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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

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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.

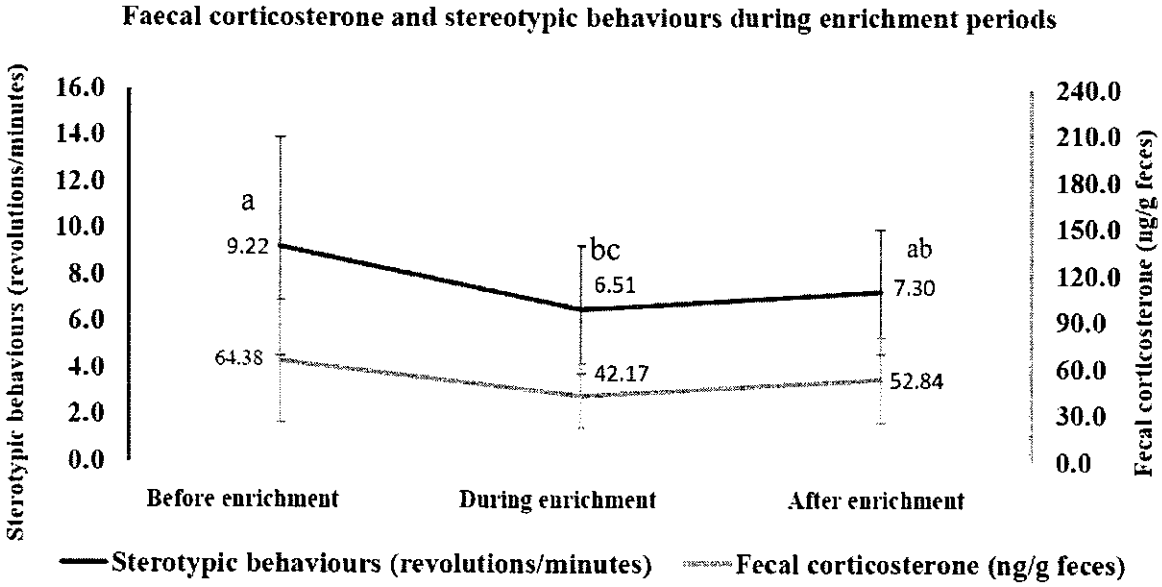

Figure 1. Average value on faecal corticosterone and stereotypic behaviours during enrichment periods. The data are shown as the least square mean. Means within the same trait with different superscript letters differ ($P<0.05$)

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≤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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Effect of behavioral enrichment on fecal corticosterone hormone levels in chimpanzees (*Pan troglodytes*) captive for conservation

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Abstract - Chimpanzees (*Pan troglodytes*) are classified as an endangered species (IUCN), therefore their conservation is important. Raising chimpanzees in a captive environments different from the natural environment may cause them to exhibit fewer natural behaviors and affect stress. Behavior enrichment is another way to help maintain animals in captivity and relieve their stress. Corticosterone is a steroid stress hormone group, that is secreted when animals are in stressful conditions with decreased immune system function. The objective of this study was to examine the effect of behavior enrichment on corticosterone levels in chimpanzees captive for conservation by collecting the fecal material of 4 chimpanzees (2 male and 2 female) before, during and after behavior enrichment, then the fecal material was dried and hormones were extracted with 90 % ethanol. The solution was analyzed for level of corticosterone hormone by ELISA technique using completely randomized design. Results of the study on corticosterone levels in male chimpanzees found that during the period of before behavior enrichment, during enrichment and after enrichment period were not significant. However, there was a continuous decrease in corticosterone levels. Corticosterone levels in female chimpanzees found that during the behavior enrichment period were higher than after enrichment and before the enrichment period, the level was not

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significant. The results of this study may be used as basic guideline information for designing behavior enrichment programs suitable for captive chimpanzees.

Keywords: Chimpanzee (*Pan troglodyte*), corticosterone, behavioral enrichment, captive

1. Introduction

Wild chimpanzees, have a potential lifespan about 60 years and experience an increase in levels of stress-related hormones as they age. This situation is similar to that of humans, as reported by NIA-supported researchers in the Proceedings of the National Academy of Sciences (Emery et al., 2020). These findings from a 20-year study of animals in their natural environment suggested that increases in stress hormones are a normal part of aging, rather than a consequence of other factors, such as the environment where the humans are studied or certain lifestyle factors such as poor nutrition or insufficient physical activity (Emery et al., 2020). Female chimpanzees are generally less gregarious than males but nevertheless are confronted with considerable intrasexual social competition (Newton-Fisher, 2006). The observations of captive female chimpanzees forming coalitions represent an inherent capacity rather than an artifact of confinement and close proximity to humans, the absence of reports of such behavior from wild populations suggests that it may occur only under appropriate ecological and demographic conditions. Chimpanzees show wide variation in many aspects of their behavior across communities and populations, which is produced or influenced by differences in ecology, demography, or local tradition (Whiten et al., 2001). Corticosterone is a steroid-based hormone of 21 carbons, classified among adrenal corticosteroids synthesized in the adrenal cortex. Corticosterone, is the main glucocorticoid in rodents, birds, reptiles, and amphibians, controlling the metabolism and stress response (Yoshinao & Michael, 2021). In humans, corticosterone is mainly synthesized in the zona glomerulosa of the adrenal cortex; however, the activity of corticosterone is weak in humans. Corticosterone is important as an intermediate product for the production of aldosterone from pregnenolone in steroidogenesis. (Yoshinao & Michael, 2021). Numerous studies have demonstrated that the magnitude of the cortisol response marshaled by an individual depends upon both the physiological and the psychological aspects of the stressor that induces it (Miller & James, 2002). Psychological stressors have generally received more attention in the literature (Abbott et al., 2003). However, experimental evidence that both unpredictability and loss of control are associated with a heightened stress response led to an early expectation among researchers that in social animals, subordinate individuals should generally maintain higher levels of circulating glucocorticoids than dominants (Creel, 2001). Tests of this hypothesis in primates have been performed primarily on captive populations, producing mixed results. For some groups, this relationship normally holds true (Sapolsky, 1992). Wild animals raised in captivity in an environment that is different from their natural environment can exhibit different behaviors and display stress symptoms. When animals are deprived of the possibility to

perform species-specific behavior, they may show signs of suffering such as behavioral disorders, chronic stress, or other pathological conditions (Wurbel et al., 1996). Behavioral enrichment is a process of stimulating the five senses: eyes, ears, nose, tongue and touch (movement) so that animals can exhibit natural behavior and reduce stress, helping animals to achieve better mental and physical health. The behavioral enrichment format is divided into two types: 1. Food Enrichment (variety more than feed or feeding change method) 2. Non-Food Enrichment (make equipment from natural material or use toys not harmful to animals) to stimulating natural behavior in animals (Panisa, 2011). Behavior enrichment programs aims to provide chimpanzees with an enjoyable and fulfilling life. Billie et al. (2016) are dedicated to improving their overall well-being by encouraging cognitive and sensory stimulation, easing stress, reducing boredom, and promoting physical and mental health (In nature, chimpanzees spend most of their time foraging for food and exploring their habitat). However, in captivity, they have a lot more leisurely time. So Billie's team of volunteers and caregivers aim to originate fun and unique ways to keep them entertained (Billie et al., 2016). The safety of the animals is paramount in all activities. In addition, the promotion of behavior is considered an indicator of the welfare of animals kept in a zoo (Young, 2003). The objective of the present study was to examine the effect of behavior enrichment on corticosterone levels in chimpanzees captive for conservation.

2. Materials and methods

2.1 Animals and housing

Subjects were four adult chimpanzees (two males and two females) from the Chiang Mai Night Safari (Table 1). The Chimpanzees had lived in captivity for about 17 years at Chiang Mai Night Safari. Chimpanzees were divided into two groups: two males and two females. Each group was housed in the same way. The enclosures they were held captive in ~9 x 12 m, and divided into two parts: an enclosed compound of 3 x 3 m, and an external "backyard" of 6 x 9 m. Inside the backyard there was a rope and tires, a timber bed for climbing and there was a bowl of water which chimpanzees could drink from at anytime.

Table 1. Data on Chimpanzees

No	Local ID	Microchip Number	Sex	Age (Year)
1	M40-001	900.012000102360	Female	33
2	M40-004	900.012000103046	Female	30
3	M40-002	900.012000103124	Male	33
4	M40-003	900.202011240342	Male	31

2.2 Behavior Enrichment Program

The behavior enrichment program designed for chimpanzees involved 15 activities, each activity lasted approximately 30 min. Details about the enrichment program are shown in Table 2.

