Effect of Stress from Cage Transfer on Fecal Corticosterone Concentration of Collared-Scope Owls (*Otus bakkamoena*)

移籠緊迫對領角鴞糞便中皮質酮濃度變化之影響

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Abstract

Corticosterone is secreted from adrenal glands in response to environmental stress and/or emotional arousal. It is regulated by hypothalamic-pituitary-adrenal axis (HPA axis). Its measurement with the non-invasive technique has become an important tool for assessing environmental or other stress in wild animals. In this study we used 14 collared-scope owls (*Otus bakkamoena*) as test animlas. They were caged together for more than six months and then transferred into individual cages for 21 days. The fecal samples were collected during the pre-transfer period and post-transfer period. Fecal corticosterone concentration was measured with EIA. The food intake was recorded during the post-transfer period. The results showed that a peak corticosterone concentration was found within first 2 days after transfer. All owls showed poor food intake, indicating that the cage transfer caused stress on the owls from handing, examining, and change in cage environment. The fecal corticosterone concentration decreased gradually on the third to sixth day after transfer in response to diminishing in the stress effect. Food intake gradually recovered. The mean corticosterone concentration decreased to a baseline level on the eighth to the twenty-first day after transfer, that was only a third of that prior to transfer. It is concluded that the collared-scope owls needed 6 to 8 days after cage transfer to adapt to a new cage environment.

摘 要

腎上腺皮質酮(corticosterone)屬於固醇類荷爾蒙，受下視丘、腦下垂體所調控，能反應環境壓力與情緒變化，因此又被稱爲「壓力荷爾蒙」。以糞便檢測動物體內的腎上腺皮質酮濃度變化，爲一種非侵入性檢測技術，在野生動物研究領域能成爲論證工具，用以評估環境對動物的影響。本試驗利用農委會特有生物研究保育中心長期收容暫時無法野放的領角鴞進行研究。試驗動物皆經混籠飼養 6 個月以上，試驗前先隨機收集移籠前之糞便樣本，之後移入隔離籠舍、個別飼養。收集移籠後 21 天內之糞便樣本，以 80%乙醇進行萃取，並以 EIA 檢測分析，比較移籠前、後糞便中的皮質酮濃度變化，並紀錄移籠後的個別進食狀況。結果發現，移籠後的 2 天內，動物因受到人爲的抓取、檢查與環境改變的緊迫所刺激，皮質酮濃度上升，且多數試驗動物個體有拒食的狀況。移籠後第 3-6 天皮質酮濃度逐漸下降，個體漸漸適應環境，所有的個體進食狀況漸漸恢復正常。移籠後的第 8-21 天，期間皮質酮濃度穩定無變化且其值低於移籠前 1/3。結果證實，以糞便檢測領角鴞的皮質酮濃度變化，能了解領角鴞對環境改變的生理反應，並發現圈養之領角鴞對新環境之適應期 6-8 天左右，移籠後的進食狀況可反映出動物的適應狀況。

Key words: stress, owl, corticosterone, caged transfer, feces

關鍵詞：緊迫、貓頭鷹、腎上腺皮質酮、移籠、糞便

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Introduction

Environmental stress may cause physiological stress of animal, often resulting in sickness or even death (Wasser *et al*. 1997; Del Hoyo *et al*. 1999). As a body experiences the stress, adrenal gland secretes glucocorticoids. There are two kinds of glucocorticoids: corticosterone for birds and rodents, and cortisol for mammalians such as primates and carnivores (Walsh *et al*. 1985; Touma and Palme 2005).

A non-invasive technique has been developed to assay corticosterone concentration in feces. It has become an important tool for measuring environmental stress of wild birds, particularly for endangered species. The method can get a correct physiological data without puncture to the birds. It has been also used in assaying glucorticoid concentrations in many of other animals (Touma and Palme 2005). It has excellent practicability, high accuracy, and many other advantages. It does not need to use invasive collection of blood that affects animal welfare (Goymann 2005; Touma and Palme 2005).

It has been known that there is a positive relationship in corticosterone level between serum and feces (Dehnhard *et al*. 2003; Ludders *et al*. 2001), Furthermore, glucocorticoid concentration in owl's fecal samples are found to be more stable than that in the urine samples (Wasser and Hunt 2005). Therefore, the measurement of fecal corticosterone concentration has been used in investigation in the fields of ecology, reproductive biology, and conservation (Wasser *et al*. 1997; Dehnhard *et al*. 2003). Up-to-date, effect of stress caused by cage transfer for captive wild-birds has not been well understood. Wasser *et al*. (1997) first used the non-invasive method to measure physiological stress from disturbance on the northern spotted owl.

