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Effects of enrichment on faecal corticosterone and stereotypic behaviour of sloth bears (*Melursus ursinus*) in captivity at Chiang Mai Night Safari

Siriporn Umsook[1]*, Tulyawat Sutthupat[1], Aratchaporn Meemey[1], Yuttana Klangnongsang[1], Pawart Jaidee[1], Nuttawach Termsnguanwong[2], Cheeranan Phuriphakdeesanong[2], Panatda Bungsrisawat[3], Savek Kiatsomphob[3] and Nanthana Pothakam[1]

[1] *Veterinary, Conservation and Research Section, Animal Management Division, Chiang Mai Night Safari, Chiang Mai, 50230, Thailand*

[2] *Animal Welfare Management Section, Animal Management Division, Chiang Mai Night Safari, Chiang Mai, 50230, Thailand*

[3] *Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand*

* *Corresponding author: n.pothakam@gmail.com*

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Abstract - Corticosterone is a hormone that responds to the level of stress in animals and to their stereotypic behaviour. In wildlife and captive animals, organising activities for behaviour enrichment and improvement of animal welfare and quality of life is necessary. This study aimed to investigate the effects on stereotypic behaviours associated with behaviour enrichment on the levels of corticosterone in faeces and the correlation between faecal corticosterone and stereotypic behaviours in captive sloth bears. The five sloth bears used were captive at Chiang Mai Night Safari. Data were collected in three periods: before, during, and after the behaviour enrichment. The faeces were collected individually to determine corticosterone using enzyme-immunoassay (EIA). Repetitive pacing in the same area was observed as stereotypic behaviour. The results showed that individual faecal corticosterone levels and the stereotypic behaviour of the sloth

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bears before and during the behaviour enrichment period were significantly different ($P<0.05$). Stereotypic behaviour was statistically significantly different before, during, and after the behaviour enrichment ($P<0.05$). Additionally, the correlation between the amount of faecal corticosterone and stereotypic behaviour was calculated using Pearson's correlation coefficient and found to be statistically significantly related ($P<0.05$). This study provides basic information about the effect of enrichment on faecal corticosterone and the stereotypic behaviour of captive sloth bears.

Keywords: Stereotypic behaviour, corticosterone, enrichment, sloth bear (*Melursus ursinus*)

1. Introduction

Sloth bears (*Melursus ursinus*) are a species highlighted as vulnerable and facing threats in The IUCN Red List (Dharaiya et al., 2016). This animal is not native to Thailand and is primarily found in the Indian subcontinent, including India, Sri Lanka, Nepal, and Bangladesh (Prater, 1965). Sloth bears are captive in specific zoos or wildlife conservation organisations for conservation, education, or research purposes. Keeping animals in captivity is a widely accepted approach to conserving threatened species. These animals receive veterinary care and be free from starvation and predation (Clubb & Mason, 2007). This was shown by various studies on different animal species, indicating that survival and fecundity rates are generally higher in captive than in wild populations (Robert, 2009). However, captive animals live in an environment different from the wild (McPhee & Carlstead, 2010). This can lead to many difficulties during captivity. One main concern for animals in captivity is the expression of abnormal behaviour, such as stereotypic behaviour. This can be one of the indicators of poor animal welfare and is often a sign of decreased animal welfare because the opportunity to express natural behaviour has been limited. (Shih et al., 2016; Clubb & Mason, 2007). Restrictions in performing their normal species-specific behaviours may lead to stress and harm their welfare in captivity. This will lead to changes in their behavioural patterns, such as developing abnormal behaviours like stereotypic behaviours (Mason et al., 2007). If an animal can show its normal, species-specific behaviour similar to those in the wild, it shows that the animal's needs in the captive environment are being met, and it could have good health (McPhee & Carlstead, 2010). However, most bear species (including sloth bears) exhibit abnormal behaviour when kept in captivity. This is due to many factors, including the size of the enclosure, a barren or unexciting environment (Morgan & Tromborg, 2007), and no opportunity for exploration and foraging (McPhee & Carlstead, 2010). The abnormality in their behaviour is a potential indicator of pain, suffering, and injury in captive animals (Düpjan & Puppe, 2016). Mason (1991); such behaviour can be described as stereotypic behaviour which is defined as repetitive, unvarying, and functionless behaviour. Stereotypic behaviour has been documented in many animal species kept in captivity, including American black bears (*Ursus americanus*) (Carlstead & Seidensticker, 1991) and giant pandas (*Ailuropoda melaleuca*) (Owen et al., 2005). This behaviour can be reduced by increasing the biological relevance of captive environments through enriching their living conditions as explained below.

