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A comparative study on physiological plasma mineral mapping of sloth bears *(Melursus ursinus)* in *in-situ* vis-a-vis semi captivity conditions

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ABSTRACT

To determine if habitat influenced the physiological plasma mineral profiles in sloth bears, a study was conducted to establish the normal physiological plasma mineral profiles of free-ranging and semi-captive Indian sloth bears. Plasma samples from 28 sloth bears were analysed for their mineral concentrations. The concentration of microminerals (mg L^{-1}) namely Zn, Co, Fe, Mn, Cr, Cu respectively were 1.56±0.90; 0.85±0.42; 3.85±1.11; 0.29±0.27; 0.45±0.01; 1.00±0.85; and macro minerals (mg dL^{-1}) like Mg, Ca, Na, K, P were 2.06±0.72; 8.89±2.28; 42.3±5.96; 10.7±2.04; 23.2±3.55, respectively. It was found that plasma of free ranging sloth bears showed higher concentrations of Zn, Fe, Mn, Cu, Mg, Na, K, whereas, samples of bears in semi captivity showed higher Co, Ca, P, and Cr levels, which signifies that free ranging animal have a choice over their feeding but in semi-captivity, it is controlled and man-made.

Key words: Free ranging, habitat, mineral profiles, semi-captivity, sloth bear

INTRODUCTION

The Sloth bear is endemic to the Indian sub-continent and found in India, Sri Lanka, Nepal, Bhutan and Bangladesh. In India, sloth bears are distributed from the southern tip of the Western Ghats to the foothills of the Himalayas. Sloth bears (*Melursus ursinus*) have been listed as vulnerable by the IUCN red list with the estimates of about 20,000 or fewer individuals and less than 10,000 adults existing in the wild. The wild population of sloth bears has declined by 30 – 49% in the last 30 years (Graesli et al., 1999). Sloth bears in India have been exploited for generations as "dancing bears". Wildlife SOS, a non-profit organization working to protect and conserve the natural resources of India, secured the end of the dancing bear trade. Relinquished and confiscated sloth bears now reside in four rescue/rehabilitation centers in India, overseen by Wildlife SOS.

Sloth bears inhabit the tropical and subtropical regions of the Indian subcontinent and are distributed from the foothills of the Himalayas to the southern end of the Western Ghats mountain range, as well as in the island of Sri Lanka (Prater, 1965). Despite long periods of evolutionary separation, sloth bears have retained their carnivorous morphology but have developed unique physiologic adaptations to live in tropical/ subtropical climates, with substantial reliance on frugivory (feeding on fruits) and myrmecophagy (feeding on insects like termites or ants) (Laurie and Seidensticker, 1977; Gokula et al., 1995; Bargali

et al., 2004). Both the micro and macro mineral content in the blood varies according to the feeding habit and available feed resources. Sloth bears have metabolic differences compared to other species of bears. They have been observed to have an overall lower metabolic rate compared to brown and polar bears, and torpor (winter sleep) is unheard of in case of sloth bears (McNab, 1992). Minerals are inorganic components which are required in very minute quantities, yet very important for the normal physiology of animals. Assessment of the plasma mineral profile is an indication of the physiological as well as nutritional status of an animal. Blood mineral profile is important in understanding the impact of disease both at an individual and population level of animals. Macro-minerals namely calcium (Ca), phosphorus (P) and magnesium (Mg) and micro-minerals namely, copper (Cu), zinc (Zn) and cobalt (Co) are essential for fundamental physiological functions like cell morphology, integrity, motility, hormone secretion and metabolism (Kong et al., 2015; Diskin, 2016). In this study, we have tried to establish the reference plasma mineral profile of sloth bears residing in Free ranging wild as well as in semi-captivity. We also have tried to observe the change in the plasma mineral profile associated with change in the habitats.

MATERIAL AND METHODS

Study site and animals

Free-ranging wild sloth bears rescued from human animal conflict situations in the state of Karnataka and captive sloth bears rehabilitated at lifetime care centre of Wildlife SOS at Bannerghatta Bear Rescue Centre were utilized for this study. The samples were collected from 28 numbers of sloth bears from both free ranging wild sloth bears and semi-captive sloth bears rehabilitated at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bangalore, Karnataka, India. Both these locations are within the documented habitat range for sloth bear species. The sloth bears in the wild were free ranging ones and the ones in captivity were individually rehabilitated under different situations and kept under captivity for more than 7 - 10 years.

All the sloth bears were apparently healthy. The adults were having normal behavioural responses, and found to be clinically healthy during examination at the time of sampling (as determined by body temperature, hydration, heart/respiration rate, and a detailed external physical examination).