Table 2. Behavior enrichment programs and response times for chimpanzees

Date	Enrichment Program	Time to Response (min.)	Time to Enrichment (min.)	Behavior Response
Day 1	Coconut supplement diet	1-5	>10	They use their hands and teeth to gnaw off coconut shells and then eat coconut water and coconut meat. Some chimpanzees play with coconuts first and then eat coconut water and coconut meat.
Day 2	Scatter feeding substituting feeding pattern	1-5	6-10	Walking to collect feed pellets takes longer than the tray feeding method.
Day 3	Hand-fed substituting feeding pattern	1-5	>10	Get more familiar with the keeper.
Day 4	Hanging-fed substituting feeding pattern	1-5	>10	They stand and hang to eat feed pellets continuously.
Day 5	Grain scattered supplement diet	non	non	Not interested in grains.
Day 6	UHT milk supplement diet	1-5	1-5	They use their hands and teeth to unwrap a milk carton. After finishing, they returned the empty milk cartons to the keeper.
Day 7	Coconut supplement diet	1-5	>10	They use their hands and teeth to gnaw off coconut shells and then eat coconut water and coconut meat.
Day 8	Frozen fruit supplement diet	1-5	1-5	Enjoy frozen fruit Reduce aggressive behavior.
Day 9	Banana trunk and leaf supplement diet	1-5	>10	The first chimpanzee that got the banana tree played with it satisfied and then passed it on to other chimpanzees to play with. Some chimpanzees eat banana tree trunks.
Day 10	Change feeding time	1-5	>10	Chang's feeding time from 2 times /day to 3 times/day affects chimpanzees not fighting and grabbing feed.

Table 2. Behavior enrichment programs and response times for chimpanzees (cont.)

Date	Enrichment Program	Time to Response (min.)	Time to Enrichment (min.)	Behavior Response
Day 11	Supplement frozen fruit dessert	1-5	>10	They use their hands to smash and find a place to sit and eat frozen fruit dessert.
Day 12	Add plastic ball	1-5	1-5	They run and pick up a ball to throw and play with.
Day 13	Add fecal other animal	non	non	Not interested in fecal material of other animals.
Day 14	Turn on Mozart's music	non	non	Not interested in Mozart's music.
Day 15	Add pile dead leaves	1-5	1-5	Walk to a pile of dead leaves, throw and tear play pile of dead leaves.

2.3 Fecal collection

Fecal samples were collected weekly before, during and after behavior enrichment during periods, when animals were individually housed for procedures required in other ongoing studies. Fecal samples were collected weekly in the morning between 09.00 to 12.00 h to avoid variation in the circadian rhythm of corticosterone secretion. Defecation was either observed or indicated to have occurred within the hour by the zoo keeper. Fecal boluses were broken open and approximately 50 g of material was taken from the middle and placed into zip-lock plastic bags labeled with the chimpanzees sex, time of collection and date. Samples were preserved at -20°C until fecal extraction and hormonal analysis.

2.4 Fecal extraction

Fecal samples were extracted as described by Brown et al., (2004). Frozen feces were thawed at room temperature (RT) and dried in a conventional oven (60°C) for 24-48 h. Mixed powdered feces (0.1 g) were placed into glass tubes, 4.5 ml (18 x 180 cm) of EtOH and 0.5 ml of distilled water were added, and tubes were vortexed briefly. Samples were extracted by boiling in a water bath (90°C) for 20 min, with 90% EtOH added to keep the volume at 5 ml, and then centrifuged at 2,500 rpm for 20 min. The fecal extracts were combined, dried in a 90°C water bath, re-suspended in 5 ml of 90% EtOH, dried down again, and finally re-suspended in 1 ml of methanol into 1.5 ml microtube and were then stored at -20°C until analysis using enzyme-linked immunosorbent assay (ELISA).

2.5 Analysis by enzyme-linked immunosorbent assay (ELISA)

Concentrations of fecal glucocorticoid metabolites (fGCM) were measured in extracts diluted 1:3 in assay buffer (0.0137 M Trizma base, 0.2 M Tris-HCl, 0.2 M NaCl, 0.2 M EDTA, 0.001% BSA, and 0.001% Tween 20; pH 7.5) using a double-antibody

EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006, Coralie Munro, UC Davis, CA) previously used in Thailand. 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 RT for 2 h, followed by 100 µl of TMB solution and incubation for 20-35 min. Stop solution was added (50 µl, 1 N HCl) and absorbance measured at 450 nm (TECAN, Männedorf, Switzerland). Assay sensitivity was 0.12 ng/ml. Intra- (at 90% binding)) and inter-assay coefficients of variation (CV, based on dose values) were < 10% (all duplicates over 10% which were reanalyzed) and 12.28%, respectively. The The level of the hormone corticosterone was determined to interpret the results in comparison with the behavior enrichment data.

2.6 Statistical analysis

All data were analyzed by using IBM SPSS statistic software version 25. Descriptive data are presented as the mean ± standard deviation (SD) for corticosterone levels. Analyses compared means One-Way ANOVA and Duncan Post Hoc tests using 3 groups: before enrichment, during enrichment and after enrichment.

3. Results and discussion

3.1 Enrichment program

The enrichment program for chimpanzees consisted of 15 activities which were divided into two categories, Food and Non-food enrichment activities. Food enrichment activities were 11 activities that responded to 10 activities and non-response to 1 activity. Non-food enrichment activities were 4 activities that responded to 2 activities and non-response to 2 activities. The results of enrichment programs in chimpanzees were that for food enrichment, there was a 73.33% response to food enrichment 90.91% and non-food enrichment 26.67% response to non-food enrichment 50% (Figure 1).

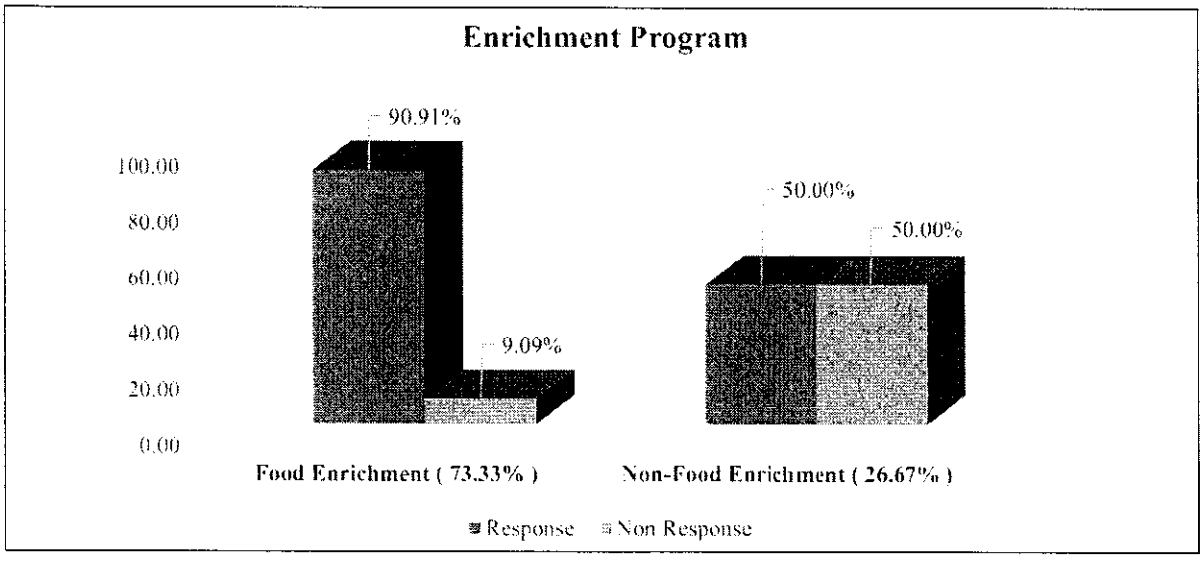

Figure 1. Results of chimpanzee responses to the enrichment program.

The positive results of this study generally corroborate the results of the few other published feeding enrichment studies. Chamove et al. (1982) reported reduced levels of aggression and abnormal behaviors and more foraging when woodchip litter seeded with food items was added to primate enclosures (Chamove et al., 1982).

3.2 Corticosterone in chimpanzees

It was found that corticosterone levels in male chimpanzees before, during and after the behavior enrichment period were not-significantly different (P = 0.895). However, there was a continuous decrease in corticosterone levels equal to 57.63±24.93 ng/g feces, 54.51±3.06 ng/g feces, and 52.25±23.41 ng/g feces, respectively. Corticosterone levels in female chimpanzees during the behavior enrichment period were higher than after enrichment and before enrichment period was non-significantly (P = 0.071). There values were 45.15±10.16 ng/g feces, 36.04±6.85 ng/g feces, and 45.05±2.35 ng/g feces during, after and before enrichment respectively (Table 3). Under the report of the relation between the level of self-mutilation and the concentration of fecal metabolites of glucocorticoids in captive chimpanzees (*Pan troglodytes*). The influence of environmental on behavioral and endocrine variables of primates, have been increasingly studied by many authors, and it has been shown that abnormal behaviors associated with increased glucocorticoids may be directly related with the impairment of the animals' well-being. This study used 22 adult chimpanzees (*Pan troglodytes*), which were kept in captivity in three different institutions. Fecal samples were collected in triplicate and mean concentrations of glucocorticoid fecal metabolites coincident with three different scores of self-mutilation were 34.65±9.35 ng/g feces, 90.34±29.08 ng/g feces and 138.82±38.45 ng/g feces respectively, showing significant differences in this study (Cristiane et al., 2015). Past academic reports indicated that glucocorticoids play an especially important role in the regulation of aggression (Soma, 2006). Aggression between males typically heightens as they compete for reproductive opportunities with estrous females, and male cortisol levels frequently increase during such aggression in many primate species (Bergman et al., 2005; Setchell et al., 2005; Setchell et al. 2010; Fichtel et al., 2007). Also, research using fecal glucocorticoids to assess stress levels in captive river otters found that corticosterone levels in male otters were higher than in female otters (Rothschild et al., 2008). Environmental enrichment is commonly advocated as an effective method to improve the psychological wellbeing of nonhuman primates and other mammals in captivity (Markowitz & Shirley, 1987; Carlstead, 1996). As with psychological wellbeing, no widely accepted definition exists about what constitutes enriched environments. It is generally acknowledged, however, that such environments include both social and physical components (Erwin & Sackett, 1990; Poole, 1991). Furthermore, research on environmental enrichment of Brown Capuchins (*Cebus apella*) advocated that behavioral fecal cortisol measures associated with environmental enrichment are effective indicators of environmental enrichment is commonly advocated as an effective method to improve psychological well-being of nonhuman primates and other mammals in captivity, Boinski et al. (1999) found that across six conditions (preexperimental,