Behavioral enrichment of captive animals can be divided into two main categories: food enrichment and non-food enrichment, which stimulate species-specific behaviour (Düpjan & Puppe, 2016). For sloth bears, stereotypic behaviour includes pacing back and forth, rocking, excessive grooming, or repetitive movements (Montaudouin & Le Pape, 2004). Stereotypic behaviour is highly related to stress in captive animals, especially bears (Dantzer, 1991). Besides the inability to perform some essential species-specific behaviour, stress can also contribute to developing animal stereotypies. Stress, specifically chronic stress, is the leading cause of poor welfare in bears, which has been widely accepted as one of the main factors that lead to stereotypic behaviours (Manteca & Salas, 2015). These stereotypic behaviours can be evaluated using direct observation methods and faecal corticosterone analysis (Shih et al., 2016). This non-invasive measurement of corticosterone in the faeces has been used repeatedly to determine individual differences in the physiological response to captive environments (Shepherdson et al., 2013). According to Sapolsky (2002), stress in animals like mammals is a complex condition controlled by the sympathetic nervous system and a steroid hormone (corticosterone). Corticosterone measurement is a robust integrator of environmental stressors and an animal's physical condition. Corticosterone levels can be measured directly from blood (such as plasma and serum), saliva, or indirectly *via* assessment of their metabolites. It is also excreted via urine and faeces (Sheriff et al., 2011) where it can be measured. The measurement of corticosterone excreted *via* the faeces is a non-invasive approach that allows for sample collections and analyses without any direct contact with the animals; thus, such an approach can minimise unnecessary stress (Wasser et al., 2000). Large mammals and endangered species where repeated capture and handling are almost impossible or with high energetic costs (Wasser et al., 2000). Thus, faecal corticosterone analysis provides a non-invasive method for studying the physiological response of wildlife to various stressors and is a ground-breaking monitoring technique in wildlife management and conservation (Wasser et al., 2000). The objective of this study was to investigate the effect of behavioural enrichment on corticosterone in faeces, the stereotypic behaviours, and the interaction of faeces corticosterone and stereotypic behaviours in captive sloth bears at Chiang Mai Night Safari

2. Materials and methods

2.1 Animal and housing

This study was certified by the Animal Care and Use Committee, the Chiang Mai Night Safari. The reference number is CNS 002/2567. The study involved five sloth bears (two males and three females), aged between 10 and 22 years old (mean age 15.20 years) at the Chiang Mai Night Safari (Table 1) and was carried out for 45 days. Sloth bears were released into the outdoor exhibit enclosure from 10.00 to 18.30. Daily, there was a daily serving of ripe bananas, papaya, sweetcorn, boiled pumpkin, boiled eggs, boiled chicken ribs, and seasonal fruits at 11.30, 15.30, and 19.30.

Table 1. Details of sloth bears (*Melursus ursinus*) at Chiang Mai Night Safari.

Name	Sex	Age (Years)	Captive time (Years)	Weights (Kg)
SB01	Female	20	17	100
SB02	Female	14	14	120
SB03	Female	10	10	120
SB04	Male	22	16	150
SB05	Male	10	10	90

2.2 Exhibit area

During daylight, the sloth bears were individually released into the exhibit area, which had an area of 468 m^2 and consisted of a 10-meter rock wall on one side and a 6-meter-deep moat on the other, which separated the animals from the visitors. The enclosure was furnished with natural soil and living vegetation, along with an artificial stone hill. A water tank for drinking was provided throughout the day.

