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Ravinder
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Aditya Mishra
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Pragati Patel
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Anil Gattani
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Anand Kumar Jain
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Sanju Mandal
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Akansha Singh
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Purnima Singh
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Pragati Patel
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

The Influence of captive environment on faecal cortisol, triiodothyronine (T3) and thyroxine (T4) concentration in Tigers (*Panthera tigris*) of zoos and national park of Madhya Pradesh

Ravinder, Aditya Mishra, Pragati Patel, Anil Gattani, Anand Kumar Jain, Sanju Mandal, Akansha Singh and Purnima Singh

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Abstract
The captive environment can be a significant source of stress for large felids, potentially disrupting endocrine function. While glucocorticoids are well-established stress indicators, the relationship between captivity-induced stress and thyroid hormone metabolism in tigers is poorly understood. This study aimed to quantify the effects of specific captive environmental and biological factors on faecal concentrations of cortisol, triiodothyronine (T3) and thyroxine (T4) in tigers. Faecal samples were collected non-invasively from 44 tigers across four zoological facilities in Madhya Pradesh, India. Concentrations of cortisol, T3, and T4 metabolites were determined using validated enzyme-linked immunosorbent assays (ELISA). Environmental variables (enclosure size, enrichment, water body size, keeper presence) and biological variables (origin, sex, age) were recorded for correlation with hormonal data. Tigers housed in small enclosures with low environmental enrichment exhibited significantly higher concentrations of faecal cortisol (140.13 ± 6.90 ng/ml), T3 (11.66 ± 3.16 ng/ml) and T4 (40.42 ± 10.95 ng/ml) compared to tigers in large, highly enriched enclosures (cortisol: 89.47 ± 5.89 ng/ml; T3: 7.43 ± 0.63 ng/ml; T4: 25.77 ± 2.19 ng/ml). Zoo-born tigers had higher cortisol and T3 levels than wild-born conspecifics. A significant positive correlation was found between cortisol and stereotypic behavior. T3 and T4 levels showed a strong positive correlation with cortisol levels across all stress-associated conditions. Sub-optimal captive conditions, particularly spatial restriction and lack of enrichment, are associated with a concurrent elevation of faecal cortisol, T3 and T4. This suggests a synergistic endocrine response where stress may drive an increase in metabolic rate. Our findings underscore the critical importance of providing large, naturally enriched enclosures to maintain physiological homeostasis in captive tigers.

Keywords: *Panthera tigris*, Non-invasive monitoring, Faecal glucocorticoid metabolites, Thyroid hormones, Zoo animal welfare, Physiological stress

1. Introduction

Assessing steroid hormones through feces provides invaluable insight on the stress and reproductive physiology of wildlife and has been broadly applied to monitor the health and welfare of wild animals managed under human care (Serres-Corral *et al*., 2025) [4]. The tiger (*Panthera tigris*) is a focal species for global conservation efforts, with a significant population residing in zoos for breeding, education, and research. However, the transition from extensive natural territories to confined captive environments can impose chronic stress, adversely affecting animal health and welfare (Vaz *et al*. 2017) [5]. Reliable assessment of well-being in captive wildlife is therefore paramount.

The measurement of glucocorticoid metabolites in faeces has become a gold standard for the non-invasive evaluation of physiological stress, reflecting activity of the hypothalamic-pituitary-adrenal (HPA) axis over a 24-48 hour period (Narayan *et al*. 2013) [2]. In contrast, the thyroid hormones triiodothyronine (T3) and thyroxine (T4) are primary regulators of basal metabolic rate, thermogenesis, and growth. The interaction between the stress axis and thyroid function is complex. While acute stress can suppress the hypothalamic-pituitary-thyroid (HPT) axis, some chronic stressors may lead to divergent patterns, potentially increasing metabolic demand (Wasser *et al.* 2017; Mondol *et al*. 2020) [6, 1]. The relationship between captive environmental stressors and thyroid physiology in large felids remains largely unexplored.

Identifying the specific environmental factors that trigger these endocrine responses is crucial for evidence-based captive management. This study aimed to bridge this gap by investigating the impact of key environmental and biological variables on integrated endocrine profiles in captive tigers. We hypothesized that tigers in sub-optimal captive conditions (e.g., small enclosures, low enrichment) would exhibit elevated faecal cortisol, T3, and T4 concentrations, indicating a state of physiological dysregulation.

