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Stress and its evaluation in wild herbivores

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Internal or external stimulus that instigates a biological response in excess to normal is known as stress (Yaribeygi *et al.,* 2017). Stressors are environmental factors that stimulate abnormal homeostatic, physiological and behavioural responses. Different stressors are anthropogenic activities; adverse climatic conditions like drought, sudden rain or wind, excessive fatigue due to predator or pain (Radostits *et al*., 2007). In response to stress, the corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and other stress mediators are released (Carrasco and Van de Kar, 2003). These hormones are metabolised by digestive enzymes in liver and gut and can be quantified by measuring faecal cortisol metabolite concentration (FCM). It is a non-invasive and cost- effective technique (Hadinger *et al.,* 2015). This short review aims to discuss the different studies involving wild animals and evaluation of stress in wild animals.

Chinnadurai *et al.* (2009) conducted a study in Tembe Elephant Park and Thanda Game Reserve, South Africa to assess the viability of commercially available Radioimmunoassay to quantify faecal cortisol metabolites (FCM) in wild herbivores. Samples were collected from both male and female animals of 6 species- Giraffe (*Giraffa camelopardalis*), Impala (*Aepyceros melampus*), Nyala (*Tragelaphus buxtoni*), Kudu (*Tragelaphus strepsiceros*), Wildebeest (*Connochaetes taurinus*), and Zebra (*Equus burchelli*). Opportunistic samples were collected during the dry (May to August) and wet season (December to February). FCM concentration was measured using commercially available corticosterone RIA kits. FCM levels are influenced by the comparative proportion of indigestible fiber in the diet. Thus, as food availability varies, FCM levels were also found to vary across seasons ranging between 30-40 ng/g dry faeces in dry season to 50-60 ng/g dry faeces in the wet season.

Konjevic *et al.* (2010) conducted a study involving free- range fallow deer (*Dama dama)* in Brijuni national park, Croatia to validate the faecal glucocorticoid metabolite estimation technique for stress evaluation and to evaluate the seasonal pattern of glucorticoid release. A total of 200 deer were physically restrained for administration of adrenocorticotropic hormone (ACTH) intramuscularly and faecal samples were collected at several intervals after that. FCM concentration was measured using 11-oxoetiocholanolone EIA. The highest FCM concentration found 18 hours after ACTH injection. Additionally, 134 samples of fallow deer collected over different months during the year. Higher FCM concentration was found during May (40-196 ng/g), March (25-315 ng/g) and November (50- 2035 ng/g) compared to July (10-195 ng/g). The author attributed the variation in FCM concentration to change in body metabolism from anabolic to catabolic type.

Corlatti *et al.* (2011) investigated the seasonal variability in FCM secretion in red deer (*Cervus elaphus*) in response to ambient climatic conditions. Faecal samples were collected over a period of one year in Danube Auen National Park, Austria and processed to extract cortisol. FCM level were evaluated using 11-oxoetiocholanolone enzyme immunoassay. The authors detected lower FCM concentration in summer and higher concentration in winter. The authors attributed this pattern to reduced feed intake and low metabolic rates in summer, while these factors are higher in winter.

Sheriff *et al*. (2011) reviewed the different techniques for gluocorticoid metabolite measurement in biological samples- blood, saliva, urine, faeces, hair and feathers. Stress evaluation by measurement of FCM level was considered advantageous as it is cheap and non- invasive. FCM levels are not altered by handling/ restraint due to delay time (6-24 hours) between application of stressor and induced release of FCM in faeces. Plasma glucocorticoids were found to be indicative of point- of- time stress and affected by circadian patterns and minor disturbances. It was found that FCM levels are highly correlated with true plasma glucocorticoid levels.

Goymann (2012) highlighted about the advantages and drawbacks regarding non- invasive stress evaluation in wild animals. Estimation of faecal cortisol metabolites (FCM) was found to be affected by

the fiber content in diet that in turn influences the gut microbiota composition that metabolises the hormone. Comparison of faecal cortisol metabolites in faecal samples collected during different seasons is not very meaningful if change in ambient temperature results in change in metabolic rate as it will lead to variable hormone metabolite excretion rate.

