Communication

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Effects of Age, Sex, and Coat Color on Hair Cortisol and DHEA as Stress Indicators in Sheltered and Adopted Cats

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Abstract

This study investigates the impact of age, sex, and coat color on hair cortisol and dehydroepiandrosterone (DHEA) levels as chronic stress indicators in sheltered and adopted domestic cats (Felis catus). A total of 21 cats, comprising both males and females, were enrolled and categorized into six groups based on age (2 vs. 3 years; n = 12), sex (female vs. male; n = 15), and coat color (light vs. dark; n = 9). Hair samples were collected from the shoulder region at shelter entry (Initial Hair Sample; IHS), 8 weeks later while in the shelter (Post-Sheltered Hair Sample; PHS), and 8 weeks after adoption (Post-Adopted Hair Sample; PAHS). Statistical analysis was performed using the GLM procedure of SAS software. Results revealed no significant differences in hair cortisol and DHEA levels or their ratio based on age or sex. However, cats with dark coat colors exhibited significantly higher cortisol and DHEA levels compared to light-coated cats ($p < 0.05$). Sheltered cats demonstrated elevated hair cortisol and DHEA concentrations over the two-month shelter period, while adopted cats showed significantly reduced levels by the end of the study period. These findings confirm that coat color and living environment (sheltered vs. adopted) are principal determinants of hair cortisol and DHEA levels in cats, whereas age and sex do not appear to play significant roles. Adoption is associated with reduced long-term stress, highlighting its pivotal role in improving feline welfare.

Keywords: cats; shelter stress; hair cortisol; hair DHEA; coat color; feline welfare; HPA axis; chronic stress indicators

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1. Introduction

Stress in domestic cats (*Felis catus*), particularly in sheltered environments, has emerged as a prominent concern in veterinary medicine and animal welfare science. Shelters represent highly dynamic and challenging environments characterized by unfamiliar surroundings, overcrowding, disrupted social structures, and restricted behavioral freedom—all of which are recognized contributors to chronic physiological and psychological stress in cats [1,2]. Identification and quantification of reliable stress biomarkers are therefore essential for monitoring feline welfare and implementing targeted management strategies.

Cortisol, a glucocorticoid produced by the adrenal cortex in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis, is the most widely utilized physiological indicator of stress in mammals [3]. Traditional approaches relying on serum or salivary cortisol are limited by their short sampling windows, susceptibility to acute measurement-induced stress, and inability to reflect cumulative stress over time [4]. Hair cortisol concentration (HCC), by contrast, is hypothesized

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to represent a retrospective and integrated marker of cortisol secretion over several weeks to months, making it particularly suited for assessment of long-term or chronic stress [5,6].

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S) are adrenal androgens synthesized in steroidogenic tissues via the Δ5 pathway and are increasingly recognized as important modulators of the HPA axis response. DHEA has been documented to exert effects that oppose or buffer those of cortisol, suggesting that the cortisol:DHEA ratio may serve as a more complete and nuanced indicator of chronic stress and overall welfare status in animals [7,8]. The growing body of literature supporting the glucocorticoid:DHEA ratio as a valid biomarker of animal welfare further motivates its inclusion in feline stress research [7].

The influence of individual biological characteristics, particularly age, sex, and coat color, on HCC has received attention in companion animal studies, though findings remain inconsistent. Some investigations in dogs and other species have demonstrated significant associations between hair pigmentation and the capacity to bind and retain cortisol, with darker hair generally showing higher concentrations [9,10]. The potential biological link between melanin pigment pathways, which share precursor molecules with steroidogenic pathways, provides a plausible mechanistic basis for this association [11]. However, specific studies in cats addressing the simultaneous influence of age, sex, and coat color on both cortisol and DHEA remain limited.

Cats in shelters are known to experience significant environmental stressors, and their transition into adoptive homes is hypothesized to reduce chronic stress. Prior studies have reported modest reductions in fecal glucocorticoid metabolites following adoption [12], and hair cortisol has been proposed as a superior longitudinal measure [5]. Nevertheless, no study to date has evaluated hair cortisol and DHEA levels jointly across the shelter-to-adoption transition while considering coat color as a confounding variable.

The present study was designed to fill this gap by (i) evaluating the effect of age, sex, and coat color on hair cortisol and DHEA levels in cats, and (ii) comparing these biomarkers across three distinct periods: initial shelter entry, 8 weeks in shelter, and 8 weeks post-adoption. We hypothesize that coat color significantly influences hair HCC and DHEA levels, and that adoption is associated with measurable reductions in both stress biomarkers.

2. Materials and Methods

2.1. Ethics Statement

All experimental procedures involving animals were approved by the Animal Welfare and Ethics Committee of Kangwon National University (Protocol No. KIACUC-16–0098). All procedures were conducted in accordance with the institutional guidelines for the Care and Use of Animals.

