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Research Article

Effects of an Indoor Lifestyle on Selected Serum and Urine Biomarkers in Domestic Cats

Erdem Gülersoy [1], Esma Kısmet [1], Mehmet Maden [2]

[1] *Harran University, Veterinary Faculty, Department of Internal Medicine, Şanlıurfa, Türkiye*
[2] *Selçuk University, Veterinary Faculty, Department of Internal Medicine, Konya, Türkiye*

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Abstract
This study aimed to assess stress in indoor and free-ranging domestic cats by measuring biomarkers (serotonin, cortisol, dopamine) in serum and urine, along with urinary hippuric acid levels, and to examine their correlation with exposure to industrial substances, particularly toluene-containing materials. A total of 244 cats underwent physical and laboratory assessments, including anamnesis, physical examination, laboratory analyses (urine analysis, urine sediment analysis, urine bacterial culture, venous blood gas analysis, complete blood count (CBC), serum biochemistry), and abdominal ultrasonographic examination. After applying inclusion/exclusion criteria, 16 cats were categorized into two groups: Free-ranging (n = 8) and Indoor (n = 8). Serum and urine serotonin, cortisol, dopamine, and urinary hippuric acid concentrations were measured to assess exposure to indoor materials. Indoor cats had a higher respiratory rate and significantly greater bladder wall thickness (0.28 ± 0.07 cm vs. 0.19 ± 0.02 cm, p = 0.002). Urine pH was lower in indoor cats, and hippuric acid crystals were found in their urine sediment. Serum serotonin levels were lower in free-ranging cats (p = 0.006), while urinary serotonin (p = 0.005), cortisol (p = 0.012), dopamine (p = 0.003), and hippuric acid (p = 0.033) levels were higher in indoor cats. The findings suggest that indoor confinement may be associated with increased physiological stress and greater exposure to toluene-containing materials, which could potentially contribute to stress-related conditions and elevate the risk of toluene-associated effects.

Keywords: Cortizol, Dopamine, Hippuric acid, Serotonin, Stress.

Introduction

Companion animals, particularly cats and dogs, coexist with humans in shared environments, leading to exposure to similar chemical agents. Research indicates that indoor pets are subjected to environmental contaminants through multiple exposure pathways, including ingestion, dermal contact, and inhalation. These contaminants originate from various household sources, such as carpets, textiles, plastic materials, pet accessories, and consumer products like cleaning agents, adhesives, candles, children's toys and personal care items [1]. Toluene, a widely used solvent in various household products, is employed for its ability to dissolve, dilute, and facilitate the drying of compounds. It is commonly found in paints, rubbers, polishes, and adhesives, as well as in the production of materials like nylon, plastics, and polyurethane. Toluene also serves as a precursor in the synthesis of chemicals such as benzene and benzoic acid, and is present in consumer goods, household products, and plasticizers [2,3]. Exposure to toluene triggers metabolic reactions that produce various metabolites, with one of the main pathways being its conversion to hippuric acid [4].

Hippuric acid, an acyl glycine derivative, is formed through the conjugation of benzoic acid with glycine and serves as a biomarker for toluene exposure. Upon ingestion of phenolic compounds, the body converts them into benzoic acid, which is then conjugated to hippuric acid and excreted in the urine [5]. As a result of this metabolic process,

* Corresponding author: E. Gülersoy, **Email:** egulersoy@harran.edu.tr

urinary hippuric acid levels can be used as a biomonitoring parameter for exposure to toxic organic compounds, including toluene [4]. In households with pets, particularly cats, the provision of essential resources is crucial for their well-being and sense of security. Similar to animals in zoological settings, domestic cats' welfare is influenced by their environment. The absence of environmental enrichments, such as toys, scratching posts, and appropriate feeding and drinking stations, can contribute to stress-related conditions and behavioral disorders [6]. Moreover, many cat owners prefer synthetic or microfiber-covered sofas made from polyester, nylon, and acrylic blends, which are resistant to scratching and easier to clean from cat fur. However, these materials often contain chemical compounds, including benzene and benzoic acid, which may contribute to environmental toxicant exposure [1,4,6].

