etorphine/ml, Pharmacy Veterinary Faculty Utrecht) with Zalopine®(10mg medetomidine/ml, Orion Pharma Animal Health). After 1 phlebotomy under general anesthesia (volume 8,5L) and an intensive successful training program for conditioned blood collection, phlebotomy could be performed without sedation. After 13 months and a 22 phlebotomies (volume varied between 0,2-6,8L) serum GGT decreased to 66U/Land the iron saturation dropped to below 80%. His general condition improved dramatically, including his appetite and activity and even hairs on the tip of his ears have grown back.

Acknowledgements:
The author would like to thank the rhino keepers and the head of the African section of Rotterdam Zoo for their support and commitment to the animal training program.

References:

1.Kock, N., C. Foggin, M.D. Kock, and R. Kock. 1992. Hemosiderosisin the black rhinoceros (Diceros bicornis): A comparison of free-ranging and recently captured with translocated and captive animals. J. Zoo Wildl. Med. 23:230-234
2. Paglia, D.E., and P. Dennis. 1999. Role of chronic iron overload in multiple disorders of captive black rhinoceroses (Diceros bicornis). Proc. Am. Assoc. Zoo Vet. Pp. 163-171.
3. ISIS reference values (ZIMS).

Validation of a rhino specific ferritin enzyme immunoassay for the black rhinoceros (*Diceros bicornis*) for assessing the impact of individual and environmental factors on serum ferritin concentrations.

Jessye Wojtusik and Terri L. Roth
Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden

Hemochromatosis (iron overload disease) is a potentially fatal disease that sometimes affects the critically endangered black rhinoceros (*Diceros bicornis*). Therefore, it is important to develop methods for monitoring the progression of iron storage (hemosiderosis), diagnosing the disease state, evaluating treatment efficacy, and assessing environmental factors that may impact iron absorption in this species. Ferritin is an acute phase protein complex that binds iron for storage and is used to measure total iron stores. Traditionally, an enzyme immunoassay (EIA) developed for horses was used to measure rhino ferritin. However, ferritin is species-specific, and recent inconsistencies in the equine EIA results prompted our lab to create a rhino-specific assay originally developed and validated for Sumatran rhinoceros (*Dicerorhinus sumatrensis*) ferritin. Our goal was to validate this assay for the black rhino and to investigate the influence of several environmental and individual factors on ferritin concentrations. Ferritin was isolated from black rhino liver then subjected to gel electrophoresis, using equine and Sumatran rhino ferritin as positive controls to verify sample purity. Staining with potassium ferricyanide confirmed the isolated protein was positive for iron. Western blot analysis and a parallelism were used to validate cross-reactivity of the Sumatran rhino ferritin antibody with black rhino ferritin. Serum samples (n= 681) collected during 1990-2016 from 28 black rhinos ranging in age from <1 to 32 years and maintained at 11 U.S. institutions were analyzed using the rhino ferritin EIA. Data were analyzed by NOVA and correlation analysis with the following factors included: individual, subspecies, location, sex, age, age group, collection date (month, year, season), captive/wild status of individual at birth and of parents, and time in captivity. All data are reported as means± SEM. The mean serum ferritin concentration for all samples was 3090.5 ± 98.4 ng/ml (range: 84.6 to 19,296.1 ng/ml). Concentrations differed among individuals ($P < 0.05$) and were higher ($P < 0.05$) in males (3539.2± 108.6 ng/ml) than females (1094.7 ± 124.0). Ferritin was lower ($P<0.05$)in pre-pubertal (< 5 years old) individuals (1761.8 ± 152.7 ng/ml) than adults(6-25 years; 3392.7 ± 118.3) and seniors (26+ years; 3570.7 ± 347.3). Ferritin levels in adults and seniors were not different ($P> 0.05$), but the senior sample size was small limiting the possibility of

significance. Overall, ferritin concentrations were not directly correlated with age (R2= 0.25). Interestingly, ferritin differed between subspecies with concentrations higher in Southern black rhinos (3264.8 ± 112.1) than in the Eastern sub-species (2322.5 ± 184.4). Location of birth (captive vs. wild) for the individual, or its parents, did not influence ferritin levels (P> 0.05) and there was no correlation found between amount of time spent in captivity and serum ferritin (R2= 0.19). Ferritin concentration was not impacted by date, month or season of serum collection (P> 0.05). The rhino-specific ferritin EIA was validated for use in measuring black rhino serum ferritin concentrations. The resulting data indicate that ferritin concentrations are variable and influenced by individual, subspecies,
sex, and age group and therefore these factors must be considered when trying to diagnose hemochromatosis.
(The PI was supported by a generous gift from an anonymous donor and the study was supported by Michael and Tucker Coombe.)

Endocrine monitoring of wild Asian elephants in modified landscapes

Sreedhar Vijayakrishnan[1,2,] M Ananda Kumar[2], R Sriram[2], Vinod Kumar[3], G Umapathy[3], Anindya Sinha[1,2]
[1]School of Natural Sciences and Engineering, National Institute of Advanced Studies, Indian Institute of Science Campus
[2]Nature Conservation Foundation, Mysore, India 3Laboratory for Conservation of Endangered Species

Biologically, stress has been defined as the response evoked in an individual when a threat or a particular stressor is perceived. Although a coping mechanism acting through secretion of glucocorticoid and related hormones, stress may have negative effects on individuals by suppressing their reproduction, altering feeding and movement patterns, as well as negatively affecting their immune responses. Our study attempted to monitor stress responses in Asian elephants in a plantation-dominated landscape and a forest-farm matrix from November 2013 to April 2014, and August 2015 to March 2016. 5 to 10 g of dung samples were collected from identified individuals on an opportunistic basis and wherever individual identification of samples was not possible, identification was ensured at least at a herd level. Samples were dried using a heater-blower setup, and were preserved in airtight containers with silica gel devoid of moisture. The hormones were later extracted from these samples using enzyme-linked immunosorbent assay (ELISA) techniques. We used hierarchical Linear Mixed Effect (LME) models to assess the factors influencing FGM concentrations in elephants under several circumstances. Through model averaging tasks, we assessed the best fitting model, which indicated that the glucocorticoid metabolite concentrations in elephants are affected by age-sex classes and occurrence of disturbance events. The data showed a stark increase in most age-sex categories post perturbations, or in other words, human disturbances had a fairly high negative influence on the physiology of elephants, characterized by elevated FGM concentration. In 2015-16, we collected 512 dung samples and preliminary results of the analysis comparing the two landscapes, shows that the mean FGM concentrations in elephants of Sathyamangalam (n=38) seem to be significantly higher ($p<0.00001$; $t=-4.958$) than that of the elephants in Valparai (n=474), irrespective of their age, sex, group composition, and habitat use. This could potentially be because of the higher frequency of interactions between elephants and humans in Sathyamangalam, where elephants come to croplands on a daily basis and indulge in crop damages as opposed to Valparai where elephants use human-use areas for movement, than for activities such as foraging. A similar result is obtained by comparing samples collected from anthropogenic habitats in Valparai (tea/coffee plantations, settlements, and roads and mud paths) and that in Sathyamangalam (banana fields and roads) where the mean FGM of the latter seem to be significantly higher than that in Valparai ($p<0.00001$; $t =-4.961$). FGM levels of males invariably were high across landscapes and this is in