Sampling procedure

Each bear was sampled only once for this study. Sloth bears were immobilized using a ketamine-xylazine combination; ketamine hydrochloride (5 mg kg^{-1} body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg kg^{-1} body weight; Troy Laboratories Pty Ltd., Smithfield, New South Wales 2164, Australia). These drugs were administered using a distance projectile drug delivery system. Blood was collected from the jugular vein within 10 min after immobilization using a 20-gauge sterile hypodermic needle in vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA), with heparin for mineral studies. Samples were immediately stored on cool packs at 4–8 °C and transported to the testing laboratory.

Laboratory analysis

1 ml of the plasma sample was taken and 1 ml of 5N HCl was added to it. The volume was made to 10 ml by adding double distilled water (1:10 dilution). Mineral contents in plasma samples were estimated using Optima 8000 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Perkin Elmer, Shelton CT#064840, USA).

Statistical analysis

After quantifying the mineral concentration, the collected data was tabulated. Descriptive statistical analysis like mean ± SD (Standard deviation), SEM (Standard error mean), range and 95% CL (Confidence level) of mean were calculated for captive, wild and entire population respectively. The significance of difference in the mean value based on habitat was performed by independent t-test for normally distributed data and non-parametric Mann Whitney U-test for variables that violated the normality. The normality test was

conducted by Kolmogorov-Smirnov and Shapiro-Wilk test. The homogeneity of variance of normally distributed variable was conducted by Levene's test and for non-parametric data by Kruskal-Willis one-way ANOVA test. All processing of data was conducted with the software packages Microsoft Excel 2010 for data storage and SPSS version 21 for statistical analysis. The P-values $P<0.05$ and $P<0.01$ with an alpha level of 95% were assumed as statistically significant (*) and highly significant (**) respectively.

RESULTS AND DISCUSSION

Basic descriptive statistical analysis on different mineral concentrations of entire (semi-captive and free range) populations (Table 1) were established as a standardised value. Mean value of each micro-mineral and macro-mineral parameters based on habitat (Table 3) were compared to evaluate the significance difference between each other. Comparing the mean of these mineral concentrations, it was found that Zn, Fe, Mn, Cu, Mg, Na and K were higher in wild population, whereas Co, Cr, Ca and P were higher in captive populations (Fig. 1 and 2). By normality test it was determined that Zn, Co, Fe, Mn, Cu, Mg, Na, K, Cr and Ca were deviating from the normal distribution curve by the Normality test as shown in Table 2. Mean (± S.D.) of Cu was found to be significantly higher in wild (1.74±0.92) as compared to captive populations (0.594±0.46) (Table 3). Mean (± S.D.) of Mg was found to be significantly higher in the wild conditions (2.57±0.79) as compared to captive. (1.78±0.51) (Table 3). Mean (± S.D.) of Na was found to be significantly higher in wild habitat (45.5±6.30) as compared to captivity (40.4±5.06) (Table 3). Mean (± S.D.) of Zn, Fe, Mn and K were found to be higher in the wild (1.68±1.35), (4.21±1.55), (0.40±0.43) and (11.6±2.53) as compared to captivity (1.49±0.56), (3.65±0.76), (0.22±.06) and (10.2±1.60), respectively but not significantly related (Table 3). Mean (± S.D.) of Co (0.93±0.40) and Cr (0.45±0.01) were found to be significantly higher in semi-captivity as compared to wild habitat (0.71± 0.42) and (0.44±.01) respectively (Table 3). Mean (± S.D.) of Ca and K were observed to be higher in captivity (9.20±2.49) and (23.8±4.22) as compared to wild habitat (8.33± 1.83) and (22.1± 1.45), respectively but not significantly related with each other (Table 3).

Table 1. Statistical analysis of mineral concentrations of entire (semi-captive and free range) Sloth bear population

Parameters	Sample size	Mean ± SD	SEM	Range		95% CL of Mean	
				Minimum	Maximum	LCL	UCL
Zn (mg L^{-1})	28	1.56±0.90	0.17	0.32	4.14	1.26	1.90
Co (mg L^{-1})	28	0.85± 0.42	0.08	0.37	1.23	0.70	1.00
Fe (mg L^{-1})	28	3.85±1.11	0.21	2.40	7.80	3.49	4.25
Mn (mg L^{-1})	28	0.29± 0.27	0.05	0.14	1.62	0.22	0.40
Cr (mg L^{-1})	28	0.45± 0.01	0.00	0.43	0.48	0.44	0.46
Cu (mg L^{-1})	28	1.00±0.85	0.16	0.01	2.76	0.70	1.31
Mg (mg dL^{-1})	28	2.06±0.72	0.14	1.11	3.81	1.83	2.32
Ca (mg dL^{-1})	28	8.89±2.28	0.43	5.97	15.4	8.09	9.72
Na (mg dL^{-1})	28	42.3±5.96	1.12	26.5	51.0	39.9	44.4
K (mg dL^{-1})	28	10.7±2.04	0.38	8.08	16.5	10.0	11.4
P (mg dL^{-1})	28	23.2±3.55	0.67	16.2	28.7	21.8	24.5