The decision to restrict a cat to an indoor environment is typically made by the owner to safeguard the animal's welfare, primarily to reduce exposure to outdoor hazards such as traffic accidents, infectious diseases, and predation. However, indoor-only cats may be exposed to higher concentrations of industrial chemicals present in household materials and furnishings, such as flame retardants and plasticizers, compared to free-ranging cats with outdoor access. Moreover, free-ranging cats naturally engage in species-typical behaviors such as hunting, climbing, and territory exploration, which are important for their mental and physical well-being. The inability to perform these activities in indoor settings, especially in the absence of adequate environmental enrichment (e.g., toys, scratching posts, climbing structures, interactive play), may result in chronic stress and contribute to behavioral disorders. Therefore, the provision of appropriate enrichment strategies is essential for maintaining welfare in indoor-housed cats and mitigating the potential negative effects of confinement [1,6,7]. Thus, this study aims to evaluate stress levels in both indoor and free-ranging domestic cats by analyzing selected biomarkers, including serotonin, cortisol, and dopamine, in serum and urine, along with urinary hippuric acid levels, which are important indicators of toluene exposure. Additionally, it investigates the potential correlation between urinary hippuric acid levels and exposure to industrial substances, particularly toluene-containing materials commonly found in cat households, such as fabrics, plastics, and cat toys and supplies.

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board Faculty of Veterinary Medicine, Selçuk University Local Ethics Committee (Approval number: 2020/42).

All cats included in the study were presented to the Selçuk University Faculty of Veterinary Medicine Animal Hospital for general examination, routine check-ups, and vaccination between March 2021 and April 2022. A total of 244 cats underwent physical and laboratory evaluations as described below. All animals first received a thorough physical examination, followed by blood sample collection. Urine samples were then obtained via cystocentesis without prior bladder filling. Finally, ultrasonographic examination of the bladder was performed after moderate bladder filling (4 mL/kg of saline administered intravesically) to ensure accurate imaging with minimal discomfort, in accordance with animal welfare guidelines. To determine the suitability of the cats for inclusion in the study, each cat was evaluated through anamnesis, physical examination, and a series of diagnostic tests. These included urine analysis, urine sediment analysis, and urine bacterial culture to assess urinary tract health; venous blood gas analysis, complete blood count (CBC), and serum biochemistry to evaluate overall health and organ function; and abdominal ultrasonographic examination to detect any underlying structural abnormalities.

All cats were kept in examination rooms free of other animal odors and cleaned with disinfectant. They were allowed 10–15 minutes to acclimate to the environment. Blood samples were collected between 9:00 and 10:00 a.m. to minimize variations in diurnal stress hormone levels [8]. The same veterinary staff collected anamnestic data, including information on the cats' lifestyle, demographic details (age, sex, breed, neutering status, medical and vaccination history), and diet. As part of the physical examination, rectal body temperature, respiration and heart rates, palpation of lymph nodes, and evaluations of the oral and mucosal tissues were performed, along with auscultation of the lungs and heart. After physical examinations, venous blood (both with anticoagulants [heparinized tubes for blood gas and electrolyte analysis and K_3EDTA for CBC] and without anticoagulants for serum biochemistry) and urine samples were collected from all cats by the same veterinary staff with minimal restraint and stress, within a time frame not exceeding 20 minutes. Venous blood samples (3–6 mL) were drawn from the vena cephalica, and urine samples (5–10 mL) were collected via

cystocentesis using a 25-gauge needle under ultrasound guidance (Mindray Z60, China). Additionally, ultrasonographic evaluation of the bladder's fullness, bladder wall thickness, and kidney morphology was conducted using a 7.5 MHz microconvex probe after shaving the abdomen and applying alcohol and coupling gel. For the CBC, the following parameters were assessed using an autoanalyzer (Sysmex pocH100i, Japan): leukocytes, lymphocytes, monocytes, granulocytes, erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, and hemoglobin levels. Blood and urine samples were then centrifuged (10 minutes at 2000 g), and the supernatant was extracted and stored at −80°C until analysis. Blood gas and electrolyte analyses (pH, pCO_2, pO_2, sodium, potassium, chloride, lactate, base excess, and bicarbonate levels) were performed within 5-10 minutes using heparinized blood samples on an ABL 90 Flex Blood Gas/Electrolyte Analyzer (Model 5700 Radiometer, USA). Serum biochemical analyses (blood urea nitrogen, creatinine, glucose, total protein, albumin, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, gamma-glutamyl transferase, cholesterol, triglycerides, calcium, and phosphorus concentrations) were conducted within 30-45 minutes using a biochemical analyzer (Biotecnica BT 3000 Plus, Italy). Urine analysis (URIT-31, Accurex Biomedical, India) and urine sediment examinations (at ×40 magnification using light microscopy, Olympus, Japan) were performed on the urine samples.