control, toy, box, box & toy, postexperimental) behavioral and fecal cortisol measures had significantly different within-subject differences across the six conditions. Research on daily and seasonal variation of basal and stress-induced corticosterone levels in captive starlings (*Sturnus vulgaris*) found changes in levels. Birds were bled four times during the daily cycle and during three different simulated seasons: under a short-day photoperiod (mimicking winter), under a long-day photoperiod (mimicking summer) and it was found that handling and restraint elicited robust increases in corticosterone at all times of the day and during all three seasons. Levels were higher at night, during the bird's inactive period, and decreased during the day (Romero & Remage-Healey, 2000).

Table 3. Effects of enrichment programs on corticosterone levels in chimpanzees

Chimpanzees	Corticosterone (ng/g feces)			SEM	P-value
	Before Enrichment	During Enrichment	After Enrichment		
Male	57.63±24.93	54.51±3.06	52.25±23.41	4.42	0.895
Female	36.04±6.85	45.15±10.16	45.05±2.35	1.90	0.071
SEM	2.79	4.15	3.99		
P-value	0.387	0.056	0.471		

SEM: Standard error of the mean in row comparison of data between corticosterone levels during period of before enrichment, during enrichment and after enrichment period.
Within a row, values with different non-significantly ($P < 0.05$).

From past reports, it is clear that older chimpanzees exhibited a significantly shallower rate of decline driven by higher levels of corticosterone at the end of the day. Males exhibited higher cortisol concentrations than females across the day. None of the interactions involving chimpanzee sex were significantly different ($P > 0.1$) (Emery et al., 2020). However, increases in fecal cortisol were observed in five of the six cases in which urinary cortisol was higher following stress. In the one case in which urinary cortisol declined following stress, a reduction also was observed in fecal cortisol, both the latter case and the one discordant case occurred in the same female (Whitten et al., 1998).

4. Conclusions

It was found that, in male chimpanzees found that before, during and after behavioura; enrichment, there were continuous decreases corticosterone levels. Enrichment programs in female chimpanzees found that during the enrichment period corticosterone levels were higher than after enrichment and before the enrichment period. Therefore, the results of this study may be used as basic guideline information for designing behavior enrichment programs suitable for captive chimpanzees.

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Ejaculate characteristics of captive felidae in conservation programs

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Abstract - Many carnivore species in the Felidae family, hold conservation significance according to the International Union for Conservation of Nature (IUCN) due to its rapid decline in natural habitats. The objective of this study was to optimize semen evaluation through electroejaculation and examine ejaculate characteristics in captive Felidae for conservation purposes. The totaling nine animals were anesthetized with medetomidine (0.02 mg/kg) and tiletamine-zolazepam (1.5 mg/kg) before semen collection, which was performed using electroejaculation. Semen characteristics, including volume, pH, sperm concentration, total sperm motility, and progressive sperm motility, varied among different species. In jaguars, the semen traits were as follows: volume 1248.57±696.29 µl, pH 6.29±0.33, sperm concentration 150±31.32 x10^6/ml, total sperm motility 70±3.42%, and progressive sperm motility 64±3.57%. Black leopards exhibited

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the following semen traits: volume 22.50±9.46 µl, pH 6.25±0.25, sperm concentration 35±7.36 x10^6/ml, total sperm motility 25.03±14.98%, and progressive sperm motility 12.50±2.50%. In leopards, semen traits were observed as volume 24.75±0.71 µl, pH 6.50±0.71, sperm concentration 49.5±0.71 x10^6/ml, total sperm motility 24.50±0.71%, and progressive sperm motility 11±1.41%. Asiatic golden cats demonstrated semen traits as volume 8.50±1.50 µl, pH 6.75±0.25, sperm concentration 17.50±2.50 ×10^6/ml, total sperm motility 12.50±2.50%, and progressive sperm motility 12.00±2.00%. Golden tigers exhibited the following semen traits: volume 105±14.14 µl, pH 6.25±0.35, sperm concentration 49.95±0.07 x10^6/ml, total sperm motility 60.25±0.35%, and progressive sperm motility 19.90±0.14%. The results of this study serve as guidelines for storing frozen semen and conducting artificial insemination in the Felidae family to aid in breeding and increasing the population for further conservation.

Keywords: Felidae, semen profile, sperm motility, electroejaculation

1. Introduction

The Felidae family, a group of carnivorous mammals, holds conservation value according to the International Union for Conservation of Nature (IUCN) due to its rapidly declining populations in the wild. Among its notable members, the jaguar (*Panthera onca*), which stands as the sole representative of the *Panthera* genus in the Americas, is listed as near-threatened on the IUCN Red List (Quigley et al., 2017). Similarly, the leopard (*Panthera pardus*), which has a widespread distribution across southern Africa, is categorized as vulnerable to extinction (Swanepoel et al., 2016). Another remarkable member, the golden tiger, first observed in the jungles of India, exhibits a distinct blonde coloration with pale golden fur and red-brown stripes instead of the typical black (Xu et al., 2017). Additionally, Asiatic golden cats (*Catopuma temminckii*), inhabiting forests across mainland and insular tropical Asia, face a near-threatened status on the IUCN Red List (Petersen et al., 2021). To safeguard the Felidae family and other imperiled felid species, effective conservation strategies are imperative, including the implementation of captive breeding and genetic management programs. These programs can utilize various assisted reproductive technologies (ART) such as genome resource banking, *in vitro* fertilization (IVF), embryo transfer (ET), and artificial insemination (AI) (Thiangtum et al., 2006). Artificial breeding emerges as a promising technique to preserve the genetic diversity of these species (Chen et al., 2007). Such endeavors are crucial for the maintenance and sustainability of these invaluable genetic resources. The initial step in applying artificial breeding techniques to wild populations involves obtaining fundamental semen characteristics (Ishikawa et al., 1998; Chen et al., 2007). Electroejaculation under surgical anesthesia has become the standard collection technique in the vast majority of wild mammalian species because many of them are intractable. The technique was used successfully in an extensive range of species (Prieto et al., 2014). Numerous investigations have explored the artificial collection of semen via electroejaculation, spanning various species such as the fishing cat (*Prionailurus viverrinus*) (Thiangtum et

al., 2006; Pothakam et al., 2023; Wittayarat et al., 2024), Amur leopard cats (Jeong et al., 2018), flat-headed cat (*Prionailurus planiceps*), Asiatic golden cat (*Catopuma temminckii*) (Wittayarat et al., 2024), peccaries (*Tayassu tajacu*) (Souza et al., 2009), serow (*Capricornis sumatraensis*) (Suwanpugdee et al., 2009), and Asiatic black bears (*Ursus thibetanus*) (Chen et al., 2007). Spermic ejaculates were recovered from all the species mentioned above, but sperm quantity and quality were variable among individuals. The objective of this study was to optimize semen evaluation through electroejaculation and characterize ejaculate traits in captive Felidae for conservation purposes.

2. Materials and methods

2.1 Animal

A total of nine mature males from the Felidae family were utilized in this study, comprising two jaguars, four black leopards, one leopard, one Asiatic golden cat, and one golden tiger (Table 1). These animals were housed in conservation captivity at the Chiang Mai Night Safari. Over the period from 1 November 2022 to 30 August 2023, a total of 18 trials were conducted involving these males.

Table 1. Details of Felidae family at Chiang Mai Night Safari

No	Animal	ID	Age (years)	Weight (kg)
1	Jaguar	6159	14	65
2				69.5
3				68
4				68
5		8920	18	95.5
6				104.3
7				103
8				107
9	Black leopard	2607	14	58
10		3500	15	58
11		2920	10	47
12		1171	9	50
13	Leopard	8188	5	39.55
14				39
15	Asiatic golden cat	3568	18	14.55
16				20.8
17	Golden tiger	8697	9	150
18				147

2.2 Anesthesia and recovery

The animals underwent a 12-hour fasting period prior to the commencement of the experiments. They were then immobilized through an intramuscular injection of a combination of medetomidine (Eurovet, Animal Health BV, Netherlands) and tiletamine-zolazepam (Zoletil®, Virbac, São Paulo, Brazil) at a dosage of 0.2 and 1.5 mg/kg, respectively. After the semen collection procedure and individual data collection, the animals were injected with atipamezole (ZooPharm) at a dosage five times the medetomidine dose (0.1 mg/kg) by intramuscular injection (Hartman et al., 2015).

