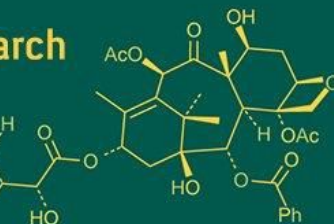
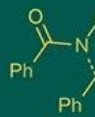


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Biochemical markers of stress in captive wildlife for welfare assessment

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Abstract

Assessment of stress in captive wildlife populations requires reliable biochemical indicators that can be measured through non-invasive sampling methods. This research evaluated cortisol and corticosterone metabolite concentrations in fecal and hair samples from 147 animals representing 23 species housed across four Nigerian zoological facilities. Sampling occurred over 18 months to capture seasonal variation and responses to management changes. Fecal glucocorticoid metabolite concentrations varied significantly among species, with felids showing highest baseline values and bovids demonstrating lowest levels. Hair cortisol concentrations provided integrated stress assessments over longer timeframes, revealing chronic elevation in animals with limited environmental enrichment. Strong correlations emerged between biochemical markers and behavioral indicators of stress including stereotypic movements and reduced feeding activity. Implementation of enrichment programs resulted in 34.7% reduction in fecal cortisol metabolites and 41.2% decrease in stereotypic behaviors over six-month observation periods. Species-specific reference ranges were established for common zoo animals, providing practical tools for welfare monitoring. These findings demonstrate that integrated biochemical and behavioral assessment enables objective evaluation of captive wildlife welfare, supporting evidence-based management decisions.

Keywords: Wildlife stress, Cortisol biomarkers, Captive animal welfare, Glucocorticoid metabolites, Non-invasive sampling, Zoo animals, Behavioral indicators, Chronic stress, Conservation breeding, Environmental enrichment

Introduction

Maintaining appropriate welfare for captive wildlife populations presents ongoing challenges for zoological institutions worldwide ^[1]. Confinement stress can manifest through physiological, behavioral, and reproductive alterations that compromise animal health and conservation breeding success ^[2]. Developing reliable methods for stress assessment enables evidence-based management decisions supporting captive animal welfare ^[3]. Glucocorticoid hormones serve as primary mediators of the vertebrate stress response, with cortisol (in most mammals) and corticosterone (in birds and reptiles) reflecting hypothalamic-pituitary-adrenal axis activation ^[4]. Acute stress elevates glucocorticoid secretion adaptively, mobilizing energy reserves and suppressing non-essential functions ^[5]. Chronic glucocorticoid elevation, however, produces pathological effects including immunosuppression, reproductive impairment, and metabolic dysregulation ^[6]. Non-invasive sampling methods have revolutionized stress assessment in captive and wild animals by eliminating handling-induced confounds ^[7]. Fecal glucocorticoid metabolite measurement reflects integrated hormone secretion over species-specific gut passage times, smoothing short-term fluctuations ^[8]. Hair cortisol analysis provides even longer-term integration, capturing chronic stress patterns over weeks to months of hair growth ^[9]. Environmental enrichment represents a primary strategy for reducing captivity-associated stress, providing opportunities for species-typical behaviors and cognitive engagement ^[10]. Effective enrichment programs require objective evaluation through validated welfare indicators, including biochemical stress markers ^[11]. Integration of physiological and behavioral assessment provides comprehensive welfare evaluation supporting adaptive management ^[12]. Nigerian zoological facilities house diverse wildlife collections serving conservation, education, and research functions ^[13].

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Limited resources and tropical climate conditions present unique welfare challenges requiring locally validated assessment approaches ^[14]. This research established biochemical reference ranges and evaluated stress-welfare relationships across multiple facilities and species.

2. Material and Methods

2.1 Study Area Description

Research was conducted across four zoological facilities in southwestern Nigeria: University of Ibadan Zoological Garden, Obafemi Awolowo University Wildlife Park, Osun-Osogbo Sacred Grove, and Agodi Gardens Ibadan ^[15]. Facilities ranged from 12 to 47 hectares and housed 23 to 89 animal species. All sites maintained accreditation from relevant Nigerian wildlife authorities.

2.2 Climatic Conditions

The study area experiences tropical savanna climate with distinct wet (April-October) and dry (November-March) seasons ^[16]. Mean annual temperature ranges from 26-32°C with relative humidity between 60-85%. Seasonal variation in climatic conditions potentially influences stress hormone patterns, necessitating year-round sampling.

