MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 42 (1): 92-105 (February 2023) https://mjs.um.edu.my

# STUDY OF FECAL GLUCOCORTICOID METABOLITE IN BEARS: A REVIEW

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**Abstract:** Fecal glucocorticoid metabolite (FGM) analysis is a non-invasive method to monitor animals' welfare in captivity and wild environments. Glucocorticoid also known as cortisol is a hormone that indicates the level of stress in animals and humans. This paper reviews the use of FGM analysis on bears and the methodologies used to study this hormone in every species of bear. The review method used was descriptive review. The bears that were included in this review are the brown bear (*Ursus arctos*), Polar bear (*Ursus maritimus*), Asiatic black bear (*Ursus thibetanus*), American black bear (*Ursus americanus*), Malayan sun bear (*Helarctos malayanus*), sloth bear (*Melursus ursinus*), Andean spectacled bear (*Tremactos ornatus*), and giant panda (*Ailuropodia melanoleuca*). Studies of FGM on polar bears showed that zoo-to-zoo transportation could cause an increase in FGM level during transportation and FGM is not suitable to be used to differentiate between pseudo-pregnancy and true pregnancy. In Malayan sun bear, FGM level is high in female bears that show agonistic behavior and is associated with low progesterone levels. In addition, studies on Malayan sun bear show that not only FGM can be analyzed from fecal samples, but also the reproductive hormones of estrogen and progesterone. In Asiatic black bears, FGM is higher in bears that live in a bile farm than forage outside the forest reserve. High parasite load in giant pandas is associated with a high level of FGM since parasite infection is considered a stressor that can elicit a stress response. Also both male and female panda have high FGM during the breeding season to increase metabolism to generate energy required for reproductive activities. The Alopecia syndrome in Andean spectacled bear has no significant relation to FGM level. Brown bears with several types of food in their diet have lower FGM than those with only one type of food. There is no specific study of FGM that focused on sloth bear and American black bear, but there were several studies on glucocorticoid in black bears that are not extracted from fecal samples. FGM can be analyzed using both enzyme-immunoassay (EIA) and radioimmunoassay (RIA) but, EIA is preferable due to safety reasons.

***Keywords:*** *Fecal glucocorticoid metabolite, bears, stress, reproduction*

## 1. Introduction

The fecal glucocorticoid metabolite (FGM) is commonly used in studying animal stress as it is a non-invasive way of investigating adrenal activities of animals either in captivity or free-ranging (Keay et al., 2006; Sheriff, et al., 2011). The advantages of using FGM in monitoring animals' adrenal activities are that samples can be collected easily as FGM comes from the fecal sample (Sheriff et al., 2011). Next, FGM represents the total fraction of the whole free (unbound) glucocorticoid in an organism. FGM is also not affected by researcher-induced biases of handling events or short-term fluctuations in glucocorticoid due to normal pulsatile changes in glucocorticoid secretion (Sheriff et al., 2011). Besides, FGM is not heavily influenced by the time of the day due to the circadian pattern of an organism unlike the plasma glucocorticoid (Sheriff et al., 2011).

Around the world, there are only 8 bear species namely the brown bear (*Ursus arctos*), Polar bear (*Ursus maritimus*), Asiatic black bear (*Ursus thibetanus*), American black bear (*Ursus americanus*), Malayan sun bear (*Helarctos malayanus*), sloth bear (*Melursus ursinus*), Andean spectacled bear (*Tremactos ornatus*), and giant panda (*Ailuropodia melanoleuca*).

The population of bears worldwide is under threat due to climate changes, loss of habitats because of urban development, agricultural activities, logging, and poaching. Humans are also hunting bears for their furs and meats (James & Serge Lariviere, 2019). Since bears are commonly being held captive as part of conservation efforts by placing them at zoos, rehabilitation centers or wildlife parks, it is important to monitor their well-being by assessing their adrenal activities to determine their stress level. As the continuous release of glucocorticoid as part of feedback mechanism controlled by the hypothalamus-pituitary-adrenal (HPA) axis can be detrimental to an organism, it is important to inspect the level of glucocorticoid of an animal in captivity (Sheriff et al., 2011). The level of glucocorticoid can be obtained by taking samples of blood, hair, saliva, urine, and fecal sample of the studied animal. However, among all of the mentioned samples, fecal samples are the easiest mean of assessing the level of glucocorticoid of an animal (Keay et al., 2006; Sheriff et al., 2011; Young et al., 2004).

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Received: February 15, 2022
Accepted: April 15, 2022
Published: February 28, 2022



DOI:https//doi.org/10.22452/mjs.vol42no1.9
Malaysian Journal of Science 42(1): 92-105 (February 2023)

Hence, this review was made to describe the study of FGM that has been done to the 8 species of bears from around the world in the past 10 years or further. This review will discuss the purpose of studying FGM in bears, the methodologies used in those studies, and the outcomes of each study. Besides discussing the use of FGM in assessing the level of stress and correlates it with other variables such as parasites infection, pregnancy and behaviour, this review will also discuss the factors that affect the level of FGM in fecal samples such as how the fecal sample is being handled and the preservation method used. The articles that were reviewed are listed in Table 1.