*SD- Standard deviation, *CL- Confidence level, *LCL- Lower confidence level, *UCL- Upper confidence level

Table 2. Normality test of mineral concentrations of Sloth bear population based on habitat

Parameters	Kolmogorov-Smirnov[a]	Shapiro-Wilk
Zn (mg L^{-1})	0.001	0.001**
Co (mg L^{-1})	0.000	0.000**
Fe (mg L^{-1})	0.180	0.002**
Mn (mg L^{-1})	0.000	0.000**
Cr (mg L^{-1})	0.000	0.002**
Cu (mg L^{-1})	0.200#	0.012*
Mg (mg dL^{-1})	0.004	0.002**
Ca (mg dL^{-1})	0.000	0.001**
Na (mg dL^{-1})	0.200#	0.040*
K (mg dL^{-1})	0.200#	0.041*
P (mg dL^{-1})	0.200#	0.359

#. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

On analysing the homogeneity of variance it was found that concentrations of Co, Cr, Cu, Mg and Na were found to be significant ($p<0.01$) with respect to habitat while other parameters were found to have no significant effect.

Literature pertaining to the wild Sloth bear plasma mineral profile is very limited. Perhaps, this is the first study of its kind where we have tried to establish the normal physiological range of sloth bears and its variation when they are free ranging or when kept in semi captivity. Mineral content in the plasma varies according to the feeding habit and available feed resources. Both the micro and macro minerals differ in blood samples of the sloth bears under different habitats, which can be attributed to the availability of the feed sources. Plasma of free ranging bears showed a higher Zn, Fe, Mn, Cu, Mg, Na, K, whereas, that in semi captivity showed higher Co, Ca, P and similar Cr level, which signifies that

