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Hair cortisol levels in cats before and during their first month at a homeless animal shelter

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Summary

Hair cortisol analysis is a helpful tool for non-invasive measurement of the long-term activity of the HPA (hypothalamic-pituitary-adrenal) axis. Long-term exposure to stressors affects, among others, animals staying in shelters for homeless animals. The study aimed to assess the hair cortisol level in cats from 8 different shelters. Hair grows on average about 1 cm per month. Therefore, it was concluded that the cortisol level in the hair closer than 1 cm to the skin reflected the cortisol level in cats during their stay in the shelter (A period). In contrast, the cortisol level in the rest of the hair corresponded to the cortisol level preceding the cat's admission to the shelter (B period). Hair cortisol levels in the cats were higher before they arrived at the shelter than during their stay. Hair cortisol levels in the period before the shelter were higher in females than they were in males. On the other hand, during the stay at the shelter, the opposite was true: the cortisol level in females was lower than in males.

Keywords: feline, animal shelter, stress

A shelter for homeless animals is an environment fraught with stressors. For cats arriving at a shelter, contact with unfamiliar people, animals and objects in a completely unfamiliar environment is a difficult experience. They react to stressful environmental conditions. However, in cats, inhibition of normal behaviour is a much more common response to stress than abnormal behaviour. They become inactive, unwilling to explore or play, eat less frequently and neglect self-care (15).

Cats in shelters are often kept in cages. Small individual cages limit the ability of cats to express natural behaviour. Such limited space prevents cats from walking, running, jumping, playing, eating and drinking freely away from the litter box. In addition, cats kept in small individual cages often have no hiding places or scratching surfaces (12, 16). One way to prevent these restrictions is to enrich the environment. Improving the environmental complexity of captive animals and meeting their behavioural needs reduces stress (4). Environmental enrichment strategies can help improve the welfare of cats in animal shelters. Moreover, environmental enrichment is fundamental for the welfare of shelter animals (17). For example, even providing cats with simple cardboard boxes as hiding places reduces their stress level (19).

Behavioural, physiological and health indicators can be used to assess shelter cats' level of well-being and stress (20). To assess stress responses for scientific purposes, physiological indicators are most often used. We can determine vital signs, such as blood pressure and heart rate, and laboratory parameters, such as cortisol levels. Cortisol is synthesized in the adrenal cortex in response to the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The body's exposure to stressors is associated with increased activity of the HPA axis. Therefore, elevated cortisol levels indicate stress (8). Acute stress can be measured by blood and saliva cortisol levels, whereas chronic stress can be assessed by analyzing the cortisol level in urine, faeces or hair.

Hair analysis for levels of endogenously produced cortisol was first performed in humans by Raul et al. (14). The free, unbound fraction of cortisol is incorporated into hair pulp during its growth from blood vessels by passive diffusion (13). Since then, hair cortisol analysis has been repeatedly confirmed as a unique tool for a non-invasive measurement of the long-term activity of the HPA axis.

The study aimed to determine and evaluate the hair cortisol level of shelter cats before arriving at shelters for homeless animals and during one month of staying there.

Material and methods

Approval for the study was obtained from the Animal Welfare Committee of the Faculty of Animal Sciences and Bioeconomy of the University of Life Sciences in Lublin, Poland (ZdsDZ/1/2022 of May 25, 2022). The research was part of the project "Reducing the stress level in shelter cats through the use of environmental enrichments" under the programme "Student science clubs create innovations" financed by the Ministry of Education and Science in Poland (contract No. SKN/SP/534344/2022).

The study involved 39 shelter cats from 8 different shelters for homeless animals in Poland. They had been brought to the shelter from the street. Each cat had been in the shelter for about 4-5 weeks when material for testing was collected. The average approximate age of the cats in the study group was 3.63. Veterinarians assessed their approximate age on admission to the shelter. Seventeen cats were female, and 22 were male. When material for testing was collected, 34 cats were already spayed/neutered, and the rest were still intact. Based on the clinical examination and laboratory tests carried out by veterinarians when the animals were admitted to the shelter, they were declared healthy.

In 7 of the 8 shelters, cats were kept in groups, in cathouses, and could use an outdoor enclosure. In one, they were kept in individual cages. The impact of housing conditions on stress levels was not the subject of this study, so we do not focus on the details of the cats' living environment in the shelters.