DOI:https//doi.org/10.22452/mjs.vol42no1.9
Malaysian Journal of Science 42(1): 92-105 (February 2023)

**Table 1.** Studies of fecal glucocorticoid metabolites that was done on 8 species of bears.

| Bear | *Species* | Title of study | Findings | Author |
|---|---|---|---|---|
| Polar bear | *Ursus martimus* | Fecal glucocorticoid metabolites as a measure of adrenocortical activity in polar bears (Ursus maritimus). | Five polar bears in captivity that have undergo five zoo-to-zoo transports show increased level of fecal glucocorticoid after been validate through enzyme-immunoassay (EIA). | Hein et. al., (2020) |
| | | Annual fecal glucocorticoid metabolite concentrations in pregnant and pseudopregnant polar bears (*Ursus maritimus*) in North American zoos. | Study to determine whether FGM level analysis via EIA can be used to differentiate true-pregnancy and pseudopregnancy in polar bear, shows that FGM level between polar bear that undergo true pregnancy and polar bear that undergo pseudopregnancy are not significantly different. | Bryant & Roth, (2018) |
| | | Individual and environmental factors associated with stereotypic behavior and fecal glucocorticoid metabolite levels in zoo housed polar bears. | Polar bears that live in captivity in zoos, that shows stereotypic pacing behaviour, is associated with an increased level in FGM. The study used radioimmunoassay (RIA) to analyze the level of FGM in studied polar bear. | Shepherdson et. al. (2013) |
| Brown bears | *Ursus arctos* | Methodological Considerations for Using Fecal Glucocorticoid Metabolite Concentrations as an Indicator of Physiological Stress in the Brown Bear (*Ursus arctos*). | Their study on to validate the use cortisol-assay in EIA to determine FGM level of brown bears living in a rehabilitation centre in Spain and Wilhelma Zoo, Germany shows that cortisol assay in EIA is the best assay to study FGM level in brown bears. | Dalerum et. al., (2020) |
| | | Effects of Exposure, Diet, and Thermoregulation on Fecal Glucocorticoid Measures in Wild Bears. | This study was done on free-ranging wild bears (brown bears and black bears) that inhabits the Montana National Park in United States of America. Their study shows that FGM levels in collected fecal samples of wild bears are affected by seasonal changes and diet quality. The FGM level was analyze through RIA. | Stetz et. al. (2013) |
| | | Factors Associated with Fecal Glucocorticoids in Alaskan Brown Bears (*Ursus arctos horribilis*). | Their study aims to validate the use of RIA to analyse the FGM level of wild brown bears in Alaska and to identify the factors affecting the level of FGM in their study subject. Their study shows that RIA can be used to study FGM level from fecal sample of wild brown bears and the factors affecting the FGM level in wild Alaskan brown bears (*Ursus arctos horriblis*) are type of diet and date of seasonal changes. | von der Ohe et. al., (2004) |
| American black bear | *Ursus americanus* | Effects of Exposure, Diet, and Thermoregulation on Fecal | This study was done on free-ranging wild bears (brown bears and black bears) that inhabit the Montana National Park in United States of America (USA). Their study shows that FGM levels in a | Stetz et. al. (2013) |



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| | | Glucocorticoid Measures in Wild Bears. | collected fecal sample of wild bears are affected by the seasonal changes and the quality of diet. The FGM level was analyze through RIA. | |
| Asiatic black bear | *Ursus thibetanus* | Increased stress in Asiatic black bears relates to food limitation, crop raiding, and foraging beyond nature reserve boundaries in China. | This study was made to determine the stress level of wild Asiatic black bears that live inside and outside of nature reserve in China, by using FGM analysis through RIA. Their finding shows that bear that live outside of nature reserve have higher FGM level compared to those living inside the nature reserve. | Malcolm et. al., (2014) |
| | | Analyses of fecal and hair glucocorticoids to evaluate short- and long-term stress and recovery of Asiatic black bears (*Ursus thibetanus*) removed from bile farms in China. | This study aims to evaluate the stress level of the Asiatic black bears that were relocated from bile farms to a rehabilitation centre in China. The stress level was analyse by using the hair and fecal sample of the studied bears. The study shows that FGM level of the bear (analyze through RIA) living in bile farms are high and decreasing after been move to the rehabilitation centre after a period time. | Malcolm et. al., (2013) |
| | | Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. | In this study, the Asiatic black bears that lives in one of North America zoos (Le Zoo de Granby and Little Rock Zoo) were studied along with other 5 animals from Order Carnivora. The study aims to determine whether RIA and EIA can both be used to analyse FGM to assess animals' stress in captivity. Their study shows that both RIA and EIA are useful to analyse the FGM levels of any captive animals. | Young et. al., (2004). |
| Andean spectacled bear | *Tremarctos ornatus* | Spectacled bear (*Tremarctos ornatus*) alopecia syndrome: An update. | This study was conducted on captive spectacled bear. The location was not mentioned specifically in the preceding paper presented by the author. However, their study stated that the alopecia syndrome in spectacled bear does not correlate with the FGM level in the studied bear. | Leclerc et. al., (2015) |
| Sloth bear | *Melursus ursinus* | Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. | The studied sloth bear that lives in one of North America zoos (Little Rock Zoo), were studied along with other 5 animals from Order Carnivora. The study aims to determine whether RIA and EIA can both be used to analyse FGM to assess animals' stress in captivity. Their study shows that both RIA and EIA are useful to analyse the FGM levels of any captive animals. | Young et. al., (2004) |
| Giant panda | *Ailuropoda melanoleuca* | Seasonal dynamics of parasitism and stress physiology in wild giant pandas. | The study aims to study the effect of sex, age, reproductive season and seasonal food availability on the parasitism and the level of stress of wild giant pandas. FGM analysis through RIA shows that parasite prevalence increased during bamboo shoot | Zhou et. al., (2020) |



