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## Comparison of Acute versus Chronic Stress Responses to Different Housing's Systems of Cats

Esterina Fazio, Adriana Ferlazzo, Cristina Cravana & Pietro Medica

### ABSTRACT

**Background:** The hypothalamic-pituitary-adrenal (HPA) axis and functional systems undergo the assessment of stress levels throughout living environments, contributing to avoid side effects to well-being in domestic animals, including pets. Cortisol represents the most important glucocorticoid found in felis and it is frequently used as standard marker in stress research. The purpose of the present study was to compare the adrenal and haematological patterns of cats, by taking into account the effects of different housing's systems. The hypothesis was to find a different coping strategies, according to different housings.

**Materials, Methods & Results:** For this study a total of 50 cats were selected on the basis of the breed: European domestic short hair cats, age ranged between 22 and 30 months and housing's systems, represented by cattery and/or households, respectively. On these basis, cats were distinguished into two groups, represented by group A: 22 cats living in cattery, and group B: 28 cats living in households. Blood samples were collected twice a week, for two consecutive days, during one month and subsequently analysed for haematological analysis and cortisol concentrations. Group B showed higher cortisol concentrations ( $P < 0.01$ ), Red Blood Cell ( $P < 0.05$ ), Packed Cell Volume ( $P < 0.001$ ), Platelets ( $P < 0.01$ ), Heart Rate and Respiratory Rate ( $P < 0.05$ ) values, and lower White Blood Cell ( $P < 0.001$ ) than group A.

**Discussion:** This observational study showed that cats housed in the households group showed higher cortisol, RBC, PCV, Plt, HR and RR values, and lower WBC rather than cattery's cats. Another point is that males showed higher RBC, PCV, WBC and Plt than females, irrespective of different housing's systems. The significant lower threshold of cortisol levels in cattery's cats than household's cats could suggest that these subjects were probably totally accustomed to cattery's environment; though these animals were daily stimulated by predictable stimuli and manipulations, characterized by handling and husbandry routine, regular feeding and cleaning times, standard caretaking. On the other hand, the higher cortisol concentrations observed in household's cats could be due to the different environmental stimuli, characterized by unpredictable handling, modified caretaking, presence of irregular talking, petting and manipulations by owners, which promote probably the expression of species and appropriate behaviour with stimulating activities.

The significant highest RBC and PCV values in subjects of group B could be explained on the basis of the more intense activity of these subjects, according to the access to outdoor area. These concomitant higher values were corroborated in the present study by the not surprising positive and significant correlation observed between corresponding RBC and PCV values. The higher PCV values observed in cats of group B could be suggest that their daily frequent activity induced a physiological erythrocytosis, compared to sedentary cattery's cats.

The hypothesis that the home represented more rousing than cattery setting was assessed by the physiological and consistent higher cortisol, RBC, PCV, Plt, HR and RR displayed in the home environment; The only difference between the two groups was that environmental stimulus (chronic stress) was cattery for group A, whereas household for group B. Obtained data indicate that there was a marked benefit in to establish a personnel-cat relationship in addition to the traditionally owner-cat relationship, providing physiological coping strategies in both cattery and home cats; this was corroborate in the present study by the wide but physiological cortisol range. This study indicates that predictability, familiarity and unpredictability are significantly associated with environmental stimuli and with quality of pets' life.

**Keywords:** cat, cortisol, blood count, functional parameters, housing's system.

## INTRODUCTION

Cat's adrenocortical activity is involved in the physiological response to a familiar, unfamiliar person, environment [20,25] and to human or equivalent nonhuman cues [35].

Cortisol represents the most important glucocorticoid found in felis [9]; it is frequently used as standard marker in stress research. It has previously been found to correlate with several physiological parameters [10] and ACTH concentrations [15]. In domestic cats, an increase of cortisol levels has been associated with unpredictable handling and husbandry [6,11], transport, veterinary clinic visits [27,37], surgical procedure [7,8,12] and postoperative pain [5,32]. Stressful experiences can have a major impact on the cats' welfare and may cause higher incidences of diseases in the shelters due to increased cortisol levels and immunodeficiency [36]; moreover, aggression and fear decline after several months of confinement into an animal shelter [2], and stress scores of group-housed cats should not differ between sexes [16].

Specific studies were performed on the physiological changes of cortisol concentrations in relation to different biological sampling [1], or different non-invasive methods for evaluating stressful conditions in cats [11,29].