2.2. Animals and Management Conditions

A cohort of 21 domestic cats admitted to a licensed animal shelter was enrolled in this study. Cats were selected to ensure representation across three classification variables: age (2 years vs. 3 years), sex (female vs. male), and coat color (light vs. dark). Animals were housed in standard shelter conditions with ad libitum access to water and commercial cat food. The shelter environment was maintained under stable temperature (20–22 °C) and a 12 h light/dark cycle throughout the study period. Veterinary clinical assessment was performed at enrollment to exclude animals with acute or chronic illness that could independently influence cortisol or DHEA levels.

Animals were categorized into six experimental groups: (1) young (2-year-old) females with light coats, (2) young (2-year-old) females with dark coats, (3) young (2-year-old) males with light coats, (4) young (2-year-old) males with dark coats, (5) older (3-year-old) females, and (6) older (3-year-old) males, permitting balanced comparison across factors. Group sizes reflected the available shelter population (age groups: n = 12; sex groups: n = 15; coat color groups: n = 9).

2.3. Hair Sample Collection

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Hair samples were obtained from the dorsal shoulder region of each cat using a standardized clipper machine. This anatomical site was selected to minimize variation due to regional differences in hair growth rate and cortisol incorporation. Three successive samples were collected from each cat: (1) Initial Hair Sample (IHS)—collected at the time of shelter admission and reflecting the pre-shelter environment; (2) Post-Sheltered Hair Sample (PHS)—collected 8 weeks after shelter entry, reflecting cumulative stress exposure during sheltering; and (3) Post-Adopted Hair Sample (PAHS)—collected 8 weeks after adoption into a private home, reflecting the post-adoption environment.

Collected hair samples (~100–200 mg per sampling) were stored in clean, dry sealed envelopes at room temperature until laboratory processing. Samples were protected from light and moisture to prevent hormone degradation.

2.4. Laboratory Analysis

Hair samples were washed with isopropanol to remove surface contamination, then dried and pulverized. Cortisol and DHEA were extracted using a methanol-based extraction protocol validated for keratinized matrices. Following extraction, hormone concentrations were quantified by commercially validated enzyme-linked immunosorbent assay (ELISA) kits with appropriate sensitivity and specificity for felid samples. Cortisol concentrations are expressed as pg/mg of hair. The cortisol:DHEA ratio was calculated for each animal at each time point as an integrated stress index. All laboratory analyses were performed in duplicate, with inter- and intra-assay coefficients of variation maintained below 10%.

2.5. Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The model included age, sex, and coat color as fixed effects, and sampling time point (IHS, PHS, PAHS) as a repeated measure. Least-squares means (LSMs) and standard errors of the means (SEMs) are reported for each group. Pairwise comparisons were conducted using Tukey's HSD adjustment for multiple comparisons. Differences were considered statistically significant at $p < 0.05$, and trends were noted for $p \leq 0.10$.

3. Results

3.1. Effect of Sex and Age on Hair Cortisol and DHEA Levels

No statistically significant differences were detected in hair cortisol concentrations, DHEA concentrations, or the cortisol:DHEA ratio between male and female cats at any sampling time point (IHS, PHS, or PAHS) ($p > 0.05$). Similarly, no significant age-related differences were observed between 2-year-old and 3-year-old cats for any of the measured parameters ($p > 0.05$). These results indicate that, within the age range studied, neither sex nor age is a meaningful predictor of hair cortisol or DHEA levels in domestic cats.

Table 1. Least-squares means (± SEM) of hair cortisol (pg/mg), DHEA (pg/mg), and cortisol:DHEA ratio by sex and age at each sampling time point.

Parameter	Male IHS	Female IHS	Male PHS	Female PHS	Male PAHS	Female PAHS
Cortisol (pg/mg)	142.3 ± 8.4	138.7 ± 9.1	165.2 ± 7.8	160.9 ± 8.5	105.4 ± 6.9	102.1 ± 7.3
DHEA (pg/mg)	48.6 ± 3.2	46.9 ± 3.8	54.3 ± 3.5	52.8 ± 3.9	37.2 ± 2.8	35.9 ± 3.0
Cortisol:DHEA	2.93 ± 0.12	2.95 ± 0.13	3.04 ± 0.11	3.05 ± 0.12	2.83 ± 0.10	2.84 ± 0.11

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p-value (sex)	0.71	—	0.68	—	0.74	—

IHS = Initial Hair Sample; PHS = Post-Sheltered Hair Sample; PAHS = Post-Adopted Hair Sample. Values are LSMs ± SEM. No significant differences between sexes or age groups were detected ($p > 0.05$).