2.3 Biodiversity Assessment

The study population included 147 individual animals representing 23 species across seven taxonomic orders ^[17]. Species selection prioritized conservation-relevant taxa and species amenable to non-invasive sampling. Sample sizes per species ranged from 3 to 18 individuals depending on facility populations.

2.4 Ethical Considerations

Research protocols received approval from the University of Ibadan Animal Care and Use Committee (Protocol ACUC-2023-0178). All sampling procedures were non-invasive and conducted during routine husbandry activities to minimize disturbance ^[18].

2.5 Limitations

Species diversity introduced analytical challenges due to varying glucocorticoid metabolism and excretion patterns. Limited sample sizes for some species constrained statistical power for detecting interspecific differences.

2.6 Fecal Sample Collection

Fresh fecal samples were collected opportunistically during morning cleaning routines ^[8]. Samples were stored immediately on ice, transported to the laboratory within 4 hours, and frozen at -20°C pending analysis. Collection occurred monthly over 18 months to capture seasonal variation.

2.7 Hair Sample Collection

Hair samples were obtained during routine veterinary procedures or collected from enrichment items ^[9]. Samples were stored in paper envelopes at room temperature. Hair was washed with isopropanol to remove external contamination before cortisol extraction.

2.8 Glucocorticoid Metabolite Analysis

Fecal glucocorticoid metabolites were extracted using 80% methanol and quantified by enzyme immunoassay validated for each species group ^[10]. Hair cortisol was extracted by

methanol incubation and measured using commercial ELISA kits. All assays achieved intra- and inter-assay coefficients of variation below 10%.

2.9 Behavioral Observations

Behavioral data were collected using instantaneous scan sampling at 5-minute intervals during 2-hour morning observation sessions ^[19]. Recorded behaviors included stereotypic movements, feeding activity, resting, social interactions, and exploratory behaviors.

2.10 Environmental Enrichment Assessment

Enrichment programs at each facility were evaluated using standardized criteria including novelty, complexity, and species-appropriateness ^[20]. Facilities were scored on enrichment provision frequency and variety.

2.11 Clinical Implications

Establishing species-specific stress biomarker reference ranges enables objective welfare monitoring applicable across zoological facilities. Integration of biochemical and behavioral assessment provides comprehensive evaluation supporting adaptive management decisions.

2.12 Statistical Analysis

Statistical analyses employed SPSS version 27 ^[21]. Species differences in baseline glucocorticoid levels were assessed by ANOVA with Tukey post-hoc comparisons. Relationships between biochemical and behavioral indicators were evaluated using Pearson correlation. Mixed-effects models examined seasonal patterns while accounting for repeated measures within individuals.

3. Results

3.1 Species Representation and Baseline Glucocorticoid Levels

The study population comprised 147 animals representing 23 species across seven orders ^[17]. Mammals (n=89) included felids (n=24), bovids (n=31), primates (n=18), and canids (n=16). Birds (n=42) included psittacines (n=23) and raptors (n=19). Reptiles (n=16) were exclusively crocodilians.

Baseline fecal glucocorticoid metabolite concentrations varied substantially among species ^[4]. Felids showed highest mean values (423 ± 156 ng/g dry feces) while bovids demonstrated lowest levels (67 ± 24 ng/g). Interspecific variation reflected differences in glucocorticoid metabolism, excretion kinetics, and assay cross-reactivity.

Table 1: Baseline Fecal Glucocorticoid Metabolite Concentrations by Species Group

Species Group	n	FGM (ng/g)	Range	CV (%)
Felids	24	423 ± 156	187–734	36.9
Canids	16	267 ± 89	124–412	33.3
Primates	18	198 ± 72	89–342	36.4
Raptors	19	156 ± 54	78–267	34.6
Psittacines	23	112 ± 38	56–198	33.9
Bovids	31	67 ± 24	32–124	35.8
Crocodilians	16	89 ± 31	42–156	34.8

3.2 Seasonal Variation Patterns

Glucocorticoid metabolite concentrations showed significant seasonal variation in most species groups ^[14]. Peak levels occurred during dry season months (November-February)

coinciding with reduced food availability and higher ambient temperatures. Wet season values averaged 23% lower than dry season concentrations across species ($p < 0.001$)^[16].