2.3 Enrichment activity

Enrichment devices were installed in the exhibit area before the bears were released each morning. Enrichment for sloth bears was divided into two main categories: (1) food enrichment, and (2) non-food enrichment. Food enrichment examples include (a) food balls, where the bear must roll the ball around to dispense food., (b) treat dispensing toys that require the bear to push, pull, or manipulate parts to access food, (c) scatter feeding, (d) frozen treats, and (e) hidden treats. Non-food enrichment examples, such as novel logs, branches, balls, or toys in the enclosure, provide sensory stimulation and encourage exploration and play. Enrichment materials were removed at the end of the day. One activity was implemented per day for all of the animals

2.4 Stereotypic behavioral observation

The stereotypic behaviour of all sloth bears was observed and recorded. The behavioural measure of stereotypic pacing was observation of the animal moving back and forth or circling repetitively (Owen et al., 2004). The recording of stereotypic behavior was done through both direct observation and video recording during three periods before, during, and after the enrichment. The data was collected from approximately 10.00 to 17.00.

2.5 Faecal samples collection

Fresh faeces from each sloth bear were collected before, during, and after the enrichment program. Sloth bear keepers collected fresh faecal samples from five individuals in the early morning (09.30 a.m.). Approximately 50 g of freshly dropped faeces were collected. Faecal samples were packed in resealable plastic bags and stored at -20°C until analysis.

2.6 Extraction of faeces.

Faecal corticosterone was extracted by modifying the methods previously used for sloth bears (Wasser et al., 2000). Samples were dried in a hot air oven (CMD-5, Charatchai

Machinery, Thailand) at 60°C for 48 h and then sieved to obtain a homogenised powdered hair-free sample. A 0.1 g sample was added to the tubes containing 5 ml of 90% ethanol (AR1380, RCI Labscan™, Ireland) and mixed using a vortex for 30 seconds. The mixture was boiled in a water bath (Z743156, Julabo®, Germany) at 90˚C for 20 min. Simultaneously, ethanol was added to prevent the mixture from boiling dry. The volume of extract was brought up to approximately pre-boil levels with ethanol. Upon removal from the boiling water bath, tubes were centrifuged (LMC-3000, Biosan, Latvia) at 2500x g for 20 min, and the supernatant was poured into a glass tube. Then, 5 ml of 90% ethanol was added to each tube, and vortexed for 30 seconds and then boiled in a water bath at 90˚C for 20 min. Subsequently, it was centrifuged at 2500x g for 20 min. The first and second extracts were combined. The extract was dried and reconstituted in 1 ml of methanol (AR1115, RCI Labscan™, Ireland), then briefly vortexed. The methanolic sample was kept at -20˚C for further analysis.

2.7 Faecal corticosterone analysis

The faecal corticosterone level (Kosaruk et al., 2020) was measured by enzyme-immunoassay (EIA). Faecal extracts were diluted 1:3 in assay buffer (0.0137 M Trizma base, 0.2 M Triz-HCl, 0.2 M NaCl, 0.2 M EDTA, 0.001% BSA, and 0.001% Tween 20; pH 7.5) and faecal glucocorticoid metabolites (fGCM) concentrations measured by double-antibody EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006, Coralie, Munro). Samples and corticosterone standards (50 µl) were added to wells in duplicate, followed by corticosterone-HRP (25 µl; 1:30,000) and anti-corticosterone antibody (25 µl; 1:100,000). Plates were incubated in the dark at room temperature for two hours before adding 100 µl of 3,3’, 5,5’-tetramethylbenzidine (TMB; Sigma-Aldrich Pte Ltd) solution, followed by incubation for 20 - 35 min, and 50 µl of stop solution was added. Absorbance was measured at 450 nm using a microplate reader (TECAN Sunrise, Salzburg, Austria).