In this study we used the non-invasive method to investigate the effect of stress caused by cage transfer on fecal corticosterone concentration of captive collared-scope owls. The objectives were to determine whether the fecal corticosterone level of the collared-scope owl is quantifiable, and to understand whether the stress of cage transfer affects the corticosterone level. The results obtained may be useful in wild animal's husbandry and also provide us an observatory period required for the owl in cage transfer to adapt a new cage environment.

Materials and methods

The collared-scope owl (*Otus lettia*) is a small nocturnal raptor, belonging to the family Strigidae of the order Strigiformes. It is a common resident bird in Taiwan and adapts to live with people. The collared-scope owls are catalogued as a rare and specious species by the Wildlife Conservation Act of Taiwan.

We used 14 captive collared-scope owls which received medical treatment at Wildlife Clinic Center, Taiwan Endemic Species Research Institute, as test animals. They were 8 males, 5 females, and 1 unknown sex with body sizes ranged from 23 to 25 cm and body weights from 164 to 188g.

Prior to the experiment, all of the owls were housed together in four common cages for more than six months. Then, each of the owls was transferred to an individual cage of 70x50x115 cm^3. Its bottom was placed 70 cm above the ground for collecting feces without interference on owls. A horizontal tree branch was placed inside 25cm above the cage bottom to provide the roosting place. Food and water were placed on the bottom of the cage. The experiment was conducted at room temperature of about 25℃ under natural light.

The experimental period was from October to November. At the beginning of the experiment, each owl was given a physical examination, and then, transferred into an individual cage on 17 October. Its feces was collected and fresh diet was given daily. Otherwise, there was no other disturbance on the test animal.

A total of 18 fecal samples were collected randomly from the owls from the common cages in October during the pre-transfer period. After transfer, fecal samples were collected from each of the individual cages at 08: 00 to 10: 00 AM on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 14th, 17th and 21st days. We collected all feces on the cage bottom, excluding white urine. When collection, no matter how many feces on the bottom, we collected all feces and classified to become a sample. The samples only separated into which owl and which day. Then, the fecal samples collected were dried at 55℃ and preserved at-20℃, the weight was varied from 2.275g (maximal) to 0.01g (minimal). When extraction, the samples did ran out on one-time extraction, and corticosterone was extracted with 80% ethanol (Wasser, *et al*., 1994; Wasser and Hunt 2005), and the volume of ethanol was 3-fold sample's volume. The corticosterone-ethanol solution was centrifuged, and the supernatant was collected. The above extracted steps were repeated twice. Then, solution dried at 55℃, and recovered with the buffer of the Corticosterone ELIA Kit (Assay designs, Inc., Ann Arbor, MI), the recovered buffer volume was sample's weight and finally preserved at-20℃ to wait assayed. The corticosterone concentration was assayed with the Enzyme-linked immunoassay (ELIA) method with the Corticosterone ELIA Kit (Assay designs, Inc., Ann Arbor, MI).

Data of fecal corticosterone concentrations were expressed as mean ± standard deviation (Tables 1, 2). Student t-test was used to compare the corticosterone concentrations between the pre-transfer period and the post-transfer period. Analysis of variance (ANOVA) was used to determine significance ($P< 0.05$) of transfer stress on the eighth, tenth, fourteenth, and twenty-first days.

Results

The corticosterone concentrations of 18 fecal samples collected prior to transfer were 158.9 ± 63.1 ng/g with the range of 69.9 to 239.3 ng/g. After transfer the fecal corticosterone concentrations rapidly increased to a peak on 1st or 2nd days after transfer (Figs. 1). The highest concentration was detected mostly on the 1st day. The concentrations of these two days were 359.4 ± 139.4ng/g with a range of 139.4 to 591.8 ng/g (Tables 1). The mean concentration after transfer was 2.3 fold higher than that of prior to transfer. Twelve owls excreted their first feces in the first two days after transfer, and remaining two owls excreted on the 4th day.

Then, the fecal corticosterone concentrations gradually declined and stabilized at a low level on 8th day, indicating the adaptation of the owls to the new cages. There was no significant difference in the mean concentration between pre-transfer and post-transfer periods on the 3rd to 6th days after the transfer (Table 1). The mean concentrations from 8th to 21st day were significantly decreased and lower than one third of that of the pre-transfer period.