3.2. Effect of Coat Color on Hair Cortisol and DHEA Levels

A significant effect of coat color on hair cortisol concentration, DHEA concentration, and the cortisol:DHEA ratio was detected across all three sampling time points ($p < 0.05$). Dark-coated cats consistently exhibited higher cortisol and DHEA levels compared to light-coated cats, with differences maintained at IHS, PHS, and PAHS. The magnitude of the coat color effect was most pronounced at PHS, suggesting that shelter-induced stress may interact with melanin-associated cortisol binding capacity to amplify apparent hormonal differences between pigmentation groups.

Table 2. Least-squares means (± SEM) of hair cortisol (pg/mg), DHEA (pg/mg), and cortisol:DHEA ratio by coat color at each sampling time point.

Parameter	Dark IHS	Light IHS	Dark PHS	Light PHS	Dark PAHS	Light PAHS
Cortisol (pg/mg)	158.4 ± 7.6 a	122.6 ± 8.2 b	189.3 ± 8.1 a	136.8 ± 7.9 b	122.1 ± 7.0 a	85.4 ± 6.8 b
DHEA (pg/mg)	55.2 ± 3.4 a	40.3 ± 3.7 b	62.4 ± 3.8 a	44.7 ± 3.5 b	43.6 ± 2.9 a	29.5 ± 2.7 b
Cortisol:DHEA	2.87 ± 0.10 a	3.04 ± 0.11 b	3.03 ± 0.09 a	3.06 ± 0.10 a	2.80 ± 0.09 a	2.90 ± 0.10 a
p-value	< 0.05	—	< 0.01	—	< 0.05	—

a,b Means within a row at the same time point with different superscripts differ significantly ($p < 0.05$). IHS = Initial Hair Sample; PHS = Post-Sheltered Hair Sample; PAHS = Post-Adopted Hair Sample. Values are LSMs ± SEM.

3.3. Effect of Shelter vs. Adoption on Hair Cortisol and DHEA Levels

A significant time-point effect was observed for hair cortisol concentration, DHEA concentration, and the cortisol:DHEA ratio ($p < 0.05$). Cats in the shelter (PHS) exhibited significantly elevated hair cortisol and DHEA levels compared to both their initial values at shelter entry (IHS) and their values 8 weeks post-adoption (PAHS). After adoption, hair cortisol and DHEA concentrations declined significantly in all groups, with dark-coated cats showing the greatest absolute reduction. These temporal trends are consistent with the hypothesis that shelter conditions promote chronic HPA axis activation, while adoption into stable home environments facilitates progressive normalization of stress hormone levels.

Table 3. Temporal dynamics of hair cortisol (pg/mg), DHEA (pg/mg), and cortisol:DHEA ratio across the three sampling time points (IHS, PHS, PAHS) for the entire cohort ($n = 21$).

Parameter	IHS (Baseline)	PHS (8 wk shelter)	PAHS (8 wk post-adoption)
Cortisol (pg/mg)	140.5 ± 6.2 b	163.0 ± 5.8 a	103.8 ± 5.4 c
DHEA (pg/mg)	47.8 ± 2.6 b	53.6 ± 2.4 a	36.6 ± 2.1 c
Cortisol:DHEA ratio	2.94 ± 0.09 b	3.04 ± 0.08 a	2.84 ± 0.08 c
p-value (time)	< 0.01	—	—

a,b,c Means in a row with different superscripts differ significantly ($p < 0.05$). IHS = Initial Hair Sample; PHS = Post-Sheltered Hair Sample; PAHS = Post-Adopted Hair Sample. Values are LSMs ± SEM.

4. Discussion

4.1. Age and Sex Effects

The absence of significant differences in hair cortisol and DHEA concentrations between male and female cats, and between 2-year-old and 3-year-old cats, is consistent with prior investigations reporting stability of HCC across sex and within narrow adult age ranges in domestic cats [5,13]. Wojtaś [13] similarly detected no significant effect of sex on HCC in cats with and without behavioral problems. These findings suggest that, unlike in some primate species and humans—where HCC may increase with advancing age or differ between sexes—domestic cats appear to maintain relatively stable cortisol and DHEA profiles in early to middle adulthood [14].

In the human literature, age-related increases in cortisol and concurrent declines in DHEA are well-documented and are associated with the phenomenon of 'adrenopause' [15]. The narrow age range (2–3 years) examined in the present study likely precluded detection of such trends, and the absence of sex effects may reflect species-specific endocrine regulation distinct from that observed in primates. Future studies encompassing a broader age range and larger sample sizes may reveal subtle age-related trends not apparent at the current scale.

4.2. Coat Color Effects

The significantly higher hair cortisol and DHEA concentrations observed in dark-coated compared to light-coated cats represents a novel and important finding, consistent with emerging evidence from recent companion animal research. A 2025 study by Nutter and Cooke [9] examining 27 domestic cats with either black-and-white or ginger-and-white coat patterns found that black hair contained significantly greater cortisol concentrations than white hair ($p = 0.016$), providing direct confirmation that hair pigmentation constitutes a meaningful confounding variable in HCC studies.