2.8 Statistical analysis.

The experimental design was arranged in a 3 x 5 factorial in a completely randomised design. The first factor was the enrichment period, and the second was the individual animal. The observed data are reported as the least square mean with standard error of the mean, utilising a general linear model (GLM) followed by Tukey’s test with a confidence interval of 95% for statistically significant differences ($p<0.05$). Correlations between the observed data were also examine using Pearson’s correlation coefficient. The statistical analysis employed SAS® On Demand for Academics (SAS Institute Inc., Cary, North Carolina, United States).

3. Results and discussion

3.1 The corticosterone level in sloth bears

A comparison of the concentrations (as ng/g) of corticosterone in dry faecal samples collected before, during, and after enrichment from five sloth bear individuals is shown in Table 2. Corticosterone levels for bear SB01 were 57.01, 71.27, and 38.99 ng/g dry faeces before, during, and after enrichment (P=0.140). For SB03, the levels were 125.62, 46.86, and 90.97 ng/g dry faeces (P=0.302). SB04 had levels of 38.18, 19.84, and 38.15 ng/g dry faeces (P=0.463), while SB05 had 76.04, 24.03, and 24.64 ng/g dry faeces (P=0.118). However, the corticosterone level of the sloth bear numbers SB02 before (25.00 ng/g dry faeces) and during enrichment (48.83 ng/g dry faeces) was significantly lower compared with after enrichment (71.46 ng/g dry faeces) (P=0.021). The individual concentrations of corticosterone before (P=0.009) and during enrichment (P=0.011) were significant. However, the concentration was not significant after enrichment (P=0.376). Measurement of faecal glucocorticoid is widely used to estimate the stress level in captive animals. (Keay et al., 2006; Sheriff et al., 2011). Many reports of utilising faecal glucocorticoid to indicate the stress level in bears have revealed the potential of using faecal glucocorticoid levels to monitor animal welfare (Shepherdson et al., 2013; Abdul-Mawah et al., 2022; Zhou et al., 2020). However, the data on sloth bears are still limited. (Zoumin et al., 2023). Only one study found that assessing faecal glucocorticoid from sloth bears measured as both cortisol and corticosterone helps evaluate the stress of sloth bears. Corticosterone is a hormone linked to stress. Measuring corticosterone levels in fecal samples allows to track stress levels in without causing them any harm (Young et al., 2004). The data from Young et al. (2004) showed that the mean baseline of corticosterone was 64.1 ng/g faeces, which is quite close to our average value before the enrichment period. Chronic stress is known to cause elevated corticosterone levels (Martin, 2009). However, post-enrichment, a decrease in corticosterone concentration was noted in all sloth bears, indicating a positive impact of enrichment on corticosterone levels.

Table 2. Faecal corticosterone (ng/g dry faeces) from individual sloth bears before, during, and after enrichment.

Animals	Faecal corticosterone (ng/g dry faeces)			SEM	P-value
	Before enrichment	During enrichment	After enrichment		
SB01	57.01^{bc}	71.27^{a}	38.99	6.91	0.140
SB02	25.00^{cyz}	48.83^{abyz}	71.46^{y}	8.82	0.021
SB03	125.62^{a}	46.86^{bc}	90.97	19.43	0.302
SB04	38.18^{bc}	19.84^{d}	38.15	6.09	0.463
SB05	76.04^{b}	24.03^{cd}	24.64	12.47	0.118
SEM	5.20	2.86	10.65		
P-value	0.009	0.011	0.376		

$^{a-d}$ Means within the same column with different superscripts indicate inter-individual differences (P<0.05)
$^{x-z}$ Means within the same row with different superscripts indicate statistical differences between treatments (P<0.05)
Data was shown as least square means with standard error of the mean (SEM) and P-value of each column and row