Commercially prepared blood agar plates (trypticase soy agar with 5% sheep blood), MacConkey agar plates, and Columbia CNA agar plates were inoculated with urine samples. Prior to use, each batch of agar media was inspected according to the manufacturer's instructions. A 0.001 mL calibrated loop was used to transfer urine samples onto the agar plates. The plates were incubated anaerobically at 35°C and examined at 18, 24, and 48 hours post-incubation. Significant bacteriuria was defined as bacterial counts exceeding 105 colony-forming units (CFUs) per plate.

After completing all physical and laboratory examinations, cats with systemic infections or comorbid diseases, including endocrinopathies, pyelonephritis, chronic kidney disease, and urolithiasis, as well as those currently receiving treatment with medications such as antibiotics, antihistamines, corticosteroids, nonsteroidal anti-inflammatory drugs, anticholinergics, antidepressants, urinary acidifiers, glycosaminoglycans, diuretics, or other drugs used to treat urinary tract diseases (either currently or within at least 2 weeks prior to admission), were excluded from the study. To minimize the likelihood of coinfection with additional viruses, additional serological tests were conducted on serum and conjunctival and/or fecal swab samples, following the manufacturers' instructions. The tests included assays for feline calicivirus antigen (FCV Ag Asan Pharm®, Korea; sensitivity and specificity: 96% and 98%, respectively, vs IFA), feline herpesvirus antigen (FHV Ag Asan Pharm®, Korea; sensitivity and specificity: 96.5% and 98%, respectively, vs IFA), feline immunodeficiency virus antibody/feline leukemia virus antigen (FIV Ab/FeLV Ag Asan Pharm®, Korea; sensitivity and specificity: 98% and 98.7%, respectively, vs IFA), and feline parvovirus antigen (FPV Ag Asan Pharm®, Korea; sensitivity and specificity: 97.8% and 98.8%, respectively, vs IFA). All these tests were negative. Additionally, cats with parasites or parasite eggs detected in stool microscopic examinations, despite the absence of clinical findings, as well as cats admitted to the hospital that had eaten food in the morning, were excluded from the study to minimize any potential effects on the results, particularly on urine hippuric acid levels. Consequently, 16 of the 244 cats initially considered for inclusion in the study were ultimately selected, following a meticulous evaluation based on predefined inclusion and exclusion criteria, which involved comprehensive physical and laboratory examinations. A post hoc power analysis was conducted using an estimated large effect size (Cohen’s d = 1.5), a significance level of $\alpha = 0.05$, and a sample size of 8 cats per group. The analysis indicated a statistical power of approximately 0.80, suggesting that the sample size was adequate to detect meaningful differences between groups. The 16 cats included in the study were subsequently divided into two groups according to their lifestyle: the Free-ranging group (n = 8) and the Indoor group (n = 8). A free-ranging cat is defined as an owned cat that spends time outdoors without physical restraint [9].

Biomarker measurements from serum and urine supernatants included serotonin (detection range: 15.63-1000 ng/mL, Elabscience, Texas, USA), cortisol, and dopamine (detection ranges: 0.50-300 ng/mL and 0.50-200 ng/mL, respectively), using feline-specific commercial ELISA test kits (code: EA0010Ge, Bioassay Technology Laboratory, Zhejiang, China), according to the manufacturers' instructions. Urinary hippuric acid levels were measured using commercial ELISA kits (detection range: 1-1000 µg/mL, sensitivity: 10 µg/mL, Bioassay Technology Laboratory, Zhejiang, China), in accordance with the

manufacturer's instructions in the Science and Technology Application and Research Center of Harran University.