Hair was collected from cats close to the skin and cut with scissors in a non-invasive way. Since hair grows approximately 1 cm per month (11), it was concluded that cortisol levels in hair closer to the skin than 1 cm reflected cortisol levels in cats during their stay at the shelter (A). On the other hand, the cortisol level in the remaining (further) part of the hair corresponded to the cortisol level in the period before the cat's admission to the shelter (B). The methodology is shown in Figure 1.

The methodology for determining the cortisol level in cats' hair was borrowed from Koren et al. (10) and Accorsi et al. (1). The hair in our study was first divided into two separate samples (as described in the text above and Figure 1). The hair from each selection was then individually chopped into 1-2 mm pieces, and 20 mg of the trimmed hair was placed in a glass vial. Methanol (Sigma-Aldrich, Poznań, Poland) was added, and the vials were incubated at 50°C with gentle shaking for 24 hours. After incubation,

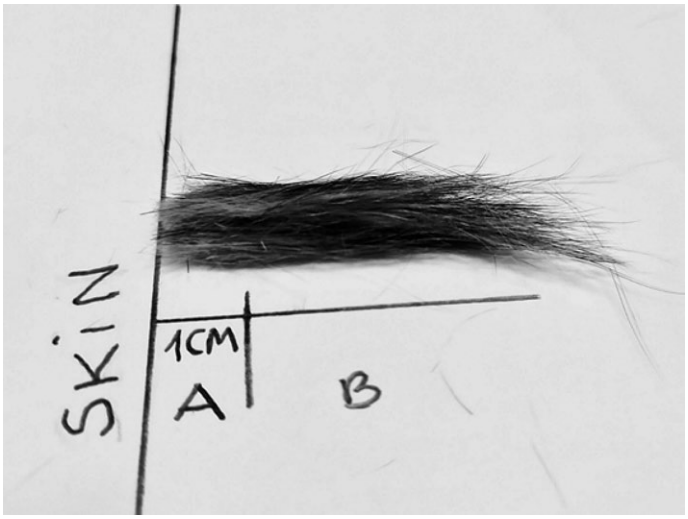

Fig. 1. Methodology adopted in the study

the supernatant was filtered to separate the liquid phase and placed in disposable glass culture tubes. This supernatant was then evaporated, and the dry residue was dissolved in 1 ml of phosphate-buffered saline (PBS) 0.05 M, pH 7.5. The samples were centrifuged until well mixed. Cortisol levels in the samples were determined using DRG Salivary Cortisol HS ELISA. The procedures followed the manufacturer's instructions. Cortisol concentrations were expressed in ng/ml.

Statistical analysis was performed using Statistica 13.1. The normality of the distribution of the features examined was determined by the Shapiro-Wilk test. Since the distributions differed significantly from the normal distribution, the non-parametric Mann-Whitney U test was used to assess differences between the means.

Limitations. The small sample size of this study limits its applicability to a broader cat population, especially regarding the number of cats broken down by individual shelters. It is possible that the results were not statistically significant due to the small number of animals. However, the results indicate that this is still an intriguing and insufficiently explored area of research.

The cats used in the study were taken from the street. We assumed they lived in the streets since they were taken from there, but we had no exact knowledge their past. We did not know if they were abandoned, ran away or lived on the streets since birth and, if not, for how long.

Results and discussion

The average hair cortisol level was 0.74 ng/ml before arrival at the shelter (period B) and 0.56 ng/ml during the one-month stay (period A). The cortisol level before arrival at the shelter was higher than it was during the stay (Fig. 2), but the difference was not statistically significant ($Z = -1.233$, $p = 0.217$).

Analysis of cortisol levels before and after admission to the shelter, broken down by individual shelters, revealed that in 6 of the 8 shelters, the cortisol level before admission was higher or unchanged (Fig. 3).