2.3 Electroejaculation

Semen was collected from each male using a standardized electroejaculation procedure (Wildt et al., 1987). The animal was restrained in a lateral position, and the pubic region was cleaned. Subsequently, the penis was washed successively with sterile physiological saline before semen collection. Semen collection was conducted using an electroejaculator (Minitube electroejaculator, Germany). The electroejaculator probe used for jaguar, black leopard, leopard, and golden tiger measured 2.54 cm in diameter and 12.7 cm in length. For the Asiatic golden cat, a smaller probe measuring 0.99 cm in diameter and 6.35 cm in length was used. The probe was soaked with lubricating jelly (K-Y gel®) and completely inserted into the rectum of the animal. The stimulatory cycle comprised 16 stimuli at each voltage, starting from 0.5 V and increasing in steps of 0.5 V up to 8 V. These stimuli were maintained for a duration of 3 seconds from the beginning of the procedure. When the animal ejaculated, the electric value at that time was noted and this electric level was maintained until semen was no longer found.

2.4 Semen evaluation

Immediately after collection, semen was evaluated for color, volume, pH, sperm concentration and sperm motility. Ejaculate volume was estimated by aspirating the semen into a calibrated positive displacement pipette; pH was measured using pH test paper; sperm concentration was determined using a Neubauer hemocytometer; the total sperm motility was evaluated by placing a drop of semen on a pre-warmed slide at 37°C without a cover slip, and the progressive sperm motility was conducted by observing spermatozoa in five different visual fields. Two experienced technicians then examined the slide under a binocular microscope (Olympus cx23, Japan) at 100X magnification, using standard techniques.

2.5 Statistical analysis

All semen characteristics were examined individually for mean and standard error of the mean (SEM) values.

3. Results and discussion

3.1 Semen collection

A total of 18 semen collections were conducted on nine males, with 1-3 ejaculates per male, spread approximately 3 months apart. Males were able to ejaculate semen 17 times, which is a success rate of 94.44%. Spermic ejaculates were obtained

from all males. All males responded to electrical stimuli with muscle contractions starting at 0.5 V and gradually increasing by 0.5 V increments until reaching 8.5 V (Table 2). The semen collection procedure was successful for every male across all species, but there was a wide variation in semen characteristics among the species and individual males. In the case of jaguars, ejaculates were obtained starting at 4 V (ranging from 4 to 7 V) stimulation. However, it has been demonstrated that jaguars store their semen using an electrical current between 5 to 9 V (Silva et al., 2019). The black leopard yielded ejaculates starting at 5 V (ranging from 5 to 7.5 V) stimulation. The leopard produced ejaculates beginning at 4.5 V (ranging from 4.5 to 6 V) stimulation. For the Asian golden cat, ejaculates were obtained starting at 5.5 V (ranging from 5.5 to 6 V) stimulation. However, it has been demonstrated that Asian golden cats store their semen using an electrical current between 2 to 5 volts (Wittayarat et al., 2024). The golden tiger yielded ejaculates starting at 8 V (ranging from 8 to 8.5 V) stimulation. Differences in response to electrical stimulation among Felidae (jaguars, black leopards, leopards, Asiatic golden cat, and golden tiger) found that golden tigers had the highest voltage requirement to trigger a response. The voltage required to trigger a response decreased in black leopards, jaguars, Asiatic golden cat, and leopards, respectively. Additionally, in the domestic cat, it has been reported that the volume and the number of sperm were different in each ejaculate when electroejaculation with stepwise increases in voltage (1, 2, 4, and 8 V) was used, and the semen volume increased as the electroejaculation trial was repeated (Fukui et al., 2013). Although, the response to the electroejaculation stimulus can vary not only between species or between males from the same species, but even between collections from the same male. Therefore, modifications to the protocol during the process, based on the male's responses, may be needed, even if one strives to follow the same stimulation protocol in all electroejaculation procedures (Wildt et al., 1987). It is important to develop a specific collection protocol for the target species, but it is no less important to have an adequate probe, sometimes specially designed, for each species collected from (Schmitt & Hildebrandt, 2000; Roth et al., 2005; Prieto et al., 2014). The general quality of seminal traits in the Felidae family and other wild animals in captivity (*ex situ*) was likely influenced by many factors such as nutrition, genetic heterozygosity, and age (Thiangtum et al., 2006; Chen et al., 2007)

Table 2. Result of electroejaculation in Felidae family

No	Animal	ID	Electro. (V)	Volume (µl)	pH	% Motility		Concentration (x10⁶/ml)
						Total	Progressive	
1	Jaguar	6159	5.5	5000	6	80	70	260
2			4	1000	7	70	65	200
3			6	1300	5	75	70	220
4			6.5	300	7	75	70	65
5		8920	7	-	-	-	-	-
6			5.5	670	6	60	50	120
7			6.5	120	7	60	55	100
8			6.5	350	6	70	68	85
9	Black Leopard	2607	7.5	10	6	10	10	20
10		3500	7.5	10	6	10.05	10	25
11		2920	6.5	20	6	20	10	45
12		1171	5	50	7	40	20	50
13	Leopard	8188	4.5	25	7	25	10	50
14			6	24.5	6	24	12	49
15	Asiatic Golden	3568	5.5	7	7	10	10	15
16			6	10	6.5	15	14	20
17	Golden Tiger	8697	8	115	6	60	20	50
18			8.5	95	6.5	60.5	19.8	49.9

3.2 Semen characteristic

Semen was collected using electroejaculation. The mean (±SEM) seminal characteristics in jaguars were as follows: volume 1,248.57±696.29 µl, pH 6.29±0.33, sperm concentration 150±31.32 ×10⁶/ml, total sperm motility 70±3.42%, and progressive sperm motility 64±3.57%. Consistent with the report by Araujo et al. (2018), jaguars exhibited a semen concentration of 347.2±295.6 µl (ranging from 2 to 800 µl), and progressive sperm motility of 77 ±11.4% (ranging from 50% to 90%). In black leopards measurements were 22.50±9.46 µl, 6.25±0.25, 35±7.36 ×10⁶/ml, 25.03±14.98%, and 12.50±2.50%, respectively. For the leopard measurements were 24.75±0.71 µl, 6.50±0.71, 49.5±0.71 ×10⁶/ml, 24.50±0.71%, and 11±1.41%, respectively. For the Asiatic golden cat measurements were 8.50±1.50 µl, 6.75±0.25, 17.50±2.50 ×10⁶/ml, 12.50±2.50%, and 12.00±2.00%, respectively. Moreover, it has been reported that Asiatic golden cat ejaculate volume and sperm concentration

were 136.0 µl. and 0.1×10^6 spermatozoa/ml, respectively (Wittayarat et al., 2024). In the golden tiger measurements were 105±14.14 µl, 6.25±0.35, 49.95±0.07 $\times10^6$/ml, 60.25±0.35%, and 19.90±0.14%, respectively (Table 3). However, it has demonstrated that Siberian tiger characteristics of semen in each ejaculate volume were 5.8±2.7 ml (range: 2.3-11.5), and motile sperm were 82.4±11.4 % (range: 50.0-95.0) (Fukui et al., 2013). Once semen is obtained, and before its use for assisted reproduction techniques (ART), the evaluation of sperm quality is a necessary step to know whether the sample of semen is suitable for conservation treatment. Sperm collection in combination with artificial insemination is a common and widely used assisted reproduction technique (ART) in animal reproduction. The methods are mostly complemented by semen cryopreservation, ART may support conservation programs for the Felidae family (jaguars, black leopards, leopards, Asiatic golden cat, and golden tiger).

Table 3. Comparison of semen characteristics of wild captive Felidae by electroejaculation

Characteristic	Jaguar (n=2)	Black Leopard (n=4)	Leopard (n=1)	Asiatic Golden (n=1)	Golden Tiger (n=1)
Volume (µl)	1248.57±696.29	22.50±9.46	24.75±0.71	8.50±1.50	105±14.14
pH	6.29±0.33	6.25±0.25	6.50±0.71	6.75±0.25	6.25±0.35
concentration ($\times10^6$/ml)	150±31.32	35±7.36	49.5±0.71	17.50±2.50	49.95±0.07
total sperm motility (%)	70±3.42	25.03±14.98	24.50±0.71	12.50±2.50	60.25±0.35
sperm progressive (%)	64±3.57	12.50±2.50	11±1.41	12.00±2.00	19.90±0.14

Data represent Mean±SEM

4. Conclusion

From this study, it can be concluded that the Felidae responded to electrical stimuli at about 4-8.5 V. The semen characteristics in Felidae were as follows: volume ranged from approximately 8.5 to 1248.57 µl, pH ranged from approximately 6.25 to 6.75, sperm concentration ranged from approximately 17.50 to 150 $\times10^6$/ml, total sperm motility ranged from approximately 12.50 to 70%, and progressive sperm motility ranged from approximately 11 to 64%. Based on the results of semen collection via electroejaculation from the Felidae family (including the jaguars, black leopards, leopard, Asiatic golden cat, and golden tiger), guidelines for storing frozen semen and artificial insemination within the Felidae family can be established to aid in breeding efforts and population augmentation for conservation purposes. The electroejaculation method shows promise and can be further refined for application in other wildlife species, contributing to sustainable wildlife conservation efforts.