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| | | | season and caused increased level of FGM and most infected age class are juvenile and sub-adult pandas. | |
| | | Rising fecal glucocorticoid concentrations track reproductive activity in the female giant panda (*Ailuropoda melanoleuca*). | The study was conducted to monitor the FGM level between parturient and non-parturient female panda that lives in Smithsonian National Zoological Park and Zoo Atlanta through EIA. The study shows that FGM level was highest during the periestrus stage in both parturient and non-parturient female panda. In contrast, FGM level was lower during the late luteal phase in the non-parturient female giant panda. | Kersey et. al., (2011) |
| | | Non-invasive determination of fecal steroid hormones relating to conservation practice of giant panda (*Ailuropoda melanoleuca*). | The author aims to validate that sex steroid level can be used to distinguish the sexes of giant panda and to observe the fecal cortisol level to monitor stress level. The study subjects are nine giant pandas which captivity in Center of Breeding and Conservation of the Rare and Endangered Wildlife, Shaanxi Province, China. The analysis of fecal sample through ELISA shows that fecal sample can be used to monitors both sex steroids and cortisol level which indicates stress level. | Yu et. al., 2011 |
| | | Parallel and seasonal changes in gonadal and adrenal hormones in male giant pandas (*Ailuropoda melanoleuca*). | The author aims to analyze the level of stress and androgen hormones by using fecal sample of male pandas in Smithsonian National Zoological Park and Atlanta Zoo. Their study shows that using fecal sample to analyze androgen is more convenient than using urine sample. Also, FGM level in male panda increased during the reproductive season. FGM and androgen analysis was done via EIA. | Kersey et. al., (2010) |
| Malayan sun bear | *Helarctos malayanus* | Social Influences on the Estrous Cycle of the Captive Sun Bear (*Helarctos Malayanus*). | The author studies on the effect of social influences on the FGM level and reproductive cycling of female Malayan sun bear in several zoos in USA. The study shows that female sun bear under high social influence possesses agonistic behaviour with high FGM level which could suppress the mating behaviour. | Frederick et. al, (2013). |
| | | Reproductive timing and aseasonality in the sun bear (*Helarctos malayanus*). | The study aims to determine whether Malayan sun bear is a seasonal breeder or non-seasonal breeder. Analysis was done on the level of progesterone and estrogen of the female Malayan sun bear via fecal sample through RIA proves that sun bear is non-seasonal breeder. The studied bears were living in captive at 8 government zoos in USA. | Frederick et. al., (2012) |
| | | Fecal steroid analysis for monitoring reproduction in the sun bear (*Helarctos malayanus*). | The study aims to establish a non-invasive technique to evaluate the reproductive cycle of female Malayan sun bear, to study the effect of porcine zona pellucida (PZP) protein on the reproductive | Schwarzenberger et. al., (2004) |



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| | | system of sun bear and to determine whether Malayan sun bear is a seasonal breeder or non-seasonal breeder. Their study shows that fecal sample can be used to observe the reproductive cycle of Malayan sun bear through hormone analysis via EIA, female Malayan sun bear is a non-seasonal breeder, and female sun bear treated with PZP have missing ovarian activity. | |
| | Annual Changes in Fecal Estradiol-17β Concentrations of the Sun Bear (*Helarctos malayanus*) in Sarawak, Malaysia. | This study was done to investigate the reproductive cycle of female Malayan sun bear through analysis of estradiol hormone from fecal sample via EIA. Their results show that female Malayan sun bear is a seasonal breeder. | Onuma et. al., (2001) |
| | Reproductive Pattern of the Sun Bear (*Helarctos malayanus*) in Sarawak, Malaysia | This study was done to investigate the reproductive cycle of female Malayan sun bear through analysis of progesterone hormone from fecal sample via EIA. Their results show that female Malayan sun bear is a seasonal breeder. | Onuma et. al., (2000) |



