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Faecal corticosterone responses of black rhinos (*Diceros bicornis*) to a transfer between housing facilities within a zoo

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Keywords:
acclimatisation, faecal corticosterone metabolites (FCM), stress

Article history:
Received: 7 July 2014
Accepted: 13 January 2015
Published online: 31 January 2015

Abstract
Faecal corticosterone metabolites (FCM) of four black rhinos (*Diceros bicornis*) at Magdeburg Zoo in Germany were investigated to assess the stress level associated with an internal transfer of rhinos between housing facilities. Moving rhinos within the zoo led to a punctual increase of FCM concentrations in three animals. We observed no signs of a period of acclimatisation, such as increased FCM levels for several days or longer periods. Increased FCM levels of one out of three rhinos re-investigated one year after the enclosure change, may be related to the reproductive status of this female (five to six months before parturition). The moderate FCM response patterns found in this study indicate that the transfer of rhinos between enclosures at Magdeburg Zoo was minimally invasive.

Background

Stress in zoo mammals is a complex phenomenon and a number of physiological and behavioural measures have been developed to evaluate the influence of potential stressors on health and reproduction of mammals in captivity (Morgan and Tromborg 2006; Mason 2010). For a number of reasons, non-invasive measurement of faecal hormone metabolites has become a widely used method for the evaluation of stress in various mammal species under free-ranging and captive conditions (Keay et al. 2006; Hodges et al. 2010).

To sustainably protect the population of the critically endangered black rhino (*Diceros bicornis*), effective management in captivity as well as in its natural environment is needed. Potential stress caused by artificial conditions may be one reason why black rhinos in captivity are not self-sustaining (Carlstead and Brown 2005). Environmental variables, such as enclosure size, noise level or public access, can affect health and cause poor reproductive performance (Carlstead et al. 1999). Faecal corticosterone metabolites (FCM) have been shown to be reliable indicators to investigate stress responses of black rhinos (Brown et al. 2001). There is scientific evidence for higher FCM levels associated with black rhinos that are exposed to the public around a greater portion of the perimeter of their enclosure (Carlstead and Brown 2005). FCM analysis has also been applied to evaluate translocation-related stress in black rhinos under semi-wild (fenced reserves with limited space) or free-ranging conditions, e.g. to assess the period of physiological post-release acclimatisation in order to optimise translocation programmes (MacDonald and Linklater 2007; Linklater 2010).

In this study, we measured FCM concentrations of black rhinos at Magdeburg Zoo in Germany to evaluate the stress level associated with an internal transfer of rhinos between enclosures and to assess possible longer-term acclimatisation responses to the new housing facilities.

Action

The study involved four black rhinos kept in Magdeburg Zoo (Germany) in 2010 and 2011. The group consisted of male 'Madiba' (born in 1992, International Studbook Number 653) and females 'Kenia' (born in 1967, ISB number 0153), 'Mana' (born in 1981, ISB number 0295) and 'Maleika' (born in 1995, ISB number 0559). The females 'Mana' and 'Maleika' were moved into new housing facilities on 1 June 2010, the rhino male followed on 23 August 2010. Female 'Kenia' was left in the old enclosure, where the animal died in October 2013 at the age of 46 years. In contrast to translocations under semi-wild or wild conditions, rhinos were not immobilised or sedated.

Instead, the animals had been trained to enter their crate on a daily basis during the two preceding weeks and were accompanied by familiar keepers during the shift between facilities and afterwards (Grothmann 2012).

The old rhino facilities were part of a large-animal enclosure built in 1967, which was originally planned for three elephants, two hippos and two rhinos (Bürger 1967). The new facilities were designed specifically for black rhinos. There is capacity for 1.2 adult breeding black rhinos with additional space for two calves/subadults. There are three off-exhibition night stalls (30m² each), one for each adult. The visitor hall is composed of a 200 m² indoor enclosure, which may be divided if necessary. This indoor facility has been proved to be very useful if calves are born during the winter. Furthermore, there are three outdoor enclosures. One covers approximately 800m² and is shared with African primates. Another one comprises 1100 m², where rhinos are presented together with African hoofstock and Marabou storks (*Leptoptilos crumeniferus*). The third outdoor enclosure is an off-show boma-like area, which can be separated into two parts of 200 m² and 600 m².