All data were analyzed using SPSS 27.0 (SPSS for Windows). The Kolmogorov–Smirnov test was applied to assess the normality of the data. Parametric data are presented as mean ± SD and analyzed using the independent t-test. Statistical significance was considered at $p < 0.05$.

Results and discussion

Animals

The mean age of the 8 cats in the Free-ranging group was 2.75 ± 0.88 years (Table 1). These cats were primarily fed a combination of home-cooked food and commercial dry food, and according to the anamnesis, they likely consumed food from outside, such as from garbage or waste. All cats in this group received routine antiparasitic treatment and vaccinations. 5 cats were male (neutered), and 3 were female (non-neutered). The mean age of the 8 cats in the Indoor group was 3.0 ± 0.92 years (Table 1). These cats were exclusively fed commercial dry cat food and were born indoors with no access to the outside environment. The indoor cats had access to enrichment materials, including scratching posts, cat beds, and toys. All cats in this group also received routine antiparasitic treatment and vaccinations. Of the eight cats, six were neutered males and two were females, one of which was neutered. Six out of the eight cats in Indoor group were fed the same commercial dry food. The food's analytical components included 36.00% protein, 16.00% fat, 8.00% raw ash, 2.50% crude fiber, 3.70% omega-6, and 0.75% omega-3. The food additives in this dry food consisted of 25,000 IU/kg of vitamin A, 1,500 IU/kg of vitamin D_3, 150 mg/kg of vitamin E, 25 mg/kg of vitamin C, 2,200 mg/kg of taurine, 50 mg/kg of niacin, 18 mg/kg of copper (as sulfate), 95 mg/kg of zinc (as sulfate), 20 mg/kg of zinc (as chelate), 30 mg/kg of manganese (as sulfate), and 0.30 mg/kg of selenium (as selenite).

Table 1. Physical examination finding results

Parameters	Free-Ranging Group n = 8 mean ± SD	Indoor Group n = 8 mean ± SD	p value
RR (breaths/sec)	**49 ± 7.63**	**81.50 ± 8.53**	**0.016ǂ**
HR (beats/sec)	150.50 ± 15.70	153 ± 7.92	0. 583
*Temperature (°C)	38.10 ± 0.48	39.03 ± 0.42	0.712
Age (years)	2.75 ± 0.88	3 ± 0.92	0.515

RR: Respiratory rate, HR: Heart rate, *Rectal, SD: Standard deviation, ǂ95% CI: 23.8 to 41.2, Cohen's d = 4.02.

The respiratory rate of cats in the Indoor group was higher than that of cats in the Free-ranging group ($p = 0.016$). No abnormalities were detected during lung and heart auscultation or in the evaluation of palpable lymph nodes. No statistically significant differences were found in the comparison of other parameters between the groups ($p = 0.538$ for heart rate, $p= 0.712$ for body temperature, $p = 0.515$ for age). Physical examination findings are presented in Table 1.

Ultrasonographic examination results

Bladder examination was performed after achieving moderate filling (intravesical administration of saline at a dose of 4 mL/kg) to ensure accurate results with minimal discomfort, in accordance with ethical guidelines for animal welfare (e.g., the Guide for the Care and Use of Laboratory Animals or European Directive 2010/63/EU). The bladder wall thickness of cats in the Indoor group was 0.28 ± 0.07 cm, while that of cats in the Free-ranging group was 0.19 ± 0.02 cm ($p = 0.002$). No morphological or echogenic abnormalities or uroliths were detected in the ultrasonographic examination of the kidneys in any of the cats.