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## 2. Fecal Glucocorticoid Metabolite Studies in All Bears

2.1 Study on fecal glucocorticoid metabolite (FGM) in captive polar bear (*Ursus maritimus*)

Hein et. al. (2020), study the practicality of enzyme-linked immunoassay to analyse the FGM level in polar bears. They aim to prove that the measuring of polar bears' FGM can be used as diagnostic tool for the long-term assessment of the polar bears' welfare either in captivity or wild. According to the authors, animals' transportation from one place to another can cause stress. This transport-related stress can be validated by conducting EIA to detect FGM levels from fecal samples of polar bears. Fecal sampling was done before transportation, during transportation, and after transportation. Their study confirmed that the FGM level in polar bears (*Ursus martimus*) undergone a significant increase after 5 zoo-to-zoo transport, especially during transportation. After transportation, the FGM level of the studied polar bears drop, but below the baseline level.

Bryant and Roth (2018) investigated if FGM can be used to distinguish between a pregnant bear and a pseudo-pregnant bear. Their objective was to compare the FGM profile between the female polar bear with true pregnancy and the female bear that undergoes pseudo-pregnancy. They hypothesized that the FGM level between a pregnant female bear and a pseudo-pregnant female bear will be different. They also predict that pregnant female bear has a higher FGM level than the pseudo-pregnant female bear or vice versa. However, after doing FGM level analysis through EIA, they found out that there was no significant difference in FGM level between the pregnant female polar bear and pseudo-pregnant female polar bear. Hence, the result of their study shows that FGM cannot be used as a mean to differentiate pregnant polar bears from the pseudo-pregnant polar bears.

Studies on the level of FGM and its relationship with the stereotypic behavior of polar bears (*Ursus martimus*) that were being held captive in several zoos in North America shows that polar bears that exhibit stereotypic pacing behavior are associated with an increased level of FGM (Shepherdson et al., 2013). Thus, living in captivity and surrounded by concrete walls has caused the polar bears to have little view outside their immediate surroundings (Shepherdson et al., 2013). Enrichment of the surrounding area where the polar bears were held, increasing the number of social engagements among individuals, and allowing the bears to view of their outer surroundings are known to reduce the pacing behavior. This will in turn lower the FGM level (Shepherdson et al., 2013).

2.2 Study on FGM level in captive Malayan sun bear (*Helarctos malayanus*)

A study on the FGM level on captive Malayan sun bears was done by Frederick et. al. (2013). Their study aims to observe the effect of agonistic interactions on the level of FGM and fecal estrogen and fecal progesterone of the female Malayan sun bear. Their study shows that agonistic interaction between female sun bears in captivity is associated with a high level of FGM. A high level of FGM will lower the level of fecal progesterone. In contrast, low agonistic behavior is associated with a high level of estrogen (Frederick et al., 2013).

Study by Frederick et. al. (2013) showed that not only glucocorticoid can be analyzed from the fecal sample, but also female reproductive hormones such as estrogen and progesterone. In 2012, they investigate whether sun bear is a seasonal breeder or a non-seasonal breeder. This was done by analyzing the level of progesterone or estrogen from the fecal sample of 13 adult female sun bears that was collected every month for a year. Their study showed that the female Malayan sun bear is not a seasonal breeder and does not exhibit pseudo-pregnancy behavior, which is common in bears that live in 4 seasons countries (Frederick et al., 2012).

Same study has also been done in the past by Schwarzenberger et. al. (2004) and Onuma et. al. (2001, 2002). Both researchers used the same method to analyze the reproductive hormone of the female Malayan sun bear by extracting the hormones from fecal samples (Onuma et al., 2001, 2002; Schwarzenberger et al., 2004). However, their results contradict each other whereby Onuma et. al. (2001, 2002) found that sun bear is a seasonal breeder, which is not the same as the result reported by Schwarzenberger et. al. (2004). In 2012, Frederick and colleagues confirmed the Malayan sun bear as a non-seasonal breeder.

These studies not only show that sun bear is a non-seasonal breeder and agonistic behavior, or interactions can suppress the reproduction hormone of the female bears, but they also proved that not only stress hormone (glucocorticoid or cortisol) can be assessed from fecal sample but can also be used to monitor reproductive hormones such as estrogen and progesterone.

2.3 Study of FGM level in Asiatic black bears (*Ursus thibetanus*) and sloth bear (*Melursus ursinus*)

In Asiatic black bear (*Ursus thibetanus*), FGM was used to study the short-term and long-term stress and recovery of the Asiatic black bears that were removed from bile farms in China

(Malcolm et. al., 2013). In the study, bears that live in the bile farm have elevated levels of FGM compared to those that live at a rehabilitation center. The Asiatic black bear that lives in bile farms has a permanent hole or fistula that was made in their abdomen and gall bladder to allow the bile to drip out freely through a collection tube. Bile collection was done when the bears were distracted by food or sometimes were restrained in a holding cage. These are the factors that cause the elevation of the FGM level of the Asiatic black bear at the bile farm. The bears that were saved from the bile farm, upon arrival at the rehabilitation center, have a high FGM level. However, the FGM level decreases over time as the bear in the rehabilitation center are being treated well, feed well, and have social interaction with other Asiatic black bears (Malcolm et. al., 2013).