Amounts of food consumed by the owls during the post-transfer period are shown in Table 2. They varied from none feeding, common feeding to high feeding. All owls showed poor appetite on the 1st day; 7 owls had none feeding, and 7 had common feeding. There was none of high feeding. The poor appetite continued to 6th day and get better on 7th day. All owls ate well on 10th day and after.

Discussion

When birds are under environmental stress, corticosterone is secreted from adrenal glands. With it also fecal corticosterone concentration increased (Wasser *et al*., 1997; Hischenhauser *et al*. 2005). When northern spotted owls (*Strix ocidentalis caurina*) had the stress induced with

Table 1. Fecal corticosterone concentrations of collared-scope owls during the pre-transfer period and post-transfer period

表 1. 比較領角鴞個體移籠前後的皮質酮濃度變化

Corticosterone (ng/g)	Pre-transfer period	Post-transfer period (day)					
		1st-2nd	3rd-4th	6th	8th	10th-14th	17th-21th
mean concentration	158.9	359.4*	222.4	201.5	80.7*	69.1*	55.5*
standard deviation	63.1	139.4	86.4	159.9	51.9	34.0	30.5
number of samples	18	16	10	10	12	23	23

* $P < 0.05$ (paired t-test)

Table 2. Food intake of collared-scope owls after transferring to individual cages (none, no food intake; high, food intake at 70% by our provision; common, between none and high; number under the feeding condition, number of owls)

表 2. 領角鴞個體於移籠後的每日進食量變化。其中“None”表示拒食，“High”表示對於我們所供給的食物進食量超過 70%，“Common”表示有進食，但進食未到達“high”的程度。再進食量下的數字代表的是個體數。

Days after transfer	Food intake		
	None	Common	High
1	7	7	0
2	1	12	1
3	3	7	4
4	2	1	11
5	2	9	3
6	1	1	12
7	0	6	8
8	0	6	8
9	2	6	6
10	0	0	14
11	0	5	9
12	0	5	9
13	0	2	12
14	0	8	6
15	0	5	9
16	0	4	10
17	0	2	12
18	0	6	8
19	0	11	3
20	0	5	9

ACTH stimulation, the highest fecal corticosterone concentration was found at 12 h postinfusion. After punctured ACTH, their fecal corticosterone concentrations increased within 2 h postinfusion and an 3 fold increase at 12 h postinfusion (Wasser *et al*. 2000). In our study the corticosterone concentration of collared-scope owls had a peak on the 1st and 2nd day after transfer with a 2.3 fold increase (Table 1), fairly similar to that observed for northern spotted owls by Wasser *et al*. (2000). The result of this study suggested that the collared-scope owls suffered with stress from cage transfer.

Cage transfer stress on captive wild-birds has not been well understood. Wasser *et al*. (1997) collected the blood and fecal samples of northern spotted owls at Woodland Park Zoological Gardens.

The results showed that both blood and fecal corticosterone concentrations had an acute increase after transfer and then returned to baseline level gradually. The similar case was found in our study; the fecal coticosterone concentration increased rapidly on the first two days after cage transfer and then decreased gradually to a stable concentration level on the 8th day (Fig. 1).