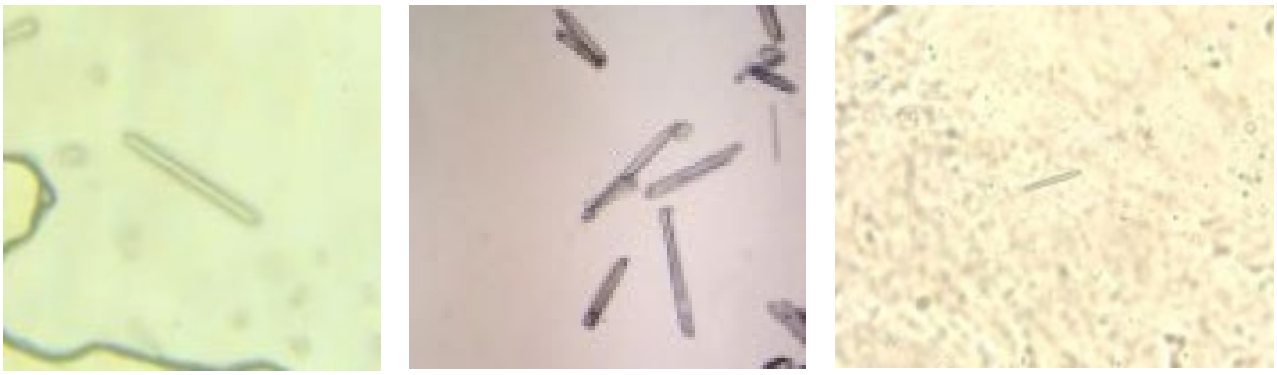
Laboratory analysis results

The CBC and serum biochemistry parameters assessed during laboratory evaluations were within reference ranges [10] and were used solely to screen for comorbid conditions as part of the inclusion and exclusion criteria. As these parameters were not part of the primary study analysis, they are not presented in detail here. However, these data are available from the corresponding author upon reasonable academic request. In the dipstick analysis of urine samples obtained via ultrasound-guided cystocentesis, only urine pH showed a statistically significant difference between the groups, being lower in the Indoor group cats ($p = 0.011$). The results of the urine dipstick analysis are presented in Table 2. Urine sediment examination revealed hippuric acid crystals in all indoor cats, identified based on their morphological characteristics (Figure 1). Urine bacterial culture results showed no bacterial growth in any of the cats included in the study.

Table 2. Urine dipstick analysis results

Parameters*	Free-Ranging Group n = 8 mean ± SD	Indoor Group n = 8 mean ± SD	p value
WBC	0.62 ± 0.51	0.75 ± 0.70	0.512
Sg	1.025 ± 0.70	1.025 ± 0.00	0.674
Protein	1.25 ± 0.70	2.12 ± 0.64	0.548
pH	**6.93 ± 0.32**	**6.12 ± 0.74**	**0.011ǂ**

WBC: Leukocyte, Sg: Specific gravity, pH: Power of hydrogen,* Readings are reported in terms of semi-quantitative values corresponding to each colour change, ǂ95% CI: 0.21 to 1.41, Cohen's d = 1.42.



x40 magnification, light microscope

Figure 1. Microscopic image of hippuric acid crystals

Biomarker measurement results

Serum serotonin levels were lower in free-ranging cats compared to indoor cats (p = 0.006). Urinary serotonin (p = 0.005), cortisol (p = 0.012), dopamine (p = 0.003), and hippuric acid levels were higher in indoor cats compared to free-ranging cats (p = 0.033). Serum and urine biomarker levels, along with urinary hippuric acid levels, are presented in Table 3.

This study evaluated the effects of exposure to household chemical substances on urinary hippuric acid levels and stress markers in urine and serum, comparing indoor cats, kept indoors throughout their lives, with free-ranging cats. The most significant findings were observed in urine samples, compared to physical examination and serum analysis results. Indoor cats had higher serum serotonin, urine serotonin, cortisol, dopamine, and hippuric acid levels than free-ranging cats. These findings suggest that indoor cats have greater exposure to household chemicals than free-ranging cats, as indicated by elevated urinary hippuric acid levels. Additionally, despite the absence of overt clinical signs such as stress-related sneezing, excessive grooming, or itching, indoor cats appear to experience subclinical stress, as reflected by alterations in serum and urine stress biomarkers [11]. This may predispose indoor cats to stress-related conditions and behavioral disorders like feline idiopathic cystitis (FIC), and they could also be at increased risk for symptoms associated with toluene toxicity, such as mucosal irritation, nausea, respiratory and neurological problems [3].