The mean hair cortisol level of female cats was 0.62 ng/ml, and that of male cats was 0.68 ng/ml

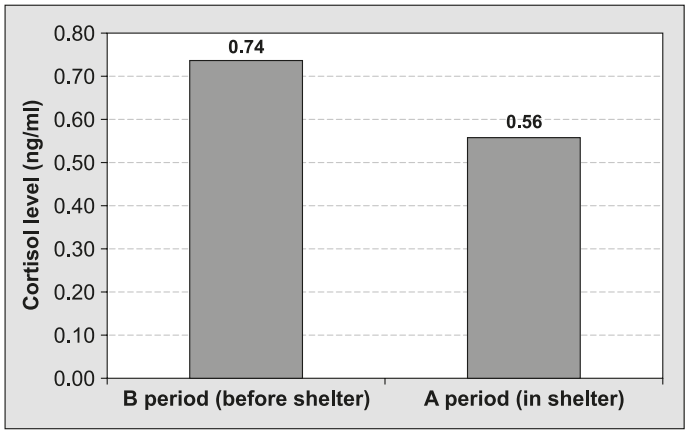

Fig. 2. Mean hair cortisol level in cats (ng/ml) before admission (B) and in the shelter (A)

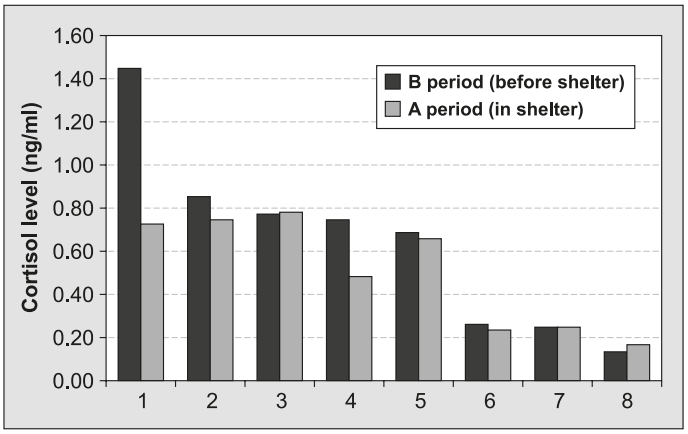

Fig. 3. Hair cortisol levels (ng/ml) in cats from individual shelters (1-8)

Tab. 1. Hair cortisol levels [ng/ml] in male and female cats before and during their stay at the shelter

	Female cats	Male cats
Before admission (period B)	0.85	0.65
In the shelter (period A)	0.51	0.60

(Z = 0.6147; p = 0.5388). Cortisol levels broken down by the cats' sex and the two periods are presented in Table 1. In female and male cats, cortisol levels decreased after arrival at the shelter compared to the preceding period, but the differences were not statistically significant (p = 0.535 and p = 0.489).

We found that cortisol levels in females in the pre-housing period were higher than in males. In contrast, during the stay at the shelter, the cortisol level in females was lower than in males (Fig. 4).

The cats in our study were found on the street, but nothing else was known about their past. Dybdall et al. (3) assessed the behavioural differences between owner surrender and stray domestic cats after entering an animal shelter. They found that cats surrendered by their owners displayed significantly higher mean stress scores than stray cats. On the other hand, in a study by McCobb et al. (2), physiological stress levels (cortisol creatinine ratio) did not differ significantly between stray and surrendered cats.

In our study, cortisol levels in cats staying at the shelter were lower than their cortisol levels before admission. Fukimoto et al. (6) assessed the stress level in cats staying at a shelter and after adoption by new owners. They determined the level of cortisol metabolites in the faeces. On average, faecal cortisol metabolite levels decreased after the cats moved to their new home, but the difference was insignificant.

For our study, we selected only cats staying at the shelter for 4-5 weeks. We only wanted to observe the change in cortisol levels after arriving at the shelter. Durman (2) found that the greatest behavioural changes occurred in cats within the first four days of admission to a homeless shelter and persisted for the first month of adaptation. Using the Cat-Stress-Score, Kessler and Turner (9) monitored adaptation to shelter conditions in 140 cats. Stress levels decreased during the observation period, with a marked reduction in stress in the first four days, and two-thirds of the cats adapted satisfactorily within two weeks. In another study of shelter cats, Rochlitz (1) observed that cats showed signs of adaptation to new conditions within two weeks. In future studies, we would like to analyze