The cortisol concentrations in the domestic cat are not affected by the different daily activities and photoperiods [15,19]. Nevertheless, the highest cortisol concentrations were found during the evening while the lowest was spotted during the morning; this is probably due to the fact that cats are nocturnal animals [30].

It is generally accepted that the stressful stimuli associated with handling during the blood sampling procedure can alter the haematological pattern in cattery's cats [24]. Likewise, it is reasonable to assume that the sympathetic stimulation in response to acute stress induced a functional reactivity, with an increase of heart rate (HR) and respiratory rate (RR).

The purpose of the present study was to compare the adrenal and haematological patterns of cats, by taking into account the effects of different housing's systems.

## MATERIALS AND METHODS

### *Study overview*

All methods and procedures used in this experiment were in compliance with the guidelines of the Italian Health Minister for the care and use of animals (D.L. 4/3/2014 n. 26) and EU Directive (Directive 2010/63).

Informed consent was collected from all owners who volunteered a cat for the study.

For this study a total of 50 cats were selected on the basis of the following criteria:

- 1) Breed: European domestic short hair cats.
- 2) Age: between 22 and 30 months of age.
- 3) Housing's systems: cattery, households.

### *Study cats*

Cats were distinguished into two groups, according to different housing's systems; group A included cattery cats (12 females and 10 males), group B included household cats (16 females and 12 males). Group A lived in the cattery for a short amount of time ( $3 \pm 1.5$  months) prior to the start of study.

The age of cats of groups A, ranged from 24 to 30 months, was estimated at intake by the cattery veterinarian with an assessment of teeth, body morphology and condition, because no reliable history was detected in the past; age of cats of groups B ranged from 22 to 24 months, as reported by their owners. All females were spayed and males were castrated. On the basis of their history, all subjects did not show clinical signs of any symptoms of disease detectable either clinically or by laboratory examinations.

Each animal was given a thorough physical examination (examination of mucosa, heart rate, respiratory rate, hydration state, body weight), and individuals did not shown signs of clinical illness. All cats after clinic check were defined in good health. Handling included weighing, routine veterinary check and blood samplings.

The cats received a commercial feedstuff (*Adult Light Sterility Cat - Trainer*: croquettes with chicken and turkey) twice a day. The composition of the diet was the same for the two groups throughout the study, to minimize the effect of diet composition; fresh water was available *ad libitum*.

### *Housing's systems studied*

Local cattery (Messina, Sicily, Italy) comprised an area of 200 m<sup>2</sup>, with seven indoor sections and a total of sixty domestic cats subjected to different lengths of time, room density and sex ratio.

Twenty-two healthy young subjects were assigned to this study and were included in group A. They were housed in the cattery for at least 6 weeks before to the study, and no cats were removed or added to the group A during the study. These cats lived in

group housing enclosures in an indoor room, ranging 3.6 m x 5.5 m (~20 m<sup>2</sup>), with a density of 0.8 cats/m<sup>2</sup>, contained shelves, beds and toys; during the study, the mean ambient temperature and humidity in the indoor room were maintained between 19-22°C and 40-50%, respectively. The cats could move freely within the room and interact with another, and showed regular activities during the day as running and playing, in daily contact with cats of the same room. Visitors were able to view the animals between 14:00 and 16:00 h, from Monday to Saturday. The cats had daily contact with humans, including handlers, veterinarians, cleaning staff and owners of the cattery, and received daily grooming and social interaction from their caretakers. Individual cats were recorded with their name and related identification number, according to hair colour and sex, and, during the study, the subjects were identified on the basis of these variables.

Twenty-eight healthy young subjects were assigned to this study and were included in group B. Household cats of group B had free outdoor access to a little garden, in contact with another one or two adult cats. Local households of groups B comprised an area ranged between 90 to 120 m<sup>2</sup>, and about the 50% of this area was utilized by the cats.

#### *Study procedure*

Peripheral blood samples (1-3 mL) were collected twice a week, for two consecutive days, during one month, by needle-cannula of the radial vein. Handler bias was minimized by using the same and usual clinician of cattery for all sampling, using the same methods and in the same order of procedures; to avoid interferences with the daily cleaning and feeding procedures (before 13.30 h), and to ensure that cortisol levels were not impacted by diurnal rhythms, samplings took place from 14.00 to 16.00 h, under quiet conditions. To ensure uniformity of handling, cats of groups B were sampled in their home environment by their family veterinarian (veterinarian in charge). Blood samples were placed into evacuated tubes (Venoject)<sup>1</sup>, and in K3-EDTA tubes<sup>2</sup> for haematological analysis. Evacuated tubes samples were subsequently (within 1 h) centrifuged for 15 min at 1500 x g, and serum was harvested and stored in polystyrene tubes at -20°C until assay for cortisol concentrations.