Surveys conducted in the United States between 2017 and 2018 reported that 68% of the American population owns pets, with cats and dogs accounting for more than 90% of this figure. Similarly, the number of pets is also increasing in Türkiye [12]. In the United States, pet care expenses, including food and veterinary services, total 72.6 billion dollars, with food accounting for 42% of this amount. While cat and dog food packages claim to be free of harmful components, studies have detected environmental pollutants such as bisphenol A (21-136 ng/gr), parabens and their metabolites (155-570 ng/gr) [13,14]. Studies have detected the highest concentrations of polybrominated diphenyl ether (PBDE), a contaminant used in various products such as building materials, electronics, furnishings, motor vehicles, airplanes, plastics, and polyurethane foams, in the blood of cats and dogs in the United States, Japan, Sweden, England, Pakistan, and Austria, respectively [14,15,16,17]. In a study conducted in Spain, organochlorine and organophosphate pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, PBDEs, and organophosphate esters were detected in the hair of cats and dogs [18]. Polyethylene

terephthalate (PET) and polycarbonate (PC) are two common components of household plastic products, with an annual production of nearly 4 million tons. PET has been associated with irritation and increased tearing upon contact and visual impairment and blurred vision upon ingestion. PC nanoplastics have been shown to increase neutrophil degranulation in vitro. Food and environmental products are important routes of exposure to these organic pollutants. Large molecules, such as microplastics (MP), are thought to be excreted in feces rather than urine [19]. A study detected PET and PC levels ranging from 1500 to 12,000 ng/g in cat foods purchased from local markets in the United States, Thailand, and Canada, with more than 80% of the foods containing PC [20]. Hippuric acid crystals are rarely seen in urine sediment. They are yellowish or colorless, long, prism- or plate-shaped, thin, and often form clusters. These crystals are observed in acidic and neutral pH and are soluble in ether. Although they are generally considered to have little clinical significance, they can be seen in cases of ethylene glycol toxicity or from exposure to substances containing ethylene glycol, such as those found in PET materials used in polyester fiber/fabric, fabrics, and bottles commonly found in domestic environments. Phenolic disinfectants and cleaners are frequently used in households. Although instructions often recommend diluting these products, they are commonly used in high concentrations due to the "more is cleaner" mentality. Additionally, cleaning equipment is often not rinsed thoroughly after use. Since water evaporates more quickly than phenols, these chemicals can remain on surfaces and cleaning tools, with their odor becoming more concentrated over time [21]. Considering that certain cat foods may contain PET and PC, and that many household products and pet items contain chemicals, including toluene [22]. The presence of hippuric acid crystals in urine sediment and the higher urinary hippuric acid levels observed in indoor cats compared to free-ranging cats in this study may be associated with both dietary factors and environmental exposure to toluene, which is abundant in household materials, despite the absence of clinical signs of irritation, respiratory and/or neurological problems [23]. Additionally, the use of scratching posts, toys, and painted food and water bowls, as well as prolonged exposure to these items, may contribute to this condition. However, since the outdoor activities and food consumed by free-ranging cats cannot be monitored, it is not possible to conclude that they are not exposed to such substances. Nonetheless, based on the results of this study, it appears that indoor cats are more exposed.

Veterinarians commonly assess respiratory rate as a key physiological parameter in various species, including cats. An elevated respiratory rate can result from a range of both physiological and pathological factors, including stress, hyperthermia, pain, and diseases affecting multiple organ systems [24]. In this study, the respiratory rate was significantly higher in indoor cats compared to free-ranging cats ($p = 0.016$, Table 1). This difference may be related to stress caused by handling, as well as the unfamiliar environment of the examination room for indoor cats [25]. Additionally, this finding is consistent with the elevated levels of stress biomarkers observed in this study, which will be discussed further.

Cats have evolved to thrive on a diet primarily consisting of animal tissues, developing a metabolism adapted to a carnivorous lifestyle, similar to other obligate carnivores such as mink and ferrets. In their natural habitats, cats mainly prey on small mammals. A study conducted in New Zealand showed that small mammals like rats, rabbits, possums, mice, and stoats comprised 93% (by weight) of the diet of feral cats over a period of three years [26]. Domestic cats, however, can develop urinary tract diseases, with diet being a significant factor in their onset. One such condition, urolithiasis, involves the formation of crystals or stones in the urinary tract. Carnivorous diets generally result in acidic urine, which is typically safe for cats. When provided with commercial diets, however, the pH of their urine can be reduced through the inclusion of acidifying agents such as ammonium chloride, calcium chloride, or methionine, to help prevent struvite urolith formation [27]. Current guidelines recommend maintaining an adult cat’s urine pH between 6.0 and 6.4 to reduce the risk of struvite urolithiasis. While urine composition is directly affected by diet, defining a "normal" reference range is challenging, as the natural diet of cats is difficult to characterize. In one study, cats fed a "semi-natural diet" consisting of rat carcasses had a mean urine pH of 6.98. The higher urinary pH levels observed in free-ranging cats in the present study may be a result of their natural or semi-natural diet [28]. Stress related to capture may also have impacted the results. Cats are often stressed during trapping, which can stimulate the sympathetic nervous system and adrenal glands, increasing metabolism and promoting protein catabolism. This process can lead to increased sulfuric acid production and a drop in urinary pH. The lower urine pH observed in indoor cats in this study ($p = 0.011$, Table 2) could also be due to