He *et al.* (2014) conducted a study on captive stress and effect of gender on stress in Forest musk deer breeding centre, China. A total of 32 captive adult musk deer (*Moschus berezovskii*) of both gender were evaluated during the non- breeding season. FCM concentration was found to be higher in female deer (23- 101.3 µg/g) and male deer (34.0- 70.8 µg/g) reared in crowded enclosure. The FCM level in deer reared in sparsely crowded enclosures was detected in the range- female deer (25.8- 114.9 µg/g) and for male deer (65.9- 96.4 µg/g). Male animals were found to have lower FCM levels than female animals. The author speculates that the difference in FCM levels may be accorded to difference in steroid metabolism and different gonadal hormone effect.

Hadinger *et al.* (2015) conducted a study on stress evaluation and factors affecting faecal cortisol metabolite (FCM) interpretation in field studies. Faecal samples of 183 adult Chamois (*Rupicapra rupicapra)* were collected during the dry season in the Totes Gebirge range, Austria. FCM were analysed using an 11, 17-dioxoandrostane enzyme immunoassay. After faecal cortisol extraction, four samples were subjected to High performance liquid chromatography (HPLC) for validation of the target EIA to identify and quantify FCM. The authors cite that the actual baseline and disturbed FCM levels are unknown in case of free range wild Chamois. FCM levels (152.3 ng/g to 1828.5 ng/g) varied with age and social status of animal in the herd. FCM levels were found minimum during summer and increased by autumn season.

Formenti *et al.* (2016) investigated the stress induced by interspecies interaction in Apennine chamois (*Rupicapra pyrenaica ornata*) in response to habitat invasion by other species like Red deer (*Cervus elaphus*) and livestock in three national parks of Italy. A total of 318 faecal samples of Apennine chamois were collected and FCM concentration was measured using enzyme immunoassay (EIA). FCM levels in chamois were found higher in areas inhabited by both chamois and Red deer compared to areas inhabited by chamois and livestock. FCM levels in chamois were found even lower in areas inhabited by chamois alone. Thus, anthropogenic activities leading to livestock grazing in protected areas induces stress in wild herbivores like chamois. Interspecies interactions are also major stressors.

Konjevic *et al.* (2016) conducted stress evaluation study in Radobojski Orehovec breeding centre, Russia involving captive fallow deer (*Dama dama*). A total of 106 fresh faecal samples were collected over the year to analyse seasonal pattern of stress. FCM concentration was measured using 11-oxoaetiocholanolone EIA. Highest concentration of FCM was measured during winter (430-2385 ng/g) and summer (186-3271 ng/g). Lower FCM concentration was measured during spring (129-2896 ng/g).

Pavitt *et al.* (2016) conducted a study to explore how faecal androgen (FAM) and faecal cortisol (FCM) metabolite concentrations change with age, season and reproductive stage in Red deer (*Cervus elaphus).* The faecal samples of 200 Red deer were collected in the Rum national nature reserve, Scotland. Mean FAM concentration was found to be 22.93±1.16 ng/g while mean FCM concentration was found to be 106.35±31.87 ng/g. FCM and FAM varied with the female's pregnancy status and spiked in the calving season. FCM levels were recorded to be higher in aged animals compared to younger animals that may be due to age related desensitization of the cortisol stress response or debility and stress.

Huber *et al.* (2017) conducted stress evaluation in non- anesthetized European roe deer (*Caproleus caproleus*) in Grimso wildlife research area, Sweden. The animals were physically restrained and handling behaviour was recorded based on resistance to handling and vocalization. Body morphological measurements were noted and blood samples were collected. Leucocyte coping capacity (LCC) technique was employed for stress evaluation and compared to other stress parameters including heart rate, total blood cortisol, neutrophil to lymphocyte ratio, body temperature and animal behaviour. LCC is a measure of reactive oxygen species (ROS) produced by neutrophil in real time.

Fattorini *et al.* (2018) conducted a study in Abruzzo, Lazio and Molise national park, Italy in the months of July- October to study aggression, vigilance and cortisol level in the Apennine chamois (*Rupicapra pyrenaica ornata*). Animals were observed for behavioural data using binoculars. Faecal samples were collected from the herd and faecal androgen metabolites and faecal cortisol metabolites were measured using multi- species testosterone enzyme immunoassay and multi- species cortisol enzyme immunoassay respectively.