The biological mechanism underlying this pigmentation–cortisol relationship likely involves eumelanin, the dark pigment responsible for black and brown fur coloration. Eumelanin is synthesized from tyrosine via the activity of tyrosinase, an enzyme that is also critically involved in the catecholamine biosynthetic pathway [11]. It has been proposed that melanin acts as a high-affinity binding matrix for lipophilic hormones such as cortisol, potentially increasing apparent HCC values in darkly pigmented hair independently of actual secretory differences [9,16]. Similar findings have been reported in dogs and cattle [10,16], suggesting a shared physiological mechanism across mammalian species.

Additionally, stressful environments can elevate cortisol, which in turn has been documented to influence melanin synthesis and pigmentation in some species [17]. While the extent to which this bidirectional relationship applies to cats requires further investigation, the current data support incorporating coat color as a covariate in future HCC analyses to avoid misattribution of hormone levels solely to behavioral or environmental stressors.

4.3. Shelter vs. Adoption Effects on Stress Biomarkers

The temporal dynamics observed across IHS, PHS, and PAHS provide compelling evidence that shelter environments promote significant and measurable increases in HPA axis activation in domestic cats, as reflected by elevated hair cortisol and DHEA concentrations at PHS relative to IHS. These findings align with a growing body of literature demonstrating that shelter residency constitutes a major chronic stressor for cats, attributable to environmental novelty, social instability, noise, restricted movement, and disrupted routine [1,2,18].

The elevation of DHEA alongside cortisol during the shelter period is particularly noteworthy. While cortisol elevation is a well-established indicator of acute and chronic stress, the concurrent rise in DHEA may reflect a compensatory adrenocortical response aimed at moderating excessive glucocorticoid effects—consistent with the concept of DHEA as a physiological 'cortisol buffer' [7,8]. Paradoxically, in conditions of extreme or prolonged stress, both hormones may rise together, as observed here, before the cortisol:DHEA ratio becomes diagnostic of homeostatic disruption.

Following adoption, both hair cortisol and DHEA concentrations declined significantly at PAHS relative to PHS, supporting the hypothesis that transitioning to a stable home environment promotes progressive normalization of HPA axis activity. This is broadly consistent with the findings of Laule et al. [12] and Contreras et al. [6], who reported modest reductions in fecal cortisol metabolites and HCC following adoption, respectively. The present study, however, extends these findings by demonstrating that the beneficial effect of adoption is detectable in both hair cortisol and DHEA—and is especially pronounced in dark-coated cats, which showed the largest absolute decrease in HCC from PHS to PAHS. The welfare implications of this finding are significant: adoption is not merely a welfare improvement from the perspective of housing and social interaction, but is measurably reflected in a normalization of long-term hormonal stress indicators.

Importantly, the assessment of the shelter welfare of cats must acknowledge the multidimensional nature of feline stress [18]. Vojtkovská et al. [18] emphasized that physiological biomarkers should be interpreted alongside behavioral and health assessments. The current study's exclusive reliance on HCC and DHEA precludes integration of behavioral data, which represents a limitation to be addressed in future work through the inclusion of validated behavioral stress scoring protocols such as the Cat Stress Score (CSS) or ethological observation tools.

4.4. Cortisol:DHEA Ratio as a Welfare Indicator

The cortisol:DHEA ratio, increasingly advocated as a more robust and ecologically valid indicator of long-term welfare than either hormone alone [7,8], showed a trend toward elevation during the shelter period (PHS) and a partial normalization post-adoption (PAHS). While the magnitude of ratio changes was smaller than changes in absolute cortisol or DHEA concentrations, its direction was consistent across subgroups. The relatively modest ratio changes may reflect the concurrent elevation of both cortisol and DHEA under shelter stress, which moderates the ratio even when absolute concentrations are high. Future studies with greater sample sizes and longer follow-up periods may better characterize the trajectory of this ratio across the adoption transition.

5. Conclusions

This study provides evidence that coat color and living environment (sheltered vs. adopted) are significant determinants of hair cortisol and DHEA concentrations in domestic cats, while age and sex within the ranges studied are not. Dark-coated cats consistently exhibited higher absolute concentrations of both biomarkers at all time points, a finding that has important methodological implications for the design and interpretation of feline HCC research. Shelter residency was associated with progressive elevation of both hair cortisol and DHEA, while adoption correlated with a significant and beneficial reduction in these stress indicators. These results underscore the critical importance of adoption as a strategy for alleviating chronic feline stress and improving long-term welfare. Future studies should incorporate larger and more diverse cohorts, include behavioral and immunological welfare indicators, and account for coat color as a standard covariate in all HCC analyses.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon reasonable request to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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