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

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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

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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/

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]

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.

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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.

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