Bangar (2019) assessed the captive Blackbuck and Spotted deer in Rajiv Gandhi zoological park Pune, for stress in terms of adrenocortical activity using non-invasive faecal samples collected during two seasons October heat (October – November) and winter (December – January). The study animals were born and raised in the zoo itself The FCM was determined using Radioimmunoassay method. The FCM in Blackbuck was within a range of 0.18 ng/g to 2.62 ng/g while that of spotted deer was within a range of 0.18 ng/g to 3.07 ng/g. It was concluded that FCM is significantly affected by visitor numbers. Temperature humidity index significantly affected the FCM in both Spotted deer and blackbuck.

Ozkan *et al.* (2019) conducted a study on stress assessment in semi- captive Reindeers (*Rangifer tarandus*) using faecal samples in Norway. A total of

eight adult animals were injected Adrenocorticotropic hormone intramuscularly and faecal samples were collected consequently at two, seven and 24 hours after the hormone administration. Simultaneously faecal samples were collected from another herd of 12- 30 captive reindeers in a rearing farm. The results were compared for biological validation of FCM levels. FCM was estimated using 11-oxoaetiocholanolone enzyme immunoassay. FCM levels reached 34- 6408 ng/g after two-six hours of ACTH administration and peaked at 7 hours thereby decreasing afterwards. While the FCM levels in captive reindeers in farm varied significantly ranging between 212- 1159 ng/g in morning to 605-4673 ng/g in evening. The authors thus successfully validated the non- invasive sampling method for study of stress in reindeers. The authors quote that faecal sample collection is non- invasive but not non- disturbing as human presence is a stressor that may however not affect the FCM level in the immediate samples but may lead to higher FCM levels in the sample collected later. Time lag of seven- eight hours was found between the exposure to stressor and resultant effect in FCM level in reindeers.

Vilela *et al*. (2020) measured cortisol in three tissue types- blood, faecal and hair samples collected from a herd of 80 Red deers (*Cervus elaphus*) to evaluate stress. Samples were collected during two hunting seasons in Lousã Mountain area of Portugal after the animals were. The FCM was measured using 11- oxoetiocholanonolone EIA. It was found that FCM level increased during months of harvesting. FCM level remained low during hunting season probably due to habituation to hunting activity. FCM concentration increased during the summer season compared to winter due to less availability of pastures and water. FCM level was found not correlated to cortisol concentration measured using hair and serum samples as different biological samples indicate stress level for different periods of time. While glucocorticoid metabolite level in plasma is indicative of immediate stress, FCM levels signify stress upto a certain time before sample collection and cortisol metabolite accumulated in hairs are indicative of chronic stress.

Donini *et al.* (2021) performed a study on stress levels in Northern Chamois (*Rupicapra rupicapra)* and Red Deer (*Cervus elaphus)* female adult animals in Stelvio National Park, Italy during the summer season over a period of seven days. Environmental temperature was also noted. FCM concentration was analysed using an 11-oxoetiocholanolone-17- CMO: BSA enzyme immunoassay. FCM level decreased after 4th day onwards of exposure to author's team. Occasional episodes of rain lead to rise in level of FCM that is probably due to increased microbial intake and consequent rise in gut microbial action to metabolize steroids (Mostl *et al.,* 1999).

REFERENCES

Bangar, N. (2019). Faecal cortisol metabolites as an indicator of stress in captive spotted deer (*Axis axis*) and Blackbuck (*Antilope cervicapra*) in India. M.Sc. thesis (Wildlife Health and Management), University of Nairobi.

Carrasco, G. and Van De Kar, L. D. (2003). Neuroendocrine pharmacology of stress. *European Journal of Pharmacology*, **463**(1):235–272.

Corlatti, L., Palme, R., Frey-Roos, F. and Hackländer, K. (2011). Climatic cues and glucocorticoids in a free-ranging riparian population of red deer (*Cervus elaphus*). *Folia Zoologica,* **60**(1): 176 - 180.