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การสำรวจความหลากหลายของชนิดพรรณพืชในพื้นที่เชียงใหม่ไนท์ซาฟารี

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บทคัดย่อ

เชียงใหม่ไนท์ซาฟารี มีพื้นที่ติดต่อกับอุทยานแห่งชาติดอยสุเทพ-ปุย โดยมีพื้นที่ทั้งหมด 819 ไร่ ทำให้พื้นที่มีความหลากหลายของพรรณพืช โดยการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสำรวจความหลากหลายของชนิดพรรณพืชในพื้นที่เชียงใหม่ไนท์ซาฟารี โดยใช้วิธีการสำรวจแบบ Line transect sampling ตามเส้นทางถนนขนาดความกว้าง 5 เมตร วางแนวสำรวจตามเส้นทางถนนทั้งหมดของพื้นที่ ทำการบันทึกชนิดพรรณ และจำนวน จากการสำรวจพบว่า ป่าในพื้นที่แบ่งออกเป็น 2 ประเภท คือ ป่าเต็งรัง (Dry deciduous dipterocarp forest) และป่าผสมผลัดใบ (Mixed deciduous forest) สามารถจำแนกชนิดพรรณพืชได้ 121 ชนิด ใน 39 วงศ์ วงศ์ที่พบจำนวนชนิดมากที่สุด 5 อันดับแรก คือ วงศ์ FABACEAE (24 ชนิด) วงศ์ MORACEAE (10 ชนิด) วงศ์ EUPHORBIACEAE (7 ชนิด) วงศ์ LYTHRACEAE (7 ชนิด) และวงศ์ COMBRETACEAE (6 ชนิด) และอื่นๆ อีก 34 วงศ์ 67 ชนิด ดังนั้นการศึกษาในครั้งนี้สามารถนำไปใช้เป็นฐานข้อมูลเบื้องต้นในการจัดการพื้นที่ และใช้ประโยชน์เพื่อเป็นพืชอาหารสัตว์ได้

คำสำคัญ: สำรวจ ความหลากหลายพรรณพืช เชียงใหม่ไนท์ซาฟารี

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การส่งเสริมพฤติกรรมหมีควาย (*Ursus thibetanus*) ในสภาพการเพาะเลี้ยงเชิงอนุรักษ์

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บทคัดย่อ

หมีควาย หรือ หมีดำเอเชีย เป็นหมีที่ใหญ่ที่สุดในประเทศไทยมีเขตการกระจายพันธุ์อยู่ในเอเชียตะวันออก ซึ่งหมีควายจัดเป็นสัตว์ที่มีแนวโน้มใกล้สูญพันธุ์ จึงมีเป้าหมายที่ต้องการเพิ่มจำนวนประชากรของสัตว์ป่าที่ใกล้จะสูญพันธุ์ โดยหมีควายที่ถูกนำมาเลี้ยงในสภาพการเพาะเลี้ยงเชิงอนุรักษ์อาจส่งผลต่อการเปลี่ยนแปลงพฤติกรรมตามธรรมชาติ และส่งผลให้หมีควายเกิดความเครียดได้ การส่งเสริมพฤติกรรม (Behavioral Enrichment) จึงเป็นขบวนการเพื่อการปรับปรุงหรือส่งเสริมสิ่งแวดล้อมและการดูแลสัตว์ โดยเน้นการกระตุ้นประสาทสัมผัสเพื่อให้สัตว์ได้ผ่อนคลายความเครียดลง ช่วยให้สัตว์มีสุขภาพจิตและสุขภาพกายที่ดีขึ้น การศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของการตอบสนองของหมีควายต่อกิจกรรมการส่งเสริมพฤติกรรมสัตว์ โดยการส่งเสริมพฤติกรรมสัตว์ของเชียงใหม่ไนท์ซาฟารีแบ่งออกเป็น 2 รูปแบบ ได้แก่ 1. การส่งเสริมพฤติกรรมโดยการใช้อาหาร (การเพิ่มความหลากหลายของอาหาร หรือ ปรับเปลี่ยนวิธีการให้อาหาร) 2. การส่งเสริมพฤติกรรมโดยการสร้างกิจกรรม (การทำอุปกรณ์จากวัสดุธรรมชาติ หรือ การใช้ของเล่นที่ไม่เป็นอันตรายต่อสัตว์) เพื่อกระตุ้นพฤติกรรมตามธรรมชาติให้แก่สัตว์ ได้ทำการส่งเสริมพฤติกรรมในหมีควายจำนวน 15 ตัว (เพศผู้ 12 และเพศเมีย 3) ของเชียงใหม่ไนท์ซาฟารีภายในระยะเวลา 12 เดือน พบว่ามีการส่งเสริมพฤติกรรมโดยการใช้อาหาร คิดเป็นร้อยละ 68.49 และการส่งเสริมพฤติกรรมโดยการสร้างกิจกรรม คิดเป็นร้อยละ 31.51 โดยผลของระยะเวลาในการตอบสนองภายในเวลา 1 - 5 นาที คิดเป็นร้อยละ 89.04 และการตอบสนองภายในเวลา 6 - 10 นาที คิดเป็นร้อยละ 10.96 ทั้งนี้การส่งเสริมพฤติกรรมสัตว์มีเป้าหมายเพื่อให้หมีควายในเชียงใหม่ไนท์ซาฟารีทุกตัว มีสุขภาพที่แข็งแรงได้รับปริมาณอาหารที่เพียงพอและเหมาะสม ได้รับการส่งเสริมพฤติกรรม และมีการพัฒนาคุณภาพชีวิตของหมีควายอยู่เสมอแม้ถูกเลี้ยงในสภาพการเพาะเลี้ยงเชิงอนุรักษ์

คำสำคัญ: การส่งเสริมพฤติกรรม หมีควาย สภาพการเพาะเลี้ยงเชิงอนุรักษ์

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การศึกษาความหลากหลายของชนิดพรรณพืชในพื้นที่เชียงใหม่ไนท์ซาฟารี เพื่อใช้เป็นพืชอาหารค่างห้าสี

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บทคัดย่อ

เชียงใหม่ไนท์ซาฟารี มีพื้นที่ติดต่อกับอุทยานแห่งชาติดอยสุเทพ-ปุย โดยมีพื้นที่ทั้งหมด 819 ไร่ ทำให้พื้นที่มีความหลากหลายของพรรณพืช โดยการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสำรวจความหลากหลายของชนิดพรรณพืชในพื้นที่เชียงใหม่ไนท์ซาฟารี และศึกษาการเลือกกินชนิดพรรณพืชของค่างห้าสี โดยใช้วิธีการสำรวจแบบ Line transect sampling แบบตามเส้นทางถนนขนาดความกว้าง 5 เมตร วางแนวสำรวจตามเส้นทางถนนทั้งหมดของพื้นที่ ทำการบันทึกชนิดพรรณ และศึกษาการเลือกกินชนิดพรรณพืชของค่างห้าสี จำนวน 6 ตัว แบบเต็มที่ (*ad libitum*) เพื่อทำการคัดเลือกชนิดพรรณพืชที่ค่างห้าสีเลือกกิน โดยทำการทดสอบวันละ 3 ชนิด ทำซ้ำ 3 วัน ทำการบันทึกข้อมูลชนิดพรรณพืชที่ค่างห้าสีเลือกกินเป็นอาหาร และข้อมูลชนิดพรรณพืชที่ค่างห้าสีไม่เลือกกินเป็นอาหาร จากการสำรวจพบว่าในพื้นที่มีป่าแบ่งออกเป็น 2 ประเภท คือ ป่าเต็งรัง (Dry deciduous dipterocarp forest) และป่าผสมผลัดใบ (Mixed deciduous forest) สามารถจำแนกชนิดพรรณพืชได้ 120 ชนิด ใน 39 วงศ์ จากการศึกษาการเลือกกินชนิดพรรณพืชของค่างห้าสี พบว่า ค่างห้าสีเลือกกินชนิดพรรณพืช จำนวน 31 ชนิด 18 วงศ์ ค่างห้าสีไม่เลือกกินชนิดพรรณพืช จำนวน 89 ชนิด 39 วงศ์ ดังนั้นการศึกษาในครั้งนี้สามารถนำไปใช้เป็นข้องมูลพื้นฐานในการจัดการพื้นที่และยังเป็นแนวทางในการศึกษาพืชอาหารสำหรับสัตว์ชนิดอื่นที่กินใบไม้เป็นอาหารหลักในเชียงใหม่ไนท์ซาฟารี อีกทั้งนำไปใช้ประโยชน์เพื่อเป็นพืชอาหารของค่างห้าสีต่อไป