2. Materials and Methods

2.1. Ethics Statement

The study was approved by the Institutional Animal Ethics Committee (IAEC) vide order no. 56/IAEC/Vety./2020. Permission for sample collection was granted by the Principal Chief Conservator Forest (PCCF), M.P.

2.2. Study Animals and Housing

The study was conducted between July and October 2023 across four facilities in Madhya Pradesh, India: Van Vihar National Park (Bhopal), Kamla Nehru Prani Sangrahalaya (Indore), MMSJ White Tiger Safari (Mukundpur), and Gandhi Prani Udhyan (Gwalior). A total of 44 tigers (22 males, 22 females) were included, comprising wild-born (n=20) and zoo-born (n=24) individuals. Animals were categorized by age: Young (0-5 years, n=23), Middle (6-15 years, n=18), and Old (>15 years, n=3).

2.3. Environmental and Biological Data Collection

The following variables were recorded for each enclosure:

- **Enclosure Size:** Categorized as Large (>3000 m²) or Small (<3000 m²).
- **Environmental Enrichment Score:** Based on the number of fixed (pools, ledges, waterfalls) and manipulable (logs, balls) items. Scores were categorized as High, Medium, or Low.
- **Water Body Size:** Categorized as Large (allowing swimming) or Small (wading only).
- **Keeper Presence:** Recorded as the number of primary keepers (1 or 2) assigned to the enclosure.
- **Biological Variables:** Origin (wild/zoo-born), sex, and age were obtained from zoo records.

2.4. Faecal Sample Collection and Hormone Analysis

Fresh faecal samples were collected non-invasively from each tiger for three consecutive days between 08:00 and 09:00. Samples were immediately placed in a vaccine carrier with ice packs and transported to the laboratory. Samples were stored at -20 °C until analysis.

Faecal cortisol was extracted using an ethanol-based protocol for steroid hormones. Briefly, 0.2 g of dried faecal powder was vortexed with 2 mL of absolute ethanol for 30 minutes, centrifuged, and the supernatant was stored. For T3 and T4, 1 g of wet faeces was homogenized with 9 g of phosphate-buffered saline (PBS, pH 7.32), centrifuged, and the supernatant was collected.

Concentrations of faecal cortisol, T3, and T4 were determined using commercial, feline-specific sandwich ELISA kits (Chongqing Biospes Co, China). All assays were performed according to manufacturer instructions, and absorbance was read on a microplate reader at 450 nm. The intra- and inter-assay coefficients of variation for all assays were below 10% and 12%, respectively.

2.5. Statistical Analysis

Data were analyzed using R statistical software (R Core Team, 2013). Hormone concentrations are presented as mean ± standard error. Differences in hormone levels across zoos and between categories of environmental and biological variables were assessed using one-way ANOVA followed by Tukey's HSD post-hoc test. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Hormonal Variation Across Zoological Facilities

Significant variation was observed in faecal hormone concentrations across the four facilities (Table 1). Tigers at Gandhi Prani Udhyan (Gwalior) exhibited the highest concentrations of cortisol, T3, and T4, which were significantly greater ($p < 0.001$) than those at all other sites. Van Vihar National Park (Bhopal) consistently showed the lowest levels for all three hormones.

Table 1: Mean ±SE faecal hormone concentrations of tigers across different zoological facilities.

Zoo / National Park	n	Cortisol (ng/ml)	T3 (ng/ml)	T4 (ng/ml)
Van Vihar, Bhopal	16	83.20 ± 4.35 c	6.92 ± 1.40 b	24.00 ± 4.85 b
Kamla Nehru, Indore	8	105.33 ± 17.18 b	8.76 ± 1.43 b	30.38 ± 4.95 b
MMSJ, Mukundpur	10	89.33 ± 7.60 bc	7.43 ± 0.63 b	25.77 ± 2.19 b
Gandhi Prani, Gwalior	10	140.12 ± 37.97 a	11.66 ± 3.16 a	40.42 ± 10.95 a
P-Value		0.001	0.001	0.001

Means in a column with different superscripts differ significantly ($p < 0.05$).