FGM can also be used to assess the well-being of animals that live in the free-ranging area and this study was done by Malcolm *et. al.* (2014). Their study shows that reducing habitat can be a factor of stress in free-ranging Asiatic black bears in China. Due to habitat reduction, the bears were forced to forage for food in the area where they are prone to be poached such as raiding a farm or stealing livestock. By analyzing the FGM level from the collected fecal sample using EIA, they found out bears that live outside of the nature reserve have a higher level of FGM than those that live in the nature reserve. Limited fall food and perceived risk of mortality due to crop raiding at human areas are factors of the stress of the free-ranging Asiatic black bear that lives outside of the nature reserve (Malcolm et. al., 2014).

The use of fecal sample to investigate FGM in sloth bear (*Melursus ursinus*) also been done by Young et. al. (2004). They investigated the ability for FGM analysis to detect adrenocortical responses to stressful stimuli. Their study was done on sloth bears and other animals from 5 families within order Carnivora which are the Himalayan black bear (*Ursus thibetanus laniger*), domestic cat (*Felis catus*), cheetah (*Acinonyx jubatus*), clouded leopard (*Neofelis nebulosa*), black-footed ferret (*Mustela nigripes*), slender-tailed meerkat (*Suricata suricatta*), and red wolf (*Canis rufus*). Their study shows that FGM levels from fecal samples can be detected by using both EIA and RIA. So far, this is the only study of FGM that was done on sloth bears.

2.4 Study of FGM level in giant panda (*Ailuropoda melanoleuca*) and spectacled bear (*Tremarctos ornatus*)

FGM has also been used in studying the effect of sex, age, reproductive season, and seasonal food availability on the parasitism and stress physiology in wild giant pandas by Zhou et al. (2020). In their study, FGM was used to measure physiological stress in pandas. Their study shows that reproductive season and seasonal food availability significantly affect the FGM level of wild panda. This is because during high food availability and mating season, the giant pandas expand their home range, and this increases their encounter with the environmental transmission stages of parasites. Age class also affected the parasite load and stress level. It was shown in this study that, juvenile and sub-adult pandas have higher parasite burden associated with high level of FGM compared to the adult and old pandas. This study confirms that higher parasite loads in the giant pandas increase their FGM level. Meanwhile, sex and reproductive season have no significant correlation with cortisol level of the studied giant pandas (Zhou et al., 2020).

A study on giant pandas in China used fecal androgen and FGM levels to determine the reproductive seasonality of the male giant panda (Kersey et al., 2010). The study states that the use of fecal samples to study the relationship of androgen level with FGM level is easier to be done than using urine samples as feces are more readily available than urine samples as giant panda defecates more than 20 times per day. Their study shows that male giant panda has elevated levels of excreted androgens during the 5-month-long breeding season together with the elevated level of FGM. High FGM level in male giant pandas during breeding season is due to the fact that they have increased metabolism that help them to sustain energy, move longer distances, and express aggressive behaviors that are important for winning breeding competitions (Kersey et al., 2010).

Another study using FGM to track reproductive activity in the female giant panda was done by Kersey et al. (2011). Their study proposes that even though FGM was used to monitor stress levels, the main function of glucocorticoid (GC) is to modulate metabolism which include the response to calorie intake, lactation and/or environmental seasonality. The study also shows that among all reproductive states, FGM level was highest during the periestrus stage in both parturient and non-parturient female panda, whereas FGM level was lower during the late luteal phase in the non-parturient female giant panda. This shows that the pattern of excreted FGM clearly reflected the adrenal responses to reproductive stimuli.

Yu et. al. (2011), study on the use of fecal sex steroids to distinguish sexes and to monitor the FGM level to measure the stress response to artificial semen collection, artificial insemination, and parturition, in nine giant pandas which live in captive at Center of Breeding and Conservation of the Rare and Endangered Wildlife, Shaanxi Province, China. Their study hypothesizes that the use of fecal sample to monitors hormone levels in giant panda can remove direct handling on the animal to draw blood to get the information of their physiological well-being. Their study shows that male pandas have higher level of fecal testosterone compared to female across three seasons (Spring, Autumn and Winter), while the level of fecal estradiol between males and females are not significantly different across the three seasons. Artificial semen collection, artificial insemination, and parturition cause increased level of FGM. In their study, Yu et al. (2011) use EIA to analyze the level of fecal testosterone, fecal estradiol and FGM. The study shows that fecal

sample not only can be used as a mean to assess the level of stress in giant panda, but also to assess the reproductive hormone of male and female giant panda.