คำสำคัญ: ค่างห้าสี พืชอาหาร ความหลากหลาย

The study of plant diversity in the Chiang Mai Night Safari and feed plant selection for the red-shanked douc langur (Pygathrix nemaeus)

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Abstract

The Chiang Mai Night Safari is located in an area connected to the National Park Doi Suthep-Pui, covering a total area of 819 rai. This region boasts a diverse range of plant species, making it an ideal location for studying the plant diversity and selection of food for the red-shanked douc langur. The study utilized line transect sampling along the trail, with a five-meter fixed-width technique used for the road route, to record species. Additionally, the feeding habits of six red-shanked doucs were observed to determine their preferred plant species. The survey revealed that the forests in the area can be classified into two types: dry deciduous dipterocarp forests and mixed deciduous forests. A total of 39 families and 120 plant species were identified. The study of the red-shanked doucs feeding habits, found that they selected 31 plant species from 18 families as their main feed source, while 89 species from 39 families were not selected. This information can be used for effective area management and as a guide for studying the feeding habits of other leaf-eating animals in the Chiang Mai Night Safari. Furthermore, it can also be used to provide suitable feed plant for the red-shanked doucs.

Keyword: Red-shanked douc langur, Feed plant, diversity

ศึกษาความสัมพันธ์ของระดับฮอร์โมนคอร์ติโคสเตอโรนต่อการแสดงพฤติกรรมของหมีควาย ในสภาพการเพาะเลี้ยงเชิงอนุรักษ์

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บทคัดย่อ

หมีควาย เป็นหมีที่มีขนาดใหญ่ที่สุดในประเทศไทย มีเขตการกระจายพันธุ์อยู่ในเอเชียตะวันออก อีกทั้งยังจัดเป็นสัตว์ที่มีแนวโน้มใกล้สูญพันธุ์ หมีควายที่ถูกนำมาเลี้ยงในสภาพแวดล้อมที่แตกต่างจากธรรมชาติ ส่งผลให้มีการแสดงพฤติกรรมที่แตกต่างกันออกไป เมื่อสัตว์ขาดการแสดงความสามารถตามธรรมชาติ อาจส่งผลให้สัตว์แสดงพฤติกรรมที่ไม่พึงประสงค์ออกมา ก่อให้เกิดความเครียดสะสม โดยฮอร์โมนคอร์ติโคสเตอโรน เป็นฮอร์โมนความเครียดในกลุ่มสเตียรอยด์ ส่งผลให้การทำงานของระบบภูมิคุ้มกันของร่างกายสัตว์ลดลง การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความสัมพันธ์ของระดับฮอร์โมนคอร์ติโคสเตอโรนที่สกัดจากมูลต่อการแสดงพฤติกรรมของหมีควายจำนวน 6 ตัว (เพศผู้ 3 ตัว และเพศเมีย 3 ตัว) โดยทำการบันทึกพฤติกรรม 5 กลุ่ม ได้แก่ กิจวัตรประจำวัน การเคลื่อนไหว การผ่อนคลาย การเข้าสังคม และพฤติกรรมที่ผิดปกติ ระยะเวลาในการบันทึกพฤติกรรม 385 ชม. ทำการเก็บตัวอย่างมูลหมีควาย จากนั้นนำมูลสกัดฮอร์โมน และวิเคราะห์หาระดับความเข้มข้นของฮอร์โมนคอร์ติโคสเตอโรน ด้วยวิธี ELISA พบว่า ระดับฮอร์โมนคอร์ติโคสเตอโรนกับพฤติกรรมกลุ่มกิจวัตรประจำวัน การผ่อนคลาย และการเข้าสังคม ไม่มีความสัมพันธ์กันทางสถิติ ($P>0.05$) แต่ระดับฮอร์โมนคอร์ติโคสเตอโรนกับพฤติกรรมการเคลื่อนไหวมีความสัมพันธ์กันทางสถิติ ($P=0.022$) และระดับฮอร์โมนคอร์ติโคสเตอโรนกับพฤติกรรมที่ผิดปกติมีความสัมพันธ์กันทางสถิติ ($P=0.002$) ดังนั้นผลการศึกษาในครั้งนี้ใช้เป็นข้อมูลพื้นฐานในการปรับปรุงสภาพแวดล้อมภายในกรงเลี้ยง และเป็นแนวทางการออกแบบโปรแกรมเสริมสร้างพฤติกรรมให้เหมาะสมต่อหมีควายเพื่อลดพฤติกรรมที่บ่งบอกความเครียดต่อไปในอนาคต

คำสำคัญ : ฮอร์โมนคอร์ติโคสเตอโรน หมีควาย พฤติกรรม

The Association of corticosterone concentration with behavior of Asiatic black bear (*Ursus thibetanus*) in captive for conservation

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Abstract

Asiatic black bears (*Ursus thibetanus*) is largest bear in Thailand. Its distribution area in East Asia. The Asiatic black bears is classified vulnerable species. Wild animals are raised in environments that are different from their natural habitats. As a result, different behaviors will be displayed. When animals cannot express their natural abilities. It may cause the animal to show unwanted behavior causing accumulated stress. Corticosterone hormone is steroid group, which is secreted when animals are in stressful conditions and affected to decrease immune system function. The objective of this study association of corticosterone concentration with behavior in six Asiatic black bears (3 Male, 3 Female). The behaviors were divided into five groups daily routine, movement, relaxation, socialization, and abnormal behaviors observations were performed for 385 hours. Collected samples of Asiatic black bears droppings. Then the fecal was extracted from the hormones, and analyze the level of corticosterone hormone by ELISA method. It was found that non-significant association between the concentration of corticosterone hormone with daily routine, relaxation, and socialization ($P>0.05$), but there was an association between the concentration of the corticosterone hormone with movement ($P=0.022$) and association between the concentration of the corticosterone hormone with abnormal behavior ($P=0.002$). Therefore, the results of this study may be used as basic guideline information for improving the environment and designing behavior enrichment programs suitable for Asiatic black bears in the future.

Keywords: Corticosterone hormone, Asiatic Black Bear, Behavior

แผนการจัดการเพาะขยายพันธุ์สัตว์ป่าสวนสัตว์ สำนักงานเชียงใหม่ไนท์ซาฟารี

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บทคัดย่อ

การเพาะขยายพันธุ์สัตว์ป่าสวนสัตว์ เป็นกระบวนการหนึ่งที่นำไปสู่การอนุรักษ์นอกถิ่นอาศัย (*ex situ*) โดยมีวัตถุประสงค์เพื่อบริหารจัดการประชากรสัตว์ป่าสวนสัตว์อย่างมีประสิทธิภาพ และลดจำนวนสัตว์ส่วนเกิน แผนการจัดการเพาะขยายพันธุ์สัตว์ป่าสวนสัตว์ สำนักงานเชียงใหม่ไนท์ซาฟารี ประจำปี 2566 จากสัตว์ 133 ชนิด 1,044 ตัว ประกอบด้วย สัตว์เลี้ยงลูกด้วยนม 88 ชนิด 786 ตัว สัตว์ปีก 24 ชนิด 178 ตัว สัตว์เลื้อยคลาน 20 ชนิด 66 ตัว และสัตว์น้ำ 1 ชนิด 14 ตัว พบว่ามีจำนวน 21 ชนิด ที่ต้องดำเนินการเพาะขยายพันธุ์ โดยพิจารณาจาก 1) จำนวนสัตว์ที่เหลือจำนวนน้อย 2) อายุสัตว์ 3) สัตว์ที่ใช้แสดงหรือโชว์ 4) สัตว์ที่มีครบทั้งเพศผู้-เพศเมีย ซึ่งมีแนวทางการเพาะขยายพันธุ์ดังนี้ 1) ดูข้อมูลพันธุ์ประวัติ (Pedigree) และทำการจับคู่ผสมพันธุ์ โดยให้สายพันธุกรรมห่างไกลที่สุด 2) บ่งชี้สัตว์ที่ใช้เป็นพ่อ-แม่พันธุ์ 3) แจ้งผู้เลี้ยง (Keeper) ทำการเทียบคู่ และปล่อยผสมพันธุ์ พร้อมทำการจดบันทึก 4) ทำการติดตามผลการเพาะขยายพันธุ์ทุกๆ 3 เดือน เพื่อทำการบันทึกผลการผสมติด และการแก้ไขปัญหาการผสมไม่ติด จากการปฏิบัติตามแผนการจัดการเพาะขยายพันธุ์ สรุปได้ว่า สามารถได้ลูกสัตว์จำนวน 7 ชนิด 10 ตัว และดำเนินการแก้ปัญหาสัตว์ที่ผสมไม่ติด ดังนี้ 1) การจัดการด้านอาหาร 2) การตรวจสุขภาพ 3) การเปลี่ยนคู่ผสมพันธุ์ 4) ใช้เทคโนโลยีช่วยการสืบพันธุ์ (Assisted reproductive technology: ART) จากแผนการจัดการเพาะขยายพันธุ์สัตว์ป่าสวนสัตว์ สำนักงานเชียงใหม่ไนท์ซาฟารี สามารถเพิ่มจำนวนประชากร และนำไปสู่การอนุรักษ์สัตว์ป่าในอนาคต รวมถึงช่วยลดจำนวนสัตว์ส่วนเกินได้