Before blood samplings, heart rate (HR bpm/min) and respiratory rate (RR breaths/min) were evaluated using stethoscope (Littman, model Classic

II SE); heart rate was measured for a period of 15 s and multiplied by four to give a result expressed in beats per minute.

Total serum cortisol concentrations were assayed by amplified Enzyme Immunoassay (EIA) following the instructions of the manufacturer<sup>3</sup>. During the first incubation, the cortisol sample competed with cortisol conjugated to horseradish peroxidase (HRP) for the specific sites of the antiserum coated on the wells. Following incubation, all unbound material was removed by aspiration and washing. The enzyme activity bound to the solid phase was inversely proportional to cortisol concentration in calibrators and samples, and it was made evident by incubating the wells with a chromogen solution (tetramethylbenzidine, TMB) in substrate-buffer. Colorimetric reading was carried out using a spectrophotometer (Sirio S)<sup>3</sup> at 450 and 405 nm wavelength. Assay sensitivity was 5 ng/mL and intra- and interassay CVs were 4.6% and 6.9% respectively.

The venous blood samples were analyzed (within 1 h) by an automatic photometer SLIM for haematological assays (SEAC), measuring Red Blood Cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell (WBC); the Platelets (Plt) were counted by conventional manual techniques using Bürker's chamber.

#### *Statistical analysis*

Student's unpaired *t*-test was applied to test the differences between groups and sexes. A linear correlation analysis (Pearson's method) was performed to analyse the relationships among physiological parameters. The level of significance was set at  $P < 0.05$ . All calculations were performed using the PRISM package<sup>4</sup>.

### **RESULTS**

Data are presented as means  $\pm$  standard deviation (sd).

Cortisol concentrations of groups A and B were reported in Figure 1. In cattery's cats (group A) cortisol concentrations ranged from 103.79  $\pm$  49.87 nmol/L in males to 85.74  $\pm$  42.83 nmol/L in females; in household's cats (group B) concentrations ranged from 154.54  $\pm$  46.04 nmol/L in males to 140.18  $\pm$  40.76 nmol/L in females. Obtained data showed higher total, male and female cortisol concentrations in cats of group B ( $P < 0.01$ ) than cattery's cats of group A.

Haematological parameters (Table 1) showed higher RBC ( $P < 0.05$ ), PCV ( $P < 0.01$ ) and Plt ( $P < 0.001$ ) values, and lower WBC ( $P < 0.001$ ) values in cats of groups B than subjects of group A. Compared to females, males of groups A and B showed higher PCV, WBC ( $P < 0.05$ ) and Plt ( $P < 0.01$ ) values. Positive and significant correlations between RBC and PCV was observed in cats of groups B ( $r = 0.86$ ;  $P < 0.01$ ), irrespective of different sex.

Functional parameters (Table 2) showed higher HR ( $P < 0.05$ ) and RR ( $P < 0.05$ ) values in cats of groups B than subjects of group A. Males of group B showed higher HR ( $P < 0.01$ ) and RR ( $P < 0.05$ ) than group A.

## DISCUSSION

The main purpose of the present study was to compare the acute versus chronic stress responses to different housing's systems of cats, by taking into account cortisol, blood count and functional changes.

Comparison of hormonal data with published values described in cats (*Felis domesticus*) revealed that cortisol concentrations were in agreement with the physiological wide ranges observed in cats and showed in Table 3. Nevertheless, slight variations might be ascribed to differences in methods and are the subject of speculation; in addition to that, some differences may also be explained by different experimental conditions and environments.

as the nature and severity of the procedure [28], stress handling techniques and familiar home versus clinic environment [23].

The most important findings of the present study were that:

1) Overall, cats housed in the households group showed higher cortisol, RBC, PCV, Plt, HR and RR values, and lower WBC rather than cattery's cats.

2) Males showed higher RBC, PCV, WBC and Plt than females, irrespective of different housing's systems.