A total of 225 rhino faecal samples were collected on a daily basis by the animal keepers during two periods: between 24 May and 22 June 2010 (including the time when the two females were moved) and between 9 June and 23 July 2011 (10–12 months after moving the three rhinos to the new housing facilities). Keepers collected fresh samples as part of stall cleaning routines in the morning (before 0900). Sample collection and faecal extraction followed a protocol that was originally developed for primates (Ziegler et al. 2000) and had been slightly adjusted for elephants (Rasmussen et al. 2008). Directly after collection, an approximately 10–15 g aliquot taken from the bolus was stored in a polyethylene tube filled with 25 ml of 90% ethanol and stored in a fridge at approximately +8° C until analysis of FCM. Faecal extraction was explained in detail by Ziegler et al. (2000). The total volume and the dry weight of individual samples were determined and FCM concentrations were given as ng/g dry weight. The analysis of FCM concentrations was conducted at the Endocrinological Laboratory of the German Primate Centre in Göttingen (Dr M. Heistermann) via a validated enzyme immunoassay using antibodies raised against corticosterone (Heistermann et al. 2006). Intra-assay coefficients of variation of high and low-value quality controls were 6.7% (high) and 8.4 % (low). Corresponding inter-assay CV values were 7.7% (high) and 12.0% (low). Analyses for regression were carried out to test the relationship between sample dry weight and FCM concentration using best subsets regression. Bivariate comparison of samples was conducted using a t-test and Mann–Whitney U-test. All statistical analyses were carried out using the Sigma Stat program (Jandel Scientific, SanRafael, California).

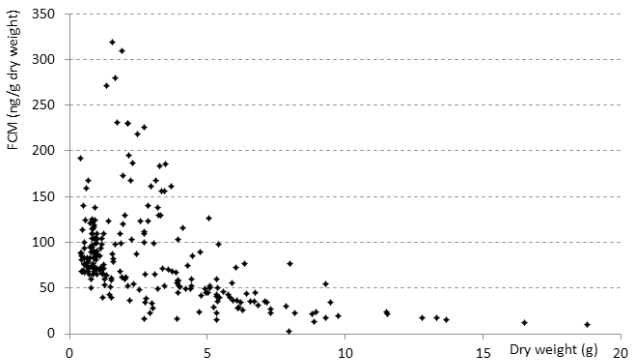

Figure 1. Influence of the original samples' dry weight on concentrations of faecal corticosterone metabolites (ng/g dry weight) calculated from all samples collected. Best criterion: $R^2 = 0.2$.

Consequences

Plotting FCM concentrations of all samples against dry weight reveals that originally heavier samples tend to have lower FCM concentrations ($R^2 = 0.2$; Figure 1). This relationship becomes more evident when looking at each individual and collection period exclusively (mean $R^2 = 0.49$, standard deviation = 0.12). The effect was not expected, as the exact dry weight and the exact volume of the samples are considered and FCM concentrations are presented as ng/g dry weight. However, a similar relationship has already been observed in small birds, where very small faecal samples (< 0.02g) resulted in proportionally higher FCM levels (Millspaugh and Washburn 2004; Goymann 2005). Although the reasons for this effect remain unknown, possible explanations are different extraction efficiencies between small and large samples (Millspaugh and Washburn 2004) or spurious correlations (Goymann 2005). However, to our knowledge, such an effect has not been reported with regard to mammals so far. The relationship between sample weight and FCM concentrations could potentially bias the results of other studies using similar data collection and faecal extraction protocols.

FCM profiles of all three females (including 'Kenia', who remained in the former rhino enclosure) show a punctual peak after three ('Mana', 'Maleika') to four ('Kenia') days after 'Mana' and 'Maleika' were moved (Figure 2). ACTH challenges, carried out by Brown et al. (2001), have shown that black rhino faecal samples reflect corticosterone levels during the previous one to three days before collection. Thus, the peaks found in our study can clearly be linked with the event of moving the rhinos to the new enclosure. The punctual response indicates that rhinos responded to a single event. The profiles do not support a period of acclimatisation in terms of increased FCM levels over several days or even longer. In contrast, rhinos captured under semi-wild or wild conditions, enduring much more invasive procedures (capture, immobilisation, transport over much longer distances and boma-management for several weeks), responded with 2.5 to five times increase in glucocorticoid levels, reducing over a period of up to six weeks after arrival of the rhinos at their destinations (Turner et al. 2002; MacDonald and Linklater 2007; Linklater et al. 2010). For example, Turner et al. (2002) emphasised that their study animals experienced extreme stress: rhinos were captured in a reserve in Zimbabwe before being crated and shipped to a zoo and a wildlife reserve in the USA. These authors suggest that the FCM profile (decreasing levels for at least six weeks post-arrival) represent the 'upper end of the physiological scale'. Although direct comparisons between such highly invasive translocations and the transfer of rhinos between housing facilities within a zoo are not appropriate, our findings may illustrate the 'lower end' of the stress-response scale of black rhinos. Owing to the relationship between dry weight and FCM concentration mentioned above (higher values when lighter samples were collected and vice versa), no transfer-peak can be identified with regard to the male rhino.