In Andean spectacled bear, the relationship between FGM and alopecia syndrome was studied by Leclerc et al. (2015). They hypothesize that alopecia syndrome in Andean spectacled bears can be associated with a high level of FGM. However, their study shows that there is no significant difference in the level of FGM between the affected and non-affected panda. Later in 2019, Horn et al. (2019) shows that most female spectacled bears in captivity have alopecia syndrome compared to the male spectacled bear. According to the study, stereotypical behaviors were common among both males and females whether they were affected by alopecia or not, but the syndrome was seen only in females who had been socially housed. They suggest that female Andean bears be housed with adult conspecifics only when the females choose to cohabitate. However, this study does not involve FGM analysis to examine whether these stereotypic behaviors are related to the FGM level.

2.5 Using FGM to study thermoregulatory demands in free-ranging brown bears (*Ursus arctos*) and American black bear (*Ursus americanus*)

The FGM can also be used to study the thermoregulatory demands in free-ranging bears. A study conducted by Stetz et al. (2013) shows that bear's FGM level increased linearly with the biological effects of temperature (early September – end of October) during fall seasons as the temperature drops (temperature between 27℃ to 35℃). Meanwhile, in summer, the FGM level in the bear's fecal sample is low for bears that are presumed to spend more time in the hottest area. Their study shows that FGM concentration also corresponds to the diet quality of the free-ranging wild bears. For fecal samples of bears with less nutritious diet, where their feces consist of coarse vegetation, FGM concentration is higher than the FGM level in the feces of bears that have a nutritious diet consisted of berries and meats. However, their study was conducted generally on free-ranging wild bears that inhabit the area of Glacier National Park, Montana, USA. In their reports, it was mentioned that the area of their study is an area where both wild brown bears (*Ursus arctos*) and black bears (*Ursus americanus*) can be found. Thus, the fecal sample that was collected may belong to brown bears or black bears.

In 2004, Ohe et. al. studied the factors affecting the FGM level in fecal samples of Alaskan brown bears. Their study shows that FGM levels in the fecal sample of Alaskan brown bear can be affected by the types of diet and date of seasonal changes. In their study, bears that their diet consists of several types of foods has a decreasing level of FGM over time compared to the bear that their diet consists of one type of food only. To explain their finding, they stated that brown bears in the wild rely on a wide range of food sources from plant matter to terrestrial and marine meat, and the selection of specific foods changes seasonally (Ohe et al., 2004).

In 2020, Dalerum et al. investigate the quantification of FGM in brown bears using the enzyme immunoassay (EIA) method. Studies of FGM were conducted by Stetz et al. (2013) and Ohe et al. (2004) using radio-immunoassay (RIA) to quantify the level of FGM contained in fecal samples. In this investigation, they found that EIA using cortisol assay is the best to be used as an assay to quantify FGM from fecal samples. However, fecal samples that will be analyzed using EIA must be frozen immediately after defecation to prevent the loss of bioreactivity of the FGM in the sample. This will be challenging if the study of FGM was conducted on-field situation.

For the study of FGM in black bears (*Ursus americanus*), there is no study of FGM that focused specifically on the species. The feces of American black bear might be collected by Stetz et al. (2013) in their study, but as mentioned in the first paragraph of this subtopic, the samples might belongs to either brown bears or American black bear. Even so, that does not mean that there is no study on the glucocorticoid metabolite (GM) level of black bears was done. There are several studies of the GM level of black bears that were conducted but the GM was not extracted from fecal samples. The following Table 2 lists the studies of GM level on black bears.

**Table 2.** Study of glucocorticoid metabolite on the black bear (*Ursus americanus*).

| Bear | Title of study | Source of glucocorticoid metabolite | Findings | Author |
|---|---|---|---|---|
| American black bear (*Ursus americanus*) | Sex, Diet, and the Social Environment: Factors Influencing Hair Cortisol Concentration in Free-Ranging Black Bears (*Ursus americanus*). | Hair | The study aims to investigate the effect of diet, sex and the social environment on American black bear past hypothalamic-pituitary-adrenal (HPA) activity via hair cortisol concentration (HCC) through EIA. The study shows that the HCC of American black bear are varied by sex which results from variability in the nutritional needs of larger-bodied males relative to smaller-bodied females. The studied bears are wild American black bears at Parsnip Plateau and Hart Ranges of the Rocky Mountains. | Lafferty et. al., (2015) |
| | Seasonal serum glucose, progesterone, and cortisol levels of black bears (*Ursus americanus*). | Blood | The study was done to investigate the relationship between the utilization of body fat and protein during a period of food deprivation, with the level of serum cortisol in 62 wild black bears. RIA analysis on serum cortisol of studied bear shows that elevated level of serum cortisol during winter not as a stimulus for protein-derived glucose, but for the catabolism of fat as an energy source. | Harlow et al. (1990) |
| | Insulin and Glucagon Responses in the Hibernating Black Bear. | Blood | This study aims to observe the hormonal changes in two hibernating black bears that were held captive in ills farm Institute and Research Center (Mayo Clinic) in Rochester, Minnesota. The study shows that the corticosteroids level is lower during active phase but higher during hibernating phase. It was not mentioned in detail in the article of what method was used to analyze the plasma corticosteroid level. | Palumbo et al., (1983) |