It is then reasonable to assume that the physical component of environmental stimuli (chronic stress) may combine with the clinical visit and blood samplings (acute stress) in order to contribute to disruption of homeostasis in the animals; in this contest cortisol may be consider as a reliable physiological marker of stress in animals, including dogs and cats [26,33]. The significant lower threshold of cortisol levels in cattery's cats than household's cats could suggest that these subjects were probably totally accustomed to cattery's environment; though these animals were daily stimulated by predictable stimuli and manipulations, characterized by handling and husbandry routine, regular feeding and cleaning times, standard caretaking. Furthermore, sufficient space, proper housing complete with stimulating physical facilities and activities, active exploratory and play behaviour, cat-to-cat socialisation, and human personnel contact were probably all impor-

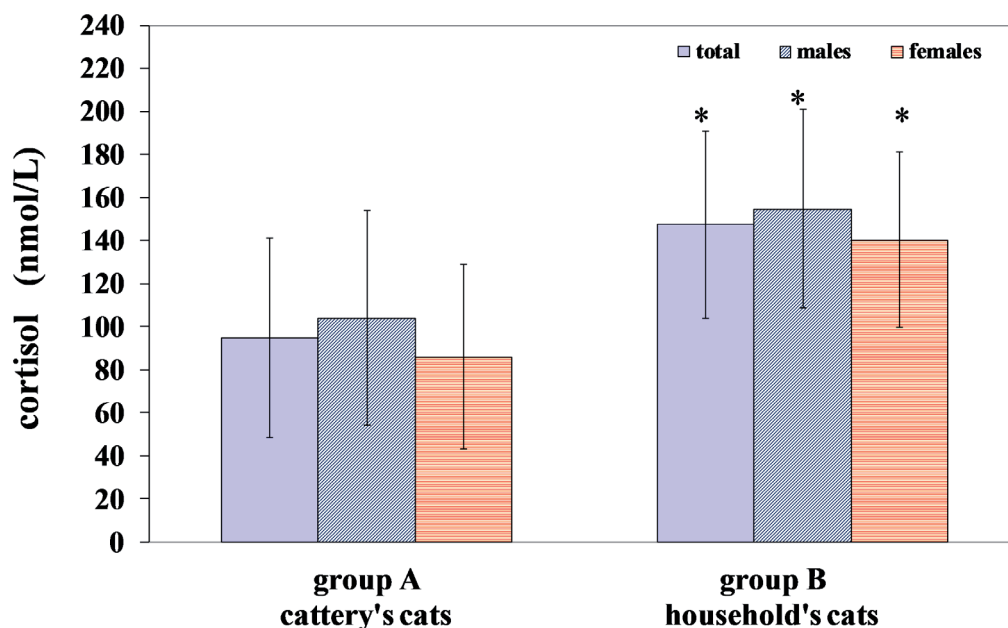


Figure 1. Circulating cortisol concentrations (mean  $\pm$  sd) of cattery cats (group A) and households (group B). vs group A: \* $P < 0.01$ .



tant elements of standard housing of group A. Evidence also suggests that the physical and mental well-beings of young domestic cats in confined facilities are greatly improved in an environment where cats can express a wide range of normal feline behaviours [13].

The lowest cortisol levels of group A becomes the major finding of this study and was attributed to the routine cattery's life, particularly under predictable conditions, as were utilized in this study.

Hence, cattery's environment, with no particular physical and emotional load, probably induced a physiological but reduced adrenocortical response. Although environmental stimuli are likely to act through the HPA axis, the neuroendocrine mechanisms involved in the cattery and household settings were different; as a matter of facts, these data suggest that housing system was, probably, a major factor contributing to cat's physiological responses.

Additionally, though behavioural data were not assessed in this study, also made note that all cats submitted to clinic visit and blood samplings showed a physiological restlessness. Furthermore, the personnel involved as well as usual clinic procedures and veterinary clinic environment should be kept as unpredictable, both in cattery's and household's cats.

Based on this evidence, however, it is possible to presume that some parameters may be more reflective of the cats' perception of their environment, rather than of underlying unpredictable stress conditions.

On the other hand, the higher cortisol concentrations observed in household's cats could be due to the different environmental stimuli, characterized by unpredictable handling, modified caretaking, presence of irregular talking, petting and manipulations by owners, which promote probably the expression of species and appropriate behaviour with stimulating activities. Hence, the opportunities to engage in a wide range of behaviours such as hiding, perching, playing with toys to simulate hunting behaviour (batting, pouncing, throwing up in the air), scratching, and playing with familiar people might induce an activation of the HPA with a significant increase in cortisol levels in household's cats, suggesting that there may be environmental stimuli-induced increase in the activity of adrenal response. This observation confirmed previous studies which showed a probably individual sensitivity to the effects of stressful stimuli, represented by different environments also in domestic cats, with a wide variability of HPA activity [7].