คำสำคัญ: แผนการจัดการ เพาะขยายพันธุ์ สัตว์ป่าสวนสัตว์

Breeding management plan in Wildlife of Chiang Mai Night Safari

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Abstract

Breeding management plan of wild animals in zoos. It is a process that leads to ex-situ conservation. The objective is to manage the population of wild animals in the zoo effectively. and reduce the number of surplus animals. Zoo wildlife breeding management plan Chiang Mai Night Safari Office, the year 2023, from 133 species (1,044 animals), consisting of 88 species of mammals (786 animals), 24 species of birds (178 animals), 20 species of reptiles (66 animals), and 1 species of aquatic (14 animals). It was found that 21 species needed to be propagated. Considering 1) the small number of animals remaining, 2) the age of the animals, 3) animals used for show, 4) animals that have both males and females. There are guidelines for breeding as follows: 1) pedigree and make breeding pairs, by keeping the genetic line as distant as possible, 2) Identify the animal used as the breeder, 3) Notify the keeper for matching, and release to breed ready to take notes, 4) Follow up on the breeding results every 3 months to record the results of breeding, and solve the problem of mixing not sticking. From following the breeding management plan, it was successful that 7 species (10 animals), could be obtained and the problem of animals that did not breed was solved as follows: 1) feed management, 2) health examination, 3) changing breeding pairs, 4) use assisted reproductive technology (ART) technique. The wildlife breeding management plan of Chiang Mai Night Safari can increase the population and lead to wildlife conservation in the future, including helping to reduce the number of excess animals.

Keywords: Breeding, Management plan, Wildlife

การใช้เครื่องหมายทางพันธุกรรมตรวจหาเชื้อ *Toxoplasma gondii* ในเสือ และสิงโต

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บทคัดย่อ

เชื้อ *Toxoplasma gondii* เป็นสาเหตุของการเกิดโรคท็อกโซพลาสโมซิส (Toxoplasmosis) หรือโรคขี้แมว โดยมีแมวเป็นโฮสต์จำเพาะ (Definitive host) และสัตว์ป่า สัตว์เลี้ยง สัตว์เศรษฐกิจ รวมทั้งมนุษย์เป็น โฮสต์กึ่งกลาง (Intermediate) สามารถติดโรคได้ และทำให้เกิดการแท้งได้ มีวัตถุประสงค์ เพื่อศึกษาความแม่นยำการนำเครื่องหมายทางพันธุกรรมตรวจหาเชื้อ *Toxoplasma gondii* ในเสือ และสิงโต โดยทำการเก็บตัวอย่างเลือดเสือจำนวน 10 ตัวอย่าง และสิงโตจำนวน 10 ตัวอย่าง ถูกนำมาสกัดดีเอ็นเอด้วยชุดสกัดดีเอ็นเอ ทำการบ่งชี้เชื้อ *Toxoplasma gondii* โดยใช้ไพรเมอร์ *bradyzoite surface antigen* (*SAG4*) ยีน ด้วยเทคนิค nested PCR พบว่า เครื่องหมายทางพันธุกรรม *SAG4* สามารถตรวจหาเชื้อ *Toxoplasma gondii* ด้วยความแม่นยำ 75% เมื่อนำเครื่องหมายทางพันธุกรรม *SAG4* ตรวจหาเชื้อ *Toxoplasma gondii* ในเสือ และสิงโต ไม่พบเสือ และสิงโตติดเชื้อ *Toxoplasma gondii* ผลการศึกษาครั้งนี้บ่งชี้ว่าเครื่องหมายทางพันธุกรรม *SAG4* ดังกล่าวสามารถนำมาตรวจหาเชื้อ *Toxoplasma gondii* ในเสือ และสิงโตได้

คำสำคัญ: เครื่องหมายทางพันธุกรรม ทอกโซพลาสมา กอนดิไอ เสือ สิงโต

Using genetic markers to detect *Toxoplasma gondii* in Tigers and Lions

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Abstract

Toxoplasma gondii is the causative agent of toxoplasmosis, or cat feces disease, with cats as the definitive host and wild animals, pets, commercial animals, and humans as intermediate hosts who can contract the disease and cause abortion. The objective was to study the accuracy of genetic markers for detecting *Toxoplasma gondii* in Tigers and Lions. Ten Tigers and ten Lions whole blood samples were collected in EDTA and DNA was extracted using the Genomic DNA Isolation Kit. The nested-PCR for *bradyzoite surface antigen* (*SAG4*) gene was identified *Toxoplasma gondii*. The *SAG4* genetic marker detected Toxoplasma gondii with 75% accuracy. The *SAG4* genetic marker was used to detect *Toxoplasma gondii* in Tigers and Lions, no Tigers and Lions were infected with *Toxoplasma gondii*. The results of this study indicate that the *SAG4* genetic marker can be used to detect *Toxoplasma gondii* in Tigers and Lions.

Keywords: Genetic markers, *Toxoplasma gondii*, Tigers, Lions

การวางรางให้อาหาร เพื่อลดความเสี่ยงถึงตายของวัววาตูซี่ จากการตกรางอาหาร

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บทคัดย่อ

วัววาตูซี่ ที่เลี้ยงเป็นฝูงที่เชียงใหม่ไนท์ซาฟารี จำนวน 30 ตัว (15.15) ประสบผลสำเร็จในการเพาะเลี้ยงจนกระทั่งพบว่า มีลูกวัวอายุราว 1-3 ปี จำนวน 5 ตัว ได้ตกลงไปในรางให้อาหารในลักษณะหลังลงและตีนชี้ฟ้า หากมิได้รับการช่วยเหลือจากผู้เลี้ยงอาจเป็นอันตรายถึงชีวิต ผู้เลี้ยงได้มีการปรับเปลี่ยนองศาของการวางรางให้อาหารวัวใหม่ จากรางให้อาหารที่มีโครงสร้างวางนอนราบและก้นรางติดพื้นดิน ลักษณะคล้ายรูปอักษร U ให้เป็นวางตะแคงเล็กน้อยคล้ายรูปอักษร C เพื่อป้องกันมิให้ลูกวัวตกลงไปในรางให้อาหาร หลังจากการปรับเปลี่ยนการวางผ่านไปราว 2 ปี ไม่พบว่าลูกวัวตกลงไปในรางให้อาหารอีกเลย

คำสำคัญ: วัววาตูซี่ ลูกวัว การจัดวาง รางให้อาหาร

Arrangement of Feeding Container to Avoid Mortal Risk in Watusi Cattle

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Abstract

Watusi cows raised in herds at Chiang Mai Night Safari, numbering 30 (15.15) were successful in breeding until found that a number of 5 calves, about 1-3 years old, fell into the feeding container with their backs down and their feet pointing skyward. If there is no help from the animal keeper, the calves may be life threatening. The keeper has changed the angle of placing the cow feeding container. The feeding container that lies flat and the bottom of the container is on the ground. It looks like the letter U and is placed on its side slightly like the letter C to prevent the calf from falling into the feeding container. After about two years of changing the placement, no calves were found to fall into the feeding container again.

Keywords: Watusi cattle, Calf, Arrangement, Feeding container.

