
12/2018	**Sample collection, Senegal** - sample collection (soil)

PROJCET PARTICIPATION

2017-2018	Bioavailability and utilization of macro- and micro- elements in soil resources for nutrition of non-domesticated large herbivores (CIGA-20175004). Investigator: Ing. Lucie Stoklasová
2017	Dynamika druhové diverzity vybraných skupin obratlovců a jejich zdrojů v klimaticky kontrastních tropických oblastech (IGA – 20205015)

LANGUAGE SKILLS	English – proficient user

APPENDIX 5

List of author's publications

Publications in scientific journals:

Stoklasová L, Váhala J, Hejcmanová P. 2020. Minerals in the blood, hair, and faeces of the critically endangered Western Derby eland under human care in two wildlife reserves in Senegal. Biological Trace Element Research 195:105-109. (IF 2.64)

Stoklasová L, Váhala J, Hejcmanová P. 2020. Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) in two conservation breeding reserves in Senegal. Animal Physiology and Animal Nutrition 00:1-4. (IF 1.6)

Hejcmanová P, Ortmann S, **Stoklasová L**, Clauss M. 2020. Digesta passage in common eland (*Taurotragus oryx*) on a monocot or a dicot diet. Comparative Biochemistry and Physiology, Part A 246: 110720. (IF 1.97)

Scientific conference contribution:

Hejcmanová P, **Stoklasová L**, Grúňová M, Brandlová K, Žáčková M. 2017. Preferences of Western Derby elands for macro- and microelements in the Fathala and Bandia reserves: potential key knowledge for conservation. Antelope, giraffe, hippo in the 21 st century: conservation action in Africa, Prague, Czech Republic, 19.2.-25.2.2017.Book of Abstracts p. 43.

Stoklasová L, Hejcmanová P. 2018. Macro- and microminerals in blood, hair, and faeces of the critically endangered Western Derby elands in Senegal. GBCC, 25th – 26th September 2018, Prague, Czech Republic.

Stoklasová L, Hejcmanová P. 2017. Biochemical and mineral blood profile of Western Derby eland in the conservation breeding population in Senegal. 11th International conference on Behaviour, Physiology and Genetics of Wildlife – 4th. – 7th. 10. 2017, Berlin, Germany, Book of Abstracts p. 123.

Stoklasová L, Hejcmanová P. 2019. Diet quality of zebra and buffalo in semi-arid and sub-humid environments in Senegal. Wildlife Research and Conservation, *30th September – 2nd October 2019,* Berlin, Germany, Book of Abstracts p. 104.