Obtained data showed that the different environments could provide a predictable or unpredictable setting to affect adrenocortical responsiveness. Hence, the combination of individual and social factors may affect the physiological cortisol threshold, being part of a generalized coping responses rather than a result of any specific stressful stimulus.

In this study, it is difficult to distinguish between stressed and/or unstressed cats, because the cortisol concentrations ranged between physiological values in both cattery's and household's cats. Therefore, it is possible to presume that the free access to an outdoor area (group B) represented a consistent and efficacious stimulus rather than no access to an outdoor area (group A), irrespective of cat-to-cat socialisation.

Another point is that the effects of aging on the regulation of cat's HPA axis response to challenge by stressful stimuli were not extensively investigated [6], and from a practical standpoint, the ages of cats were almost considered homogeneous in this study.

Additionally, it is worth noting that cortisol concentrations were not significantly different between males and females inside the single group, according to Kessler and Turner [16].

Certainly, the presence or absence of a circadian rhythm has not been tested experimentally and cannot be ascribed to the time of day, since all blood sampling for the different groups were performed at the same times (at 14.00 h and 15.00 h).

Related to hematological variables, ranges showed in Table 1 were in agreement with physiological values reported in literature for this species [3,4,22,23,38].

The significant highest RBC and PCV values in subjects of group B could be explained on the basis of the more intense activity of these subjects, according to the access to outdoor area. These concomitant higher values were corroborated in the present study by the not surprising positive and significant correlation observed between corresponding RBC and PCV values. The higher PCV values observed in cats of group B could be suggest that their daily frequent activity induced a physiological erythrocytosis, compared to sedentary cattery's cats.

It is generally accepted that the low pituitary-adrenal axis reactivity would be accompanied by high sympathetic reactivity [17,18]; hence, although we did not analyze the intensity and duration of daily regular

**Table 1.** Hematological variables (mean  $\pm$  sd) of male and female cats lodged in different housing systems.

Group A	Total	Males	Females
RBC (millions/ $\mu$ L)	7.70 $\pm$ 0.83	8.19 $\pm$ 1.16*	7.22 $\pm$ 0.50
Hb (g/dL)	10.16 $\pm$ 1.16	10.20 $\pm$ 1.21	10.13 $\pm$ 1.13
PCV (%)	32.27 $\pm$ 1.91	32.30 $\pm$ 2.20	32.25 $\pm$ 1.62
MCV (fL)	41.50 $\pm$ 2.82	41.00 $\pm$ 3.00	42.00 $\pm$ 2.64
MCH (g/dL)	12.74 $\pm$ 1.27	12.83 $\pm$ 0.70	12.66 $\pm$ 1.85
MCHC (g/dL)	31.45 $\pm$ 1.44	31.40 $\pm$ 0.72	31.50 $\pm$ 2.17
WBC (K/ $\mu$ L)	17.99 $\pm$ 1.11	18.73 $\pm$ 0.32*	17.26 $\pm$ 1.90
Plt (K/ $\mu$ L)	216.19 $\pm$ 2.13	246.33 $\pm$ 3.27**	186.00 $\pm$ 1.00
Group B	Total	Males	Females
RBC (millions/ $\mu$ L)	8.03 $\pm$ 0.68*	8.45 $\pm$ 0.92*	7.62 $\pm$ 0.44
Hb (g/dL)	10.69 $\pm$ 0.87	10.94 $\pm$ 1.45	10.45 $\pm$ 0.30
PCV (%)	33.88 $\pm$ 1.70**	35.62 $\pm$ 2.88*	32.16 $\pm$ 1.54
MCV (fL)	46.65 $\pm$ 1.99	46.55 $\pm$ 2.69	46.75 $\pm$ 1.31
MCH (g/dL)	15.17 $\pm$ 0.85	14.70 $\pm$ 1.10	15.65 $\pm$ 0.62
MCHC (g/dL)	33.02 $\pm$ 0.33	32.41 $\pm$ 0.11	33.65 $\pm$ 0.55
WBC (K/ $\mu$ L)	10.37 $\pm$ 0.77***	10.95 $\pm$ 0.83*	9.80 $\pm$ 0.71
Plt (K/ $\mu$ L)	325.63 $\pm$ 2.63***	335.46 $\pm$ 2.91*	315.80 $\pm$ 2.35

vs group A: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

physical activity in the present study, it is reasonable to assume that the presumptive hemoconcentration could be due to adrenergic stimulation after acute exposure to physical activity, as described in exercise-trained rats rather than sedentary subjects [31].