ศึกษาการนำใบไม้แห้งมาใช้เป็นภาชนะใส่อาหารสัตว์

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บทคัดย่อ

การแปรรูปใบไม้แห้งเป็นภาชนะใส่อาหารสัตว์ เป็นกิจกรรมหนึ่งภายใต้โครงการพันธมิตรสีเขียว ของสำนักงานเชียงใหม่ไนท์ซาฟารี โดยมีวัตถุประสงค์เพื่อนำใบไม้แห้งในพื้นที่มาใช้ให้เกิดประโยชน์ และรณรงค์แก้ไขปัญหาหมอกควัน และไฟป่าในพื้นที่ ใบไม้แห้งสามารถนำมาใช้เป็นภาชนะสำหรับใส่อาหารสัตว์ทดแทนภาชนะพลาสติกหรือกระเบื้องดินเผาที่ใช้ในปัจจุบัน เพื่อลดปัญหาการแตกร้าวหรือฉีกขาดจากการกระทำของสัตว์ และลดขั้นตอนการทำความสะอาดภาชนะใส่อาหารสัตว์ ใบไม้แห้งในพื้นที่ที่เหมาะสมในการนำมาผลิตจานใบไม้แห้งมากที่สุด คือ ใบตองตึง หรือ ใบพลวง *(Dipterovarpus tuberculstus Roxb)* เนื่องจากใบมีขนาดใหญ่และมีเส้นใยที่เหนียวง่ายต่อการนำมาขึ้นรูป และสามารถหาได้ง่ายในพื้นที่ของสำนักงานเชียงใหม่ไนท์ซาฟารี โดยภาชนะใส่อาหารสัตว์ที่ทำจากใบไม้แห้ง มี 2 รูปแบบ คือ 1) แบบถ้วย ขนาด 5 นิ้ว และ 6 นิ้ว 2) แบบจาน ขนาด 8 นิ้ว และ 9 นิ้ว สัตว์แต่ละชนิดเหมาะสำหรับใช้ภาชนะที่ทำจากใบไม้แห้งในรูปแบบ และขนาดที่ต่างกัน ได้แก่ 1) แบบถ้วย ขนาด 5 นิ้ว เหมาะสำหรับสัตว์ในกลุ่มนกกระตั้ว และนกแก้ว เป็นต้น 2) แบบถ้วย ขนาด 6 นิ้ว เหมาะสำหรับ สัตว์ขนาดเล็ก เช่น เมียร์แคต ลิงกระรอก และลีเมอร์ เป็นต้น 3) แบบจาน ขนาด 8 นิ้ว เหมาะสำหรับ สัตว์กินเนื้อขนาดเล็ก เช่น เสือปลา และเสือลายเมฆ เป็นต้น 4) แบบจาน ขนาด 9 นิ้ว เหมะสำหรับ สัตว์กินเนื้อขนาดใหญ่ เช่น เสือโคร่ง และสิงโต เป็นต้น จากการนำใบไม้แห้งมาใช้เป็นภาชนะใส่อาหารสัตว์ทดแทนภาชนะพลาสติกหรือกระเบื้องดินเผา พบว่า สามารถลดงบประมาณในการจัดซื้อภาชนะพลาสติกหรือกระเบื้องดินเผาลงได้ และสัตว์มีการตอบสนองกับภาชนะที่ทำจากใบไม้แห้งได้เป็นอย่างดี อีกทั้งการนำวัสดุจากธรรมชาติมาใช้ให้เกิดประโยชน์เป็นการสร้างจิตสำนึกให้กับผู้เลี้ยงสัตว์ รวมทั้งเป็นการอนุรักษ์ทรัพยากรธรรมชาติอีกทางหนึ่ง

คำสำคัญ: ใบไม้แห้ง ภาชนะ อาหารสัตว์

Study the use of dry leaves as containers for animal feed

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Abstract

The transformation of dry leaves into animal feed containers is one of the activities under the Green Partnership Project of the Chiang Mai Night Safari. The objective is to make use of dry leaves in the area and to campaign to solve the problem of smog and forest fires in the area. Dry leaves can be used as containers for animal feed, replacing the plastic containers or clay tiles currently used, to reduce the problem of cracking or tearing from animal actions and to reduce the steps of cleaning animal food containers. The most suitable dried leaves in the area for producing dried leaf plates are the Gurjan leaves, Pluang leaves (*Dipterovarpus tuberculstus Roxb.*) because the leaves are large and have tough fibers that are easy to shape and can be easily found in the area of the Chiang Mai Night Safari. The animal feed containers made from dry leaves come in 2 styles: 1) Cup, 5 inches and 6 inches; 2) Plate, 8 inches and 9 inches. Different animals are suitable for containers made from dry leaves in different styles and sizes: 1) Cup style (5 inches) suitable for animals such as cockatiels and parrots, etc. 2) Cup style (6 inches) suitable for small animals such as meerkats, squirrel monkeys, and lemurs, etc. 3) Plate style (8 inches) suitable for small carnivores such as fishing cats and clouded leopards, etc. 4) Plate style (9 inches) suitable for large carnivores such as tigers and lions, etc. By using dry leaves as animal feed containers instead of plastic containers or clay tiles, it was found that the budget for purchasing plastic containers or clay tiles could be reduced, and animals responded well to containers made from dry leaves. Furthermore, using natural materials to create awareness for animal owners, as well as conserving natural resources in another way.

Keywords: Dry leaves, Containers, Animal feed

การสำรวจพื้นที่ก่อโรคเฉพาะถิ่น (Endemic area) ของเชื้อ *Clostridium perfrigens* และชนิดของสารพิษ Clostridial toxin จากตัวอย่างในพื้นที่ส่วนแสดงและคอกกักสัตว์ในเชียงใหม่ไนท์ซาฟารี

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บทคัดย่อ

Clostridium perfringens เป็นเชื้อที่สามารถพบได้ทั่วไปในธรรมชาติและทางเดินอาหารสัตว์ ซึ่งจากรายงานสัตว์ป่วยในช่วงที่ผ่านมา พบว่ามีสัตว์หลายชนิดในพื้นที่เชียงใหม่ไนท์ซาฟารีป่วยจากการติดเชื้อและได้รับสารพิษจากเชื้อ *Clostridium perfringens* ทั้งนี้การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจหาเชื้อ *Clostridium perfringens* และชนิดของสารพิษที่เชื้อสร้างขึ้นจากตัวอย่างดินในส่วนแสดงและคอกกักสัตว์ของเชียงใหม่ไนท์ซาฟารี โดยทำการเก็บตัวอย่างดิน ในระหว่างเดือนมกราคม-มีนาคม 2566 จำนวน 15 จุด ได้แก่ พื้นที่ส่วนแสดง จำนวน 11 จุด และคอกกักสัตว์ จำนวน 4 จุด และนำตัวอย่างดินมาเพาะเลี้ยงเชื้อในอาหารเลี้ยงเชื้อแบคทีเรียในสภาวะไร้อากาศ ทำการตรวจหาเชื้อจากการตรวจดูด้วยกล้องจุลทรรศน์ และตรวจหาเชื้อร่วมกับดูการสร้างสารพิษของเชื้อด้วยเทคนิคทางชีวโมเลกุล (Multiplex PCR) จากผลการศึกษาพบว่าตรวจพบเชื้อ *Clostridium perfringens* ด้วยวิธีการส่องกล้องจุลทรรศน์ จำนวน 7 ตัวอย่าง จากทั้งหมด 15 ตัวอย่าง และตรวจพบเชื้อ *Clostridium perfringens* ด้วยเทคนิค multiplex PCR จำนวน 10 ตัวอย่าง จากทั้งหมด 15 ตัวอย่าง และพบสารพิษที่เชื้อสร้างขึ้น 2 ชนิด ได้แก่ Alpha-toxin (*cpa* gene α-toxin) และ Beta2-toxin (*cpb2* gene β2-toxin) โดยพบเชื้อที่สร้างเฉพาะ α-toxin จำนวน 6 ตัวอย่าง และเชื้อที่สร้าง α-toxin ร่วมกับ β2-toxin จำนวน 4 ตัวอย่าง ซึ่งผลจากการศึกษาครั้งนี้สามารถนำมาใช้ในการควบคุมและเฝ้าระวังการแพร่ของเชื้อ *Clostridium perfringens* ที่สะสมอยู่ในดินสู่สัตว์ในพื้นที่ที่ทำการสำรวจ รวมทั้งเป็นแนวทางและมาตรการในการจัดการการควบคุมป้องกันและเฝ้าระวังการก่อโรคของเชื้อ *Clostridium perfringens* ในพื้นที่ส่วนแสดงและคอกกักสัตว์ชนิดต่างๆ ในเชียงใหม่ไนท์ซาฟารี

คำสำคัญ: คลอสตริเดียม เพอร์ฟริงเจนส์ คลอสตริเดียม ท็อกซิน มัลติเพล็กซ์พีซี อาร์ ดิน

A survey from endemic area of *Clostridium perfringens* and Clostridial toxin in the soil from the exhibition area and animal enclosure at Chiang Mai Night Safari

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Abstract

Clostridium perfringens is commonly found in an environment, especially in the soil. It is considered a common enteric microbiota in animals and humans. From previous case reports, many animal species at Chiang Mai Night Safari were infected with *Clostridium perfringens* and exposed with Clostridial toxins. The objective of this study was to detect *Clostridium perfringens* and Clostridial toxin in the soil from the exhibition area and animal enclosure at Chiang Mai Night Safari. From January to March 2023, fifteen soil samples were collected from 11 areas of the exhibition and 4 from the animal enclosures. Total soil samples were inoculated in culture media under anaerobic conditions. The detection of *Clostridium perfringens* and Clostridial toxin were confirmed through microscopic examination and multiplex PCR respectively. From the results of this study, *Clostridium perfringens* was detected, by microscopic examination in 7 soil samples out of 15 soil samples. From the multiplex PCR method, *Clostridium perfringens* was detected in 10 soil samples out of 15 soil samples. Two types of Clostridial toxin were identified by multiplex PCR including Alpha-toxin (*cpa* gene α-toxin) and Beta2-toxin (*cpb2* gene β2-toxin). Six soil samples were produced specifically α-toxin and four soil samples were produced both α -toxin and β2-toxin. These results can be used to control the transmission of *Clostridium perfringens* from the soil to the animals within the sampling areas. Furthermore, these results can be as a guideline and preventive measure for controlling *Clostridium perfringens* infection in various animal species at Chiang Mai Night Safari.

Keywords: *Clostridium perfringens*, Clostridial toxin, Multiplex PCR, Soil