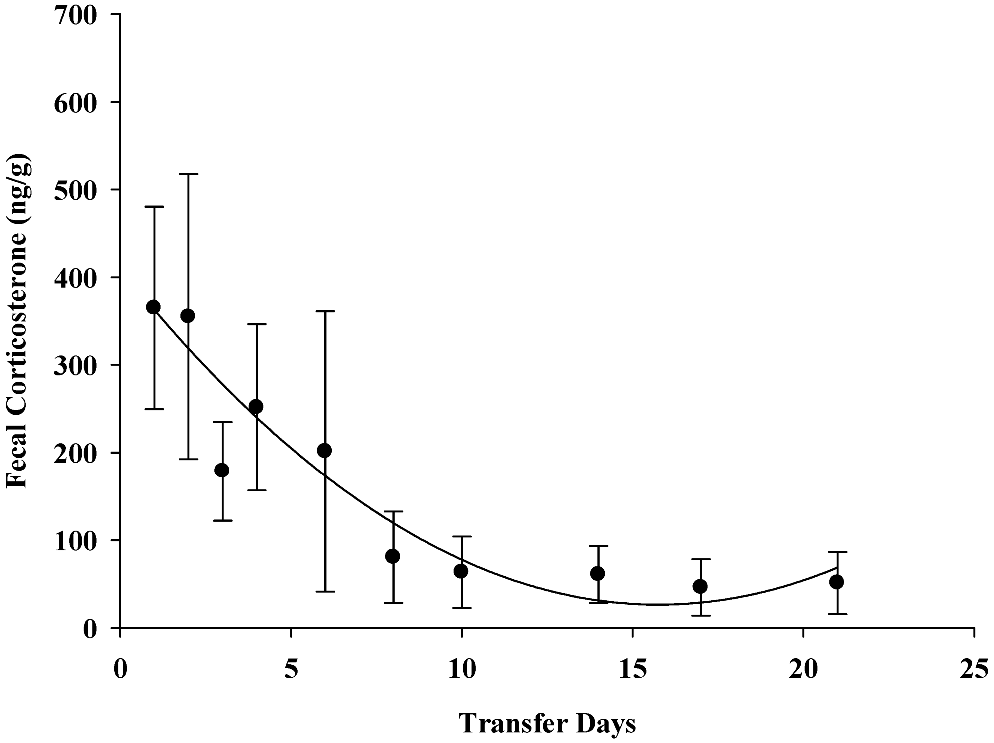

Fig. 1. Fecal corticosterone concentrations (solid circles, means; vertical lines, standard deviations) on 1st day to 22 days after cage transfer. $Y = 445.509 - 67.945X + 3.768X^2 - 0.068X^3$; $r^2 = 0.615$.

圖 1. 移籠後的皮質酮濃度的平均值與標準差，回歸曲線爲 $Y = 445.509 - 67.945X + 3.768X^2 - 0.068X^3$；$r^2 = 0.615$。圖中可明確地發現，在第 8 天之後個體適應新環境，生理上皮質酮濃度分泌穩定，形成基礎值濃度。

The *ad lib* feeding models was used in this study. "High feeding" is defined as a good food intake, "common feeding" as normal food intake, and "none feeding" as anorexia (Table 2). None feeding was observed on the first day when a peak concentration of fecal corticosterone was detected (Fig. 1). Then, the owls were gradually adapted to the new cage environment. With it the corticosterone concentration gradually declined and food intake improved from 3rd to 6th days. The corticosterone concentration decreased to the baseline level on 8the day, while the food intake returned to common or high feeding after 6th days. The results of our study found that the response in cage transfer-induced stress was in correspondent to the change in food intake. After the corticosterone concentration decreased to baseline level, the food intake also returned normal at the same time. It took 6-8 days for the captive wild owls to return normal from the cage transfer-induced stress.

Because of old cages were used to house several owls together prior to transfer, we were unable to collect fecal samples from each of the owls. When the mean fecal corticosterone concentrations of pre-transfer period when the owls were caged together were compared to those after transfer when the owls were caged individually, we found that the concentrations on the 8th to the 21st days after transfer were only a third of those prior to transfer. This may be attributable to two reasons. Firstly, the collared-scope owl is a territorial bird, and competes for foraging and survival (Carere *et al.*, 2003). Prior to transfer, several owls were housed together in old cages that they competed for the territories. After transfer, each of the owls was housed in an individual cage that was fairly similar to a natural condition with independent territory. In other words, in the period prior to transfer, the owls already had the stress from competition for the territory, and thus, the corticosterone concentrations were higher than the baseline level on 8th to 21 th days after transfer. Secondly, the lower concentrations on the 8th-21st days might be due to individual difference or the HPA axis negative feedback. Carere, *et al*. (2003) showed that there was correlation between territorial behavior and fecal corticosterone level in great tits (*Parus major*); some individuals would restrain the corticosterone secretion through HPA axis negative feedback. It is a body's protective mechanism for regulating the adrenal cortex activity after excitement and for avoiding deleterious effects after high secretion of corticosterone by stress (Kitaysky *et al.*, 2001).

Conclusion

The trend of the change in fecal corticosterone concentration during the post-transfer period may be divided into three phases: (1) the acute increase phase on the 1st or 2nd day after transfer; the fecal corticosterone concentration reaches to a peak level, (2) the gradual decline phase on 3rd to 6th day; the corticosterone level gradually decreased from the peak level and the owls started to adapt the new cage environment, and (3) the stabilized phase on 8th-21th day; the corticosterone concentrations decreased to a new baseline level; the owls have adapted to the new cage.

This study first demonstrated that the stress of cage transfer can be shown on the food intakes of the birds. After the corticosterone concentration decreased to baseline level, the food intake also returned to normal at the same time. It took 6-8 days for captive wild owls to adapt new cages after stress induced by the cage transfer. The individual difference in corticosterone concentration may be due to individual difference in adaptivity or in physiology, particularly in HPA axis regulation. A further research is needed in the future.

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