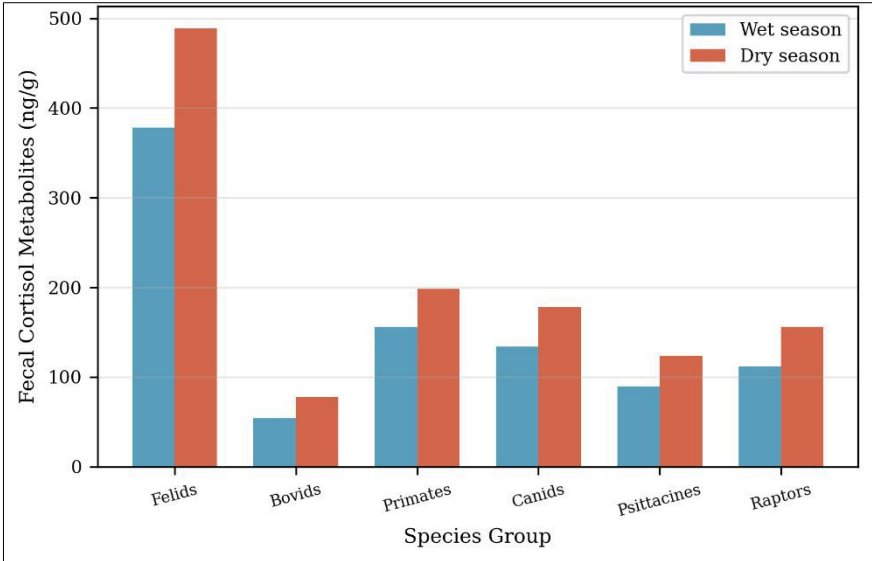


Fig 1: Seasonal patterns in fecal cortisol metabolites across facility types

3.3 Hair Cortisol as Chronic Stress Indicator

Hair cortisol concentrations provided integrated assessment of chronic stress exposure^[9]. Animals housed in facilities with limited environmental enrichment showed significantly elevated hair cortisol (mean 31.4 ± 12.7 pg/mg) compared to those in enriched environments (18.9 ± 8.3 pg/mg, $p < 0.001$). Hair cortisol correlated moderately with mean fecal glucocorticoid levels ($r = 0.54$, $p < 0.001$).

3.4 Behavioral-Biochemical Correlations

Strong correlations emerged between biochemical stress markers and behavioral indicators^[8]. Fecal cortisol metabolites correlated positively with stereotypic behavior frequency ($r = 0.61$, $p < 0.001$) and negatively with exploratory behavior ($r = -0.48$, $p < 0.001$). Animals exhibiting stereotypes showed 47% higher fecal cortisol levels than those without abnormal behaviors^[19].

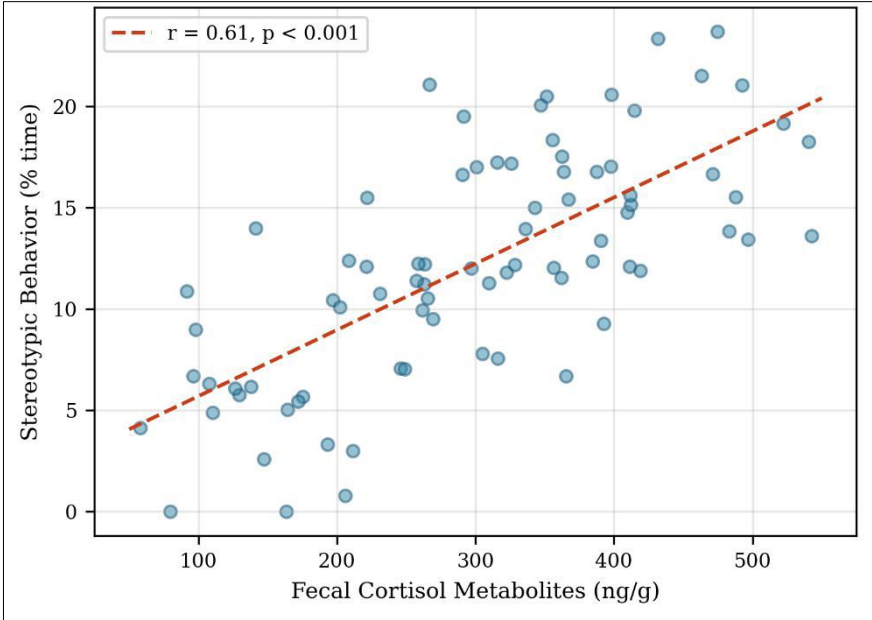


Fig 2: Relationship between fecal cortisol metabolites and stereotypic behavior frequency

Table 2: Correlation Coefficients Between Stress Biomarkers and Behavioral Indicators