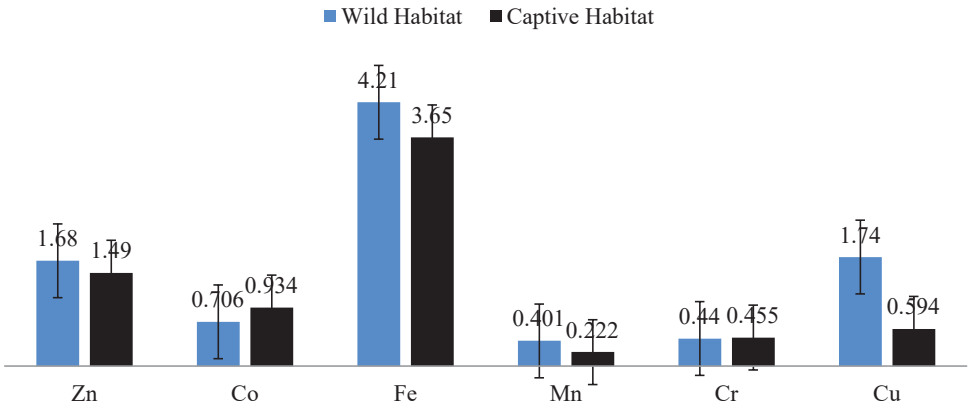

Fig. 1. Comparative study of micro-minerals with respect to habitat

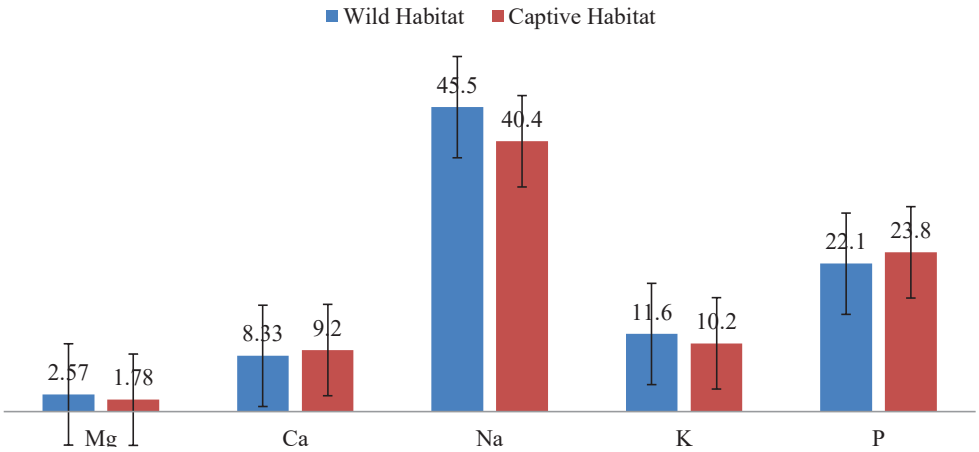

Fig. 2. A comparative study of macro-minerals with respect to habitat

Table 3. Analysis of Homogeneity of Variance and comparison of mean mineral value of sloth bear population based on habitat

Parameters	Free Ranging Wild				Captive				f-value	p-value
	Sample Size	Mean±S.D.	95% CL of Mean		Sample Size	Mean±S.D.	95% CL of Mean			
			LCL	UCL			LCL	UCL		
Zn (mg L^{-1})	10	1.68±1.35	0.98	2.57	18	1.49±0.56	1.25	1.75	[a]0.737	[d]0.759
Co (mg L^{-1})	10	0.710.42±	0.46	0.95	18	0.93±0.40	0.75	1.12	[a]0.026*	[d]0.024*
Fe (mg L^{-1})	10	4.21±1.55	3.35	5.17	18	3.65±0.76	3.33	4.00	[a]0.314	[d]0.332
Mn (mg L^{-1})	10	0.40±0.43	0.24	0.69	18	0.22±0.06	0.19	0.25	[a]0.061	[d]0.064
Cr (mg L^{-1})	10	0.44±0.01	0.44	0.44	18	0.45±0.01	0.45	0.46	[a]0.005**	[d]0.004**
Cu (mg L^{-1})	10	1.74±0.92	1.17	2.30	18	0.59±0.46	0.38	0.79	[a]0.003**	[d]0.003**
Mg (mg L^{-1})	10	2.57±0.79	2.09	3.06	18	1.78±0.506	1.58	2.02	[a]0.003**	[d]0.003**
Ca (mg L^{-1})	10	8.33±1.83	7.27	9.40	18	9.20±2.49	8.16	10.3	[a]0.472	[d]0.494
Na (mg L^{-1})	10	45.5±6.30	40.9	48.6	18	40.4±5.06	37.8	42.7	[a]0.005**	[d]0.003**
K (mg L^{-1})	10	11.6±2.53	10.1	13.0	18	10.2±1.60	9.51	11.0	[a]0.137	[d]0.146
P (mg L^{-1})	10	22.1±1.45	21.3	22.9	18	23.8±4.22	21.8	25.7	[b]0.001	[c]0.131

*p<0.05, **p<0.01, *SD- Standard deviation, *CL- Confidence level, *LCL- Lower confidence level, *UCL- Upper confidence level,

a Parameteric independent One-way ANOVA to analyse the homogeneity of Variance of Mineral concentrations based on habitat

b Non-parameteric Mann Kruskal-Wallis test to analyse the homogeneity of Variance of Mineral concentrations based on habitat

c Parameteric independent t-test to analyse the difference between mean (±S.D) of Mineral concentrations based on habitat

d Non-parameteric Mann Whiteney U-test to analyse the difference between mean (±S.D) of Mineral concentrations based on habitat

the animals in free ranging have a choice over their feeding, but in semi captivity it is somewhat controlled. However, in semi captivity, they had free access to the forest apart from being fed.

Graesli et al. (2014, 2015) studied the change in the macro-mineral profile in brown bears. The plasma calcium content in this study was lower than the reported values of Graesli et al. (2015) in brown bears inhabiting in Sweden which might be due to the change in the geographical location or the type of bear studied. This can be correlated to the availability of feed and the animal's feeding habits as well. Regarding Mg, the result obtained in the present study corroborates with the reported values of Graesli et al. (2014, 2015).

CONCLUSION

Considering the plasma mineral status in free ranging and semi-captive habitat, a significant change in Co ($P<0.05$), Cr ($P<0.01$), Mg ($P<0.01$), Na ($P<0.01$) was observed, which may be attributed to the availability of feed resources and the selective feeding behaviour of the animal. There was no change ($P>0.05$) in other mineral status.

For assessing the animal health, plasma mineral status is an important indicator. An attempt was made to establish the reference values of plasma mineral profile for apparently healthy Indian sloth bears.

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