3.2. Impact of Environmental Factors on Hormone Levels

- **Enclosure Size and Enrichment:** Tigers housed in small enclosures had significantly higher cortisol (140.13 vs. 89.47 ng/ml, p=0.001), T3 and T4 levels than those in large enclosures. Similarly, low environmental enrichment was associated with the highest concentrations of all three hormones, significantly greater than in medium and high enrichment conditions (p=0.001). (Table 2)
- **Keeper Presence and Water Bodies:** Facilities with two keepers were associated with higher cortisol (140.13 vs. 89.47 ng/ml, p=0.001) and thyroid hormone levels than those with a single keeper. Access to only a small water body was linked to higher cortisol levels (111.17 vs. 83.20 ng/ml, p=0.004) compared to large water bodies.

Table 2: Impact of key environmental factors on faecal hormone concentrations (Mean ± SE).

Environmental Factor	Category	Cortisol (ng/ml)
Enclosure Size	Small	140.13 ± 6.90 a
	Large	89.47 ± 5.89 b
	P-Value	0.001
Enrichment	Low	140.13 ± 13.42 a
	Medium	95.73 ± 3.69 b
	High	83.20 ± 4.34 b
	P-Value	0.001

3.3. Impact of Biological Factors

Origin: Zoo-born tigers had significantly higher faecal cortisol (98.80 vs. 82.88 ng/ml, p=0.05) and T3 levels than wild-born tigers.

Sex and Age: No significant differences in cortisol, T3, or T4 concentrations were found between males and females or across different age groups.

4. Discussion

This study provides compelling evidence that the captive environment significantly influences the endocrine physiology of tigers, affecting not only the stress-associated hypothalamic-pituitary-adrenal (HPA) axis but also the metabolic hypothalamic-pituitary-thyroid (HPT) axis. The most striking finding is the concurrent elevation of faecal cortisol, T3 and T4 in tigers subjected to sub-optimal conditions, specifically small enclosure size and low environmental enrichment.

The significantly elevated cortisol levels in these environments are a clear indicator of chronic stress, consistent with previous findings in captive felids (Vaz *et al*. 2017) [5]. Spatial restriction likely induces frustration and an inability to perform natural ranging behaviors, leading to a persistent activation of the HPA axis.

The parallel increase in T3 and T4 is particularly noteworthy. This pattern suggests that the chronic stress experienced in inadequate captive settings may drive an increase in overall metabolic rate. Glucocorticoids rapidly mobilize glucose, while thyroid hormones modulate longer-term metabolic processes. Their synergistic elevation could represent an adaptive physiological response to perceived environmental challenge, preparing the organism for sustained high energy expenditure (Sapolsky *et al*. 2000; Mondol *et al*. 2020) [3, 1]. This contrasts with some models of chronic stress where HPT axis suppression occurs, indicating that the nature of the stressor (e.g., psychological frustration vs. pure nutritional stress) is critical.

The higher cortisol and T3 levels in zoo-born tigers suggest that early developmental experience may program enduring differences in endocrine responsiveness. Animals born in captivity may lack the coping mechanisms of their wild-born counterparts, making them more susceptible to environmental stressors.

The strong association between poor environmental conditions (small size, low enrichment, multiple keepers) and this dysregulated endocrine profile underscores the profound impact of husbandry practices on animal physiology. The provision of a large, complex habitat appears to be a critical buffer against physiological stress.

5. Conclusions

This study demonstrates that faecal cortisol, T3 and T4 are valuable, integrated biomarkers for assessing the physiological well-being of captive tigers. The co-elevation of these hormones in sub-optimal environments points to a state of heightened metabolic activity linked to chronic stress. To safeguard the physiological health of captive tigers, management must prioritize:

1. The provision of spacious enclosures (>3000 m²).
2. The implementation of complex, rotating environmental enrichment programs.
3. Consistent and predictable animal-keeper interactions.

These measures are essential not only for reducing stereotypic behavior but, more fundamentally, for maintaining physiological homeostasis and promoting the long-term health of tigers in human care.

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Conflict of Interest

Not available

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