DOI:https//doi.org/10.22452/mjs.vol42no1.9
Malaysian Journal of Science 42(1): 92-105 (February 2023)

## 3. Methods Used to Analyze Fecal Glucocorticoid Metabolite (FGM) in Bears

### 3.1 Extraction of FGM from fecal sample

To quantify the FGM levels of an organism, the FGM must be extracted from the fecal samples that were collected as described by Keay et al. (2006) in which 90% methanol is commonly used as the extraction agent. The common methods of FGM extraction are dry extraction and wet extraction methods. In the dry extraction method, the moisture on fecal samples was removed either by drying or using a lyophilizer. The purpose of using a lyophilizer is to freeze-dry the fecal sample. The dried fecal sample will then be pulverized into powder using mortar and pestle. These fecal powders will be vortexed by mixing them with ethanol or methanol and centrifuged to form an aliquot. In the wet extraction method, the frozen fecal sample will be thawed before mixing it into a centrifuge tube containing methanol. The sample will be vortexed, centrifuged followed by serial dilution using phosphate buffer solution (PBS) (Keay et al., 2006; Vimalraj & Jayanthagaraj, 2012).

Studies by Malcolm et al. (2014) and Malcolm et al. (2013) on the stress level of Asiatic black bears by the use of FGM from fecal samples of Asiatic black bears used the dry extraction method. The fecal samples were dried using an oven at 100℃ for 12 hours (Malcolm et al., 2013). After drying, the fecal samples were pulverized into powder and those fecal powders were inserted into a tube and mixed with 90% ethanol. The mixture in the tube was vortexed before being boiled at 85℃ for 15 minutes and then centrifuged. After centrifuge, the pellet formed was mixed with ethanol, vortexed, boiled, and centrifuged again. The combined ethanol supernatant was then dried under open air before being stored in a freezer before analysis.

Most of the studies that were being reviewed in this review have their fecal sample lyophilized (freeze-dried) as a method to preserve the fecal sample before the FGM was extracted from the sample. Bryant & Roth (2018) in their study on polar bears used the freeze-dried fecal sample that was pulverized into powder. However, to extract FGM from the fecal sample, they used 90% methanol solution, and they did not boil the mixture of the methanol and fecal powder. The mixture was vortexed and then centrifuged followed by serial dilution using PBS. Studies by Kersey et al. (2010) and Deng et. al. (2014) on giant pandas used the dry extraction method on the lyophilized (freeze-dried) fecal sample using ethanol as the extraction agent. Studies by Stetz et al. (2013) on wild bears and Ohe et al. (2004) on Alaskan brown bears extracted FGM from the lyophilized fecal sample by dry extraction method using methanol solution as the extraction agent.

Shepherdson et al. (2013) used the wet extraction method on polar bears where the fecal sample was shaken overnight in a solution containing PBS, 50% methanol, and bovine serum albumin (BSA) before being centrifuged. A method by Shepherdson et al. (2013) does not involve vortexing the fecal sample as the fecal sample was shaken overnight. Frederick et al. (2013) and Schwarzenberger et al. (2004) studies were on fecal steroid analysis in Malayan sun bear but Schwarzenberger et al. (2004) study does not include FGM, but only involves fecal estrogen and progesterone. Both studies have almost the same fecal hormone extraction method, which is wet extraction. Prior to extraction, the frozen fecal samples were thawed to room temperature before being lyophilized. Their samples were freeze-dried as removal of moisture from the fecal sample will increase the concentration of the hormone in the fecal samples by four to five-fold (Schwarzenberger et al., 2004). After lyophilization, the samples were then mixed with 90% methanol solution, vortexed, centrifuged followed by serial dilution using PBS (Frederick et al., 2013; Schwarzenberger et al., 2004).

### 3.2 Quantification of FGM using immuno-assay

After the extraction method, the FGM will then be quantified using RIA or EIA commonly known as enzyme-linked immunoassay (ELISA). The selection of antibodies to be used in the immunoassay method is important because the type of glucocorticoid metabolite excreted varies among species (Keay et al., 2006). Different antibodies have different cross-reactivity for other steroids or steroid metabolites in the fecal samples (Keay et al., 2006). Antibodies that are specific for a steroid hormone may have little cross-reactivity with other hormones (Keay et al., 2006).