stress-related protein breakdown and hyperventilation [29]. This theory is supported by the higher respiratory rate noted in indoor cats ($p = 0.016$, Table 1). Furthermore, lower urine pH may enhance the formation of urinary hippuric acid crystals (Figure 1). Despite efforts to minimize stress during physical examination and sample collection, the home environment of the cats and the fact that vaccinations were administered at home, as indicated in their anamnestic and medical history, might not have entirely prevented subclinical stress.

Coping mechanisms are fundamental responses to stress and are critical in determining an individual's resilience. These strategies are generally categorized into two types: active coping, which involves direct engagement with the stressor, and passive coping, which relies on avoidance or internal regulation without active intervention. Serotonin (5-hydroxytryptamine, 5-HT) is essential in modulating coping behaviors, as evidenced in both animal and human studies. Stress activates a range of neuroendocrine and neurochemical pathways, many of which affect serotonin function, prompting research into specific components of the stress response. Notably, cortisol and dopamine have been extensively studied for their regulatory effects on serotonergic activity [30]. Both physical and emotional stressors contribute to the pathophysiology of chronic relapsing disorders such as interstitial cystitis/painful bladder syndrome (IC/PBS) in humans. FIC exhibits similar pathophysiological traits to IC/PBS, including increased serotonin release from mast cells, which plays a central role in inflammation and pain regulation. In addition, elevated expression of tyrosine hydroxylase in IC/PBS patients leads to the release of catecholamines, particularly dopamine. Interestingly, higher morning salivary cortisol levels are linked to less severe symptoms of IC/PBS [31]. However, cats with FIC show notable increases in plasma norepinephrine and dihydroxyphenylglycol concentrations, as well as a tendency toward higher epinephrine levels. Excessive concentrations of biomarkers—such as cortisol, serotonin, epinephrine, and dopamine—are commonly associated with physiological and psychological states like stress, euphoria, and illness. Recent research has focused on measuring biomarkers in bodily fluids (e.g., blood, urine, sweat, and saliva) to assess stress responses. Key stress biomarkers include serum and urine concentrations of cortisol, serotonin, and dopamine. Previous studies have suggested that urine serotonin levels could serve as an effective marker for stress in cats with FIC. Furthermore, urinary serotonin and dopamine concentrations have been proposed as potential biomarkers to distinguish FIC-affected cats from healthy ones [29,31]. Similarly, urinary cortisol levels are not influenced by hepatic metabolism and better reflect endogenous cortisol concentrations than serum levels. Likewise, measuring dopamine concentrations in urine may provide more useful clinical information than serum measurements due to its longer excretion period in urine and short half-life in serum [32]. In the present study, serum serotonin, urine serotonin, cortisol, and dopamine levels were significantly higher in indoor cats than in free-ranging cats ($p = 0.006$, $p = 0.005$, $p = 0.012$, $p = 0.003$, respectively (Table 3)). These results may reflect increased activation of neuroendocrine and neurochemical pathways in indoor cats, possibly due to restrictions on their natural behaviors. Furthermore, the structured and predictable home environment may contribute to elevated stress levels [30,33,34]. Limited cat-owner interaction or short play sessions could also increase stress, even when owners report providing a sufficient number of pet toys. A detailed survey of indoor cat owners may help identify and mitigate stress-related factors [11]. Additionally, the observed differences in urine versus serum stress markers may be due to the short half-life of these markers and their exposure to hepatic metabolism in serum. This finding suggests that urine samples may be preferable for future studies [35].