The dry weight of samples collected in 2010 was lower than in 2011, which applies to each individual investigated: 'Madiba' ($U = 335.0$; $P \leq 0.001$), 'Maleika' ($U = 494.0$; $P \leq 0.001$) and 'Mana' ($U = 500.0$; $P \leq 0.001$). While 'Madiba' had slightly (but not significantly) lower FCM concentrations in 2011 ($t = 1.83$, $df = 62$, $P = 0.072$), 'Maleika' showed clearly lower metabolite concentrations one year after the enclosure change ($U = 1420.0$; $P \leq 0.001$). The opposite trend reveals with regards to 'Mana', showing significantly increased FCM concentrations in 2011 ($U = 535.5$; $P \leq 0.001$; Figure 3). The dry weight-FCM concentration

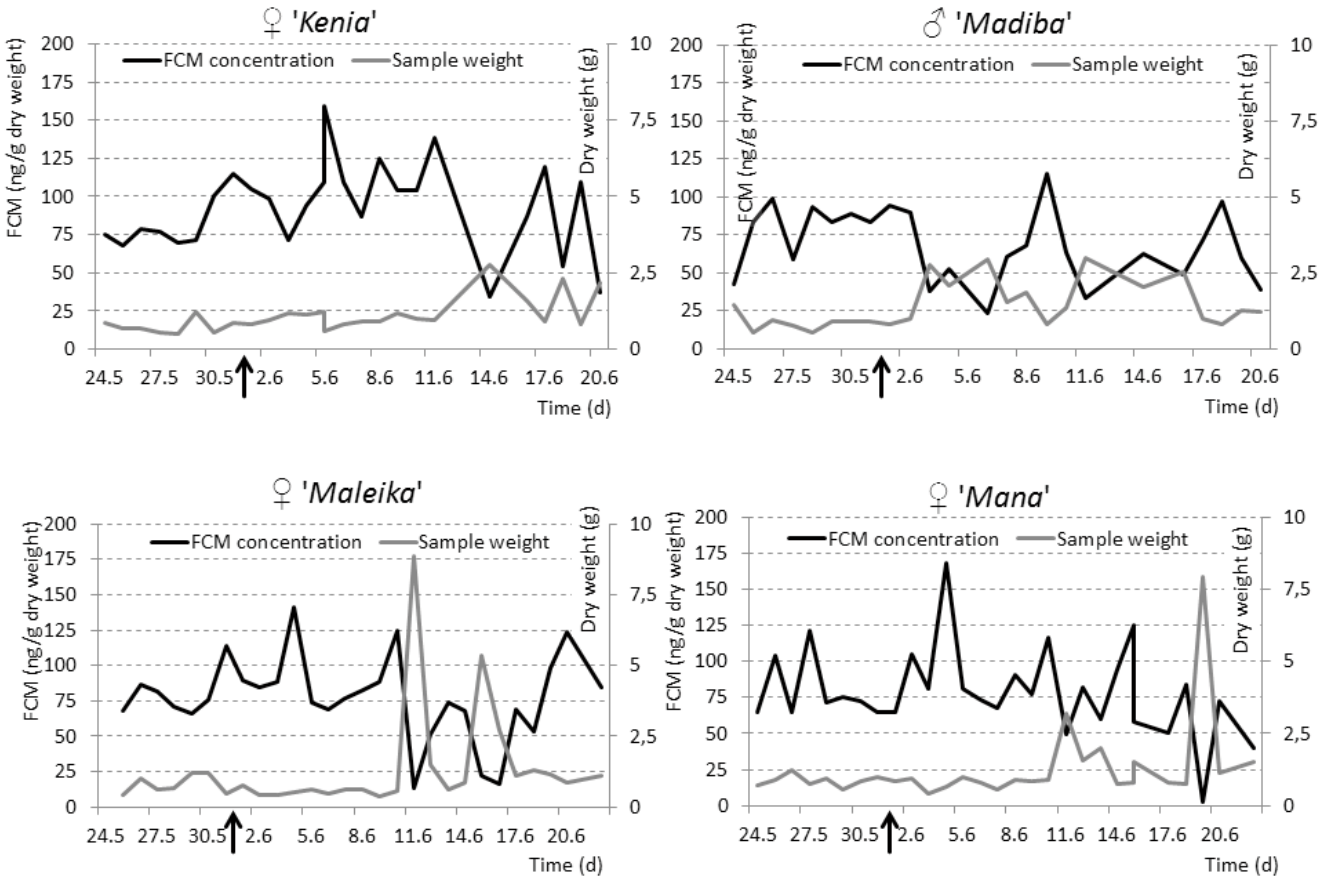

Figure 2. Profiles of FCM concentrations and the respective sample's dry weight for each individual investigated during the collection period in 2010. The date of moving for females 'Maleika' and 'Mana' is marked with an arrow.