For studies that use RIA to quantify the FGM levels in bear, the researchers are known to use the double-antibody $^{125}$I Corticosterone RIA kit as the antibodies in the kit are cross-reactive with the cortisol in the fecal sample for the RIA method (Frederick et al., 2013; Ohe et al., 2004; Shepherdson et al., 2013; Stetz et al., 2013). For studies that use EIA or ELISA, researchers use the single-antibody cortisol EIA kit in order to determine the FGM level in the fecal samples (Bryant & Roth, 2018; Deng et al., 2014; Malcolm et al., 2013; Malcolm et al., 2014). According to Sheriff et al. (2011), the use of EIA is known to be advantageous compared to RIA as it is possible to develop group-specific antibodies for FGM and it does not involve the use of radioactive substances.

### 3.3 Method of preserving fecal sample for studying FGM level in bear

Another factor that needs to be taken into consideration in quantifying the FGM from fecal samples is the way the sample is preserved. Since FGM levels in fecal samples can be affected by environmental temperature and degradation by microbial activities, it is then needed to preserve the fecal sample (Sheriff et al., 2011). Collecting fecal samples of free-ranging animals in the field has been proven to be a challenge for wildlife researchers if the collection was done in an area where the distance from the laboratory or any facilities that have a freezer is far (Sheriff et al., 2011). The way the fecal sample is being preserved could affect the FGM level in the sample. For example,

fecal sample of a brown bear that was preserved in ethanol and then stored at -20℃ will completely eliminate the FGM level in the fecal sample (Hunt & Wasser, 2003). A study done by Hunt & Wasser (2003) shows that for bear fecal samples, it is best for the sample to be frozen without the use of any solution (methanol, ethanol, and others) or the fecal sample is being lyophilized (freeze-dry).

Studies done by Kersey et al. (2010), Ohe et al. (2004), Shepherdson et al. (2013), and Stetz et al. (2013) are known to use lyophilizer in their study as a way to preserve their fecal sample before being extracted and assayed. Malcolm et al. (2014), Malcolm et al. (2013), and Frederick et al. (2013) have their fecal sample frozen at -20℃. However, during processing (extraction of FGM), Frederick et al. (2013) lyophilized their fecal sample to increase the concentration of FGM by four to five folds. The advantage that can be seen for preserving fecal samples using a lyophilizer is that it could increase the detection of FGM by increasing the concentration of FGM through the removal of moisture. The time and the condition where the fecal sample was collected must also be taken into consideration. Malcolm et. al. (2014) studied the free-ranging Asiatic black bear in China's nature reserve, collected their samples, stored them in a zip-lock plastic bag before placing the samples into the freezer within 12 hours. For Asiatic black bear that lives in captivity, the fecal samples are collected and immediately frozen at -20℃ before being processed for extraction (Malcolm et al., 2013). Fecal samples can be combined with preservatives such as sodium azide, ethanol or methanol as an effort to prevent bacterial growth since it has been demonstrated that naturally occurring bacteria and their enzymes will degrade steroid metabolites within a few hours (Hunt & Wasser, 2003; Keay et al., 2006). However, the types of preservatives used must be chosen carefully as they also can affect the FGM level reading when stored for a long-term (Hunt & Wasser, 2003).

## 4. Conclusion

In this review, FGM is known to be used to study the adrenocortical activities in several species of bear, which are the brown bears, polar bears, sun bears, Asiatic black bears, giant pandas, and spectacled bears. It is known that FGM cannot be a reliable indicator to differentiate the pregnant polar bear from the pseudo-pregnant polar bear. However, FGM can be used to measure the level of stress by observing the presence of agonistic behavior in female sun bears and correlating the level of FGM (cortisol) with the level of reproductive hormone (estrogen and progesterone) in female sun bears. The level of FGM in bears are also known to be influenced by a variety of food and environmental temperature in different seasons. Bears with high parasite load and possess stereotypic behavior has elevated level of FGM.

For quantification of FGM via RIA, double antibodies $^{125}$I Corticosterone RIA kit is commonly used. While for quantification of FGM by EIA or ELISA, the use of single-antibody cortisol EIA kit is common.

For storage of fecal samples before processing, it is recommended that the sample is immediately lyophilized or frozen at -20℃. If the sample was collected in a very secluded area, the addition of a preservative is necessary to prevent microbial degradation in the fecal sample. As for the extraction method, fecal samples can be extracted by the dry extraction method or the wet extraction method. In the wet extraction method, lyophilizing the sample can increase the concentration of FGM by four to five folds.

The fecal glucocorticoid metabolite (FGM) is commonly used in studying animal stress as it is a non-invasive way of investigating adrenal activities of animals either in captivity or free-ranging (Keay et al., 2006; Sheriff, et al., 2011). The advantages of using FGM in monitoring animals' adrenal activities are that samples can be collected easily as FGM comes from the fecal sample (Sheriff et al., 2011). Next, FGM represents the total fraction of the whole free (unbound) glucocorticoid in an organism. FGM is also not affected by researcher-induced biases of handling events or short-term fluctuations in glucocorticoid due to normal pulsatile changes in glucocorticoid secretion (Sheriff et al., 2011). Besides, FGM is not heavily influenced by the time of the day due to the circadian pattern of an organism unlike the plasma glucocorticoid (Sheriff et al., 2011).

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