Outdoor cats navigate complex and often hazardous environments, covering extensive territories that typically include diverse landscapes such as forests, farmland, and urban gardens. In these varied environments, outdoor cats engage in activities like exploring, hunting, foraging, and occasionally interacting with other cats. In contrast, indoor cats live in a controlled, safe environment but are often limited in their range of activities. Without regular interaction with humans or other cats, indoor cats may experience boredom. As obligate carnivores, cats possess an inherent drive to hunt and stalk prey, behaviors rooted in their evolutionary instincts rather than human-like tendencies. The findings of this study suggest that environments lacking variation—particularly those that are predictable and monotonous—can contribute to stress in indoor cats [34]. While many indoor cats adjust to suboptimal living conditions, cats with neuroendocrine disorders may struggle to adapt, making them a distinct population with greater needs. Veterinarians often focus on improving indoor environments, aiming to exceed the minimal conditions required for survival [33]. Although no clinical signs of FIC were observed in

the cats studied, the results imply that indoor cats may face a higher risk of developing FIC and other stress-related conditions over time. The higher risk of developing FIC is further supported by the thicker urinary bladder wall observed in indoor cats compared to free-ranging cats in the present study (p = 0.002). The confinement inherent in indoor environments may act as a stressor, as suggested by the elevated serotonin levels measured in this study, potentially contributing to the development of FIC [31,36]. The findings of this study may highlight the importance of addressing the specific needs of indoor cats [11].

Table 3. Serum and urine biomarker and urinary hippuric acid level measurement results

Parameters	Free-Ranging Group n = 8 mean ± SD	Indoor Group n = 8 mean ± SD	p value
Serum Serotonin (ng/mL)	**51.94 ± 32.95**	**156.91 ± 104.16**	**0.006***
Serum Cortisol (ng/mL)	32.81 ± 4.78	33.52 ± 6.62	0.536
Serum Dopamine (ng/mL)	1.34 ± 0.18	1.42 ± 0.26	0.519
Urinary Serotonin (ng/mL)	**13.85 ± 2.05**	**59.56 ± 4.49**	**0.005****
Urinary Cortisol (ng/mL)	**27.94 ± 2.58**	**35.86 ± 7.10**	**0.012*****
Urinary Dopamine (ng/mL)	**0.91 ± 0.08**	**1.33 ± 0.43**	**0.003+**
Urinary hippuric acid (µg/mL)	**146.25 ± 101.12**	**506.25 ± 419.45**	**0.033++**

*95% CI, 22.15 to 187.79, Cohen's d = 1.36, **95% CI, 41.97 to 49.45, Cohen's d = 13.10, ***95% CI, 2.19 to 13.65, Cohen's d = 1.49, +95% CI, 0.09 to 0.75, Cohen's d = 1.36, ++95% CI 32.9 to 687.1, Cohen's d = 1.20

This study has several limitations. First, tracking the exact food consumption of free-ranging cats was not possible due to their nature, as the areas they frequent and the animals they interact with remain unknown. Another limitation is the reliance on owner-reported data for the anamnestic and signaling information of indoor cats, as their living environments were not directly observed. Additionally, while most indoor cats consumed the same food brands, the lack of standardization in food quantities and the inability to access food content information for two cats represent further limitations. The study's scope was also limited by the low number of animal materials included. Lastly, there was an absence of research on benzoic acid, the primary precursor of hippuric acid, both in the cats and in the food they consumed. Despite these limitations, the findings of this pioneering study may provide valuable insights for future research that addresses these gaps.

Conclusions

The findings of this study suggest that cats kept exclusively indoors experience a higher physiological stress burden. Indoor cats exhibited significantly greater urinary acidity, along with elevated serum and urinary serotonin, urinary cortisol, dopamine, and hippuric acid levels compared to free-ranging cats. The increased urinary hippuric acid may reflect greater exposure to certain indoor environmental compounds, potentially associated with synthetic materials. While the study did not directly assess environmental pollutants or toluene exposure, the observed metabolic changes raise concerns about possible links between indoor environments and stress-related conditions, including FIC and behavioral disorders. These results highlight the need for further research to clarify environmental and lifestyle factors influencing feline health and stress.

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