3.2 The stereotypic behaviour levels in sloth bear

A comparison of the stereotypical behaviours of a sloth bears walking in circles at a revolutions per minute counted before, during and after enrichment from five sloth bears is tabulated Table 3. For bear SB01, the revolutions per minute before, during, and after enrichment were 8.94, 9.89, and 5.57, respectively (P=0.060). For SB03, they were 16.46, 7.70, and 10.56, respectively (P=0.163). For SB04, they were 5.91, 3.21, and 6.37, respectively (P=0.369). Lastly, for sloth bear SB05, the values during enrichment and after enrichment, i.e., 4.19 revolutions per minute and 4.37 revolutions per minute, were significantly lower than before enrichment at 10.39 revolutions per minute (P=0.032). However, the stereotypic behaviours of the sloth bear number SB02 shown before enrichment and during enrichment, i.e., 4.39 revolutions/min and 7.57 revolutions per minute, were significantly lower than after enrichment at 9.62 revolutions per minute (P=0.001). The stereotypic behaviours of five sloth bears before and during enrichment were significantly different (P<0.05). Before enrichment, the stereotypic behaviors demonstrated a significant difference (P=0.004); during enrichment, they continued to exhibit a significant difference (P=0.005). However, following enrichment, the stereotypic behaviors did not maintain a significant difference (P=0.259). Stereotypic behaviour, a potential indicator of compromised welfare of captive animals, is taken seriously as a warning sign of possible suffering. Still, it is not the sole index of welfare (Mason & Latham, 2004). Although not all stereotypic behaviours are a stress response (Carlstead, 1996), stereotypic behaviour in captive animals is usually associated with elevated corticosterone levels (Carlstead, 1998). Shepherdson (1998) studied a captive American black bear (*Ursus americanus*) in order to reduce stereotypic behaviour commonly seen in zoo animals. Using enrichment programs can significantly decrease the occurrence of stereotypic behaviour. Enrichment programs can dramatically reduce the time pandas spend performing stereotypic behaviour (Swaisgood et al., 2001; Liu et al., 2003). However, age has been implicated in the cause of stereotypic behaviours (Vickery & Mason, 2004).

Table 3. Observed stereotypic behaviours from individual animals before, during, and after enrichment.

Animals	Stereotypic behaviours (revolutions / min)			SEM	P-value
	Before enrichment	During enrichment	After enrichment		
SB01	8.94[bc]	9.89[a]	5.57	0.902	0.060
SB02	4.39[dz]	7.57[ay]	9.62[x]	0.967	0.001
SB03	16.46[a]	7.70[a]	10.56	1.947	0.163
SB04	5.91[cd]	3.21[b]	6.37	0.919	0.369
SB05	10.39[bx]	4.19[by]	4.37[y]	1.359	0.032
SEM	0.52	0.32	0.88		
P-value	0.004	0.005	0.259		

[a-d] Means within the same column with different superscripts indicate inter-individual differences (P<0.05)
[x-z] Means within the same row with different superscripts indicate statistical differences between treatments (P<0.05)
Data was shown as Least square means with standard error of the mean (SEM) and P-value of each column and row

3.3 Effect of enrichment on faecal corticosterone level and stereotypic behaviours

Figure 1 shows the average value of faecal corticosterone and stereotypic behaviours in sloth bears. The average vlaue of faecal corticosterone tended to be highest before and lower during the enrichment. However, after enrichment, the faecal corticosterone became higher than during enrichment but remained lower than before enrichment. Similarly, the stereotypical behaviours of sloth bears revealed a similar value trend between the three enrichment periods. However, there was a significant difference, which showed that there was a considerable difference compared to before and after enrichment. Stereotypic behaviour might be a response to the elevated faecal corticosterone level before the enrichment period, presumably due to stress caused by the lack of naturalistic stimuli in the enrichment (Owen et al., 2004). Enrichment is significant for maintaining popular animals with visitors, which have to face more visitors daily (Shepherdson et al., 1993; Carlstead & Shepherdson, 1994). It is a critical factor that cannot be ignored in enrichment programs.