In addition to that, the highest Plt observed in cats of group B, could be due to the non splenic pool, confirmed that these values could be submitted to an increase according to physical activity, as observed in dogs [21].

Therefore, the lowest WBC values and the highest cortisol levels, observed in group B, remain questionable; this trend could be related to the level of endogenous (or exogenous) cortisol levels, and would represent a classical glucocorticoid/leukocyte response, characterized also by lymphopenia [34]; nevertheless, no significant correlations between cortisol concentrations and WBC counts were obtained. Moreover, it is recognized that the stress associated with handling during the blood sampling procedure can alter the RBC and increase the WBC count [24], as described in cattery's cats.

Related to functional variables, ranges showed in Table 2 were in agreement with physiological values reported in literature for this species.

The hypothesis that the home represented more rousing than cattery setting was assessed by the physiological and consistent higher cortisol, RBC, PCV, Plt, HR and RR displayed in the home environment; circumstantial evidence suggests that in this familiar environment cats are more likely to make escape attempts; this is plausible because they are likely to know the setting of the best escape routes and hiding places.

As the environmental and social conditions needed for good health and welfare to vary from individual to individual depending on personality, previous learning experience, life stage, and so on [13]. On the basis of physiological hormonal, haematological and functional ranges, no definite conclusions can be produced; hence, the question of physiological relevance concerning the effects of different environmental stimuli in cats will be established. The only difference between the two groups was that environmental stimulus (chronic stress) was cattery for group A, whereas household for group B. The only likeness between the two groups was that veterinary check and blood sampling stimuli (acute stress).

**Table 2.** Heart Rate (HR) and Respiratory Rate (breaths/min) (mean  $\pm$  sd) of male and female cats lodged in different housing systems.

Group A	Total	Males	Females
HR (bpm/min)	146.5 $\pm$ 13.30	140.2 $\pm$ 16.19*	152.8 $\pm$ 6.05
RR (breaths/min)	53.2 $\pm$ 5	53.2 $\pm$ 6.37	53.2 $\pm$ 3.96
group B	Total	males	females
HR (bpm/min)	149.27 $\pm$ 4.33*	151.8 $\pm$ 4.26**	147.16 $\pm$ 3.37
RR (breaths/min)	56.36 $\pm$ 7.06*	59.6 $\pm$ 5.31*	53.66 $\pm$ 7.60

vs group A: \* $P < 0.05$  vs females: \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 3.** Mean physiological cortisol ranges (nmol/L) of male and female cats (*Felis domesticus*)

Cats	Total	Males	Females	References
Intact			31.80 - 135.0	Chatdarong <i>et al.</i> [7]
Ovariohysterectomyzed			31.80 - 135.0	Chatdarong <i>et al.</i> [7]
Home environment	13.79 - 264.8			Nibblett <i>et al.</i> [23]
Clinic environment	27.50 - 331.0			Nibblett <i>et al.</i> [23]
Clinically healthy	38.00 - 209.0			Higgs <i>et al.</i> [14]
Domestic		275.0 - 1379.5	496.6 - 551.8	Genaro <i>et al.</i> [11]

## CONCLUSION

In conclusion, this is an observational study that showed that here is a consistent relationship between physiological responses to stressful stimuli, according to acute or chronic stressors.

Obtained data indicate that there was a marked benefit in to establish a personnel-cat relationship in addition to the traditionally owner-cat relationship, providing physiological coping strategies in both cat-tery and home cats; this was corroborate in the present study by the wide but physiological cortisol range. This study indicates that predictability, familiarity and unpredictability are significantly associated with environmental stimuli and with quality of pets' life.

## MANUFACTURERS

<sup>1</sup>Terumo Corp. Leuven, Belgium.

<sup>2</sup>APTACA s.r.l. Canelli, Italy.

<sup>3</sup>SEAC/RADIM. Florence, Italy.

<sup>4</sup>GraphPad Software Inc. San Diego, CA, USA.

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**Ethical approval.** All procedures were formally approved by Ethical Committee for the care and use of animals of Department of Veterinary Sciences, and were performed in compliance with the guidelines of the Italian law on the care and use of animals (D.L. 4/3/2014 n. 26) and EU Directive (Directive 2010/63).

**Declaration of interest.** The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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