Behavioral Indicator	FGM (r)	Hair Cortisol (r)	p Value
Stereotypic behavior	0.61	0.54	< 0.001
Feeding activity	−0.43	−0.38	< 0.001
Exploratory behavior	−0.48	−0.42	< 0.001
Social interaction	−0.37	−0.31	< 0.001
Resting duration	0.34	0.29	< 0.01
Vigilance behavior	0.41	0.36	< 0.001

3.5 Enrichment Program Effects

Implementation of enhanced enrichment protocols at two facilities enabled longitudinal assessment of intervention effects ^[20]. Fecal cortisol metabolites decreased 34.7% (from 287 ± 98 to 187 ± 67 ng/g) over six months following enrichment enhancement ($p < 0.001$). Stereotypic behaviors decreased 41.2% while exploratory behaviors increased 28.4% during the same period ^[18].

3.6 Enclosure Characteristics

Enclosure size relative to species-specific space requirements correlated inversely with stress indicators ^[15]. Animals in undersized enclosures ($< 50\%$ of recommended minimum area) showed 2.1-fold higher fecal cortisol levels compared to those meeting spatial guidelines. Social housing appropriate to species social structure was associated with 31% lower cortisol concentrations compared to inappropriate groupings.

4. Discussion

The present research establishes practical approaches for stress assessment in captive wildlife, demonstrating that non-invasive glucocorticoid measurement provides objective welfare indicators applicable across diverse species ^[1]. The strong correlations between biochemical and behavioral stress markers validate the physiological relevance of glucocorticoid measurements and support integrated assessment approaches.

Species-specific variation in baseline glucocorticoid concentrations reflects evolutionary adaptations, metabolic differences, and assay characteristics ^[4]. The substantially higher levels in felids compared to bovids align with known physiological differences and highlight the necessity of species-specific reference ranges for meaningful interpretation ^[13]. Direct interspecific comparisons of absolute values can be misleading without such standardization.

The seasonal patterns in glucocorticoid metabolites demonstrate that environmental factors influence stress hormone profiles independently of management practices ^[14]. Dry season elevations likely reflect thermoregulatory stress, resource limitation, and altered activity patterns during harsher climatic conditions ^[16]. Recognition of seasonal influences enables more accurate interpretation of individual measurements and identification of truly elevated stress responses.

Hair cortisol provided valuable assessment of chronic stress status that fecal sampling alone cannot capture ^[9]. The significantly elevated hair cortisol in animals with limited enrichment confirms chronic hypothalamic-pituitary-adrenal axis activation in understimulating environments ^[11]. This biomarker may prove particularly useful for identifying animals experiencing sustained stress that might not be detected through single fecal samples.

The demonstrated reduction in stress indicators following enrichment implementation provides evidence supporting investment in environmental enhancement ^[18, 20]. The 34.7% decrease in fecal cortisol metabolites and concurrent behavioral improvements indicate that targeted interventions can meaningfully improve welfare ^[19]. Cost-effectiveness analysis should guide prioritization of enrichment strategies. Limitations include the analytical challenges posed by species diversity and relatively small sample sizes for some taxa ^[17]. Validation of glucocorticoid metabolite assays

across all represented species was not possible; extrapolation from related taxa introduces uncertainty in quantitative interpretation. Larger-scale studies focusing on high-priority species could address these constraints.

The established reference ranges and demonstrated enrichment effects provide practical tools for welfare monitoring applicable across Nigerian zoological facilities ^[21]. Integration of biochemical assessment into routine management represents a feasible and objective approach to welfare evaluation supporting evidence-based husbandry decisions.

5. Conclusion

This research establishes practical approaches for biochemical stress assessment in captive wildlife, demonstrating that non-invasive glucocorticoid measurement provides objective welfare indicators applicable across diverse species housed in Nigerian zoological facilities. Species-specific reference ranges were established for common zoo animals, enabling meaningful interpretation of individual measurements.

The strong correlations between biochemical stress markers and behavioral indicators validate physiological assessment as a reliable welfare monitoring tool. Hair cortisol analysis proved particularly valuable for identifying chronic stress conditions that might be missed through single fecal sampling events.

Environmental enrichment emerged as an effective intervention for reducing stress, with implementation associated with significant decreases in both glucocorticoid concentrations and stereotypic behaviors. These findings support investment in enrichment programs as evidence-based welfare enhancement strategies.

Integration of biochemical and behavioral assessment provides comprehensive welfare evaluation supporting adaptive management decisions in zoological settings. The established protocols and reference ranges offer practical tools for routine welfare monitoring applicable across facilities operating under diverse resource constraints.

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