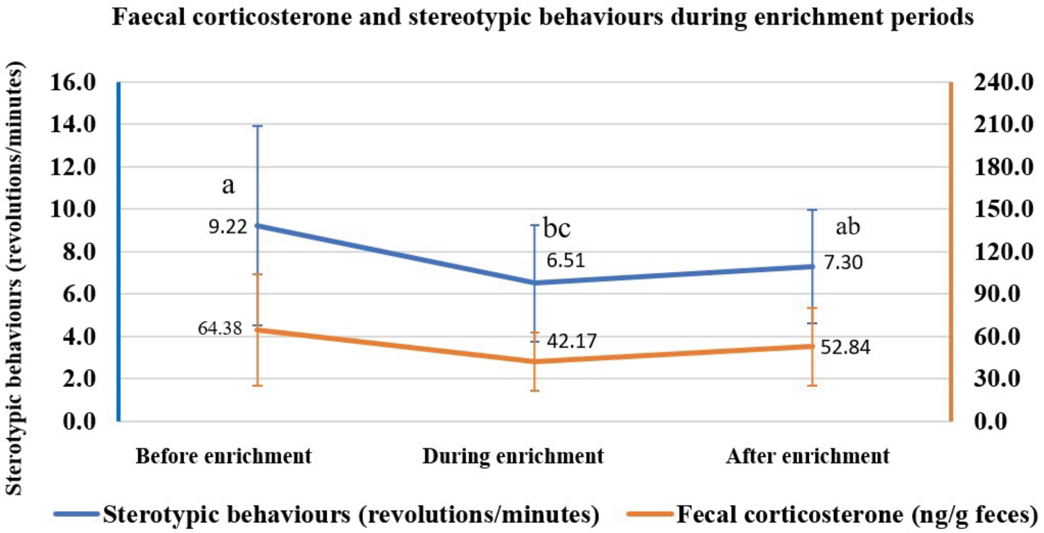

Figure 1. Average value on faecal corticosterone and stereotypic behaviours during enrichment periods. The data are shown as the least square mean. Means within the same trait with different superscript letters differ (P<0.05)

3.4 Interaction of enrichment period with individual animals

The enrichment period and individual animals were assigned as factors for the factorial study. Moreover, the interaction of faecal corticosterone and stereotypic behaviours was also elaborated for each factor (Table 4). The results displayed a significant difference in the stereotypic behaviours of sloth bears during the enrichment period. However, there were no significant differences in the level of faecal corticosterone during the enrichment period.

Regarding individual animals, the level of faecal corticosterone had a significant difference in SB03, which had the highest value. The SB03 was the youngest female sloth bear in this study. Young et al. (2004) inferred that mate introduction or breeding did not increase the faecal corticosterone level. Instead, the increasing level of faecal corticosterone was associated with abnormal defecations and deworming treatments. Interestingly, two male sloth bears have lower faecal corticosterone levels, which differed from SB03. Accordingly, the stereotypic behaviours in each animal showed such differences with the faecal corticosterone, but only the SB02 showed a significant difference compared to SB03, which was a female. Studies of the influence of sex on monitoring and assessing welfare might explain these results. For the Alaskan brown bear, sex did not influence the level of faecal corticosterone (Von der Ohe et al., 2004). Interestingly, a study in black bears focusing on hair cortisol concentration (HCC), reported variation in cortisol concentration, with males having higher HCC than females but the levels were not significantly different (Lafferty et al., 2015).

The interaction between the enrichment period and individual animals displayed the significance between the two factors (Table 4). Treatment Be x 3 had the highest value in faecal corticosterone and stereotypic behaviours, while treatment Du x 4 had the lowest value. This emphasises the influence of the enrichment time and individual animals on the level of faecal corticosterone and the observed stereotypic behaviours.

We further investigated the correlation between faecal corticosterone and stereotypic behaviours (Table 5). The result showed that faecal corticosterone was highly significantly positively correlated with stereotypic behaviours. Even though the faecal glucocorticoid metabolite is commonly used to assess the stress and welfare of the animal, the technique of radio immune assay uses radioactive substances and enzyme immune assay (EIA) is preferable for safety reasons (Zoumin et al., 2023).