effect discussed above fits with lower FCM levels associated with two rhinos in 2011. It does not explain the significantly higher FCM concentrations for 'Mana' one year after the transfer between housing facilities.

However, increased FCM levels for 'Mana' in 2011 are also not necessarily related with an increased stress level caused by the new housing facilities. It needs to be considered that the animal successfully copulated immediately after the male was moved into this new enclosure and 'Mana' gave birth to a calf in December 2011 (Schwarz et al. 2014). Her increased FCM level in 2011 could therefore also be related to her reproductive status at this time (five to six months before calving). Interestingly, a peak in the level of faecal corticoids of a gravid white rhino (*Ceratotherium simum*) female was reported to occur five months before calving (Bowers

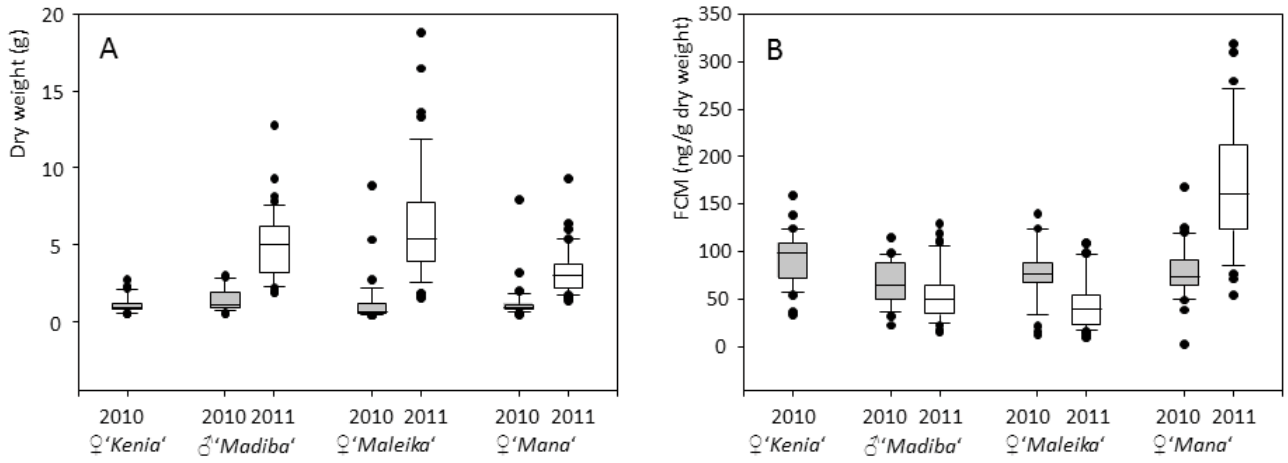

Figure 3. A: Median and inter-quartile range (IQR) of sample dry weight (g) per individual and collection period. B: Median and IQR of concentrations of FCM (ng/g dry weight) per individual and collection period.

et al. 2005). Further research into the relationship between faecal adrenal and gonadal hormone metabolites during the gravidity of black rhinos needs to be conducted to better understand FCM profiles for an appropriate assessment of stress responses.

In summary, the moderate FCM response patterns found in this study indicate that the transfer of rhinos between enclosures at Magdeburg Zoo was minimally invasive.

Acknowledgements

This study would not have been possible without the kind support of the director of Magdeburg Zoo, Dr K. Perret, as well as the zoo's curator for mammals and species conservation, K. Ruske. Special thanks go to the rhino keepers of Magdeburg Zoo, namely J. Bannier, D. Drewes, T. Haupt, A. Knaebel, S. Paelecke, E. Schulke, and V. Seilz for kindly collecting the samples. Further, the support of J. Zeller from the Museum für Naturkunde in Berlin, who assisted with the extraction of samples, as well as Dr M. Heistermann and A. Heistermann from the Endocrinological Laboratory of the German Primate Center in Göttingen, who conducted the enzyme immunoassays, is greatly acknowledged. This study was partially funded by a research grant to Prof. Zeller via the HUMBOLDT-INNOVATION GmbH.

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