Di-Francesco, J., Kwong, G. P. S., Deardon, R., Checkley, S. L., Mastromonaco, G. F., Mavrot, F., Leclerc, L. and Kutz, S. (2021). Qiviut cortisol is associated with metrics of health and other intrinsic and extrinsic factors in wild muskoxen (*Ovibos moschatus*). *Conservation Physiology*, **10**(1): 1-20.

Di-Francesco, J., Kwong, G. P. S., Deardon, R., Checkley, S. L., Mastromonaco, G. F., Mavrot, F., Leclerc, L. and Kutz, S. (2021). Qiviut cortisol is associated with metrics of health and other intrinsic and extrinsic factors in wild muskoxen (*Ovibos moschatus*). *Conservation Physiology*, **10**(1): 1-20.

Donini, V., Iacona, E., Pedrotti, L., Macho-Maschler, S., Palme, R. and Corlatti, L. (2022). Temporal stability of fecal cortisol metabolites in mountain-dwelling ungulates. *The Science of Nature*, **109**(2):109-119.

Formenti, N., Bonfanti, M., Viganò, R., Fraquelli, C., Trogu, T., Ferrari, N., Palme, T. and Lanfranchi, P. (2016). Effects of interspecies interactions: increased hormonal stress response of Apennine chamois (*Rupicapra pyrenaica ornata*) induced by livestock and red deer (*Cervus elaphus*). *Hystrix:* **27**(1):18- 19.

Hadinger, U., Haymerle, A., Knauer, F., Schwarzenberger, F. and Walzer, C. (2015). Faecal cortisol metabolites to assess stress in wildlife: evaluation of a field method in free-ranging chamois. *Methods in Ecology and Evolution*, **6**(11):1349–1357.

He, L., Wang, W., Li, L., Liu, B., Liu, G., Liu, S., Qi, L. and Hu, D. (2014). Effects of crowding and sex on fecal cortisol levels of captive forest musk deer. *Biological Research*, **47**(1):1-10.

Hing, S., Northover, A. S., Narayan, E. J., Wayne, A. F., Jones, K. L., Keatley, S., Thompson, R. C. A. and Godfrey, S. S. (2017). Evaluating Stress Physiology and Parasite Infection Parameters in the

Translocation of Critically Endangered Woylies (*Bettongia penicillata*). *EcoHealth*, **14**(1):128–138.

Huber, N., Vetter, S. G., Evans, A. L., Kjellander, P., Küker, S., Bergvall, U. A. and Arnemo, J. M. (2017). Quantifying capture stress in free ranging European roe deer (*Capreolus capreolus*). *BMC Veterinary Research*, **13**(1):127-136.

Konjevic, D., Janicki, Z., Slavica, A., Severin, K., Krapinec, K., Božić, F. and Palme, R. (2010). Non-invasive monitoring of adrenocortical activity in free-ranging fallow deer (*Dama dama* L.). *European Journal of Wildlife Research*, **57**(1):77–81.

Konjevic, D., Janicki, Z., Slavica, A., Severin, K., Krapinec, K., Želježić, D. and Božić, F. (2016). Monitoring cortisol metabolites in the faeces of captive fallow deer (Dama dama L.). *Veterinarski Arhiv*, **86**(3):363–371.

Mostl, E., Messmann, S., Bagu, E., Robia, C. and Palme, R. (1999). Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. *Journal of Veterinary Medicine*, **46**(1) :621–631.

Ozkan, S., Jorgensen, G. H. M., Eilertsen, S., Hansen, I., Hagen, S. B., Floystad, I. and Palme, R. (2019). Measuring faecal glucocorticoid metabolites to assess adrenocortical activity in reindeer. *Animals*, **9**(11): 987-997.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007). Veterinary Medicine, 10th Edn., Elsevier Publishing Co., Edinburg, pp 107-110.

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R. and Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia*, **166**(4): 869–887.

Vilela, S., Da Silva, A. A., Palme, R., Ruckstuhl, K. E., Sousa, J. P. and Alves, J. (2020). Physiological stress reactions in red deer induced by hunting activities. *Animals*, **10**(6): 1003- 1013.

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P. and Sahebkar, A. (2017). The impact of stress on body function: A review. *PubMed*, **16**(1): 1057–1072.