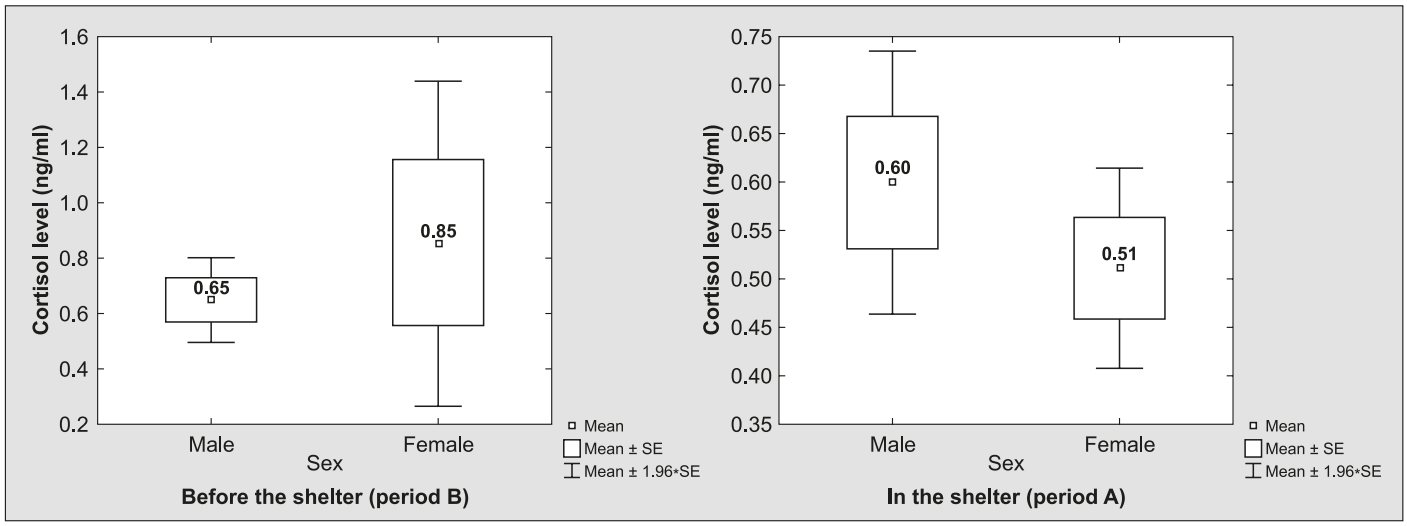

Fig. 4. Hair cortisol levels [ng/ml] in male and female cats before and during their stay at the shelter

hair cortisol in cats staying at shelters for a long time and to assess changes in cortisol levels relative to the time spent there. In a study by McCobb et al. (2), there was almost no correlation between the number of days a cat spent at the shelter and its stress level (measured by the cortisol-creatinine ratio).

In the shelters selected for this study, cats were kept mainly in multi-cat cathouses. Only in shelter no. 1 were cats kept individually in cages. Due to the small number of cats in this study, we did not analyze differences between cortisol levels and housing conditions in the individual shelters. In cats housed alone in cages, the decrease in hair cortisol levels after admission to the shelter was most pronounced. However, those cats also had the highest average cortisol levels before arrival at the shelter. Therefore, it would be necessary to examine environmental conditions in which they lived previously, in the streets. Uetake et al. (18) assessed the effects of single caging and cage size on the behaviour and stress level of domestic neutered cats housed in an animal shelter. Significant differences in the time spent on locomotion and social play were found between cats in different housing conditions. This is important because other research shows that more active cats are more likely to be adopted (5). Moreover, research by Gouveia et al. (7) shows that the longer cats stay at the shelter, the less active they become. Cats residing at the shelter for at least seven years spent significantly more time inactive than those residing there for 1-6 years (7).

Our research analyzed hair cortisol levels in cats of different sexes. It should be remembered that sex is not the only factor affecting the hair cortisol level, and not only in cats. Influencing factors may include age, sex, pregnancy, season, and even hair colour or the region of the body from which hair was collected (8). In addition, how intensively cats groom themselves can sometimes affect their hair, and thus its composition.

Overcrowded shelters for homeless animals are often places where cats experience traumatic experiences due to long-term stressors. As our study suggests, staying in a shelter can be less stressful for cats than staying in the street. In our research, the cortisol level in cats was higher before arrival at the shelter than during the stay. Although cortisol values decreased during adaptation to shelter life, no statistically significant differences were found.

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