Some studies have demonstrated the effectiveness of using cameras to investigate captive polar bear behavior. Using video sequences of polar bears, we were able to achieve an accuracy of 96.6%. (Pastorino et al., 2021). However, it was found that stereotypic behaviour was rare. The application of cameras with the power of artificial intelligence might lead to advancements in animal welfare assessment (Zhang et al., 2023). Our study revealed that the observation of stereotypic behaviour was significantly influenced by enrichment. Artificial intelligence could be used to improve animal welfare for the better life of captive animals by monitoring and assessing animal welfare simultaneously.

Table 4. Least squares mean and standard errors of means (LSM ± SEM) for effects of faecal corticosterone on stereotypic behaviour and their interaction for the enrichment period and animals.

Treatment	**Faecal corticosterone**	**Stereotypic behaviour**
Enrichment time (A)		
Before enrichment (Be)	64.38	9.22^{a}
During enrichment (Du)	42.17	6.51^{b}
After enrichment (Af)	52.84	7.30^{ab}
SEM	7.04	0.62
P - value	0.116	0.021
Animals (B)		
SB01 (1)	55.76^{ab}	8.13^{ab}
SB02 (2)	48.43^{ab}	7.19^{b}
SB03 (3)	87.82^{a}	11.57^{a}
SB04 (4)	32.06^{b}	5.16^{b}
SB05 (5)	41.57^{b}	6.31^{b}
SEM	9.09	0.79
P - value	0.006	0.001
A x B		
Be x 1	57.02^{ab}	8.94^{ab}
Be x 2	25.01^{b}	4.39^{b}

Table 4. Least squares mean and standard errors of means (LSM ± SEM) for effects of faecal corticosterone on stereotypic behaviour and their interaction for the enrichment period and animals. **(cont.)**

Treatment	Faecal corticosterone	Stereotypic behaviour
Be x 3	125.63[a]	16.46[a]
Be x 4	38.16[ab]	5.91[b]
Be x 5	76.05[ab]	10.39[ab]
Du x 1	71.27[ab]	9.89[ab]
Du x 2	48.83[ab]	7.57[b]
Du x 3	46.86[ab]	7.70[b]
Du x 4	19.85[b]	3.21[b]
Du x 5	24.04[b]	4.19[b]
Af x 1	38.99[ab]	5.57[b]
Af x 2	71.46[ab]	9.62[ab]
Af x 3	90.97[ab]	10.56[ab]
Af x 4	38.15[ab]	6.37[b]
Af x 5	24.64[b]	4.37[b]
SEM	15.74	1.38
P - value	**0.042**	**0.004**

[a-d] Means within the same column with different superscripts indicate inter-individual differences ($P<0.05$)
Data was shown as Least square means with standard error of the mean (SEM) and P-value of each column

Table 5. Pearson correlation coefficient between faecal corticosterone and stereotypic behaviour.

Factor	Hormone	Behaviour
Hormone	1.00000	0.96508**
Behaviour	0.96508**	1.00000

** = highly significant ($p \leq 0.01$)

4. Conclusion

The comparison of corticosterone and stereotypic behavior before, during, and after the enrichment showed that they all tended to have the highest value before the enrichment and get lower during the enrichment. However, after enrichment, the faecal corticosterone became higher than during enrichment but was still lower than before enrichment. This finding emphasizes that enrichment could not lower the faecal corticosterone and did not show a significant difference across the three periods. However, observing stereotypic behavior demonstrated a correlation to faecal corticosterone during the periods of enrichment, though statistically different. Our findings suggest that the correlation between faecal corticosterone levels and stereotypic behavior was significantly positive. Moreover, these two parameters might be used to investigate stress in sloth bears or even combined to estimate animal welfare. The major shortcoming in our study was small sample size, despite the fact that we used all available sloth bears in the Chiang Mai Night Safari.

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